Multiplicity of time scales in blood cell formation and leukemia: Contributions of computational disease modeling to mechanistic understanding and personalized medicine.

Thomas Stiehl

Abstract Blood cell formation (hematopoiesis) is a complex and tightly regulated process. It is maintained by hematopoietic (blood forming) stem cells and serves as a paradigmatic example for tissue maintenance, regeneration and cancer. Quantitative modeling can provide relevant insights into the dynamics of hematopoiesis in health and disease. This chapter provides an introduction to mechanistic mathematical and computational modeling of blood cell formation and its disorders. Starting with an introduction to the biological background and the concepts of mechanistic modeling a broad spectrum of questions and applications is discussed and illustrated using examples from own previous works. The considerations start with a simple model of white blood cell production which is stepwisely extended to a account for acute myeloid leukemia, one of the most aggressive cancers. The covered aspects range from basic biological questions such as stem cell regulation and interactions in the bone marrow niche to application-driven considerations including bone marrow transplantation, cancer stem cell dynamics, clonal evolution and blood cancer relapse. The role of mechanistic models for personalized medicine is discussed and illustrated. An important reason for the inherent complexity of hematopoiesis is the fact that it is comprised of a multitude of sub-processes which evolve on different time scales. This chapter provides an overview of the most important sub-processes and their time scales which range from minutes to years. The impact of the different time scales on system dynamics and model development are highlighted.
Keywords: Hematopoiesis, leukemia, hematopoietic stem cell, leukemic stem cell, cancer stem cell, feedback signaling, time scales, disease progression, phase transition, mechanistic modeling, prognosis, personalized medicine, disease modeling

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1 Introduction

Blood cells fulfill important functions such as transport of oxygen (red blood cells, erythrocytes), coagulation (platelets, thrombocytes) and immune defense (white blood cells, leukocytes). They are formed during a multi-step process which is referred to as *hematopoiesis* and takes place in the bone marrow. Every day 10^{11}–$2 \cdot 10^{11}$ mature blood cells of each type are produced by the hematopoietic (blood forming) system [68]. Blood cell formation is tightly regulated by feedback mechanisms which ensure an immediate response to perturbations such as blood loss or infection [45, 68, 85, 128, 138, 163, 204].

Hematopoiesis and Adult Stem Cells

Hematopoiesis (blood cell formation) is a paradigmatic example for tissue homeostasis and regeneration. The hematopoietic system replaces blood cells which are lost due to physiological turnover or injury [217] (p. 147).

Hematopoiesis is a multi-step process which is driven by *hematopoietic stem cells (HSC)* [163], see Fig. 1. Hematopietic stem cells are adult stem cells (tissue stem cells) which are characterized by two key properties [163]: (i) HSC are multipotent, i.e., they possess the ability to give rise to all types of mature blood cells, (ii) HSC have the capacity to self-renew, i.e., through division HSC can give rise to HSC. This enables them to maintain or even expand the size of their population over the whole life-span of an organism.

Upon division HSC produce HSC (self-renewal) and early progenitor cells (differentiation). The latter transit through multiple increasingly committed progenitor and precursor cell stages before they turn into mature blood cells. Compared to HSC, progenitor cells divide faster and are more specialized in the sense that they give rise only to a limited subset of mature blood cell types. Precursor cells are even more specialized fast dividing cells which give rise to one blood cell type [178]. Progenitor and precursor cells have a limited self-renewal capacity, i.e., they further differentiate after a finite number of divisions [102, 217] (pp. 154-155 and pp. 241-242, respectively). Consequently, they cannot maintain life-long blood cell formation in the absence of stem cells. All mature blood cell types, except some subsets of white blood cells, are post-mitotic, i.e., they do not divide [103, 156, 185].

In the bone marrow HSC occupy specific micro-environments, referred to as *stem cell niches*, which they require to maintain their function [48, 155, 177]. HSC rarely divide [34, 182] and their number is small compared to the mature blood cell counts. Depending on the markers and assays used to define HSC, the estimates of their frequencies in humans vary (e.g., 1 HSC per $3 \cdot 10^6$ mononuclear bone marrow cells in

the so-called *in vivo SCID repopulating assay* [214], $1-2$ HSC per 10^4 mononoclear cells using *long-term culture initiating cell assays* [153, 203] and similar numbers using CD34+, kit+, rhodamine123^{low} cells [161]). The quantification is challenging, since cell surface markers allow to enrich for stem cells, however, not each cell carrying a specific marker combination is a stem cell. The number of HSC actively contributing to human blood cell formation is estimated to be 50,000-200,000 [116].

Recently, the view of hematopoiesis as a hierarchy of discrete maturation stages has been challenged. Experimental results of hematopoietic differentiation, mostly relying on gene expression studies, are reminiscent of a continuous process. This has led to the concept that differentiation rather resembles a continuous change than a transition through discrete maturation stages [36, 209]. Taking into account the limited knowledge about the relation of gene expression and cell function / phenotype it remains unknown under which circumstances the continuous model of hematopoiesis has to be taken into account and when the discrete view is a good approximation. Potentially, biochemical switches may translate continuous changes of gene / protein expression into phenotypic changes that resemble discrete transitions. For many clinical applications the discrete model of hematopoiesis is a good representation of reality, especially since routine clinical data, such as bone marrow cytology (i.e., counts of immature cells in bone marrow), is discrete [175].

Acute myeloid leukemia (AML) is a cancer of the blood forming system [60, 104, 119]. Despite aggressive treatment protocols most patients eventually relapse [66, 79]. This indicates that our understanding of the disease and the arsenal of clinical interventions are still limited. The high inter-patient heterogeneity requires the development of personalized risk-scoring and treatment approaches [60, 66, 79, 80]. Similar as their benign counterparts, malignant cells in AML are hierarchically organized. The malignant cell bulk is maintained by the *leukemic stem cell (LSC)* population which gives rise to leukemic progenitor and precursor cells that differentiate into so-called post-mitotic blasts [29, 92]. LSC are one example for so-called *cancer stem cells* (also referred to as cancer initiating cells).

Cancer Stem Cells

Similar as healthy tissues, cancers are composed of different cell types. *Cancer stem cells (CSC)*, also referred to as cancer initiating cells (CIC), are the malignant counterparts of adult stem cells. They possess the abilities to self-renew and to give rise to all cell types present in the heterogeneous malignant cell bulk. In opposite to other malignant cell types, which can divide only a limited number of times, CSC are required for long-term maintenance and expansion of the cancer cell population [22, 163, 222]. Besides AML, CSC have been described in various malignancies such cancers of colon, pancreas, liver, lung, brain and skin [222]. In many cases they are highly resistant to treatment, survive therapy and trigger relapse of the

disease. Eradication of the CSC population is the main goal of curative treatment approaches [222]. LSC are a prominent example for CSC, see Fig 1.

This chapter provides examples for mathematical models of hematopoiesis and leukemia. Mainly it is a summary of own previous works. It discusses the underlying biological processes and pays special attention to the time scales on which they evolve. Clinical and mathematical implications of the different time scales are highlighted.

