Pathogenesis, treatment effects, and resistance dynamics in chronic myeloid leukemia - insights from mathematical model analyses

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Abstract Mathematical models and simulation studies are powerful tools to investigate dynamic properties of complex systems. Specifically, they can be used to test alternative hypotheses on underlying biological mechanisms for their consistency with real data and therefore to effectively guide the design of new experimental strategies or clinical trials. In this study, we present an overview of recently published mathematical approaches applied to the description of chronic myeloid leukemia (CML). We discuss three different fields relevant to clinical issues: the pathogenesis of the malignancy, the treatment effects of the tyrosine kinase inhibitor *imatinib*, and the process of acquired treatment resistance highlighting both the differences and the consistencies in the proposed hypotheses and the resulting conclusions. The mathematical models presented agree that CML can adequately be described as a clonal competition between normal and leukemic stem cells for a common resource. Furthermore, a certain therapeutic effect of *imatinib* on leukemic stem cells turned out to be necessary to consistently explain clinical data on the long-term response of CML patients under *imatinib* treatment. However, the approaches described cannot resolve the question whether or not this effect is sufficient to ultimately eradicate malignant stem cells. A number of different hypotheses have been proposed concerning the initiation and the dynamics of treatment-resistant malignant stem cell clones. The theoretical results clearly indicate that further experimental effort with the particular focus on the quantitative monitoring of resistant clones will be required to definitely distinguish between these hypotheses.

Keywords Chronic myeloid leukemia (CML) · Imatinib treatment · Resistant clone dynamics · Mathematical model · Simulation analysis

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Introduction

Our increasing knowledge concerning the molecular mechanisms underlying the pathogenesis of many types of cancers is providing the basis for the development of a new generation of therapeutic strategies. One promising trend in cancer treatment involves therapies that apply specifically targeted compounds (e.g., small molecules) that are able to interfere with signaling pathways controlling the development of malignant cells. In contrast to classical chemotherapies, these molecularly targeted treatment protocols allow for a highly specific growth inhibition of the malignant cells while mostly sparing the healthy tissue. Although, in many cases, much detail of the molecular properties of the therapeutic molecules is known, the resulting effects and the regulatory responses on the cell population level (i.e. the system dynamics) remain unclear.

At this point, mathematical model analyses and simulation studies provide powerful complementary means to achieve a deeper understanding of treatment effects and to foster a comprehensive understanding of the regulatory mechanisms. Most importantly, such approaches allow for the virtual testing of new experimental settings or clinical protocols and can lead to the formulation of new, testable hypotheses. Furthermore, they are able to rigorously check the consistency of proposed biological mechanisms with experimental and clinical data. For an overview on theoretical approaches with respect to stem cell organization, cancer progression, molecular regulation, and disease modeling in the hematopoietic system, the reader is referred to recent review articles [1–4].

It is the objective of this review to provide a systematic overview of recently published theoretical results and their biological implications with a particular focus on the disease dynamics of *imatinib*-treated chronic myeloid leukemia (CML). To elucidate the contribution of the theoretical methods for the identification of general regulatory principles of disease dynamics and treatment effects, our own results will be discussed in relation to the works of other groups. In addition to describing CML pathogenesis, the models considered focus on a quantitative description of treatment effects and of the generation and expansion of treatment-resistant clones.

CML is a clonal hematopoietic disorder that is characterized in the majority of cases by the Philadelphia (Ph) chromosome generated by a reciprocal translocation of chromosomes 9 and 22. This genetic aberration results in the formation of the *BCR-ABL1* fusion gene, which codes for a constitutively active form of the ABL1 kinase [5–7]. Because the BCR-ABL1 protein activity is no longer under the control mechanisms for ABL1, it induces the unregulated activation of multiple cytoplasmic and nuclear signaling pathways that are respon-

sible for cell proliferation and apoptosis regulation [8–11]. Furthermore, BCR-ABL1 impairs cellular regulation by external growth factors [12], modifies cell adhesion properties [13, 14], and affects various DNA repair mechanisms [15, 16]. For a detailed review of the molecular effects of BCR-ABL1, the reader is referred to Kantarjian et al. [17]. As the result of these multiple effects, the translated BCR-ABL1 fusion protein is responsible for an expansion of the malignant clone and the ultimate displacement of normal hematopoiesis.

