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Mathematical modeling of the impact of cytokine response of acute myeloid leukemia cells on patient prognosis

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Acute myeloid leukemia (AML) is a heterogeneous disease. One reason for the heterogeneity may originate from inter-individual differences in the responses of leukemic cells to endogenous cytokines. On the basis of mathematical modeling, computer simulations and patient data, we have provided evidence that cytokine-independent leukemic cell proliferation may be linked to early relapses and poor overall survival. Depending whether the model of cytokine-dependent or cytokine-independent leukemic cell proliferation fits to the clinical data, patients can be assigned to two groups that differ significantly with respect to overall survival. The modeling approach further enables us to identify parameter constellations that can explain unexpected responses of some patients to external cytokines such as blast crisis or remission without chemotherapy.

Acute myeloid leukemias (AML) comprise a heterogeneous group of malignant diseases. Since major clinical symptoms originate from impairment of healthy blood cell production, it is important to understand how leukemic cells interfere with healthy hematopoiesis. Clinical and genetic observations reveal a strong heterogeneity among individual patients. One reason for the observed heterogeneity may be differences in cytokine dependence of leukemic cells, i.e., cells of some patients require cytokines to expand (cytokine-dependent leukemic cells) whereas others exhibit autonomous (cytokine-independent) growth.

The idea that cytokine dependence of leukemic cells differs between patients is supported by experimental results. Xenotransplantation assays reveal that some leukemia samples exclusively engraft in mice transgenic for human cytokines and not in standard NSG mice[1,2]. Similarly, *in vitro* studies imply that leukemic cells of some patients exhibit autonomous growth in cell cultures whereas others require cytokines to expand[3–5]. The correlation between cytokine-dependence in cell culture and patient survival suggests that cytokine dependence of leukemic cells may be a clinically meaningful parameter[4,5]. However, it can depend on the culture conditions whether a leukemia sample exhibits autonomous growth or not[3]. Clinical trials also suggest that cytokine dependence of leukemic cells differs between patients. In principle, exogenous cytokine administration could recruit cytokine-dependent leukemic cells into cell cycle and thus increase efficacy of S-phase specific cytotoxic drugs[3]. However, clinical trials show that this approach, also referred to as "priming", works in some but not in all patients. Some trials report an improved rate of complete remission, disease free survival and rarely also overall survival after priming[6], whereas others report no effect[7–9]. A direct measurement of the increase of blasts in S-phase after cytokine administration confirms this heterogeneity[10]. More detailed studies suggest that the impact of priming may depend on the patient subgroups defined e.g., by risk scores[11–14].

Cytokine administration has become a widely used supportive strategy to prevent chemotherapy-related neutropenia[6]. In this context the question arises whether cytokines could potentially stimulate leukemic cells that survived therapy and trigger relapse. Although studies in AML patients suggest that leukemic cells can be recruited into cell cycle in response to administered cytokines[6,10,15], multiple clinical trials imply that supportive cytokine treatment has no negative effects on relapse free survival[6]. Nevertheless, there exist trials and case reports stating that in some patients administration of cytokines or their analogues increases leukemic cell load or reduces relapse free survival[16–18]. Different genetic hits accounting for that have been identified so far[17,19,20].

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On the other hand, there exist reports of patients achieving complete remission solely by cytokine administration without chemotherapy[21–24]. Both phenomena, negative and positive impact of cytokines on leukemic cell load, are so far not well understood.

The aim of this work is to study if cytokine dependence of leukemic cells has an impact on the clinical course of the disease. For this purpose, we compare disease dynamics in case of cytokine-dependent (i.e. leukemic cells require endogenous cytokines to expand) and cytokine-independent (i.e. leukemic cells can expand in absence of endogenous cytokines) AMLs using mathematical models. We focus on the following questions: (i) How does time evolution of blasts differ in mathematical models of cytokine-dependent and cytokine-independent AML? (ii) Does it have a prognostic impact if patient data fits to the model of cytokine-dependent or to the model of cytokine-independent AML? (iii) Which cell parameters determine whether cytokine administration may have negative, neutral or positive effects on the leukemic cell load?

To approach these questions, we develop new mathematical models of cytokine-dependent and cytokine-independent AML and apply them to patient data showing time changes of bone marrow blast counts between first remission and relapse. Comparing the two models we identify key dynamic features that may help to distinguish between both scenarios. Model-based patient data analysis suggests that the overall survival may depend on the type of regulatory feedback governing cancer stem cell behavior and that it could be significantly worse in case of cytokine-independent AML. Mathematical models provide potential explanations for unexpected responses of patients to cytokines described in literature[16–18,21–24].

Mathematical models are a useful tool to understand processes that cannot be manipulated or measured experimentally. They allow rigorous comparison of different hypothetical scenarios and estimation of unknown parameters[25,26]. Studies from literature demonstrate that mathematical modeling is a suitable approach to investigate the dynamics of cancer cells subjected to regulatory feedbacks or treatment interventions[25–30]. Especially in case of ambiguous experimental results or in systems where the observables strongly depend on experimental conditions, a model-based interpretation of patient data can provide additional insights.

