Cell Division Patterns in Acute Myeloid Leukemia Stem-like Cells Determine Clinical Course: A Model to Predict Patient Survival

Thomas Stiehl1,2,3, Natalia Baran4, Anthony D. Ho4, and Anna Marciniak-Czochra1,2,3

Abstract

Acute myeloid leukemia (AML) is a heterogeneous disease in which a variety of distinct genetic alterations might occur. Recent attempts to identify the leukemia stem-like cells (LSC) have also indicated heterogeneity of these cells. On the basis of mathematical modeling and computer simulations, we have provided evidence that proliferation and self-renewal rates of the LSC population have greater impact on the course of disease than proliferation and self-renewal rates of leukemia blast populations, that is, leukemia progenitor cells. The modeling approach has enabled us to estimate the LSC properties of 31 individuals with relapsed AML and to link them to patient survival. On the basis of the estimated LSC properties, the patients can be divided into two prognostic groups that differ significantly with respect to overall survival after first relapse. The results suggest that high LSC self-renewal and proliferation rates are indicators of poor prognosis. Nevertheless, high LSC self-renewal rate may partially compensate for slow LSC proliferation and vice versa. Thus, model-based interpretation of clinical data allows estimation of prognostic factors that cannot be measured directly. This may have clinical implications for designing treatment strategies.

Major Findings

Mathematical modeling and model-driven patient data analysis suggest that proliferation and self-renewal rates of leukemia stem-like cells (LSC) have greater impact on clinical dynamics of acute myeloid leukemia than self-renewal and proliferation rates of non-stem leukemia cells. The proposed mathematical model allows deriving estimates of LSC properties of individual patients that predict overall survival.

Introduction

Acute myeloid leukemias (AML) comprise a heterogeneous group of diseases (11–12). Evidence accumulated that AMLs are maintained by a population of leukemic stem cells (LSC, leukemia initiating cells, leukemia stem-like cells) that are resistant to conventional chemotherapy, and likely are responsible for relapses (7–8, 13). Division kinetics and self-renewal rates of LSC and less primitive leukemia blast cells are poorly understood, because they cannot be monitored in vivo (8, 14).

This issue is, however, of biologic and clinical significance as not only the total count of leukemic cells may determine the clinical course, but also the LSC count and LSC dynamics such as proliferation and self-renewal rates. This is demonstrated by the following two hypothetical scenarios. (i) A small number of LSC surviving induction chemotherapy drastically reduce overall survival if they rapidly expand after cessation of the treatment. (ii) A small number of LSC surviving induction therapy but remaining dormant or slowly cycling after cessation of therapy leads to relapse after many years and a longer period of survival than in scenario (i). According to this reasoning, even if it were possible to measure LSC numbers, for example, based on surface markers, it would be important to know their division kinetics and self-renewal rates. Thus far, these parameters have remained undefined (8, 14).

A growing number of genetic (11–12, 15), epigenetic (16), and regulatory aberrations (17–18) relevant for leukemogenesis and risk stratification has been described. Despite this knowledge, the impact of these factors on clinical course and on cell properties is not well-defined (12, 19, 20). In general, the impact of a given parameter may depend on the absence or presence of other, still unknown, parameters (12, 21–23). Genetic studies suggest that leukemogenetic hits vary considerably among patients (24–26). Variability in survival of patients with the same risk factors underscores the complexity of the interplay of different detected aberrations.

We propose that estimation of LSC properties in the terms of self-renewal and proliferation rates may serve as a complementary and more direct approach to gain insights into the mechanisms governing leukemia dynamics (19). In this work, we have applied...
a combination of mathematical models established by our group (5, 27). In conjunction with clinical parameters, we have studied the impact of LSC proliferation and self-renewal rates, compared with proliferation and self-renewal of less primitive leukemia blast cells or leukemia progenitor cells (LPC), on clinical outcome.

Mathematical approaches have been used many times to improve understanding of the hematopoietic system and its diseases; for review see refs. 28–33. They offer the possibility to comprehend processes not yet accessible by experimental measurements (5).
Achievements of the current work are 3-fold: (i) quantitative estimation of the impact of the proliferation and self-renewal rates of LSC as well as of the less primitive LPCs on the clinical course of disease, (ii) model-based estimation of LSC proliferation and self-renewal rates in relapsing patients, (iii) using estimated LSC proliferation and self-renewal rates to predict patients’ prognosis.

In this study, we have outlined the principles of our mathematical model and we have defined the parameters of division kinetics and self-renewal rate. Using computer simulations, we have related LSC proliferation and self-renewal rates to the clinical course of relapse. We then have applied the proposed framework to estimate surrogate LSC proliferation and self-renewal rates of 41 patients with relapsed AML and to relate them to long-term clinical outcomes.