In the following, we mainly focus on models describing the production of white blood cells (leukopoiesis), since leukemias are mostly diseases of the white blood cell lineages. More specifically, we consider neutrophil granulocytes, which are part of the innate immune system and constitute the largest fraction of white blood cells in adults [174, 185]. The models we discuss adopt the classical view of hematopoiesis as a transition through discrete maturation stages. Examples for models with a continuous description of differentiation / maturation can be found in [2, 24, 38, 61, 69, 83, 148].

2 Relevant Time Scales in Hematopoiesis and Leukemia

The production of mature blood cells results from the interplay of multiple processes which evolve at different time scales. This section summarizes the most relevant processes and their characteristic time scales. An overview is provided in Fig. 2.

2.1 Cell division

The division rate of hematopoietic cells depends on the maturation stage. Human HSC are mostly quiescent and divide on a slow timescale of approximately 1-2 divisions per year [34, 182]. The division rate of progenitors and precursors tends to increase with their maturity. Precursor division rates approximately range from once per day to once per three days [51, 70, 134, 162]. The low division frequency of HSC might serve to reduce the accumulation of mutations. Furthermore, it makes stem cells resistant to chemotherapeutic agents interfering with genome replication and cell division.

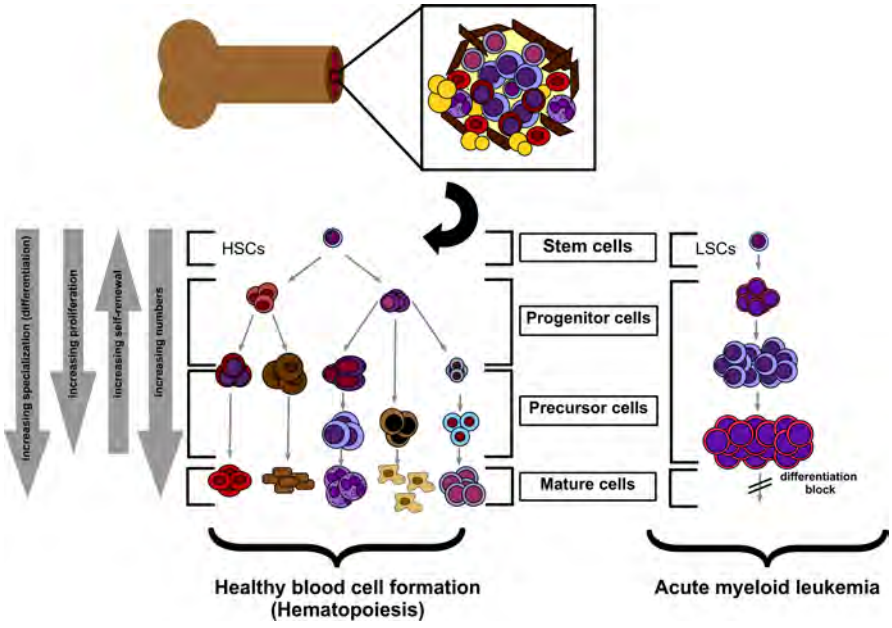

Fig. 1 Overview of hematopoiesis and leukemia. Hematopoiesis takes place in the bone marrow. It is hierarchically organized and maintained by hematopoietic stem cells (HSC). All types of blood cells are derived from the HSC population. Acute myeloid leukemia (AML) cells show a similar organization. All of them are derived from the leukemic stem cell (LSC) population. In AML patients blasts, i.e., cells with an immature phenotype, accumulate in the bone marrow and / or blood stream. Healthy blood cell formation is impaired. Leukemic blasts do not acquire a mature cell phenotype. This observation is referred to as *differentiation block*. (own figure)

2.2 Cell maturation

The transition time from the early precursor stage to the circulating mature cell stage is relatively similar for the main blood cell types. In case of platelets it is 4-7 days [103] (p. 1721) and in case of erythrocytes it is approximately 7 days [156] (p. 436). For neutrophils the transition times from the early precursor stage to circulation are reported as 5-7 or 8-14 d [185] (p.892), depending on the experimental setup. However, in case of a high demand of mature cells the transition can be much faster [121, 157]. Potentially, for some cell types there even exist shortcuts from the stem cell state to the mature cell state [84].

2.3 Cell Half-Life in Circulation

The half-life of mature blood cells in circulation significantly differs among cell types. For erythrocytes it is around 120 days [27], for platelets it is 10 days [103]

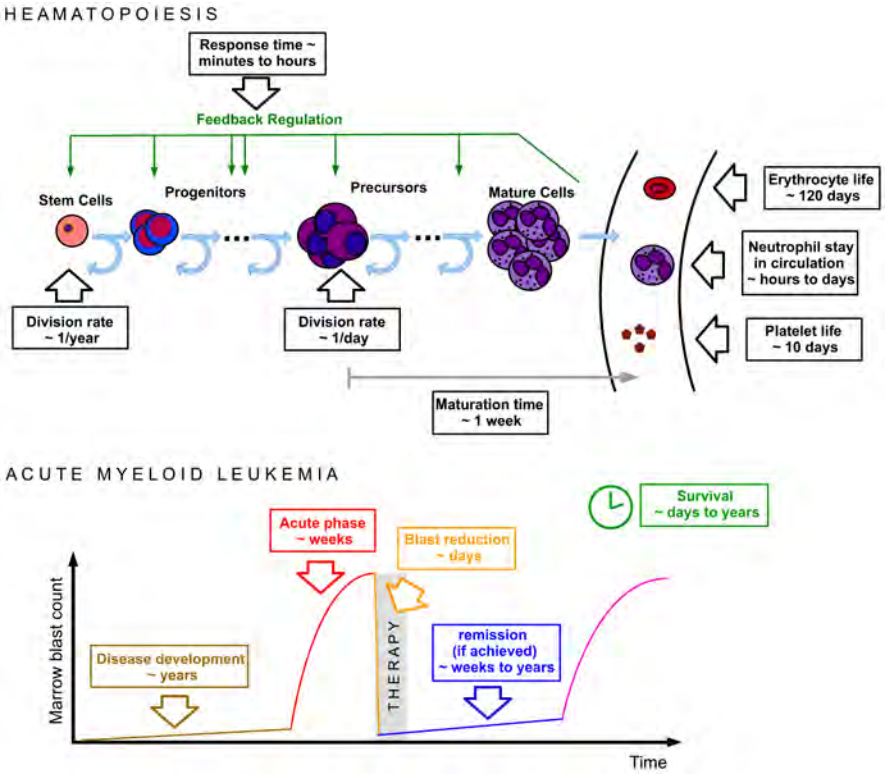

Fig. 2 Time scales in hematopoiesis and AML. Blood cell formation and leukemia emerge from the interplay of many different sub-processes. The figure provides an overview of key processes and the time scales on which they evolve. (own figure)

and for neutrophils it is between 6.7 hours and 5.4 days, depending on the used labeling strategy [154, 185]. Subsets of the white blood cells migrate into tissues to accomplish tasks of immune defense [185].