Methods and Model Description

Mathematical models. The interaction of healthy and leukemic cells by cytokine feedbacks and consumption of environmental resources, such as niche spaces, makes it necessary to model both healthy and leukemic cells. To study the potential impact of these interactions on the clinical course of the disease our models incorporate two different modes of feedback, namely cytokine-dependent leukemic cells and cytokine-independent leukemic cells. Cytokine-dependent leukemic cells expand only in presence of cytokines, whereas cytokine-independent (autonomous) leukemic cells have the ability to expand without cytokine stimulation. In this work we develop a new mathematical model for cytokine-independent leukemic cells and compare it to a model of cytokine-dependent leukemic cells proposed by Stiehl *et al.*[31] and applied to data from AML patients[25]. Both models are an extension of a model of hematopoiesis[32], which has been validated on the basis of patient data and applied to clinical questions[25,33–35].

In the following we introduce the biological system underlying our models. In a multi-step process, the hematopoietic stem cell (HSC) population gives rise to all types of mature blood cells[36]. Since acute myeloid leukemia (AML) is a disease of the myeloid lineage, our models focus on the granulopoietic branch of the hematopoietic system. A complex network of cytokine signals adjusts cell production to the need of the organism[36]. Time evolution of the non-linear cytokine feedback in the models are inspired by G-CSF, the main cytokine of granulopoiesis[36], and were proposed by Marciniak-Czochra *et al.*[32]. It has been reported that cytokine concentration influences properties of stem cells[37] and more mature cells[36], we therefore assume in the model that feedback signals act on all mitotic cell compartments.

In case of AML the leukemic cell population shows a similar hierarchical organization as the hematopoietic system with the leukemic stem cell (leukemia stem cell, LSC, leukemia initiating cell, LIC) population at the top of the hierarchy[38,39]. To investigate the potential impact of leukemic cell cytokine dependence on disease dynamics we consider two models, which are summarized in Fig. 1 and Table 1.

In Model 1 (cytokine-dependent AML), it is assumed that leukemic cells depend on the same endogenous cytokines as healthy hematopoietic cells. This assumption is justified by the following biological findings: (1) Leukemic cells express the same cytokine receptors as hematopoietic cells[40]. (2) Leukemic cells of some patients expand only in the presence of cytokines and engraft only in mice transgenic for human cytokines[1,2,4,5]. (3) Cytokine administration in some AML patients recruits leukemic cells into S-phase[10,15]. Since hematopoietic and leukemic cells absorb and degrade cytokine molecules by receptor-mediated endocytosis, the two cell lineages interact through competition for the cytokine[36,40].

Model 2 (cytokine-independent AML) is based on the evidence that in some patients malignant cells are autonomous with respect to physiological hematopoietic growth factors[4,5,41,42]. The cytokine-independent leukemic cell growth is then limited by a competition for the bone marrow space that results in an increased cellular degradation due to overcrowded bone marrow space. It is modeled by a feedback loop in death rates depending on the total immature cell population. A model assuming increased cell differentiation in case of marrow overcrowding leads to similar results. These assumptions are justified by the following evidence: (1) Different mechanisms of cytokine-independent leukemic cell expansion have been identified. One mechanism is autocrine sinalling where leukemic cells express the hematopoietic cytokines required for their survival. The cytokine expression profile of leukemic cells shows high inter-individual variability[43,44] and includes multiple growth factors such as GM-CSF[45,46], G-CSF[43], M-CSF[47], and IL-1[48]. Secretion of these factors together with expression of the corresponding receptors leads to activation of the leukemic cells[43,47]. Another mechanism of factor-independent growth is constitutive activation of key signaling components due to mutations such as JAK/STAT or MAP-Kinase[41,42]. (2) Markers for cell death such as LDH, are increased in bloodstream of leukemic patients[49,50] and enhanced cell death is observed in marrow samples of many patients[51]. The increased cell death

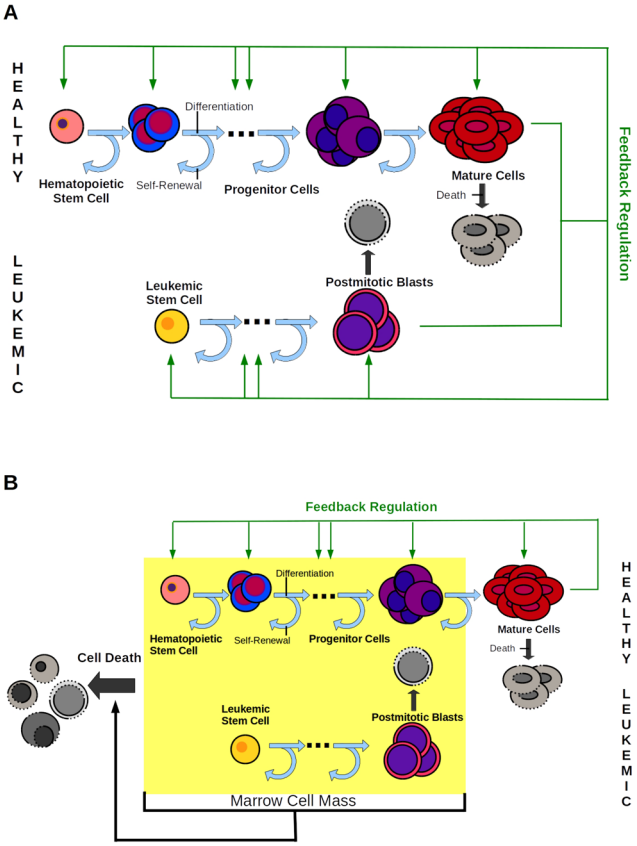

Figure 1. Models of cytokine-dependent and cytokine-independent AML. (**A**) Model 1: Hematopoietic and leukemic cells depend on the same cytokine. (**B**) Model 2: The leukemic cells are independent of cytokines. Crowding in marrow space results in increased apoptosis. The fraction of self-renewal assigned to non-stem cells is a measure of the average number of cell divisions performed before a cell becomes post-mitotic under homeostatic conditions[33].

