Title: Cell division patterns in acute myeloid leukemia stem-like cells determine clinical course: a model to predict patient survival

Authors: Thomas Stiehl¹-³, Natalia Baran⁴, Anthony D Ho⁴, Anna Marciniak-Czochra¹-³

¹Institute of Applied Mathematics, University of Heidelberg, Im Neuenheimer Feld 294, 69120 Heidelberg, Germany

²Bioquant Center, University of Heidelberg, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany

³Interdisciplinary Center for Scientific Computing (IWR), University of Heidelberg, Im Neuenheimer Feld 368, 69120 Heidelberg, Germany

⁴Department of Medicine V, Medical Center, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany.

Running title: Estimation of leukemic stem cell properties

Keywords: Acute Myeloid Leukemia, Leukemia Stem Cell, Mathematical Models, Stem Cell Properties, Prognosis
Financial Support: All authors are supported by the Collaborative Research Center, SFB 873 ‘Maintenance and Differentiation of Stem Cells in Development and Disease’ from the German Research Council (DFG).

Corresponding Author: Thomas Stiehl, Im Neuenheimer Feld 294, 69120 Heidelberg, Germany, Tel: +49-6221-548761. Fax: +49-6221-545331, Email: Thomas.Stiehl@iwr.uni-heidelberg.de

The authors declare no conflict of Interest.

Number of Figures: 6; Number of Tables: 0; Word count: 5991 (excluding title page and references)
Cell division patterns in acute myeloid leukemia stem-like cells determine clinical course: a model to predict patient survival

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

¹Institute of Applied Mathematics, University of Heidelberg, Im Neuenheimer Feld 294, 69120 Heidelberg, Germany

²Bioquant Center, University of Heidelberg, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany

³Interdisciplinary Center for Scientific Computing (IWR), University of Heidelberg, Im Neuenheimer Feld 368, 69120 Heidelberg, Germany

⁴Department of Medicine V, Medical Center, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany.

Abstract

Acute myeloid leukemia is a heterogeneous disease in which a variety of distinct genetic alterations might occur. Recent attempts to identify the leukemia stem-like cells (LSCs) have also indicated heterogeneity of these cells. Based on 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, i.e. 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. Based on 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 have greater impact on clinical dynamics of acute myeloid leukemia than self-renewal and proliferation rates of non-stem leukemic cells. The proposed mathematical model allows deriving estimates of leukemia stem-like cell properties of individual patients that predict overall survival.

Keywords: Acute Myeloid Leukemia, Leukemia Stem Cell, Mathematical Models, Stem Cell Properties, Prognosis

Quick Guide to Equations and Assumptions

We consider a mathematical model describing dynamics of leukemic and hematopoietic cells in acute myeloid leukemia. The model includes one hematopoietic and one leukemic cell line. The hematopoietic line consists of hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPCs) and post-mitotic mature cells, the leukemic line of leukemia stem
cells (LSCs), leukemia progenitor cells (LPCs) and post-mitotic leukemic blasts. Both cell

lines interact via feedback due to hematopoietic cytokines.

The main assumptions are:

- Mitotic cells are characterized by their proliferation rate, apoptosis rate and self-
  renewal rate. The latter determines what fraction of progeny cells originating from
  division adopts the same fate as the parent cell.

- Leukemic and hematopoietic cells respond to the same cytokines and compete for
  them (ref. 1-3). Cytokine densities depend mainly on post-mitotic cell densities (ref.
  4.)

- Shortage of post-mitotic cells leads to high cytokine concentrations (ref. 4). High
  cytokine levels lead to enhanced self-renewal (ref. 5-6).

- All mitotic cell types have the ability to self-renew; the self-renewal potential of stem
  cells is higher than that of non-stem cells (ref. 7-10).

- Stem cells divide less frequently than progenitor cells (ref. 8-10).

We denote by $c_1$ the density of hematopoietic stem cells, by $p_{1c}$ their proliferation rate, by $d_{1c}$
their apoptosis rate, and by $a_{1c}$ their maximum possible self-renewal rates. The flux to mitosis
is then $p_{1c} c_1$. Of the originating $2p_{1c} c_1$, the fraction $a_{1c}$ remains in the stem cell compartment
and the remainder belongs to the HPC compartment. The density of hematopoietic
progenitor cells is denoted by $c_2$, that of mature blood cells by $c_3$. The respective properties
are denoted by $p_{2c}$, $d_{2c}$ and $a_{2c}$, $d_{3c}$. The densities of leukemic stem cells, progenitor cells and
post-mitotic blasts are denoted by $l_1$, $l_2$ and $l_3$. Their respective properties as $p_{1l}$, $d_{1l}$ and $a_{1l}$
and so forth.