Materials and Methods

Mathematical model

In the following, we introduce the mathematical model. The model is an extension of a model of hematopoiesis (6, 34), which has been validated on the basis of patient data and applied to clinical questions (5, 35).

On the basis of the classical understanding of hematopoiesis (36), we assume that the hematopoietic system consists of an ordered sequence of discrete maturation states (compartments), which are sequentially traversed (36). We treat each compartment as a “well-mixed tank” and describe its time evolution using ordinary differential equations. The large count of cells forming the hematopoietic system (36, 37) justifies this approach. The model includes one leukemic cell lineage and one healthy cell lineage.

For simplicity, we assume that each lineage consists of three different cell types. In healthy hematopoiesis, we distinguish among hematopoietic stem cells (HSC), hematopoietic progenitor cells (HPC), and nondividing mature cells, whereas the leukemic lineage includes leukemic stem cells (LSC), an intermediate population, corresponding to the progenitor cell population of healthy hematopoiesis (“leukemic progenitor cells,” LPC, that is, dividing leukemic non-stem cells) and nondividing leukemic blasts.

Each cell type is characterized by the following cell properties:

- Proliferation rate, describing the frequency of cell divisions per unit of time.
- Fraction of self-renewal (self-renewal rate), 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 (5–6, 38), 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 becomes postmitotic under homeostatic conditions (5).
- Death rate, describing the fraction of cells dying per unit of time. For simplicity, we assume that dividing cells do not die and nondividing cells die at constant rates.
- Formation of blood cells is regulated by a negative feedback (4, 39), mediated by a system of lineage- and stage-specific cytokines (4, 36, 39). If there is a need for more blood cells of a certain type, the concentration of cytokine molecules increases and stimulates formation of mature cells. Numerical solutions of the model of hematopoiesis, validated on the basis of clinical observations (5–6, 35, 38), indicate that the regulation of self-renewal is a more efficient mechanism than the regulation of proliferation rates. Similar conclusions were drawn using the models of multistage cell lineages applied to regeneration and maintenance of the mouse olfactory epithelium (40). Therefore, in this article, we assume that the regulatory mechanism is based on feedback inhibition of self-renewal by mature cells. For each dividing population, a maximal self-renewal rate is prescribed. Depending on the concentration of the feedback signal, self-renewal is downregulated. We postulate that healthy and leukemic cells respond to the same feedback signals. This assumption is supported by the finding that leukemic cells express receptors for hematopoietic cytokines (1) and interact with the bone marrow microenvironment (2–3). We further presume that the level of the feedback signal decreases if mature cell counts or leukemic blast counts increase. This form of feedback can be interpreted as competition between healthy and leukemic cells for environmental factors or bone marrow niche space (38, 41). The competition of healthy and leukemic cells for environmental factors makes it necessary to model both lineages. Especially during the early phase of the disease, when leukemic cell numbers are still small, consumption of resources by healthy cells is not negligible, because it is higher than consumption of resources by leukemic cells. A model including a different mechanism of competition between leukemic and hematopoietic cells has been proposed in (41) and shows similar dynamic properties. Figure 1 gives a schematic representation of the model. Derivation of the equations can be found in the Supplementary Data (Section 1).

Simulations

Impact of LSC properties on clinically observable progress of the disease is investigated using model simulations. As a symptom of the progress, we consider impairment of hematopoiesis, which is a common feature of acute leukemias. We presume that under physiologic conditions, the hematopoietic cells are in a dynamic equilibrium, that is, production of each cell type equals its clearance. We start computer 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. Initial conditions for the other leukemic cell types (LPCs and blasts) are equal to zero. In the next step, we evaluate the period of time until mature blood cell counts are reduced by 20%. Choosing different cutoff values between 10% and 90% does not change the results; alternatively, marrow blast fractions can be used to define the time point of diagnosis. We perform these simulations for a wide range of leukemic stem and progenitor cell properties. Parameters of the hematopoietic lineage have been calibrated on the basis of the data from the literature (see Section 2 in Supplementary Data). Simulations have been performed using standard ODE-solvers from MATLAB (Version 7.8; The MathWorks, Inc.).