2.4 Feedback mechanisms

To adapt blood cell formation to the current need of the organism, the hematopoietic system is regulated by a complex network of feedback signals, referred to as *cytokines* [138]. The feedback signals respond to perturbations of blood cell counts and other stimuli, such as infections. The main regulator of red blood cell production is *erythropoietin (EPO)*. EPO measurements after phlebotomy demonstrate that EPO concentrations raise within hours after blood cell loss [128]. Similar observations have been made for *thrombopoietin (TPO)*, the main regulator of platelet formation. In healthy subjects TPO levels increase significantly during platelet donation

(plateletpheresis) [85]. The major regulator of granulocyte formation is *granulocyte colony stimulating factor (G-CSF)*. Similar as the other key cytokines, G-CSF concentrations raise within hours in response to trauma or infection [45,204]. Compared to the rate of cell division and differentiation kinetics, cytokines evolve on a fast time scale.

2.5 Preclinical phase of AML

The current knowledge about the pre-clinical phase of hematopoietic cancers is relatively sparse. There is evidence that many cancers develop based on a condition referred to as *clonal hematopoiesis* [15, 208]. Clonal hematopoiesis means that a significant part of all blood cells is derived from a single clone (i.e., a sub-population of genetically identical stem cells characterized by specific mutations) [15]. Clonal hematopoiesis in absence of hematological disorders is referred to as *clonal hematopoiesis of indeterminate potential (CHIP)*. CHIP is observed in many elderly patients. The clinical impact of clonal hematopoiesis is a matter of current research [168].

Genetic studies suggest that AML key mutations arise years before clinical manifestation of the disease [53]. Similarly, the risk of AML can be accurately predicted based on genetic aberrations years before onset [1]. This observation suggests a long pre-clinical phase of the disease. Similar observations exist for other hematological cancers [218].

2.6 Acute phase of AML

The acute phase of AML is considered as a hematological emergency. In absence of suitable treatment it leads to the death of the patient within days to months [146].

2.7 AML therapy response and relapse

Upon therapy leukemic blasts in bone marrow and peripheral blood reduce to normal counts within days [65,81,91,184]. A fast reduction of blasts seems to be associated with good prognosis [65]. Relapses occur in many patients. The times to relapse (relapse free survival) or death (overall survival) differ significantly among patients. They range from weeks to years [88, 129].

3 Mechanistic Modeling

Mechanistic models

Models are approximations of reality. They are designed to study a specific question or to predict dynamics of a system under well characterized conditions. Mechanistic mathematical / computational models are translations of relevant *processes* into formal objects. Examples for such processes are division of cells, reaction of molecules or drug effects. The formal objects are equations or algorithmic descriptions, which can be studied by mathematical and computational tools. It depends on the question under investigation which processes are considered and which can be neglected. Mechanistic models make complex systems accessible to mathematical and computational techniques. This facilitates their efficient and systematic investigation.

Modeling is an *iterative process*, see Fig. 3. Models are built based on specific *assumptions* and *simplifications*. The comparison of model output to available data and the prediction of experimental results help to *validate* the model, i.e., to assess how closely it approximates reality. Whenever the agreement of model and data is insufficient, the model has to be revised, e.g., model assumptions have to be modified and additional processes have to be considered. Model revision and validation are *iterated* until the model is sufficiently accurate to be applied to a posed question. Although models can formally not prove whether a hypothesis is correct, they can substantially contribute to the understanding of complex phenomena and to the falsification of hypotheses.

Mechanistic models have a broad range of applications. They are a powerful tools to investigate processes that cannot be studied experimentally, e.g., due to technical or ethical limitations. The latter bears a great potential in the life-sciences. The following list summarizes important applications of mechanistic models in interdisciplinary research.

- *Mechanistic understanding:* Mechanistic models allow to systematically study the dynamic properties of a system and to characterize the contribution of individual sub-processes to observed phenomena.
- *Complexity reduction:* Model analysis and simulation help to assess which processes are required to trigger a phenomenon of interest and which processes could be neglected during further investigation. They allow to quantify how sensitively system dynamics depend on process parameters and input variables. Processes evolving on a time scale which is faster or on a spatial scale which is finer than the scale of interest can often be described by simplified sub-models [19, 26, 40].
- *Bridging between scales:* Models are useful to characterize how processes which are defined on the level of individual components (e.g., individual molecules, individual cells, individual organisms) impact on properties of a large system

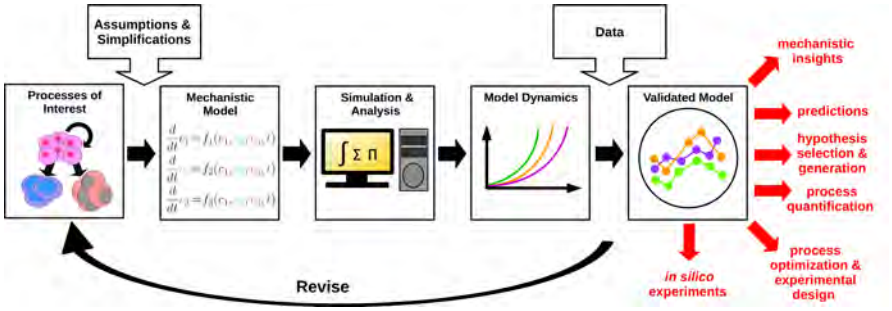

Fig. 3 Development and applications of mechanistic models. Mechanistic modeling is an iterative process. Models are validated by comparing their output to data. They are continuously revised until they recapitulate reality sufficiently well. Mismatches of models and data provide insights into the contribution of different processes to an observed phenomenon. Validated models can be used to predict or optimize the behavior of a system. (own figure)

(e.g., metabolic pathway, organ, population of organisms). In this sense models integrate processes evolving on different scales (e.g., individual versus population, short-term versus long-term, local versus global) and deduce how they impact on the characteristics of a complex system [26, 40].

- *Inference:* Systematic comparison of different models to given data using statistical tools helps to select the best fitting out of multiple competing hypotheses (so-called *model selection*). Model simulations can contribute to design experiments which allow to discriminate between competing hypotheses (*optimal experimental design*) [18, 32, 106, 158].
- *Quantification:* Fitting of models to available data (*parameter estimation*) can help to quantify processes which cannot be measured directly.
- *Prediction:* A validated model can be used to predict the behavior of a complex system.
- *Optimization:* Simulations of validated models can help to optimize processes and to design optimal strategies to reach a goal.
- *In silico studies:* A validated model can be used to simulate the outcome of new experiments or of experiments which cannot (yet) be performed. The latter may contribute to the *generation of new hypotheses*.

For a more detailed overview and examples from biology, see [9, 62, 86, 99, 108, 197, 198, 200, 224]. Specific examples will be discussed in the remainder of this chapter.

4 Models of Blood Cell Formation and Potential Applications

We start our considerations with a simple model of white blood cell formation which was published in [131] and analyzed in [73, 107, 195]. The model is illustrated in Fig. 4(A).