The density of cytokine molecules is denoted by $s$, and normalized between zero and one. It
is given by
where $k_c$ and $k_l$ are positive constants.

Time evolution of hematopoietic cells is then given by

$$s(t) = \frac{1}{1 + k_l l_3(t) + k_c c_3(t)}.$$  

For the leukemic cell line we obtain analogous equations. A schematic of the model is depicted in Fig. 1. We calibrate this model to clinical data and use it to study the influence of the different parameters on the dynamics of healthy and leukemic cells.

**Introduction**

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

This issue is however, of biological and clinical significance as not only the total count of leukemic cells may determine the clinical course, but also the LSC count and LSC dynamical properties such as proliferation and self-renewal rates. This is demonstrated by the following
two hypothetical scenarios. (1) A small number of LSC surviving induction chemotherapy drastically reduce overall survival if they rapidly expand after cessation of the treatment. (2) A small number of LSC surviving induction therapy but remaining dormant or slowly cycling after cessation of therapy lead to relapse after many years and a longer period of survival than in scenario (1). According to this reasoning, even if it were possible to measure LSC numbers, e.g., based on surface markers, it would be important to know their division kinetics and self-renewal rates. Thus far, these parameters have remained undefined (ref. 8, 14).

A growing number of genetic (ref. 11-12, 15), epigenetic (ref. 16) and regulatory aberrations (ref. 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 (ref. 12, 19-20). In general, the impact of a given parameter may depend on the absence or presence of other, still unknown, parameters (ref. 12, 21-23). Genetic studies suggest that leukemogenetic hits vary considerably among patients (ref. 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 (ref. 19). In this work we have applied a combination of mathematical models established by our group (ref. 5, 27). In conjunction with clinical parameters we have studied the impact of LSC proliferation and self-renewal rates, compared to proliferation and self-renewal of less primitive leukemia blast cells or leukemia progenitor cells, on clinical outcome.
Mathematical approaches have been used many times to improve understanding of the hematopoietic system and its diseases; for review see (ref. 28-33). They offer the possibility to comprehend processes not yet accessible by experimental measurements (ref. 5).

Achievements of the current work are threefold: (1) Quantitative estimation of the impact of the proliferation and self-renewal rates of LSC as well as of the less primitive leukemia progenitor cells (LPC) on the clinical course of disease, (2) model-based estimation of LSC proliferation and self-renewal rates in relapsing patients, (3) 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 (ref. 6, 34) which has been validated based on patient data and applied to clinical questions (ref. 5, 35).

Based on the classical understanding of hematopoiesis (ref. 36), we assume that the hematopoietic system consists of an ordered sequence of discrete maturation states (compartments), which are sequentially traversed (ref. 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 (ref. 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 3 different cell types. In healthy
hematopoiesis we distinguish among hematopoietic stem cells (HSC), hematopoietic
progenitor cells (HPC) and non-dividing mature cells, whereas the leukemic lineage includes
leukemic stem cells (LSCs), an intermediate population, corresponding to the progenitor cell
population of healthy hematopoiesis ('leukemic progenitor cells', LPC, i.e., dividing leukemic
non-stem cells) and non-dividing 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.
  Based on our earlier work and on compatibility with clinical data (ref. 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 post-mitotic under
  homeostatic conditions (ref. 5).
- Death rate, describing the fraction of cells dying per unit of time. For simplicity, we
  assume that dividing cells do not die and non-dividing cells die at constant rates.

Formation of blood cells is regulated by a negative feedback (ref. 4, 39), mediated by a
system of lineage- and stage-specific cytokines (ref. 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 based on clinical observations (ref. 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 (ref. 40). Therefore, in this paper 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 down-regulated. 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 (ref. 1) and interact with the bone marrow microenvironment (ref. 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
(ref. 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, since 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 (ref. 41) and shows similar dynamic properties. Fig. 1 gives a
schematic representation of the model. Derivation of the equations can be found in the
Supplement (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
physiological conditions the hematopoietic cells are in a dynamic equilibrium, i.e. 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 based on the data from the literature, see Supplement (Section 2). Simulations have been performed using standard ODE-solvers from Matlab (Version 7.8, The MathWorks, Inc, Natic, MA).