	Cytokine-dependent AML (Model 1)	Cytokine-independent AML (Model 2)
Leukemic cells	need endogenous hematopoietic cytokines for expansion	expand independently of hematopoietic cytokines
Healthy cells	regulated by cytokine feedback	regulated by cytokine feedback
Interaction	(i) competition for cytokine molecules, decreased self-renewal in case of low cytokine concentrations, (ii) competition for bone marrow space, death in case of marrow overcrowding	competition for bone marrow space, death in case of overcrowded marrow space

Table 1. Comparison of AML models.

is included in Model 2. Several mechanisms for spatial competition have been described such as (i) physical stress owing to overcrowding leading to extinction of cells[52], (ii) competition for a limited niche surface expressing certain receptors (contact molecules) necessary for survival of healthy and leukemic cells and apoptosis or differentiation occurring, if no contacts to these molecules can be established[53].

The latter niche competition mechanism is also valid for the cytokine-dependent AML in case of overcrowded bone marrow. Therefore, we include it also in the Model 1. As shown by numerical simulations this mechanism is relevant in Model 1 only under external cytokine stimulation. In absence of external cytokine stimulation, the feedback mechanism prevents significant overcrowding of the bone marrow space in cytokine-dependent AMLs.

Both considered models include one leukemic cell lineage and one healthy cell lineage. Dynamics of the different healthy and leukemic cell types are given by ordinary differential equations. We assume that each lineage can be described by 2 compartments: dividing cells (including stem cells and progenitors) and post-mitotic cells. The resulting four-compartment model architecture is based on a simplified description of the multi-stages differentiation process, which reduces the complexity of the differentiation process to focus on mechanisms and effects of competition between different cell lines. The previous studies showed that reduction of the number of compartments do not change the behavior of the model after the modification of the parameters corresponding to the new interpretation of the compartments[25].

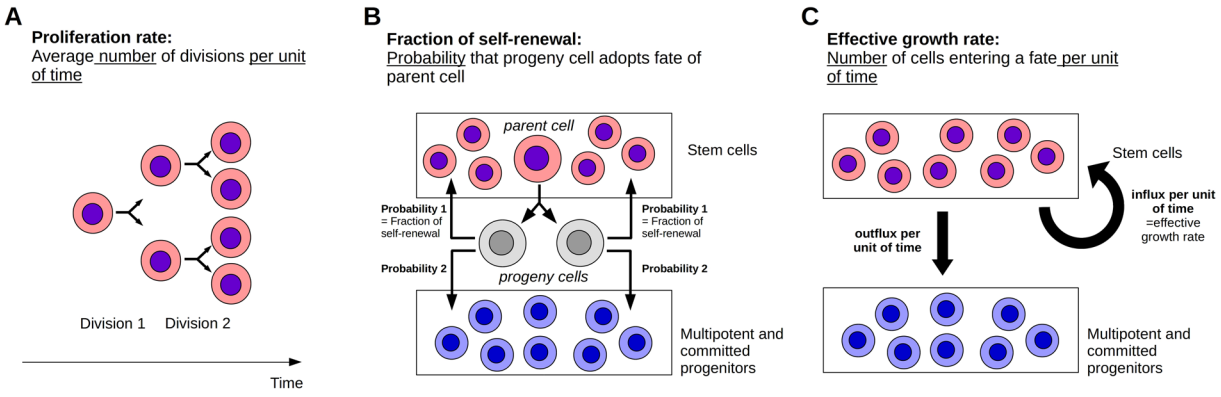

Figure 2. Important model parameters. (**A**) The proliferation rate describes the average number of divisions performed by a cell of given type per unit of time. (**B**) The fraction of self-renewal describes the probability that a progeny cell originating from division adopts the fate of its parent cell, e.g., that a daughter cell of a stem cell is again a stem cell. (**C**) The effective growth rate describes how many cells enter a given cell fate per unit of time, e.g., the number of stem cells that are generated per unit of time. The effective growth rate depends on the proliferation rate and the fraction of self-renewal.

Each cell type is characterized by the following cell parameters, which are summarized in Fig. 2:

- *Proliferation rate*, describing the number of cell divisions per unit of time.
- *Fraction of self-renewal*, describing the fraction of progeny cells returning to the compartment occupied by the parent cells that gave rise to them. On the basis of our earlier work and on compatibility with clinical data[25,26,32–35], we assume that the fraction of self-renewal is regulated by feedback signaling. The fraction of self-renewal assigned to non-stem cells is a measure of the average number of cell divisions performed before a cell differentiates under homeostatic conditions[33].
- *Death rate*, describing the fraction of cells dying per unit of time. For simplicity, we assume that under physiological conditions dividing cells do not die and non-dividing cells die at constant rates.

The effects of cytokines on hematopoietic and leukemic cells are complex. In our models, we assume that cytokines regulate the ratio of self-renewing versus differentiating cells. We neglect the impact of cytokines on the proliferation rates. Our previous quantitative modeling works, see references[25,32,33,35], have shown that additional regulation of proliferation rates has little impact on model dynamics. A system with a regulation of proliferation rates but constant self-renewal /differentiation is insufficient to explain hematopoietic reconstitution[32,33,35]. The simulations depicted in Supplemental Fig. 1 show that additional regulation of proliferation rates has little impact on the blast dynamics. The obtained models are in good qualitative and quantitative agreement with clinical dynamics during hematopoietic stress[32,33,35] and in leukemias[25].