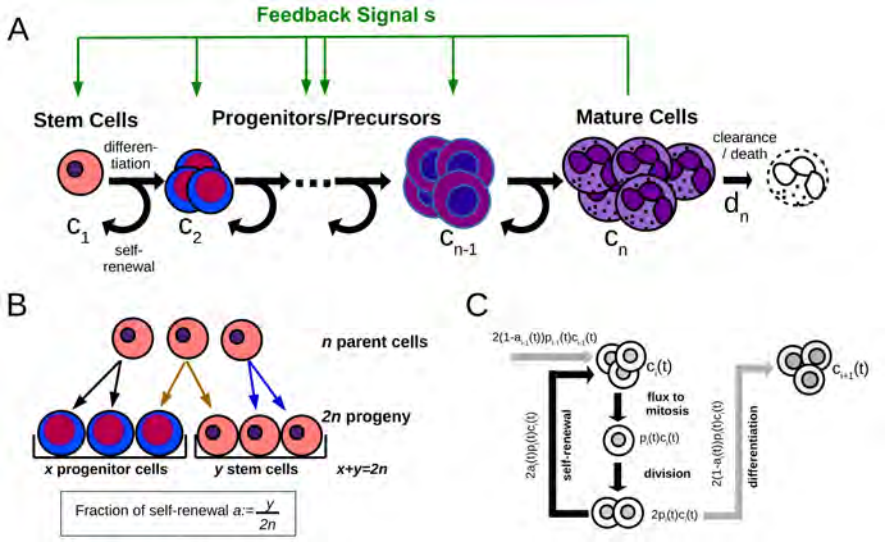

Fig. 4 Model of white blood cell formation. (A) The model describes the formation of neutrophils, the major subset of white blood cells. The model accounts for an arbitrary number of n discrete maturation steps which are sequentially traversed. HSC are denoted by c_1, mature cells by c_n. Progenitors and precursors are denoted by $c_2, \ldots, c_{n-1}$. Mature cells do not divide and die at a constant rate d_n. Key cell properties such as proliferation and self-renewal are regulated by a feedback signal s which depends on the mature cell count. (B) The fraction of self-renewal is defined as the fraction of progeny adopting the same fate as their parents. It corresponds to the probability with which a daughter cell originating from division belongs to the same cell type as the parent cell (self-renewal probability). The fraction of self-renewal is defined on the level of a cell population. It emerges from the respective frequencies of symmetric self-renewal (both daughter cells are identical to the parent cell, blue arrows), symmetric differentiation (both daughter cells are more mature compared to the parent cell, black arrows) and asymmetric divisions (one daughter cell is identical to the parent, the second daughter cell is more mature, brown arrows). (C) Summary of cell fluxes in the model, proliferation rates are indicated by p_i, where i denotes the maturation stage, fractions of self-renewal by a_i. (own figure)

4.1 A Model of Stem Cell Self-Renewal and Differentiation

We denote the number of HSC per kilogram of body weight at time t as $c_1(t)$ and that of mature cells as $c_n(t)$ $(n > 1)$. For $n > 2$ the respective amounts of progenitor and precursor cells are denoted by $c_2(t), \ldots, c_{n-1}(t)$, see Fig. 4(A). We assume that cells of maturation stage i $(1 \leq i \leq n-1)$ divide at rate $p_i(t) > 0$. Since mature neutrophils do not divide, we set $p_n \equiv 0$. For simplicity we assume that non-mature cells do not die and mature cells die at a constant rate $d_n > 0$.

Upon division a cell of maturation stage i splits into two progeny. The progeny can be identical to the parent cell, i.e., they belong to maturation stage i (symmetric self-renewal), they can both be more mature compared to the parent cell and belong to maturation stage $i+1$ (symmetric differentiation) or the cell division can be asym-

metric, i.e., one progeny belongs to maturation stage i and one to maturation stage $i+1$. We denote the probability that a progeny belongs to the same maturation stage as its parent as *self-renewal probability (fraction of self-renewal)* $a_i(t)$, see Fig. 4(B).

At time t the amount of cells of maturation stage i ($i < n$) entering division is given by $p_i(t)c_i(t)$. We neglect the duration of cell division which is short compared to the time scale of blood cell maturation. The number of cells originating from division equals $2p_i(t)c_i(t)$ of which the fraction $a_i(t)$ belongs to maturation stage i and the fraction $1-a_i(t)$ enters maturation stage $i+1$. These processes result in the following system of equations, see Fig. 4(C).

$$\begin{aligned}
\frac{d}{dt}c_1(t) &= \underbrace{-p_1(t)c_1(t)}_{\text{flux to division}} + \underbrace{\underbrace{2\cdot p_1(t)c_1(t)}_{\text{output from division}}\cdot a_1(t)}_{\text{influx due to self-renewal}} && (1)\\
&= (2a_1(t)-1)p_1(t)c_1(t)\\
\frac{d}{dt}c_2(t) &= \underbrace{2(1-a_1(t))p_1(t)c_1(t)}_{\text{influx due to differentiation}} + (2a_2(t)-1)p_2(t)c_2(t) && (2)\\
&\cdots \quad \cdots\\
\frac{d}{dt}c_{n-1}(t) &= 2(1-a_{n-2}(t))p_{n-2}(t)c_{n-2}(t) + (2a_{n-1}(t)-1)p_{n-1}(t)c_{n-1}(t) && (3)\\
\frac{d}{dt}c_n(t) &= 2(1-a_{n-1}(t))p_{n-1}(t)c_{n-1}(t) - d_n c_n(t) && (4)
\end{aligned}$$

These equations describe the proliferation, self-renewal and differentiation of hematopoietic cells. Differentiation is modeled as transit through a finite sequence of maturation stages. The equations are supplemented by non-negative initial conditions.

4.2 Modeling Feedback Regulation

It is known that cell kinetics are regulated by feedback signals. As feedback signal we consider G-CSF, the key regulator of neutrophil formation. In agreement with biological knowledge we assume (in absence of infection) constant production of G-CSF at rate $\alpha > 0$. G-CSF is eliminated via a cell-independent path at rate $\mu > 0$ and it is degraded by binding to receptors (via receptor-mediated endocytosis) on the surface of neutrophils and their precursors [63, 114]. Since neutrophils express more G-CSF receptors than their precursors we neglect G-CSF degradation by immature cells [183]. This results in the following equation for the G-CSF concentration S:

$$\frac{d}{dt}S(t) = \alpha - \beta c_n(t)S(t) - \mu S(t), \tag{5}$$

where β quantifies the elimination of G-CSF by mature neutrophils.

It is known that cytokine dynamics evolve on a faster time scale than cell division and maturation [45, 204]. This allows us to simplify the model (using a quasi steady state approximation) and express the cytokine concentration as a function of mature cells, i.e., $S(c_n(t)) = \alpha/(\mu + \beta c_n(t))$, which assumes values in $(0, \alpha/\mu]$. Division by α/μ yields $s(t) = 1/(1 + kc_n(t)) \in (0, 1]$, where k is a positive constant [131]. This expression assumes values in $(0, 1]$ and describes the strength of the feedback signal as a function of mature neutrophil counts.

In agreement with biological observations the value of the feedback signal increases if there is a shortage of mature cells. Similar functions, also referred to as Hill-type functions, are widely used to model feedbacks in the hematopoietic system [25, 42, 46]. Using the framework of renormalization groups the time-scale separation can be made rigorous and error estimates can be derived for a broad class of models [130].

It is know that G-CSF affects neutrophil progenitors and precursors. Furthermore there is evidence that G-CSF also impacts on stem cells [78, 113, 141, 180, 219]. Therefore, we assume that all non-mature cell stages respond to G-CSF.

To study effects of the feedback signal we consider a scenario which corresponds to a strong perturbation of the homeostatic equilibrium. Such a scenario is *hematopoietic stem cell transplantation (bone marrow transplantation)*.