Currently the standard therapy for CML is the tyrosine kinase inhibitor *imatinib* [18–20], which represents a prominent example of the molecularly targeted approaches mentioned above. *Imatinib* selectively inhibits BCR-ABL1 action by blocking the adenosine triphosphate-binding site of the ABL1 domain, thereby repressing the tyrosine kinase activity [21]. On the cellular level, these mechanisms lead to a selective inhibition of cell proliferation [22, 23] in BCR-ABL1-positive cells. An additional apoptosis-inducing effect of *imatinib* (preferentially in actively dividing cells) is discussed controversially in the literature [24–27]. However, there is evidence that *imatinib*-mediated apoptosis can be enhanced by growth factor stimulation of progenitor cells [28].

Particularly for newly diagnosed patients in chronic-phase CML, *imatinib* shows a very good therapeutic efficiency, inducing complete hematologic and cytogenetic remission in the majority of patients [17, 29, 30]. Despite these encouraging results, acquired (secondary) resistances to *imatinib* treatment represent a major problem [29, 31]. A number of different mechanisms underlying the development of resistance have been suggested. They comprise *BCR-ABL1* gene mutations [32, 33], overexpression and amplification of the *BCR-ABL1* locus [32, 33], activation of BCR-ABL1–independent pathways (e.g., including the Src kinase family [34]), binding of *imatinib* to serum-1 acid glycoprotein [35], increased drug efflux (e.g., by the multidrug resistance protein [36, 37]), or pharmacokinetic resistance [31]. Within this spectrum of possible resistance mechanisms, those mutations in the ABL1 kinase domain that inhibit the binding of *imatinib* molecules appear to play a prominent role [31, 33]. Currently, about 50 different mutations of the ABL1 domain have been identified by in vitro screening methods [38]. Whereas some of these mutations hamper *imatinib* binding directly by the substitution of individual ABL1 kinase domain residues, other mutations alter the spatial conformation of the protein.

To cope with the problem of acquired *imatinib* resistance, enormous effort is being invested in the design of novel therapeutic compounds able to target malignant cells that exhibit resistance to *imatinib* with the emphasis on new, targeted tyrosine kinase inhibitors with activity against, e.g.,

BCR-ABL1 and Src kinases (for an overview see [17]). The most advanced studies in this respect to date are related to the compounds *dasatinib* and *nilotinib* [17, 39].

So far, it is still unclear whether molecularly targeted therapies are in general able to cure the disease or whether they can only (transiently) control the expansion of the leukemic clone. In other words, we do not know whether the therapeutic compounds are able to target leukemic stem cells effectively. Closely linked to this problem is the question of why some subpopulations of leukemic cells are treatment insensitive and whether the drug sensitivity of leukemic cells can be deliberately manipulated. Furthermore, it is currently not known whether different malignant clones (e.g., clones with distinct resistance mutations) interact with each other or with the non-resistant clone(s) and whether the eradication of one malignant clone might facilitate the expansion of other, potentially more aggressively growing clones.

Motivated by these clinically relevant questions, we will focus the discussion on mathematical models of CML dynamics to three different fields: the pathogenesis of the malignancy, the treatment effects of the tyrosine kinase inhibitor *imatinib*, and the process of acquired treatment resistance. We will highlight not only the qualitative differences but also the areas of overlap between the proposed hypotheses and consider the resulting conclusions.

CML pathogenesis

There is a broad consensus that CML is a clonal disorder (i.e., induced by a chromosomal translocation of a single cell) on the level of hematopoietic stem cells. As demonstrated by a number of different mathematical modeling approaches [40–43], the assumption of a single leukemia-initiating cell can consistently explain the expansion kinetics of the malignant clone in myeloproliferative disorders such as CML. However, the assumed differences in the cellular properties induced by the molecular defect differ considerably between the proposed models.

Within a model based on the development of two distinct cell populations (i.e., the normal and the malignant clone), Catlin et al. [40] suggest that cellular parameters underlying amplification, differentiation, and apoptosis are identical for all cells. The simulation results demonstrate that the growth advantage necessary to achieve the ultimate dominance of the malignant clone can be achieved solely by additional microenvironmental resources that can be utilized by the malignant stem cells only. To be more precise, Catlin et al. suggest that the growth of the normal stem cell population is restricted to a certain upper limit, while the malignant stem cell population is able to grow without bounds. Because the restricted resources for normal stem cells are also used by the malignant cells, a competition with an ultimate displacement of the normal clone is induced. Due to the fact that Catlin et al. describe cellular development as a stochastic process, using probabilities of amplification, differentiation, and apoptosis rather than deterministic rates, only about 15% of all initiated malignancies (i.e., induced by the alteration of a single cell) lead to the manifestation of the disease. It should be noted that the model by Catlin et al. is based on considerations of general myeloproliferative disorders in mice and cats. A direct application of these results to the particular situation of human (chronic myeloid) leukemia should therefore be regarded with caution. However, they still provide important conceptual insights into generic mechanisms that may potentially lead to malignancies, and in this sense, the results are relevant also for the human situation.