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

- Leukemic stem cells proliferate slowly compared to leukemic progenitor cells (ref. 8-10).
- Leukemic stem cells have higher self-renewal rates than other leukemic cells (ref. 7-10).

From mathematical analysis (ref. 34) and numerical studies (ref. 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 (ref. 8-10). As a consequence, maximal self-renewal of leukemic progenitor cells has to be smaller than that of HSCs (ref. 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 (ref. 41).
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). 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: (1) at least one documented relapse of the disease in the bone marrow, (2) achievement of complete remission (less than 5% blasts in marrow) after treatment of primary diagnosis, (3) successful bone marrow examination at relapse, and (4) documented date of death or patients were still alive at the day of data collection. Criterion (4) 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. 2 a-e, see below). Therefore, we can apply the model to estimate LSC properties based on clinical data. Based on 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 to 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 (ref. 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.
residing in bone marrow. The system is initialized with steady state hematopoietic cell counts and a small number of LSCs (1 LSC per kg of body weight). Other choices of initial LSC counts lead to similar dynamics, see Fig. 3 (a, b).

Statistics

Survival distributions of different patient groups are compared using the logrank test (ref. 43). We perform an exact logrank test which is based on explicit calculation of the test statistic (ref. 44). In all considered cases, the test yields significant results (p<0.05).

Results

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 fixed LPC parameters and varied LSC proliferation rate between 50% of HSC proliferation and 1000% of HSC proliferation. LSC self-renewal rate was varied between 105% of HSC self-renewal and a value close to the maximal possible fraction of self-renewal, which is 1 (corresponding to the scenario that all progeny cells are identical to their parent cells). 105% has been chosen since there is evidence that LSC possess higher self-renewal potential than HSC (ref. 20, 34, 41). Simulation results are depicted in Fig. 2 (a). Simulations suggest that
LSC properties have a strong impact on clinical dynamics. The time needed for 20% reduction of mature cell counts varies by more than 250% for the chosen set of LSC properties. Fig. 2 (a) suggests that the same dynamics can be obtained for LSC with different self-renewal and proliferation rates. Fast impairment of healthy hematopoiesis requires large LSC self-renewal rate or fast LSC division kinetics or a combination of both.

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 2000% 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 biological interpretation of this condition is that relapse of the disease can only occur if LSCs survive chemotherapy (ref. 9-10). LSC properties were kept fixed. The results are depicted in Fig. 2 (b)-(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 to the impact of LSC described above, see Fig. 2 and 3 (a).

Properties of the LSC May Differ Between Individuals

The results demonstrated in the previous section suggest that dynamics of disease, i.e., 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 in order to estimate their respective LSC properties. We considered the following idealized scenario: A small number of LSC (less than 100 per kg of body weight) survived chemotherapy, hematopoiesis was fully restored after treatment and expansion of leukemic cells eventually led to relapse. This approach is justified by recent sequencing data showing that in many cases leukemia cells at relapse are genetically related to the leukemic cells detected at primary diagnosis (ref. 8, 15, 45).

The observed dynamics in 31 of the 41 patients considered is in agreement with this scenario. Estimated LSC properties are depicted in Fig. 4 (a) for all 31 patients. The results suggested that there was a considerable interindividual heterogeneity of LSC properties among patients. This was also true for the subgroup of FLT3-ITD positive patients, see Supplemental Fig. 2. The results also suggest that different LSC self-renewal and proliferation rates might lead to an identical individual course. Based on the model it is possible to systematically describe all combinations of LSC self-renewal and proliferation compatible with an observed course of the disease. The results suggest that high self-renewal rate was 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 Supplemental Fig. 1.

**Estimated Individual LSC Properties Might Predict Survival**

Grouping patients based on the estimated LSC self-renewal and proliferation rates (i.e., assigning patients with “high” estimated LSC self-renewal and proliferation rates to one group and patients with “low” estimated LSC self-renewal and proliferation rates to a second
group) reveals that patients surviving more than one year after the first relapse have different estimated LSC self-renewal and proliferation rates than patients surviving less than one year. Fig. 4 (a, b) provides evidence that patients could be categorized into good prognosis versus poor prognosis groups. Fig. 4. shows how these groups were defined in terms of estimated LSC self-renewal and proliferation rates. The parameter ranges for both groups were defined based on a test group. Survival curves of the two groups differ significantly (p=0.003 by the logrank test). Fig. 4 (b) shows survival curves for both prognostic groups. Results are similar if only FLT3-ITD positive patients are considered, see Supplemental Fig. 2. In the good prognosis group median overall survival after the first relapse was approximately 2 years while in the bad prognosis group it was approximately 3 months. The correlation between the estimated LSC parameters and survival suggests that the estimated LSC self-renewal 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: (1) impairment of hematopoiesis or microenvironment, (2) inefficiency of therapy or resistant LSC, (3) autonomous cell expansion, i.e., expansion of cells independently of environmental signals. For each of the 10 patients one of Scenarios 1 - 3 was compatible with clinical observations, see the Supplement (Section 3). 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