Bone marrow transplantation (Blood stem cell transplantation)

Bone marrow (blood stem cell) transplantation is a clinical intervention to cure various hematological disorders and malignancies. After eradication of the recipient's bone marrow (referred to as *conditioning*) by high dose chemotherapy or radiochemotherapy hematopoietic stem and progenitor cells are infused into the blood stream [118]. The infused cells home to the bone marrow where they proliferate and restore the hematopoietic system. In case of *autologous* transplantation the donor and the recipient are the same person. The stem cells are collected before conditioning, in case of *allogeneic* stem cell transplantation donor and recipient are different persons [118]. The transplant can be collected either from the donor's bone marrow or from stem and progenitor cells mobilized to the donor's peripheral blood. In case of allogeneic transplantations umbilical cord blood is an alternative cell source [118].

Since neutrophils have a short half-life in circulation, neutrophil counts drop shortly after eradication of the recipient's bone marrow. For this reason infections are frequent complications after transplantation. Since feedback regulations are active

during recovery from transplantation, we use this scenario to calibrate the feedback of our model.

It is known that cell proliferation rates increase due to cytokine stimulation [10]. We model this by the following equation:

$$p_i(t) = p_{i,max} s(t). \tag{6}$$

Furthermore, we assume that each cell type has a maximal self-renewal fraction $a_i = a_{i,max}$. We consider two possible regulations of self-renewal, namely (i) self-renewal increases if there is a shortage of mature cells, i.e.,

$$a_i(t) = a_{i,max} s(t) \tag{7}$$

and (ii) self-renewal decreases, i.e., differentiation increases, if there is a shortage of mature cells,

$$a_i(t) = a_{i,max}(1 - s(t)), \tag{8}$$

where

$$s(t) = \frac{1}{1 + k c_n(t)}. \tag{9}$$

We choose k such that stem cells are in equilibrium for a realistic mature cell count $\bar{c}_n$ which results in $k = (2a_{1,max} - 1)/\bar{c}_n$ for (i) and $k = 1/(\bar{c}_n(2a_{1,max} - 1))$ for (ii). In both cases the maximal possible self-renewal is $a_{i,max}$ and the minimal self-renewal is 0.

The motivation of equation (7) is that an increase of self-renewal leads to a growth of the immature cell population. The more stem and progenitor cells exist, the more mature cells they produce per unit of time. On a very short time scale the increase of self-renewal and the concomitant reduction of differentiation may lead to a reduction of mature cell output. However, the expansion of the immature cell populations outweighs this effect on clinically relevant time scales.

The motivation of equation (8) is that a decrease of self-renewal leads to an increase of differentiation which implies an increase in the output of mature cells.

We simulate the following scenarios:

- Scenario 1: increased proliferation $p_i(t) = p_{i,max} s(t)$, constant self-renewal $a_i(t) \equiv a_i$,
- Scenario 2: constant proliferation $p_i(t) \equiv p_i$, increased self-renewal $a_i(t) = a_{i,max} s(t)$,
- Scenario 3: increased proliferation $p_i(t) = p_{i,max} s(t)$, increased self-renewal $a_i(t) = a_{i,max} s(t)$,

- Scenario 4: constant proliferation $p_i(t) \equiv p_i$, decreased self-renewal $a_i(t) = a_{i,max}(1 - s(t))$.

The results are depicted in Fig. 5. We observe that recovery of white blood cell counts is very slow in Scenario 1 compared to Scenarios 2 and 3, Fig. 5(A)-(B). Scenario 3 is in good agreement with clinical observations and Scenario 2 is a reasonable approximation of reality. Scenario 4 results in a very quick but transient pulse of mature cells due to increased differentiation. However, the pulse is followed by a depression of mature cell counts since stem and immature cell counts decline due to increased differentiation, Fig. 5(C)-(D). The same result is obtained using a more detailed and carefully calibrated model of neutrophil formation [191, 192].

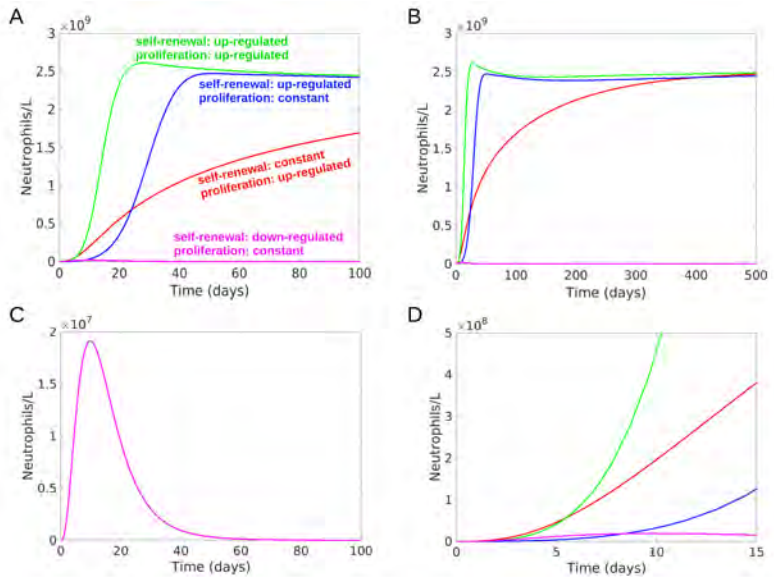

Fig. 5 Impact of feedback regulations on neutrophil engraftment (recovery) after bone marrow transplantation. (A) Only when the fraction of self-renewal increases during recovery blood cell counts increase within a clinically realistic time frame. When the fraction of self-renewal decreases (i.e., differentiation increases), no recovery is observed. (B) Same scenarios as in (A) but on a longer time scale. (C) Increased differentiation during recovery leads to a pulse of mature cells early after transplantation and a subsequent depletion of the stem cells due to a lack of self-renewal. The total amount of produced cells is small. (D) Same scenarios as in (A) but different scales of axes. Increased self-renewal (and therefore reduced differentiation) lead to a short delay of mature cell production (green and blue curves below red curve) in the first days after transplantation. However, due to increased self-renewal stem and progenitor cell populations expand. This results in an amplification of mature cell production (the more stem and progenitor cells, the more mature cells are produced per unit of time) which outweighs the observed initial delay and implies a faster recovery of mature cell counts. Model parameters were taken from [131].

We learn from the simulations that increased self-renewal is crucial to guarantee an efficient recovery (*engraftment*) after bone marrow transplantation. Mathematical analysis reveals that, whenever immature cell division rates are positive, mature cells die at positive rates and the self-renewal fraction of stem cells is above 0.5 and larger than that of all other cell types, there exists a unique positive equilibrium for the feedback scenarios 2 and 3 [195].

The simplest version of the model consists of two maturation stages (immature and mature). This two-compartment version has been studied in [73]. It has a unique homeostatic (healthy) equilibrium and a trivial equilibrium (death). Whenever immature cells have the ability to expand (positive proliferation rate and maximal self-renewal probability above 0.5) the system approaches the homeostatic equilibrium. This observation fits well to robustness of the hematopoietic system to perturbations such as blood cell loss.