Using another mathematical approach that had been adapted specifically for the situation of human CML, Michor et al. describe the disease dynamics on the basis of a deterministic compartment model assuming a completely independent development of normal and malignant cells [41]. Their approach relies on the assumption of a unidirectional differentiation hierarchy of normal and malignant cells, consisting of four consecutive cell populations: long-term repopulating stem cells, progenitor cells, differentiated cells, and terminally differentiated cells. Whereas normal cells establish a homeostatic equilibrium with constant population size, malignant cells expand exponentially without an upper limit. Whereas the cell death (e.g., apoptosis) rates are considered to be identical for normal and malignant cells, different parameter values are assumed for the control of amplification and differentiation of these two cell types. Within this paradigm, every induction of a malignant clone by a single cell inevitably leads to an ultimate dominance of the leukemic cells. To account for a random component in the process of leukemia induction, it is appropriate to describe the initial mutation as a stochastic event, as described for the resistance initiation within the same model [41].

Building on the same general concept, Dingli and Michor [42] presented another deterministic model of cancer stem cell development that, in contrast to the earlier approach, assumes a clonal competition of normal and malignant stem cells. Notably, they included the assumption that all stem cells compete for a common resource. Induced by a more effective use of available resources by the malignant cells, this model (in common to that proposed by Catlin et al.) predicts a slow but unavoidable displacement of the normal cell population. As for the earlier differential equation model proposed by Michor et al. [41], the deterministic description of leukemogenesis leads to the manifestation of the disease whenever a single stem cell has experienced a leukemic transformation.

A different model approach to describe CML pathogenesis has been suggested by our own group [43]. Similar to Catlin et al., the model assumes a stochastic competition of normal and malignant stem cells for a common resource (e.g., available stem cell supporting niches). However, additional resources for the malignant clone are not assumed. Within our single-cell-based description, the growth advantage of the malignant clone is induced by different regulatory control characteristics of stem cells. Specifically, two differences are assumed: First, malignant cells have a slightly altered regulation of stem cell/microenvironment interaction, inducing an advantage in the competition for the common microenvironmental support. Whereas this assumption is sufficient to generate clonal expansion with an ultimate displacement of the normal cells, it is not able to explain an increased production of differentiated cells, as observed in CML. To account for an increased production of differentiated cells, we make a second assumption of an impaired regulation of proliferating activity in malignant cells resulting in malignant, but not normal stem cell proliferation being independent of the number of stem cells. As in the model of Catlin et al., the stochastic nature of cellular development leads to the prediction that only a minor proportion of initial single cell mutations (about 20%) develop into a macroscopically detectable leukemia.

Although the majority of the models discussed agree that there is a competition process between normal and malignant stem cells with a competitive advantage for the malignant clone, alternative explanations for the nature of this growth advantage are suggested (cf. Table 1). Furthermore, the available model descriptions demonstrate that both limited and unlimited growth potential of the malignant cells are in principle compatible with the clinical phenomenology. Partly due to the (inherent) lack of available quantitative data on the growth kinetics of leukemic clones in patients before diagnosis, it is not possible to distinguish the proposed concepts without considering further phenomena. In the following sections, we will therefore describe some recent simulation studies (partly based on the aforementioned models) analyzing the dynamic effects induced by therapeutic interventions.

Therapeutic effects of *imatinib*

As already mentioned in the introduction, the tyrosine kinase inhibitor *imatinib* is the current standard therapy for patients diagnosed with CML. Although the molecular mode of action of this compound is fairly well known, the induced effects at the systemic level, particular with respect to the clonal dynamics, are incompletely understood. The discussion centers on the question of whether or not the pool of malignant, long-term repopulating stem cells can be effectively targeted by *imatinib*. This question has a strong clinical impact, as an inherent insensitivity of CML stem cells to *imatinib* implies that this type of therapy could not result in a definite cure. If this is true, one could speculate that other *imatinib*-derived compounds will have similar deficiencies. There are three recent publications [41, 43, 44] that discuss *imatinib* sensitivity of long-term repopulating stem cells from a theoretical perspective. These simulation studies build on mathematical models of the CML pathophysiology that have been discussed in the previous section.