16
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 to the first relapse. Corresponding estimated parameter ranges of the patients are shown in Fig. 5 (a-d) and Supplemental Fig. 3. 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 acute myeloid leukemias. 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 (ref. 8-10).

Based on the modeling experiments, we propose the following mechanism: LSC possess higher self-renewal potential than LPCs. Since 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, i.e. the average number of LPC divisions before differentiating, was high, then only a small fraction of LPCs gave rise to post-mitotic 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 post-mitotic 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 rise to post-mitotic 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 (ref. 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 since they speed up production of leukemic blasts, but this effect is approximately independent of their self-renewal behavior.

Based on 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 AML patients. Patients could be assigned to two significantly different prognostic groups (p=0.003 by the logrank test), based on estimated LSC properties. In the good prognosis group, the median overall survival after first relapse was approximately 2 years while 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.
Since LSC properties may change over time (ref. 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 (ref. 10, 45) and thus determine the outcome of the disease (ref. 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. Due to 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, since in many patients there exists a constant outflow of leukemic non-stem cells from marrow to bloodstream (ref. 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 (ref. 48, 49). Therefore, differently from pre-metastatic 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 (ref. 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, i.e. proliferation and self-renewal rates that may be used to predict patient prognosis. This constitutes a novel approach to risk stratification. Since LSC properties may emerge from selection due to therapeutic regimens (ref. 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.

Acknowledgements: This work was supported by the Collaborative Research Center, SFB 873 'Maintenance and Differentiation of Stem Cells in Development and Disease'.

References


5. Stiehl T, Ho AD, Marciniak-Czochra A. The impact of CD34+ cell dose on engraftment after Stem Cell Transplantations: Personalized estimates based on mathematical modeling. *Bone Marrow Transplant* 2014; **49**: 30-37.


42. Savitskiy VP, Shman TV, Potapnev MP. Comparative measurement of spontaneous apoptosis in pediatric acute leukemia by different techniques. Cytometry B Clin Cytom 2003; 56: 16-22.


Figure Legends

Figure 1: Schematic of the Model. The model describes time evolution of one leukemic and one hematopoietic cell lineage. Arrows indicate negative feedback depending on the level of post-mitotic cells.

Figure 2: Impact of LSC and LPC dynamics on the clinical course: (a) Impact of LSC properties on impairment of hematopoiesis. The vertical axis depicts the time elapsed between appearance of one LSC per kg of body weight with the properties indicated on the x axis (self-renewal) and y axis (proliferation) and reduction of mature healthy cell counts by 20%. Time T is defined as the time coordinate of the minimum of the depicted graph. We introduce this time unit to compare the impact of stem and progenitor cell properties on leukemia dynamics. The time scale of the vertical axis is the same in panels (a)-(e). In (a) LPC parameters are fixed (proliferation: 10 x HPC proliferation, self-renewal: 0.6). (b)-(e): Impact of LPC properties on impairment of hematopoiesis is relatively small compared to impact of LSC properties. The vertical axis depicts the time elapsed between appearance of one LSC per kg of body weight and reduction of mature healthy cell counts by 20%, the x and y axes indicate properties of the LPC population. For each of panels (b)-(e), LSC properties have been set to different fixed values: (b) LSC proliferation: 0.5 x HSC proliferation, LSC self-renewal: 0.87; (c) LSC proliferation: 5 x HSC proliferation, LSC self-renewal: 0.9; (d) LSC proliferation: 10 x HSC
proliferation, LSC self-renewal: 0.99; (c) LSC proliferation: 1 x HSC proliferation, LSC self-renewal: 0.86. Parameters for the hematopoietic lineage: see Supplement (Section 2).