For analysis of the model dynamics, we define also the *effective growth rate* of a mitotic cell population. It describes how many mitotic cells are generated per unit of time, see Fig. 2. This quantity depends on the proliferation rate and the fraction of self-renewal of the immature cell population. A high proliferation rate and a low fraction of self-renewal, as long as it is larger than one half, can lead to the same effective growth rate as a combination of high self-renewal and slow proliferation. The supplementary information contains model details (Section 1) together with analytical studies (Section 2) and model parametrization (Section 3).

Simulations. Simulations have been performed using standard ODE-solvers in MATLAB (Version 7.8, The MathWorks, Inc.). We start simulations with equilibrium cell counts in the hematopoietic lineage and a small number of LSC (1 per kg of body weight), mimicking the appearance of LSC due to a mutation or survival of LSC after therapy. Parameters describing the healthy cells (fraction of self-renewal, proliferation rate, death rate) are taken from the previous work[34] (based on model fitting to data of hematopoietic reconstitution) and are assumed to be the same for all patients. Leukemic cell properties (fraction of self-renewal, proliferation rate, death rate of post-mitotic cells) and parameters describing interaction of leukemic and healthy cells (death rates due to overcrowding, interaction of the leukemic cells with the feedback signals) are considered to be patient specific. We use the least square method to fit the model to the patient data. The purpose of the parameter fitting is to determine which of the two models are able to capture the individual dynamics of a given patient. During the fitting procedure we restrict patient-specific parameters to biologically plausible ranges: (i) The leukemic cell proliferation rates can vary between one division per two years and one division per day. The latter is considered the upper bound taking into account the DNA replication time of a eukaryotic cell[54]. (ii) Leukemic cell self-renewal may vary between zero and one. (iii) Blast half-life is chosen between 25% and 100% of leukocyte half-life, motivated by literature[55]. More details are provided in Section 3.2 of the Supplement.

To decide which of the models fits to the data of a given patient better, we find the best fit for each of the two models and calculate the RMSE (root mean square deviation) for each model. If RMSE for Model 1 is 5% larger than that for Model 2, we conclude that Model 2 fits the data better than Model 1 and vice versa. The results are robust with respect to these choices.

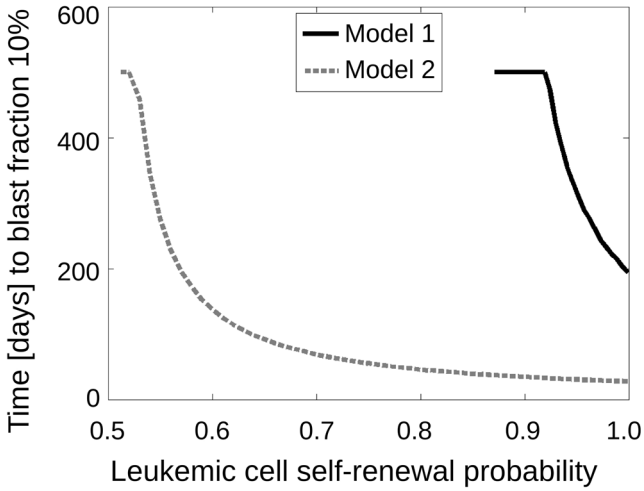

Figure 3. Leukemic cell dynamics in Model 1 and Model 2. At $t = 0$ one leukemic cell per kg of body weight is added to the healthy equilibrium. The number of days elapsing until a marrow blast fraction of 10% is depicted depending on leukemic cell self-renewal. The simulations imply that leukemic cell expansion is faster in Model 2 (dashed line) compared to Model 1 (solid line).

Application to patient data. We use bone marrow aspiration data from patients participating in clinical trials at the University Hospital of Heidelberg (Department of Medicine V; Heidelberg, Germany). Written consent for usage of clinical data for scientific purposes was obtained from each patient. We consider the data of 41 randomly chosen patients. Patients meet the following criteria: (i) at least one documented relapse of the disease in the bone marrow, (ii) achievement of complete hematological remission after treatment of primary diagnosis, (iii) successful bone marrow examination at relapse, and (iv) documented date of death or patients were still alive at the day of data collection. Criterion (iv) limited the number of considered patients.

Statistical analysis. Survival distributions of different patient groups are compared using standard Kaplan-Meier survival analysis[56]. In all considered cases, the test yields significant results ($p < 0.05$).

Data availability. All data analyzed in this study are included in this article, its supplementary information and in the cited references.

Results

Cytokine-dependence of leukemic cells has an impact on blast expansion rates. To study how the dependence of leukemic cells on endogenous cytokines can impact on the clinical course of AML, we introduce two different mathematical models. In one model leukemic cells need endogenous cytokines to expand (cytokine-dependent AML) whereas in the other model leukemic cells can expand independently of endogenous cytokines (cytokine-independent AML). The models are summarized in Fig. 1 and in Table 1. They are extensions of previous works and have been parameterized based on patient data[25,31–35]. For details see section "Methods and Model Description" and Sections 1 and 3 of the Supplement.