Michor et al. [41] analyzed the disease dynamics under the assumption that the malignant stem cell population continues exponential expansion even under *imatinib* therapy. To achieve a consistent explanation of clinical data on *BCR-ABL1* transcript levels of *imatinib*-treated CML patients, they assume that *imatinib* reduces the production rate of progenitor and differentiated cells, but not of long-term repopulating stem cells. Specifically, this assumption is based on the simultaneous explanation of the observed treatment-induced bi-phasic decline in the *BCR-ABL1* transcript levels during the first year of therapy and the rapid relapse after treatment cessation, which had been described in a small number of patients. The consistency of their model results with the clinical observations led the authors to conclude that leukemic stem cells are *imatinib*-insensitive.

However, this model fails to account for more recent clinical data on the long-term dynamics of *BCR-ABL1* transcript levels that show a continued decline at least until the fourth year of imatinib therapy [45]. The model

Table 1 Alternative model assumptions for the pathogenesis of clonal disorders

	Catlin et al. [40]	Michor et al. [41]	Dingli and Michor [42]	Roeder et al. [43]
Limited resources for malignant stem cells	No	No	Yes	Yes
Differences in cellular growth parameters between normal and malignant stem cells	No	Yes	Yes	Yes
Clonal competition for common stem cell supporting resources	Yes	No	Yes	Yes

assumptions used by Michor et al. [41] lead to an unperturbed exponential growth of the malignant stem cell clone under therapy. This expansion is responsible for the predicted relapse of the *BCR-ABL1* levels beyond 1 year of treatment, even under continuing *imatinib* administration. In response to this, it has been demonstrated by Michor [44] that the clonal competition model by Dingli and Michor [42] described above can be extended in such a way to be consistent with the available long-term data. However, the adopted model does need to assume a treatment effect of *imatinib* on the growth kinetics of malignant stem cells to simultaneously account for reasonable growth rates of the malignant clone before diagnosis and for the long-term treatment dynamics. It is important to note that even without the occurrence of any resistance mutation, the remaining competitive advantage of the malignant stem cells, as described in [44], predicts an ultimate, albeit delayed, increase of *BCR-ABL1* levels (Fig. 1a).

There is an alternative explanation for the clinical data. As demonstrated by our group, a direct *imatinib* effect on stem cells that induces a long-term exhaustion of the malignant clone is compatible both with the short- and long-term *BCR-ABL1* transcript dynamics observed under *imatinib* and with the relapse dynamics after treatment cessation [43]. The key assumption of this model is the possibility of reversible changes in the proliferative status of long-term repopulating stem cells which, although mostly in a quiescent state, are recruited into cell cycle from time to time. At least for murine systems, there is strong evidence for such a stem cell turnover, as demonstrated by continuous bromodeoxyuridine (BrdU) labeling studies [46, 47]. Based on this type of functional heteroge-

neity within the stem cell population, we assume two *imatinib* effects that selectively target proliferative, but not quiescent, stem cells: a cell kill or degradation and an inhibition of the proliferative activity of BCR-ABL1 positive stem cells. These assumptions lead to a moderate but continuing decline of the malignant stem cell clone, completely consistent with the clinically observed *BCR-ABL1* transcript dynamics. Contrasting the above described models, a continued decrease of the BCR-ABL1 levels with an ultimate eradication of the malignant clone by *imatinib* treatment is predicted (Fig. 1a). However, the average time to such a cure event has been estimated to be more than 20 years. Another important implication of this model is the predicted possibility to augment the therapeutic benefit of *imatinib* by a combination with compounds that stimulate the proliferative activity of otherwise quiescent stem cells. This hypothesis is supported by experimental evidence that *imatinib* sensitivity of quiescent stem cells might be enhanced by growth factor stimulation [28, 48, 49]. Simulations of such combination treatments predict the feasibility of significantly enhancing the treatment response and reducing the average time to exhaustion of the malignant clone to about 3 years [43].

Although the models by Michor [44] and by Roeder et al. [43] provide qualitatively different predictions regarding the potential exhaustion of the malignant clone by *imatinib*, it should be noted that both are consistent with the clinically observed phenomena of a minimal residual disease [50–52]. After achieving a reduction in *BCR-ABL1* transcript levels to below 0.1%, both models predict that these levels remain (under continued *imatinib* administration) in the range of 0.001 to 0.1% for about 10 years
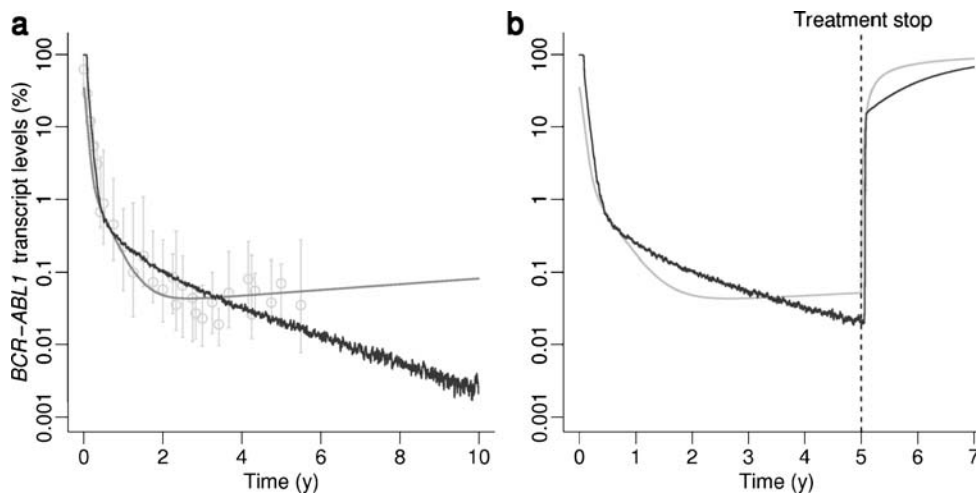