For all simulations, we assume the following, in accordance with biologic hypotheses:

- Leukemic stem cells proliferate slowly compared with leukemic progenitor cells (8–10).
- Leukemic stem cells have higher self-renewal rates than other leukemic cells (7–10).
From mathematical analysis (34) and numerical studies (38), the capacity for self-renewal of LSCs must be larger than that of HSC to observe the expansion of a LSC-derived leukemic cell population. We hypothesize that a leukemic progenitor cell cannot establish a leukemic cell line in the absence of LSC (8–10). As a consequence, maximal self-renewal of leukemic progenitor cells has to be smaller than that of HSCs (34). Furthermore, we postulate that clearance rates of blasts are constant over time. This might be accurate, provided that there exists still unoccupied bone marrow space (41).

Application to patient data

We apply the proposed model to obtain novel insights into cell properties at relapse of AML. 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. Of the considered 41 patients, 22 showed a FLT3-ITD at diagnosis. Patients had to meet the following criteria: (i) at least one documented relapse of the disease in the bone marrow, (ii) achievement of complete remission (less than 5% blasts in marrow) 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.

From the data we obtained the time elapsed between complete remission of primary disease and first relapse as well as the marrow blast fractions over time. Computer simulations indicate that dynamics of the disease are approximately independent of LPC properties (Fig. 2A–E, see below). Therefore, we can apply the model to estimate LSC properties based on clinical data. On the basis of the assumptions that LSC number at complete remission is small (less than 100 per kg of body weight) and that hematopoietic recovery occurs fast in comparison with relapse, we seek LSC proliferation and self-renewal rates that can explain the observed expansion of marrow blasts.

For this purpose, we vary LSC generation time between half a day and several months and self-renewal fraction between 0.501 and 0.999 (a fraction of self-renewal equal to 1 means that all progeny cells are of the same type as the parent cell). Within this parameter range, we find all possible combinations compatible with clinical data. Blast half-life is chosen between 25% and 100% of leukocyte half-life, motivated by literature (42). As simulations show, this choice has little impact on leukemia dynamics. In the model, blast fractions are calculated by dividing the number of all leukemic cell types by the number of all hematopoietic cell types.
LSC properties are crucial for clinical dynamics

We used computer simulations to study the impact of LSC proliferation and self-renewal rates on the dynamics of disease. As a marker of the clinical course, we chose the impairment of healthy hematopoiesis. Using the proportion of marrow blasts as a diagnostic marker led to equivalent results.

In our simulations, we measured the time from the origin of a leukemic stem cell population until reduction of mature cells by 20%. To detect its dependence on LSC properties, we simulated the impact of LPC and postmitotic cell properties. Curves corresponding to the same LSC properties have the same color (black, LSC proliferation equals 1.8 x HSC proliferation, LSC self-renewal equals 1.4 x HSC self-renewal; gray, LSC proliferation equals 2 x HSC-proliferation, LSC self-renewal equals 1.6 x HSC self-renewal) but differ with respect to LPC and postmitotic cell properties (LPC properties are varied by a factor of ten, LPC proliferation is between 0.5 x HPC proliferation and 5 x HPC proliferation, LPC self-renewal is varied between 0.09 x HPC self-renewal and 0.9 HPC self-renewal, and the death rate of postmitotic leukemia cells varies between 0.1 x death rate of mature hematopoietic cells and 0.5 x death rate of mature hematopoietic cells). Curves of the same color are very similar, although HPC properties vary by a factor of 10, while black curves differ strongly from gray curves, although LSC properties differ only by 15%. This shows that small variations of LSC properties have a large influence on clinical course, while changes of LPC and postmitotic cell properties exert much less influence.

Results

LPC properties have little impact on the course of the disease

For a range of LSC properties, we simulated the impact of LPC properties on dynamics of disease. For this purpose, we varied LPC proliferation between 50% of HPC and 2,000% of HPC proliferation and LPC self-renewal between 1% and 99% of HSC self-renewal. The latter condition assured that LPC self-renewal was smaller than HSC self-renewal rate. The biologic interpretation of this condition is that relapse of the disease can only occur if LSCs survive chemotherapy (9–10). LPC properties were kept fixed. The results are depicted in Fig. 2B–E. Simulations indicate that these variations had little impact on dynamics of the disease. Only if the self-renewal capacity of leukemic progenitor cells approaches the self-renewal capacity of HSC or LSC, the influence of LPC properties on leukemia dynamics becomes visible. For the chosen parameter ranges, the time needed for reduction of mature cells by 20% changes by less than 15% if LPC properties are varied. This value is small in comparison with the impact of LSC described above (see Figs. 2 and 3A).