Interestingly the latter is not always true for the version of the model accounting for three maturation stages (stem, progenitor, mature). For the model with three maturation stages there exist parameter ranges where cell counts converge to the homeostatic equilibrium after perturbations and there exist parameter ranges where cell counts periodically oscillate [107]. Periodic oscillation of blood cell counts are a rare class of disorders than can be observed in patients and that have been extensively studied [3, 4, 42, 55, 87, 124, 125].

4.3 Applications of Models of Stem Cell Transplantation

A more detailed version of this model [191, 192] is able to reproduce clinical observations for different protocols of hematopoietic stem cell transplantation. This quantitative version of the model can be used to obtain insights into various clinical questions.

The model allows to simulate how the composition of the transplant, i.e., the ratio of stem to different progenitor stages, impacts on the kinetics of mature cell recovery. Simulations suggest that mature cell production in the first weeks after transplantation is mainly driven by the transplanted progenitors and not by the stem cells. This finding is supported by experimental data [167] and is in line with the different time scales of cell division for stem and progenitor cells.

Another important question is how the number of transplanted cells impacts on the kinetics of neutrophil recovery. According to clinical guidelines a transplant dose of $2 - 3 \cdot 10^6$ $CD34^+$ cells per kg of body weight is sufficient for safe engraftment in autologous transplantation [72] ($CD34^+$ cells comprise stem and early progenitor cells, autologous refers to the setting where donor and recepient are the same person). A clinical approach to address the impact of cell dose on engraftment (i.e.,

recovery) is to compare neutrophil kinetics between patient groups that have been transplanted with different doses of $CD34^+$ cells. This approach results in an average reduction of engraftment time due to a defined increase of cell dose. However, clinically it is impossible to measure how a given specific individual responds to two different doses of transplanted cells. This information, however, is relevant to answer the question whether all patients respond in a similar way or whether patients with an inherently slow recovery may benefit stronger compared to patients with inherently fast recovery. Since the determinants of engraftment kinetics are only partially understood and, therefore, it cannot be predicted which patients are prone to delayed engagement, this question can hardly be addressed by clinical trials.

Using our mathematical model we ask how patients who receive a transplant of dose X would benefit from increasing the transplant by ΔX. Model simulations with many different parameter sets suggest that for a given stem cell dose X the *relative* but not the absolute reduction of recovery time due to an additional cell dose ΔX is conserved between individuals and given by

$$\%\text{ reduction of engraftment time} = \left(\frac{\log(X+\Delta X)-C}{\log(X)-C}\right)\cdot 100\%, \qquad (10)$$

where C is a positive constant which depends on the transplantation protocol [192]. This finding suggests that patients who are prone to delayed engraftment would benefit stronger form larger transplant doses compared to average patients. We notice that the logarithm is a slowly increasing function. Therefore this formula is in line with the overall clinical observation that engraftment kinetics in average do not change significantly if the transplanted cell dose exceeds a threshold of $2-3\cdot 10^6$ $CD34^+$ cells per kg of body weight [72].

In the following sections we extend the model of healthy white blood cell formation to account for acute myeloid leukemias. Healthy blood cell formation has been modeled by many authors. Important aspects include chemotherapy and transplantation [47, 98, 147, 148, 179], cellular organization and regulation [24, 25, 28, 34, 46, 49, 50, 52, 56, 76, 105, 132, 143, 166, 170, 216] or periodic oscillations of cell counts [3, 4, 42, 55, 87, 124, 125]. The hematopoietic system is a paradigm for stem cell driven tissue maintenance and repair. Questions such as robustness of these systems to perturbations, the stabilizing role of feedbacks and mechanisms to protect against cancer have also been studied using mathematical and computational models [14, 109, 164, 181, 206, 223].

5 Models of Acute Myeloid Leukemia and Potential Applications

In this section we extend the model of white blood cell formation to account for acute myeloid leukemia. Fig. 6(A)-(B) provides an overview of the models we consider.

Leukemias

Hematopoietic non-stem cells can be subdivided into a myeloid series and a lymphoid series. The most well known mature cells from the myeloid series are red blood cells, platelets and granulocytes. All of them originate from the so-called common myelpoid progenitors (CMP) which are descendants of the hematopoietic stem cells. The CMP and all cells which originate from them form the myeloid series. The most well known mature cells of the lymphoid series are B cells and T cells, which are important players of the immune system. The lymphoid series consists of the so-called common lymphoid progenitors (CLP) and all cells which are derived from them [178]. Leukemias form a heterogeneous group of malignant diseases which lead to the formation of so-called *blasts*. Blasts are abnormal cells with an immature phenotype accumulating in the bone marrow, peripheral blood and lymphatic organs. The expansion of leukemic cells in the marrow impairs healthy blood cell formation which leads to severe symptoms (e.g., anemia, coagulation disorders and immune deficiency). Depending on the cell of origin leukemias are subdivided into myeloid (myelogenous, derived from the myeloid series) and lymphocytic (lymphoid, derived from the lymphoid series) [101, 120, 133]. Based on the speed of disease progression both sub-types are subdivided into acute and chronic. Common subtypes are: AML - acute myeloid (myelogenous) leukemia, CML - chronic myeloid (myelogenous) leukemia, ALL - acute lymphocytic (lymphoid) leukemia, CLL - chronic lymphocytic (lymphoid) leukemia. Mostly, leukemias are diseases of the white blood cells, rarely so-called erythroleukemias are observed which are derived from immature red cells. They are classified as a subtype of AML [133]. Treatment approaches and prognosis differ considerably between the leukemia subtypes.

5.1 Cytokine-Dependent Acute Myeloid Leukemia

The model of AML introduced in this section is based on the following clinical and experimental observations: (i) In AML patients healthy blood cell counts are reduced [119]. This implies that malignant cells impair healthy blood cell formation. (ii) AML cells of many patients proliferate in cell cultures in presence of hematopoietic cytokines, conversely they do not or rarely proliferate in absence of cytokines [93, 117, 123]. If transplanted into mice, human AML cells expand with a higher probability if the mouse strain expresses human cytokines [67, 205]. From this we conclude that, at least in a subset of patients, leukemic cells require the cytokine signal and cannot be maintained in its absence. For this reason we denote this scenario as *cytokine-dependent acute myeloid leukemia.* (iii) AML cells of many patients express G-CSF receptors [110, 183] and respond to cytokines *in vitro* [117]. From this we conclude, that the AML cells interact with the G-CSF-like feedback signal which is considered in the model of healthy hematopoiesis and has been in-

troduced in Section 4.2.