We perform numerical simulations to compare blast expansion rates in the models of cytokine-dependent and cytokine-independent (autonomous) leukemic cells. To simulate the fastest possible expansion of leukemic cells, we set their proliferation rate to one division per day, which is an upper bound[54]. We then measure the time span from origin of one LSC per kg of body weight to a marrow blast fraction of 10% for different LSC self-renewal fractions, see Fig. 3. The simulations show that, for a given leukemic cell self-renewal, leukemic cell expansion is faster for the model of cytokine-independent leukemic cells compared to the model of cytokine-dependent leukemic cells: Whereas for cytokine-dependent leukemic cells a minimum of 200 days is needed to obtain a marrow blast fraction of 10%, 30 days are sufficient in case of cytokine-independent leukemic cell expansion. This observation can serve as a discriminator between both models and suggests that fast relapses can be explained only by autonomous leukemic cell growth or treatment failure. The latter can be excluded based on bone marrow biopsy or aspiration.

Cytokine-dependence of leukemic cells contributes to inter-individual heterogeneity of clinical courses. To check whether the observed clinical courses are covered by our models, we fit both models to patient blast counts between complete remission (CR) and relapse, see Fig. 4. Time evolution of blast counts of 22 of the considered 41 patients are compatible with both models (Fig. 4A). Evolution of blast counts of 17 patients are compatible only with the model of cytokine-independent AML (Fig. 4B) and blast counts of 2 patients are compatible only with the model of cytokine-dependent AML (Fig. 4C). The fitting results demonstrate that our models capture clinical data and that cytokine dependence of leukemic cells can contribute to the observed clinical heterogeneity of acute myeloid leukemias.

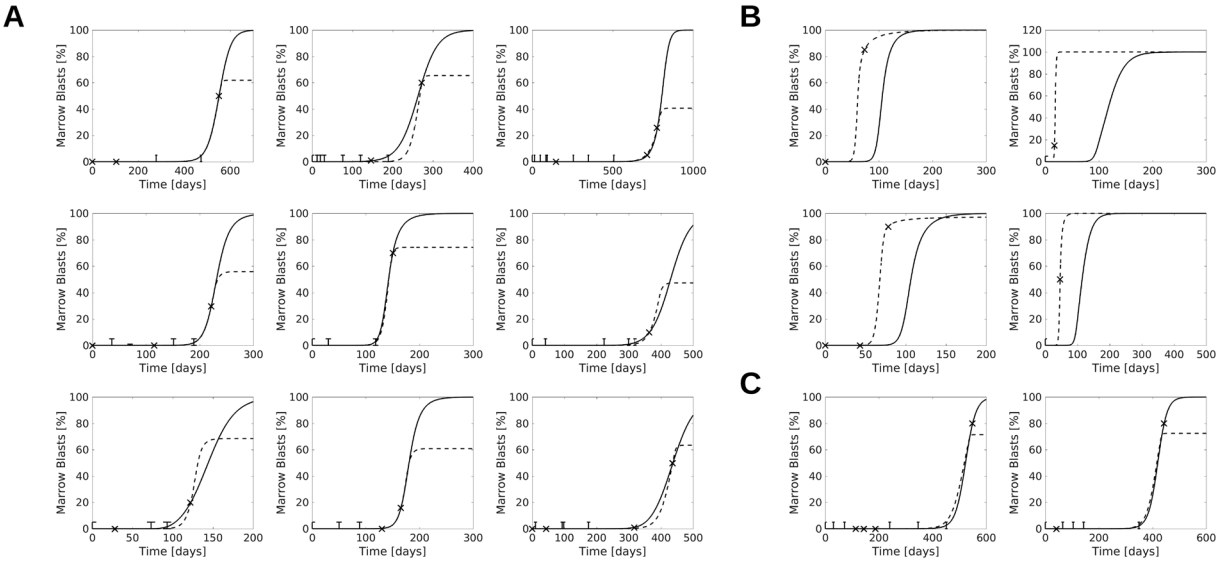

Figure 4. Fit of models to patient data. Models are fitted to marrow blast fractions of AML patients between remission and relapse (solid line: Model 1; dashed line: Model 2). Bone marrow blast fractions are marked by "x" if exact values were reported, if intervals, such as "less than 5% blasts" have been reported, the data is shown as a bar. Panels: Examples for patient data compatible with Models 1 and 2 (**A**), Model 2 but not Model 1 (**B**), or Model 1 but not Model 2 (**C**).

Prognostic significance of autonomous leukemic cell growth. We ask whether cytokine-dependence of leukemic cells can have impacts on patient prognosis. For this purpose, we compared overall survival of patients compatible only with the model of cytokine-independent AML (group 2, $n = 17$) to patients compatible with the model of cytokine-dependent AML (group 1, $n = 24$). Survival analysis yields a significantly longer overall survival for group 1 ($p = 7 \times 10^{-3}$, Fig. 5, median overall survival 700 days) compared to group 2 (median overall survival 350 days). The survival after the first relapse is also significantly increased for group 1 ($p = 0.03$). These results are in line with reports from literature stating that autonomous leukemic cell growth has a negative impact on patient prognosis[4,5] and also with the finding that in some trials patient subgroups with unfavorable risk do not respond to cytokine priming before chemotherapy[11,12].