Figure 3: Impact of LPC and LSC properties on clinical course. (a) The figure shows time evolution of bone marrow blast fractions depending on LSC, LPC and post-mitotic 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 post-mitotic cell properties (LPC properties are varied by a factor of ten, LPC proliferation is between 0.5 x HPC proliferation and 5x 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 leukemic 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. (b) Minimum time from remission to relapse, given that a small number of LSC survived chemotherapy and if hematopoiesis fully recovered after therapy. The curve depicts the maximum possible bone marrow blast count depending on the time elapsed since complete remission. The number of surviving LSC had little impact as long as it was smaller than 100 LSC per kg of body weight. The curves were obtained for LSC self-renewal of 0.999 and LSC division rate of approximately twice per day, which we considered as the upper bound; \( p_{LSC}, p_{HSC}, p_{LPC}, p_{HPC} \): proliferation rates of LSC, HSC, LPC, HPC; \( a_{LSC}, a_{HPC}, a_{LPC} \): fractions of self-renewal of LSC, HSC, LPC; \( d_H, d_L \): clearance rates of postmitotic hematopoietic or leukemic cells.

Figure 4: Estimated LSC properties and prognosis. (a) Possible combinations of proliferation rates and...
self-renewal fractions of 31 relapsing AML patients. The estimation is not unique, i.e. different combinations of self-renewal and proliferation fit equally well. Therefore each patient is represented by a line connecting possible combinations of self-renewal fractions and proliferation rates of the LSC population responsible for relapse in the respective patient. Estimated properties correlate with overall survival after first relapse. Continuous lines: survival shorter than one year, dotted lines: survival longer than one year. Cell parameters located in the gray area correlate with poor prognosis. Supplemental Figure 2 shows the corresponding plot for the subset of FLT3-ITD positive patients. The plot shows that LSC properties vary among patients and that high self-renewal may partially compensate for slow proliferation and vice versa. (b) 31 Patients (13 of them are FLT3-ITD positive) were subdivided into 2 groups based on the estimated LSC parameters. If estimated LSC parameters were located in the gray area of panel (a) the corresponding patient was assigned to the poor prognosis group otherwise the patient was assigned to the good prognosis group. The plot shows the survival curves of the good (Group 1) and the poor (Group 2) prognosis group. Survival was measured from diagnosis of the first relapse until death. The difference between the two groups is significant (p=0.003 by the logrank test).

Figure 5: Estimated LSC properties in the first and second relapse. (a, 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, d) Estimated properties of the LSC responsible for the first and second relapse. Figure (b) corresponds to the patient data in (a), Figure (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.
Figure 6: Impact of the LSC and LPC properties on clinical course. Low LPC self-renewal leads to a small LPC population but to a large probability that LPCs develop into post-mitotic blasts, high LPC self-renewal leads to a large LPC population but to a small probability that LPCs develop into post-mitotic blasts. The influx of post-mitotic cells is equal to the number of mitotic LPCs times probability to become post-mitotic. A large number of mitotic cells multiplied by a small probability leads approximately to the same result as a small number of mitotic cells multiplied by a large probability. For this reason the flux from the LPC to the blast compartment is approximately independent of the LPC self-renewal.
Figure 1
Figure 2
Figure 3

(a) Marrow Blasts [%] vs Time [days]

LSCs:
- $p_{LSC} = 1.8 \cdot p_{HSC}$, $a_{LSC} = 1.4 \cdot a_{HSC}$
- $p_{LSC} = 2 \cdot p_{HSC}$, $a_{LSC} = 1.6 \cdot a_{HSC}$

LPCs:
- $p_{LPC}$: between $0.5 \cdot p_{HPC}$ and $5 \cdot p_{HPC}$
- $a_{LPC}$: between $0.09 \cdot a_{HSC}$ and $0.9 \cdot a_{HSC}$

Postmitotic cells:
- $d_L$: between $0.1 \cdot d_H$ and $0.5 \cdot d_H$

(b) Marrow Blasts [%] vs Time [days]

- 1 LSC/kg
- 100 LSC/kg
- 1000 LSC/kg
Figure 4
Figure 5

Diagram showing the change in Marrow Blast Fraction and Time over days for relapses 1 and 2. The graphs compare the proliferation rates with Self-Renewal percentage.
Low LPC self-renewal

LSC-Population

Differentiation  Self-renewal

LPC-Population

Self-renewing
cells

Differentiating
cells

Blast-Population

Self-renewing
cells

Differentiating
cells

High LPC self-renewal

Figure 6
Cell division patterns in acute myeloid leukemia stem-like cells determine clinical course: a model to predict patient survival

Thomas Stiehl, Natalia Baran, Anthony D Ho, et al.

*Cancer Res* Published OnlineFirst January 22, 2015.