The purpose of the model described in this section is to study under which conditions leukemic cells can destabilize healthy blood cell formation. For this reason we characterize leukemic cells by their kinetic properties (proliferation, self-renewal and death). We do not model the acquisition of mutations, instead we ask how leukemic cells have to differ from their benign counterparts to successfully expand. The model is illustrated in Fig. 6(A)

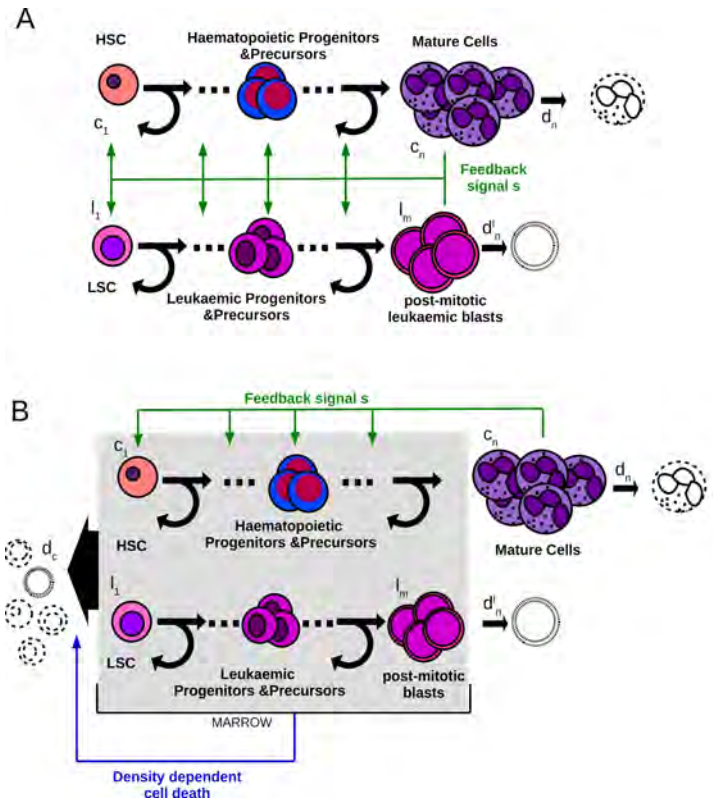

Fig. 6 Models of cytokine-dependent and cytokine-independent AML. (A) Model of cytokine-dependent AML. Healthy and leukemic cells both require cytokines to expand and compete for them. Self-renewal and proliferation of mitotic cells depend on the feedback signal. Hematopoietic (leukemic) stem cells are denoted by c_1 (l_1), the most differentiated stages of hematopoietic (leukemic) cells are denoted by c_n (l_m). Progentiors and precursors correspond to $c_2, \ldots, c_{n-1}$ and $l_2, \ldots, l_{m-1}$ (if $n,\ m > 2$). (B) Model of cytokine-independent AML. Leukemic cells expand independent of cytokines (e.g., due to constitutive activation of signaling pathways). Hematopoietic cells are subjected to the physiological feedback. Hematopoietic and leukemic cells compete for space in the bone marrow. Marrow-crowding induces cell death.

We denote by $l_i(t)$, $1 \leq i \leq m$ the number of leukemic cells of maturation stage i per kg of body weight at time t. Here, l_1 corresponds to *leukemic stem cells (LSC)* also referred to as *leukemia initiating cells*, and l_m to so-called post-mitotic blasts, the most differentiated stage of leumkemic cells. Compared to mature healthy cells the most differentiated leukemic cell stage still shows characteristics of immature cells. This phenomenon is referred to as *differentiation block*. Proliferation rate and self-renewal probability of leukemic cells of stage i at time t are denoted by $p_i^l(t)$ and $a_i^l(t)$. The death rate of post-mitotic blasts is referred to as $d_n^l > 0$.

In analogy to Section 4.2, we make a quasi steady state approximation to obtain the feedback signal as a function of healthy mature cells c_n and post-mitotic blasts l_m. This results in

$$s(t) = \frac{1}{1 + k_l l_m(t) + k_c c_n(t)}, \tag{11}$$

where k_l and k_c are positive constants [189, 196]. For many considerations we assume $k_l = k_c$, corresponding to a comparable density of G-CSF-receptors on mature cells and leukemic blasts.

Assuming that healthy and leukemic cells interact via the feedback signal we obtain the following model from [196]. The derivation is analogous to that of the model of healthy white blood cell formation in Section 4.1 and Fig. 4(C):

$$\frac{d}{dt}c_1(t) = (2a_1(t) - 1)p_1(t)c_1(t) \tag{12}$$

$$\frac{d}{dt}c_2(t) = 2(1 - a_1(t))p_1(t)c_1(t) + (2a_2(t) - 1)p_2(t)c_2(t) \tag{13}$$

$$\cdots \quad \cdots$$

$$\frac{d}{dt}c_{n-1}(t) = 2(1 - a_{n-2}(t))p_{n-2}(t)c_{n-2}(t) + (2a_{n-1}(t) - 1)p_{n-1}(t)c_{n-1}(t) \tag{14}$$

$$\frac{d}{dt}c_n(t) = 2(1 - a_{n-1}(t))p_{n-1}(t)c_{n-1}(t) - d_n c_n(t) \tag{15}$$

$$\frac{d}{dt}l_1(t) = (2a_1^l(t) - 1)p_1^l(t)l_1(t) \tag{16}$$

$$\frac{d}{dt}l_2(t) = 2(1 - a_1^l(t))p_1^l(t)l_1(t) + (2a_2^l(t) - 1)p_2^l(t)l_2(t) \tag{17}$$

$$\cdots \quad \cdots$$

$$\frac{d}{dt}l_{m-1}(t) = 2(1 - a_{m-2}^l(t))p_{m-2}^l(t)l_{m-2}(t) + (2a_{m-1}^l(t) - 1)p_{m-1}^l(t)l_{m-1}(t) \tag{18}$$

$$\frac{d}{dt}l_m(t) = 2(1 - a_{n-1}^l(t))p_{m-1}(t)l_{m-1}(t) - d_n^l l_m(t) \tag{19}$$

$$a_i(t) = \frac{a_{i,max}}{1 + k_c c_n(t) + k_l l_m(t)} \tag{20}$$

$$p_i(t) = \frac{p_{i,max}}{1 + k_c c_n(t) + k_l l_m(t)} \quad \text{or} \quad p_i(t) \equiv p_i \tag{21}$$

$$a_i^l(t) = \frac{a_{i,max}^l}{1 + k_c c_n(t) + k_l l_m(t)} \tag{22}$$

$$p_i^l(t) = \frac{p_{i,max}^l}{1 + k_c c_n(t) + k_l l_m(t)} \quad \text{or} \quad p_i^l(t) \equiv p_i^l. \tag{23}$$

The model is supplemented by non-negative initial conditions. The feedback dependence of proliferation rates has little impact on the qualitative system dynamics and the model still shows realistic behavior if proliferation rates are assumed to be constant in time.

As initial condition we choose homeostatic cell counts for the healthy subsystem (i.e., cell counts observed in healthy individuals) and a small number of leukemic stem cells. This state corresponds to the situation where a LSC population has just emerged due to mutations [196]. A similar model has been studied in [212] in the context of myelodysplastic syndroms, another malignant hematologic disease.

Mathematical analysis implies that in the considered model an increased self-renewal probability of LSC compared to HSC ($a_{1,max}^l > a_{1,max}$) is sufficient for the destabilization of the homeostatic equilibrium, even if leukemic cells proliferate slower compared to hematopoietic cells [196]. This suggests that increased self-renewal is a key driver of LSC expansion. If all parameters, except stem cell self-renewal, are identical for hematopoietic and leukemic cells, healthy cells will eventually be out-competed.