Potential effects of supportive cytokine administration. Depending on the underlying leukemic cell properties and feedbacks, external cytokine administration may have divergent effects. In some patients, external cytokines stimulate blast expansion at the expense of healthy cells[16], whereas in other patients leukemic cells are out-competed by stimulated healthy cells which results in a reduction of blast counts[21]. To understand which cell parameters are crucial for the observed dynamics we run model simulations for multiple parameter combinations. Representative results are depicted in Fig. 6. All parameters used for the simulations shown in Fig. 6 are within the parameter distributions obtained from fitting of patient data. The results can be classified using the effective growth rate of mitotic healthy and leukemic cells. It describes how many mitotic cells of the respective type are produced per unit of time (Fig. 2). In the model of cytokine-dependent AML, cytokine administration is beneficial if the effective growth rate of stimulated leukemic cells is smaller than the corresponding rate for the stimulated healthy cells (Fig. 6A) and harmful otherwise (Fig. 6B). Note that in the model of cytokine-dependent AML a higher self-renewal of leukemic cells compared to hematopoietic cells is sufficient for leukemic cell expansion, independent of the relation between the corresponding effective growth rates. If the effective growth rates of healthy and leukemic cells are similar, cytokine administration has no relevant effect. The simulation depicted in Fig. 6A is in line with the observation that in some patients one cycle of cytokine administration can result in complete remissions that are stable for time intervals between several months and more than one year[21,22,24]. The simulation depicted in Fig. 6B is in line with the observation that cytokine stimulation can result in blast crisis[11]. A fit of our models to the data from Duval *et al.*[11] is depicted in Fig. 7.

Also in the model of cytokine-independent AML the effect of cytokine administration depends on the effective growth rate of mitotic leukemic and hematopoietic cells. If the effective growth rate of leukemic cells is larger than that of stimulated hematopoietic cells, cytokine administration cannot reduce leukemic cell load, it only leads to a negligible reduction of the speed of leukemic cell expansion (Fig. 6C). In the opposite scenario cytokine administration can temporarily reduce the leukemic cell burden but remission, if achieved at all, lasts shorter than in the case of cytokine-dependent AML. The scenario depicted in Fig. 6D fits to the clinical observations reported by Xavier *et al.*[23]. Repeated cytokine administration can be used in this case to control the leukemic cell burden[23].

The mechanism behind the observed dynamics is as follows: In Fig. 6A healthy and leukemic cells depend on cytokines and thus effective growth rates increase under cytokine stimulation. However, for the chosen parameters, the effective growth rate of stimulated healthy cells is larger than that of stimulated leukemic cells. Since overcrowding of the bone marrow space leads to increased healthy and leukemic cell clearance, the cell population with a higher effective growth rate (healthy cells) out-competes the population with a smaller effective

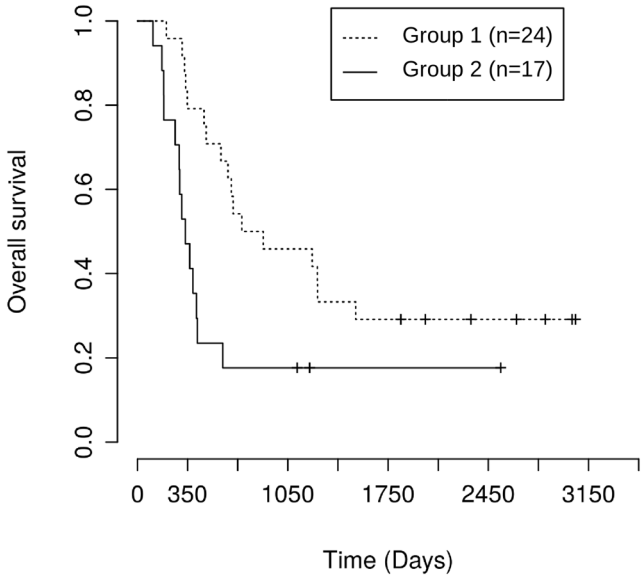

Figure 5. Patient data compatible only with model of cytokine independent AML correlates with poor overall survival. Overall survival of two patient groups is compared. Group 1: Patients compatible with model of cytokine dependent AML, group 2: Patients compatible with model of cytokine independent AML and incompatible with model of cytokine dependent AML. Survival of both groups differs significantly ($p = 7 \times 10^{-3}$). Similar results are obtained if survival after first relapse is compared ($p = 0.03$).

growth rate (leukemic cells). The scenario in Fig. 6B is similar, with the only difference that leukemic cells have a higher effective growth rate compared to healthy cells. In Fig. 6C,D only healthy but not leukemic cells respond to cytokines. Cytokine administration, therefore, results in an increased effective growth rate of healthy cells only. If the stimulated healthy cells have a higher effective growth rate than the leukemic cells, the competition will lead to a reduced leukemic cell count and to an increased healthy cell count, as shown in Fig. 6D. If the stimulated healthy cells have a smaller growth rate than the leukemic cells, cytokine stimulation cannot reduce the leukemic cell load, as shown in Fig. 6C.

Discussion

In this work we propose mathematical models to investigate a possible impact of cytokine-dependence of leukemic cells on the course of the disease. We focus on two scenarios. In Scenario 1 (cytokine-dependent AML) leukemic and healthy cells compete for endogenous cytokines, which they require for expansion. In Scenario 2 (cytokine-independent AML) leukemic cells expand independently of cytokines but cells compete for the bone marrow space and die in case of overcrowding. Both scenarios are supported by experimental evidence[3–5,41,42].

Unlike many previous models, the models considered in this work explicitly include dynamics of healthy cells. Animal experiments show that hematopoietic stem cells isolated from leukemic organisms can lead to normal hematopoiesis if transplanted to a non-leukemic host[57]. This finding suggests that a better understanding of the disease mechanisms could allow to derive clinical strategies that support healthy hematopoiesis and reduce expansion of leukemic cells.