Fig. 1 Comparison of simulation results on *BCR-ABL1* transcript dynamics. Data points represent medians and interquartile ranges of *BCR-ABL1/ABL1* percentages in subjects in the German cohort of the IRIS study, previously published in [43]. *Solid lines* represent predicted *BCR-ABL1* transcript levels (population median) based on the two different mathematical models proposed by Michor [44] (*gray*) and by Roeder et al. [43] (*black*). Whereas **a** illustrates the long-term dynamics for continued *imatinib* treatment, **b** shows the corresponding model predictions for treatment cessation after 5 years of treatment

(cf. Fig. 1a). The two models also provide qualitatively similar results regarding the relapse dynamics after treatment cessation (Fig. 1b), and it is questionable whether the predicted quantitative difference could be detected by the presumably low number of patients with treatment cessation. A related phenomenon is the leveling off of median *BCR-ABL1* transcript levels in the patient population beyond 3.5 years of treatment, which might also be explained by either of the two models. On the one hand, such an observation could result from a slowly increasing pool of malignant stem cells as predicted by the model of Michor [44]. On the other hand, it might also be the result of an increasing degree of patient heterogeneity due to the occurrence of acquired treatment resistance and further relapse-inducing transformations as discussed by Roeder et al. in [43]. Based on the currently available data, a distinction of these two hypotheses is not possible and would require more detailed data from simultaneous monitoring of individual *BCR-ABL1* transcript levels and of resistance mutations. A summary of the model assumptions and predictions discussed is given in Table 2.

It should be noted that all the model scenarios discussed above are based on the assumption that no treatment resistance exists. The following section will demonstrate how the effects of treatment-resistant clones can be incorporated into the mathematical modeling.

Dynamics of resistant clones

Although the use of tyrosine kinase inhibitors, such as *imatinib*, has lead to a dramatic improvement in the treatment of CML, the occurrence of acquired treatment resistance still presents a major challenge. Among the different mechanisms of treatment resistance (see "Introduction"), the generation of resistant clones initiated by point mutations in the ABL1 kinase domain makes a major contribution. A key question with relevant clinical implications is whether the resistance mutations are initiated after treatment start or whether they already exist before treatment. The latter option is suggested

by observations that mutated clones, detected at relapse, were also found in samples taken before *imatinib* treatment in some patients [53, 54]. A closely related issue is the question of whether treatment sensitive and resistant clones grow independently of each other or whether clonal competition influences the regulation of the clonal dynamics. Regarding this problem, two hypotheses are under discussion. The first suggests the independent growth of resistant clones, possibly induced by additional, treatment-independent growth advantages [33, 53]. In contrast, the second hypothesis proposes that *imatinib* therapy might favor or even initiate the expansion of resistant clones, possibly due to the selective pressure induced by the treatment [55]. Clonal competitions of this nature might also explain the observation that the interruption or cessation of *imatinib* therapy can induce a beneficial effect in some patients. In such cases, the treatment discontinuation resulted in a decreasing size of the resistant clone [55] or in a reversion to chronic phase after previous progression to blast crisis [56]. Following the same line of argument, one might ask whether a possible combination of tyrosine kinase inhibitors with cytotoxic compounds or cytokines augments the therapeutic success or whether it can potentially promote the expansion of treatment resistant clones. An additional unresolved and related issue is whether *imatinib* should be substituted by new types of tyrosine kinase inhibitors (such as, e.g., *dasatinib* or *nilotinib*) or whether a combination would be more efficient. In the following, we will discuss three recently proposed mathematical model analyses that aim at a deeper understanding of the dynamics of treatment resistant clones.