Properties of the LSC may differ between individuals

The results demonstrated in the previous section suggest that dynamics of disease, that is, the time interval between generation of LSC and outbreak of leukemia or time between treatment and relapse, depends predominantly on proliferation and self-renewal rates of LSC while the respective parameters of all other leukemic cell types exert a negligible influence. If we...
assume that this is true, it will be possible to infer LSC properties from clinical data. We have applied the model to clinical data from 41 patients with relapsed AML to estimate their respective LSC properties (see Supplementary Fig. S2). The results suggest that different LSC survival and proliferation rates might lead to an identical individual course. On the basis of the model, it is possible to systematically describe all combinations of LSC survival and proliferation compatible with an observed course of the disease. The results suggest that high survival and proliferation rates were required for leukemia relapse in the observed patients, whereas fast proliferation rate was not always required. Model fits to data of selected individual patients are depicted in Supplementary Fig. S1.

**Estimated individual LSC properties might predict survival**

Grouping patients based on the estimated LSC survival and proliferation rates (i.e., assigning patients with "high" estimated LSC survival and proliferation rates to one group and patients with "low" estimated LSC survival and proliferation rates to a second group) reveals that patients surviving more than 1 year after the first relapse have different estimated LSC survival and proliferation rates than patients surviving less than 1 year. Figure 4A and 4B provides evidence that patients could be categorized into good prognosis versus poor prognosis groups. Figure 4 shows how these groups were defined in terms of estimated LSC survival and proliferation rates. The parameter ranges for both groups were defined on the basis of a test group. Survival curves of the two groups differ significantly \( P = 0.003 \) by the log-rank test. Results are similar if only FLT3-ITD-positive patients are considered (see Supplementary Fig. S2). In the good prognosis group median, overall survival after the first relapse was approximately 2 years, whereas in the bad prognosis group it was approximately 3 months. The correlation between the estimated LSC parameters and survival suggests that the estimated LSC survival and proliferation rates might serve as clinically meaningful parameters to predict relapses.

**The model allows distinguishing between different mechanisms of relapse**

Ten of the 41 included patients showed fast relapses that were incompatible with the assumption that a small number of LSC survived under complete reconstitution of hematopoiesis upon induction chemotherapy. The model proposes the following reasons for fast increase of leukemic burden: (i) impairment of hematopoiesis or microenvironment, (ii) inefficiency of therapy or resistant LSC, and (iii) autonomous cell expansion, that is, expansion of cells independently of environmental signals. For each of the 10 patients, one of scenarios (i)–(iii) was compatible with clinical observations (see Section 3 in Supplementary Data). The overall survival of the fast relapsing patients was similar to that of the poor prognosis group in Fig. 4 (median survival of 7 months).

**Multiple relapses**

Among the included patients, 8 relapsed twice. In 6 cases, our assumptions that a small number of LSC survives chemotherapy and that healthy hematopoiesis is fully restored after treatment...
can recapitulate the observed data. Our results indicated that LSC properties might vary between relapses. In five of these cases, LSC proliferation and/or self-renewal rates have increased at second relapse as compared with the first relapse. Corresponding estimated parameter ranges of the patients are shown in Fig. 5A–D and Supplementary Fig. S3. In two cases, the observed dynamics were not covered by the model. This might be due to therapy resistance, autonomous cell expansion, or hematopoietic impairment.

Discussion

Our mathematical model has provided evidence that LSC properties have a significant impact on the clinical course of AMLs. This was validated by matching of the proposed model to the clinical data of 41 patients. LPC properties on the other hand have much less influence on the clinical outcome. This result was based on the assumptions that LPC proliferated faster than LSC but that LSC had higher self-renewal rates than LPC (8–10).

On the basis of the modeling experiments, we propose the following mechanism: LSC possess higher self-renewal potential than LPCs. Because stem cells have lower proliferative activity than other mitotic cell types, their replication is the rate-limiting process during expansion of leukemic cells. The self-renewal potential of LPCs determines the average number of divisions before LPCs differentiate. When LPC self-renewal rate is high, then only a small fraction of LPCs gave rise to postmitotic blasts after each division and a large number gave rise to mitotic LPCs. Therefore, a high LPC self-renewal rate leads to a large LPC compartment, but the number of originating postmitotic blasts per LPC division remains small. If LPC self-renewal rate is small, LPCs are able to perform only a small number of divisions before differentiation. In this case, only a small fraction of LPCs gives rise to mitotic LPCs after each division, whereas a large number gives

Figure 5.
Estimated LSC properties in the first and second relapse. A and C, marrow blast dynamics of two patients. Horizontal arrows at the bottom of the graph denote treatment duration (in the case of chemotherapy from the beginning of the first until end of last cycle; in the case of targeted therapy from the beginning until the end of drug administration). B and D, estimated properties of the LSC responsible for the first and second relapse. B corresponds to the patient data in A, and D to that in C. The results demonstrate that LSC properties changed between the relapses. Higher self-renewal and/or proliferation in the second relapse corresponded to selection of a more aggressive, faster expanding LSC clone than in the first relapse.