The proposed mathematical models provide criteria that might allow discrimination between cytokine-dependent and cytokine-independent AMLs. Our results suggest that rapid leukemic cell expansion and early relapses may be typical for cytokine-independent AML. The models suggest that if time between complete remission and 10% marrow blasts at relapse is shorter than 200 days, only the model of cytokine-independent AML is able to explain the dynamics.

Fitting of the developed models to patient blast counts between complete remission and relapse demonstrates that patients compatible only with the model of cytokine-independent AML have a significantly poorer overall survival compared to patients compatible with the model of cytokine-dependent AML. This observation is in line with data from cell culture studies showing that autonomous cell growth is correlated with a poor prognosis[4,5].

Another important difference between the models lies in the reaction of the patient to external cytokine administration. Cytokine administration is a commonly used treatment strategy to increase healthy cell counts and to reduce complications of chemotherapy[6]. Although considered safe in general, there exist multiple reports of patients showing unexpected increase or reduction of the leukemic cell burden after cytokine administration. Increasing leukemic cell counts can be explained by cytokine-mediated blast expansion[16], whereas decreasing leukemic cell counts result from stimulation of hematopoiesis and out-competition of leukemic clones[21]. Our modeling approach suggests the effective growth rate of leukemic and hematopoietic mitotic/stem cells as a crucial parameter to understand divergent reaction of patients to cytokine administration. The effective growth rate of a cell population describes how many cells of that population are produced per unit of time. It can potentially be estimated based on mathematical models. In our model simulations cytokine stimulation can induce complete remissions even in cytokine-dependent AML provided the effective

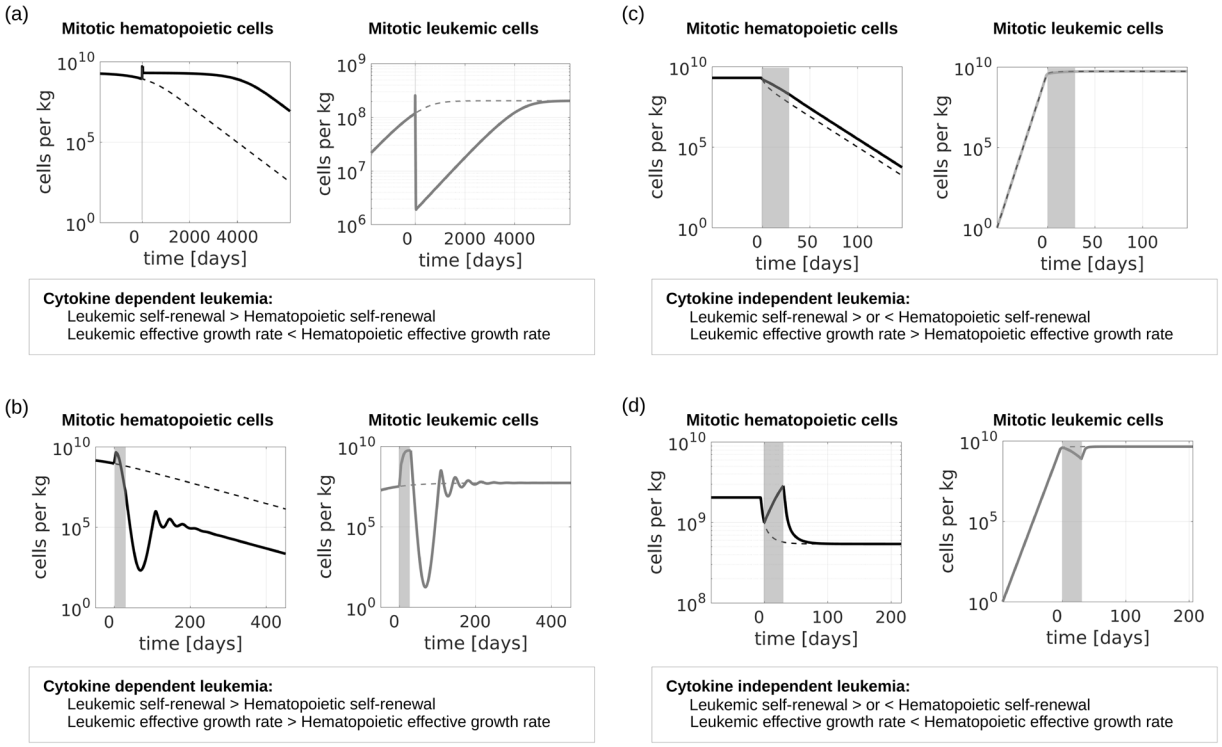

Figure 6. Effect of cytokine stimulation on leukemic cell burden. When mature cell counts are reduced by 50% due to leukemic cell load we simulate cytokine stimulation for 30 days. Dotted lines show dynamics in absence of cytokine stimulation, solid lines show dynamics during and after cytokine stimulation. (**A**) Cytokine dependent AML, the self-renewal of leukemic cells is higher than the self-renewal of hematopoietic cells and the effective growth rate of leukemic cells is smaller than the effective growth rate of hematopoietic cells. Cytokine administration reduces the leukemic cell burden. (**B**) Cytokine dependent AML, the self-renewal of leukemic cells is higher than the self-renewal of hematopoietic cells and the effective growth rate of leukemic cells is higher than the effective growth rate of hematopoietic cells. Cytokine administration increases the leukemic cell burden. (**C**) Cytokine independent AML, the effective growth rate of leukemic cells is larger than the effective growth rate of hematopoietic cells, leukemic cell self-renewal can be larger or smaller than hematopoietic cell self-renewal. Cytokines cannot reduce the leukemic cell burden. (**D**) Cytokine independent AML, the effective growth rate of leukemic cells is smaller than the effective growth rate of hematopoietic cells, leukemic cell self-renewal can be larger or smaller than hematopoietic cell self-renewal. In this case leukemic cell counts can be reduced by cytokine administration. Simulation details are provided in the Supplement (Section 3.5). All parameters are listed in Supplemental Table 2.