Komarova and Wodarz [57, 58] published an elaborate analysis of resistance emergence within the context of a conceptual framework that uses a stochastic birth and death process to describe the development of the malignant clone. Similar applications of stochastic processes within mutation networks have also been proposed for more general biomedical interventions [59]. Komarova and Wodarz apply these general results to the particular example of *imatinib*-treated CML. Herein, they assume fixed birth and death intensities (probabilities of cell division/cell death per time

Table 2 Alternative model assumptions and predictions for the *imatinib* mode of action

	Michor et al. [41]	Roeder et al. [43]	Michor [44]
Model assumptions on *imatinib* effects on (long-term repopulating) leukemic stem cells	No effect on growth kinetics	Proliferation inhibition and degradation (for actively proliferating cells only)	Alteration of competition characteristics or growth rate[a]
Prediction of *imatinib*-induced net reduction of leukemic stem cell population	No reduction (instead: continued exponential expansion)	Slow, but sustained reduction	Slow, but sustained expansion

[a] To derive a scenario for CML pathogenesis with reasonable latency times based on the given model, an additional alteration of the net growth rate of malignant stem cells is necessary. This can be achieved by changing either the growth rate coefficient or the competition characteristic, which has to be interpreted as an *imatinib* effect on leukemic stem cells. The relevant pathogenesis scenario has not explicitly been shown in [44].

step) to characterize the development of the malignant clone. Treatment effects are modeled by an additional component of the cell death intensity. Resistant clones, which do not experience this additional death component, are initiated by a secondary mutation occurring with a certain probability per cell division. Under the assumption of an efficient therapy, the cumulative number of cell divisions in the malignant clone decreases rapidly so that the probability of secondary mutations leading to resistance is reduced. The authors draw the conclusion that the probability of treatment failure (i.e., the establishment of at least one resistant clone) is higher for resistance mutations occurring before treatment starts than it is for resistance mutations induced during therapy. Mutations occurring during therapy only become relevant if the time under treatment is longer than the time from tumor induction to treatment start. This condition has been assessed as unrealistic by Komarowa and Wodarz. However, with respect to *imatinib*-treated CML, the time to the eradication of the malignant clone (if at all possible) is expected to be extremely long. Therefore, it is questionable whether the general conclusion drawn by Komarova and Wodarz applies to this particular situation. On the other hand, it should also be considered that *imatinib* treatment is assumed to decrease considerably the proliferative activity of malignant cells [23]. Thus, it can be expected that the number of cell divisions within the malignant clone might be dramatically reduced, which would in principle decrease

the rate of resistance initiation under therapy. Another important point that is discussed by Komarova and Wodarz is the application of drug combinations to prevent resistance occurrence. For the particular case of *imatinib*-treated CML, the authors find that a combination of *imatinib* with two further drugs that target different types of *imatinib*-resistant clones should prevent resistance and ensure successful therapy even for advanced disease stages. However, this result is based on the assumption that there is no cross-resistance of the drugs. Because at least a partial cross-resistance has to be assumed for currently available compounds (such as *dasatinib* or *nilotinib*), the calculations would have to be modified. Still, it will be possible to apply the proposed theory to predict optimal drug combination regiments as soon as appropriate clinical data is available.

Michor et al. [41] also discuss the situation of treatment resistance by considering stochastic mutation events within the context of their above-described deterministic disease model. Assuming that resistance mutations do not induce an additional growth advantage before therapy, they calculated that about 13% of patients harbor at least one (out of 40 different) resistance mutations at the time of diagnosis. Because the probability of accumulating resistance mutations is assumed to be related to cell cycle activity and therefore to the expansion of the leukemic stem cell population (which is a presumption of the underlying model), it can be expected that at some point in time, all patients will have acquired at least one secondary mutation.
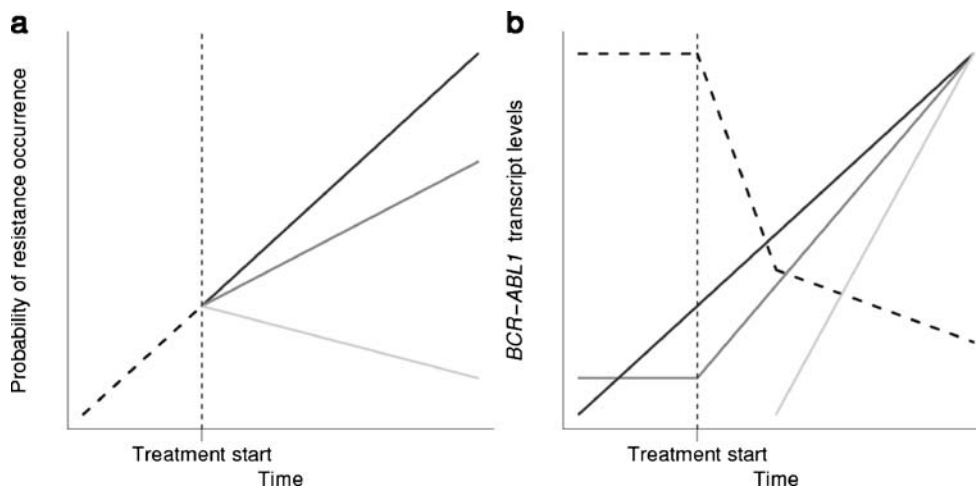