Stiehl et al.
rise to postmitotic blasts. In this case, the LSC population is small but the number of originating blasts per LSC division is high. These two opposite effects lead to approximately the same blast production in both cases (see Fig. 6). This explains why LPC self-renewal rate has a small impact on blast dynamics. This finding is new and cannot be directly deduced from existing experimental data. Importantly, our model allows self-renewal of progenitor cells, as it is crucial after bone marrow transplantation (5, 46, 47).

Our results propose that in contrast to hematopoietic reconstitution after chemotherapy, progenitor cells, despite their ability to self-renew, have no influence on short-term dynamics during leukemic cell expansion. As explained above, this effect is due to dynamic properties of the system leading to different sizes of the progenitor populations depending on LPC properties. It is important to note that LPCs play a major role because they speed up production of leukemic blasts, but this effect is approximately independent of their self-renewal behavior.

On the basis of our model, we estimated the LSC proliferation and self-renewal rates of 31 patients with relapsed AML. The results indicate that LSC proliferation and self-renewal rates show inter-individual variability. This may explain at least a portion of the clinically observed heterogeneity of patients with AML. Patients could be assigned to two significantly different prognostic groups (P = 0.003 by the log-rank test), based on estimated LSC properties. In the good prognosis group, the median overall survival after first relapse was approximately 2 years, whereas in the other group it was approximately 3 months (see Fig. 4).

Different modifications of our model suggest that the reported findings are robust with respect to model assumptions. Although absolute values of estimated LSC properties may depend on the model assumptions, the relations between LSC properties of different patients and the existence of two significantly different prognostic subgroups remain conserved. Because LSC properties may change over time (15, 26) due to mutation and selection processes, the estimated LSC parameters reflect LSC behavior averaged over time. Mounting evidence suggests that LSC are responsible for relapses (10, 45), and thus determine the outcome of the disease (19). The correlation between estimated LSC properties and overall survival supports our hypothesis that the division and self-renewal behavior of LSC significantly determines the clinical course of the disease. Because of the complexity of the mechanisms leading to evolution of AML on the one hand and simplifications in the models on the other, the estimated LSC parameters should be understood as surrogates for LSC behavior that significantly correlate with clinical outcome. As such, they should not be regarded as quantitative estimates of the kinetic properties of LSCs.

Multiple relapses in the same individual patient permitted monitoring of the estimated LSC self-renewal and proliferation rates between relapses. In most of the cases, LSC shifted towards higher estimated self-renewal rates and/or higher estimated proliferation rates from first to second relapse. Further research is required to link estimated LSC properties to detected mutations.

Our model is based on the assumption that bone marrow cells are well mixed and that spatial inhibition of cell division plays a minor role. This assumption is justified in a first approximation, because in many patients there exists a constant outflow of leukemic non-stem cells from marrow to bloodstream (12). Already in the early stages, leukemias are disseminated diseases affecting marrows of multiple bones. Similar as their benign counterparts, leukemic stem cells seem to enter bloodstream and travel between marrow cavities of different bones (48, 49).

This core, differently from premetastatic growth of solid tumors, there exists no strict spatial confinement of leukemic cells. This difference may explain why models of solid tumors incorporating spatial inhibition of cell division predict an impact of non-stem cell properties on tumor growth kinetics (50), which is not observed in our model.

Our approach can be only applied to patients after first relapse. Nevertheless, a more careful MRD (minimal residual disease) monitoring will allow applying our framework to MRD data after therapy of the primary disease in the future. The current work has provided a framework to obtain surrogate parameters for LSC division behavior, that is, proliferation and self-renewal rates that may be used to predict patient prognosis. This constitutes a novel approach to risk stratification. Because LSC properties may emerge from selection due to therapeutic regimens (15), a better knowledge of individual LSC properties will facilitate the choice of appropriate treatment strategies. Furthermore, the assignment of relapsing patients to different prognostic subgroups due to model-based estimation of individual LSC properties will help to personalize the individual schedules of follow-up examinations.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: T. Stiehl, N. Baran, A.D. Ho, A. Marciniak-Czochra
Development of methodology: T. Stiehl, N. Baran, A. Marciniak-Czochra
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Baran, A.D. Ho
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Stiehl, A.D. Ho
Writing, review, and/or revision of the manuscript: T. Stiehl, A.D. Ho, A. Marciniak-Czochra

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References


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