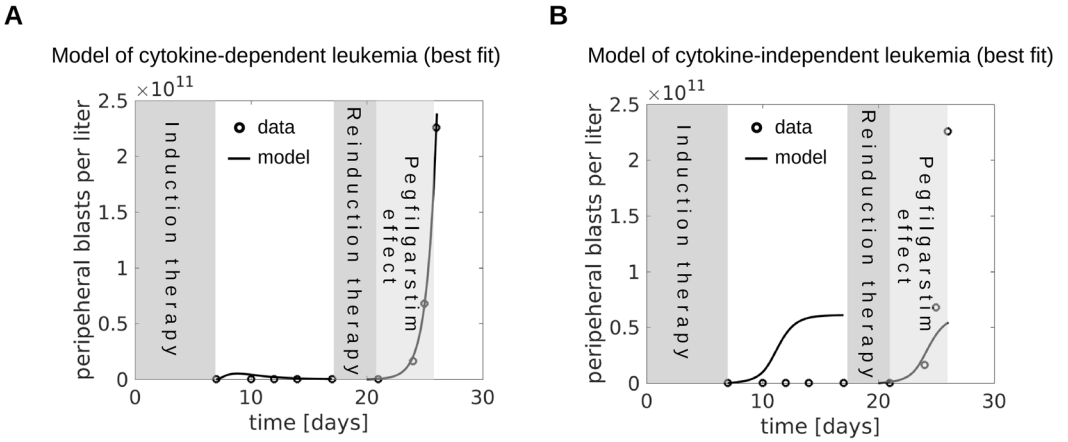

Figure 7. Blast crisis after pegfilgrastim. Blast crisis after pegfilgrastim administration can only be explained by cytokine sensitive leukemic cells. The figure shows the best fits of the models of cytokine-dependent (**A**) and cytokine independent (**B**) AML to the data from Duval[16].

growth rate of mitotic leukemic cells is smaller than that of mitotic hematopoietic cells (note that an effective growth rate of leukemic cells larger than zero is necessary and sufficient for their expansion). This scenario is in line with multiple clinical observations[21,22,24]. If the effective growth rate of leukemic cells is higher compared to hematopoietic cells, cytokine administration can result in blast expansion as it has been clinically observed[16].

Similarly, leukemic cell load can be temporarily reduced in the model of cytokine-independent AML if the effective growth rate of hematopoietic cells is larger than that of leukemic cells. Nevertheless, this reduction is less efficient compared to the case of cytokine-dependent AML, since remaining leukemic cells expand fast. Model dynamics in this case also fit to clinical reports[23]. The observation that a given treatment can be beneficial in some patients and harmful in others demonstrates the need to distinguish between the regulation modes present in different patients. The criteria developed in this work can be helpful for this task.

The models considered in this manuscript describe two opposite extremes of a continuum, namely cytokine-dependence and cytokine-independence. However, more complex dynamics may emerge in more complex scenarios, e.g., in scenarios where leukemic cells are not fully independent of cytokines but they need less stimulation than their benign counterparts or leukemic cells are less sensitive to cytokine stimulation than healthy cells. These scenarios can be included in future versions of the presented modeling framework.

From an experimental point of view, it is challenging to decide if leukemic cells depend on cytokines or not. One possibility is to study if leukemic cells show autonomous growth in cell cultures. However, the readout can depend on the culture conditions[3]. Another possibility is assessment of cell surface receptors, however experimental data suggest that presence of growth factor receptors not automatically implies response of cells to these factors[58]. Further complications arise from evidence suggesting that hematopoietic growth factors can interact with their receptors inside the cell, which would imply that autocrine loops not necessarily depend on surface receptor expression[59].

As previously shown our models can be generalized to a multi-clonal setting, see refs[34,60]. A multi-clonal version of the model of cytokine-dependent leukemia with stochastic mutation acquisition can be found in reference[60]. These extensions allow to study the impact of leukemic cell parameters on clonal evolution in absence and presence of therapy[34,60]. If the frequencies of different leukemic clones at multiple time-points are available for individual patients, the analysis presented in this work can be applied to compare cell properties of different clones. However, such data is not available in clinical routine so far.

Our mathematical modeling approach allows to assign patients to different risk groups based on time evolution of their individual blast counts. This strategy is complementary to the clinically used risk stratifications which rely mostly on genetic hits. In the future this approach could be used to identify patients with adverse and beneficial response to exogenous cytokines and to improve risk stratification approaches.

Our results support the hypothesis that cytokine-dependence of leukemic cells impacts on the course of the disease. Fitting the models to data of individual patients suggests differences in the response of AML cells to cytokines. The models propose a distinction between patients with cytokine-dependent and cytokine-independent AML cell dynamics which leads to significant differences in patients' prognosis. The modeling insights suggest that this topic may be of clinical relevance and merits further investigation.

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