Fig. 2 Schematic illustration of the probability of resistance occurrence and of the growth dynamics of treatment resistant clones depending on the underlying model. **a** Total probability of resistance occurrence (on logarithmic scale) in the leukemic stem cell population: Exponential increase due to expanding clone size before treatment start (*dashed line*); unrelieved increase under treatment if the leukemic stem cell population continues to expand (*black line*); quantitative reduction but still increasing in case of a proliferation inhibition of leukemic stem cells that does not induce a net reduction of the cell population (*dark gray line*); decrease of the total probability

of resistance occurrence if therapy reduces the proliferative activity together with an effective reduction of the number of leukemic stem cells (*light gray line*). **b** Typical qualitative growth kinetics (on logarithmic scale) of resistant clones in relation to the "native" treatment-sensitive clone (*dashed line*): Treatment pre-existing clone that harbors an inherent growth advantage (*black line*); treatment pre-existing clone without inherent growth advantage competing with "native" malignant clone for a common resources (*dark gray line*); Resistant clone with (strong) inherent growth advantage initiated during treatment (*light gray line*)

This would explain the increasing incidence of resistance in patients starting *imatinib* therapy at later stages of the disease. Because the model by Michor et al. [41] assumes an ongoing expansion of the leukemic stem cell population even under *imatinib* therapy, it necessarily predicts that the accumulation of resistance mutations cannot be decelerated by the treatment (cf. Fig. 2a). The same model has been applied to the case of inherent growth advantages of resistant clones. Because these clones expand faster than other leukemic stem cells, this might explain accelerated tumor growth during therapy. As another possible reason for this behavior, the authors propose a somewhat reduced growth rate of leukemic stem cells induced by the *imatinib* treatment.

The model of Roeder et al. [43] offers an alternative explanation for the accelerated growth of resistant clones under therapy, without assuming a growth advantage of the resistant clone (despite the resistance itself). Specifically, it can be shown that treatment initiation is able to cause clonal expansion of pre-existing resistant clones (cf. Fig. 2b). Without treatment effects, the growth of these clones is restricted by the neutral competition with the much larger "native" malignant clone. During treatment, however, the selective pressure on the non-resistant (native) clone leads to a rapid expansion of the resistant clone. Moreover, in contrast to the situation during primary disease initiation, the number of non-malignant competitor stem cells is already dramatically reduced, allowing for an accelerated expansion of the resistant clone compared to primary disease induction. Furthermore, the application of this model demonstrates not only that resistant clones arising before treatment but also those generated during treatment are compatible with clinically observed *BCR-ABL1* dynamics [43]. The existence of different types of resistant clones might relate to the observed patient heterogeneity with respect to treatment efficiency and relapse dynamics. The model results suggest that this heterogeneity can be attributed to varying degrees of resistance and to different time points of resistance initiation. However, a confirmation of this hypothesis would require further clinical data on the dynamics of resistant clones. A quantitative assessment of resistance occurrence in terms of probability distributions has not been realized within this study. A summary of the model assumptions and predictions discussed is given in Table 3.

Concluding remarks

Although each of the presented mathematical models considered is consistent with particular clinical observations, they still remain hypotheses. Indeed, it is the aim of such modeling studies to generate alternative, testable hypotheses and to provide a "road map" for distinguishing the hypotheses, which can effectively guide further experimental and clinical research.

Despite the fact that the models partly rely on different assumptions, there is clearly some overlap in the explanations that they provide. Such overlaps between independently derived conclusions strengthen the evidence for the proposed explanation. Specifically, there is consensus that CML can adequately be described as a clonal competition of stem cells for a common resource, an example of which might be the stem cell niche space. This interpretation is also supported by the experimental observation that leukemic stem cells exhibit an altered interaction with their local (stroma) environment [13, 14]. All the same, to definitely decide whether normal hematopoiesis is ultimately displaced by the malignant clone, it will be necessary to obtain quantitative measurements of the absolute stem cell numbers within the bone marrow. Furthermore, it remains impossible to distinguish whether or not the competitive advantage of leukemic stem cells is determined by additional resources (e.g., different niche environments) based on the current model analyses.

The question of whether *imatinib* targets leukemic stem cells remains unresolved. However, particularly to achieve a consistent model explanation of both CML genesis and long-term *BCR-ABL1* transcript dynamics under *imatinib*, the assumption of a certain therapeutic effect on leukemic stem cells has turned out to be necessary within the context of the proposed models. Whether the same conclusion can be derived for a scenario with an independent (i.e., non-competitive) but limited growth potential of leukemic and normal stem cells still needs to be analyzed, as this

Table 3 Alternative model assumptions/predictions for initiation and growth characteristics of resistant clones

	Komarova and Wodarz [57]	Michor et al. [41]	Roeder et al. [43]
Probability of occurrence of resistant clones in the population of stem cells	High prior to treatment due to large number of cell division events	Continuously increasing due to exponentially growing stem cell population	Not analyzed
Model assumptions on interaction of individual clones	Clonal independence	Clonal independence	Clonal competition

particular scenario has not been considered in the models currently available.

The strength and, therefore, the clinical relevance of the proposed *imatinib* effect on leukemic stem cells remain controversial. In this study, it is still an open question whether or not the *imatinib* effect is sufficiently pronounced to induce a decrease of the leukemic stem cell burden. Current clinical data do not allow a distinction of these two hypotheses because a detectable difference in the *BCR-ABL1* transcript dynamics under both scenarios is predicted to become obvious only after more than 5–6 years of treatment (cf. Fig. 1). Beside the direct determination of leukemic stem cell numbers in the bone marrow over time (which is problematic in patients), a promising strategy suggested by the theoretical results to resolve this question is a detailed analysis of the patient heterogeneity with respect to the *BCR-ABL1* transcript dynamics and, in particular, their correlation with the occurrence of resistance mutations. If the absence of resistance mutations can be shown to be a prognostic factor for a sustained decrease of *BCR-ABL1* transcript levels in individual patients, this would clearly favor the hypothesis of a relevant *imatinib* effect that is able to decrease the pool of leukemic stem cells. A sensitive test of the particular hypothesis that *imatinib* is able to eliminate proliferating cells even in the leukemic stem cell population [43] would be a clinical test of a combination of *imatinib* with growth factors that stimulate proliferation. There is increasing experimental evidence [28, 49] that this is a promising strategy.

With respect to the occurrence of treatment-resistant leukemic clones, two major questions have been addressed by the mathematical approaches discussed in this paper: the time point of resistance occurrence and the growth characteristics of these clones relative to non-resistant cells. Regarding their clinical implications, the theoretical analyses demonstrate that these questions are closely linked and need to be considered simultaneously to assess potential risks. To illustrate this, it is useful to consider the case in which resistance is initiated by cell cycle-related mutation events of leukemic stem cells. Here, the cumulative probability of new resistance mutations can be diminished by reducing the population of leukemic stem cells. However, for the situation in which the expansion of a particular, pre-existing resistant clone strongly depends on the size of its leukemic competitor clones (see Fig. 2b, dark gray line), a maximal reduction of the tumor burden might be fatal in cases where no effective treatment for this particular resistance type is available. In contrast, if those non-treatable clones could be identified as late-appearing (i.e., arising during therapy), the rapid reduction of the "native" leukemic clone would still be most beneficial in reducing the risk of treatment failure. This reasoning relies on the assumption that resistance mutations are generated solely within the leukemic clone. Although this seems likely, the possibility that mutations also arise from normal cells cannot be excluded without the analysis of unique clonal markers.

It should be noted that the variety of possible resistance scenarios proposed by the mathematical models clearly point to the current lack of adequate clinical data for a strict testing of the alternative theoretical results. This is particularly true of a systematic (quantitative) monitoring of resistant clones. However, a number of new experimental methods are currently being developed [60–63], and it can be expected that they will allow a better adaptation and verification of the mathematical models within the next year.

Summarizing the theoretical results presented, we have demonstrated that the application of mathematical models is able to enhance the understanding of general disease mechanisms and to elucidate the modes of action of therapeutic compounds. This is particularly true for the assessment of effects at the systemic level. The formulation of alternative model assumptions (i.e., working hypotheses) that equally well explain the same datasets foster the scientific process by providing the rational for specifically targeted experimental and clinical studies. However, it should be stated clearly that the practical relevance and the predictive power of mathematical models crucially depend on the available knowledge of the basic biological mechanisms and that the validation or invalidation of model assumption requires carefully designed experiments and clinical trials.

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