The opposite scenario, where proliferation of malignant cells is increased compared to the healthy cells but all other parameters do not differ is not always sufficient to trigger efficient leukemic cell expansion. To illustrate this let us assume that stem cells do not die and that the system is in its healthy equilibrium. Under these conditions the self-renewal probability of stem cells is 50%, i.e., in average one offspring of a stem cell is again a stem cell and the other is a progenitor. This ensures that

the stem cell population is constant over time. If the self-renewal probability of LSC is identical to that of HSC the LSC population cannot expand if a small number of LSC emerges in the healthy system. In this case the self-renewal probability of the LSC is also 50% which implies that their number remains constant [197]. Phrased differently, if stem cells do not die and if LSC self-renewal is identical to HSC self-renewal the LSC population cannot out-compete the HSC population. At each given time the self-renewal of both populations is either larger than 50%, then both populations expand, equal to 50%, then both populations do not change or smaller than 50%, then both populations shrink. In none of these cases the LSC population expands while the HSC population declines. Only if there exists a non-negligible stem cell death rate and if the death rate of LSC is not larger than that of HSC increased proliferation rates alone are sufficient to trigger efficient leukemic cell expansion [196].

The considered simple model is able to reproduce the biphasic disease dynamics consisting of a long pre-manifestation phase with a slow increase of the malignant cell burden followed by an acute phase with rapid leukemic cell growth, see the red curve in Fig. 7(A).

5.2 Cytokine-Independent Acute Myeloid Leukemia

In the previous section we have assumed that leukemic cells depend on the cytokine feedback. However, this assumption is not fulfilled for all patients. Leukemic cells of some patients exhibit autonomous growth, i.e., they proliferate in cell cultures in absence of cytokines [123]. In the following we consider the model from [193] which accounts for this observation. It is illustrated in Fig. 6(B). In the model healthy cell dynamics are governed by a cytokine feedback, as described above. Leukemic cells are assumed to grow independent of the cytokine signal. The unregulated growth of leukemic cells leads to an overcrowded bone-marrow. We assume that the overcrowding results in increased death of healthy and leukemic cells. This assumption is supported by increased markers of cell death such as LDH [186]. Whereas in the model described in Section 5.1 healthy and AML cells compete for the feedback signal, in the model considered in this section they compete for space.

The crowding-induced death rate is denoted by d_c and it is a function of the total amount of cells in the bone marrow. In healthy individuals approximately 50% of the bone marrow consist of empty space filled by fat tissue [144] which allows nearly a doubling of the cells without physical compression. Motivated by this we assume that d_c equals zero as long as the marrow cell count is below a critical threshold x^* and strictly monotonously increasing for larger cell counts. This results in the following system of equations.

$$\frac{d}{dt}c_1(t) = (2a_1(t) - 1)p_1(t)c_1(t) - d_c(x(t))c_1(t) \tag{24}$$

$$\frac{d}{dt}c_2(t) = 2(1 - a_1(t))p_1(t)c_1(t) + (2a_2(t) - 1)p_2(t)c_2(t) - d_c(x(t))c_2(t) \tag{25}$$

$$\cdots \quad \cdots$$

$$\frac{d}{dt}c_{n-1}(t) = 2(1 - a_{n-2}(t))p_{n-2}(t)c_{n-2}(t) \tag{26}$$

$$+ (2a_{n-1}(t) - 1)p_{n-1}(t)c_{n-1}(t) - d_c(x(t)c_{n-1}(t)) \tag{27}$$

$$\frac{d}{dt}c_n(t) = 2(1 - a_{n-1}(t))p_{n-1}(t)c_{n-1}(t) - d_n c_n(t) \tag{28}$$

$$\frac{d}{dt}l_1(t) = (2a_1^l - 1)p_1^l l_1(t) - d_c(x(t))l_1(t) \tag{29}$$

$$\frac{d}{dt}l_2(t) = 2(1 - a_1^l)p_1^l l_1(t) + (2a_2^l - 1)p_2^l l_2(t) - d_c(x(t))l_2(t) \tag{30}$$

$$\cdots \quad \cdots$$

$$\frac{d}{dt}l_{m-1}(t) = 2(1 - a_{m-2}^l)p_{m-2}^l l_{m-2}(t) + (2a_{m-1}^l - 1)p_{m-1}^l l_{m-1}(t) - d_c(x(t)) \tag{31}$$

$$\frac{d}{dt}l_m(t) = 2(1 - a_{n-1}^l)p_{m-1}l_{m-1}(t) - d_m^l l_m(t) - d_c(x(t))l_m(t) \tag{32}$$

$$x(t) = \sum_{i=1}^{n-1} c_i(t) + \sum_{i=1}^{m} l_i(t) \tag{33}$$

$$d_c(x) = d_0 \cdot \max(0, x - x^*) \tag{34}$$

$$a_i(t) = \frac{a_{i,max}}{1 + k_c c_n(t)} \tag{35}$$

$$p_i(t) = \frac{p_{i,max}}{1 + k_c c_n(t)} \quad \text{or} \quad p_i(t) \equiv p_i, \tag{36}$$

supplemented by non-negative initial conditions. The above formulation of the model assumes that all post-mitotic leukemic blasts stay in the bone marrow. For cases, where post-mitotic blasts appear in circulation, the model can be modified accordingly. In the following we refer to this model as *cytokine-independent* leukemia and to the model from Section 5.1 as *cytokine-dependent leukemia*.

In case of autonomous cell growth the condition $a_1^l > 0.5$ is sufficient to destabilize physiological homeostasis. This condition means that if upon division LSC give rise to more LSC than progenitors, the LSC population will eventually out-compete the HSC population. Unlike the model from section 5.1 the model considered here allows stable coexistence of mutated and healthy cells. This occurs if the self-renewal fraction of the mutated cells is smaller than the maximal possible self-renewal frac-

tion of HSC, i.e., $0.5 < a_1^l < a_{1,max}$. The observed coexistence may play a role in clonal hematopoiesis or slowly evolving hematological diseases [193].

When comparing both AML models using biologically plausible parameters it becomes evident that the model of cytokine-independent leukemia shows a faster progression of the disease, Fig. 7(A). This can be explained by the autonomous leukemic cell growth. In case of cytokine-dependent leukemia the LSC respond to the feedback signal in the same way as the healthy cells. Therefore, if the system is close to the homeostatic state, proliferation and self-renewal of healthy and leukemic cells are below their maximal values which are only assumed under maximal stimulation. The excess production of leukemic blasts leads to a further reduction of the feedback signal which results in a decrease of self-renewal and thus in a reduced growth rate of leukemic cells, Fig. 7(B)-(C). In case of cytokine-independent leukemia leukemic cells expand at their maximal rate until the onset of crowding-related cell death, Fig. 7(C)-(D).

Fitting of the two models to longitudinal bone marrow blast counts obtained during follow-up examinations of AML patients reveals that the data of many patients can be explained by both models. The data of a subgroup of patients only fits to the model of cytokine-dependent AML. Examples are depicted in Fig. 7(E)-(F). It turns out that patients whose marrow cell blasts only fit to the model of cytokine-independent AML have a poorer prognosis compared to the patients fitting to both models. This is in line with the finding that autonomous cell growth is linked to a poor prognosis [123] and is further supported by studies suggesting that high LDH (i.e., high cell death) is associated with poor prognosis [74, 186, 221].

The models from Sections 5.1 and 5.2 were successfully applied to clinical data [190, 193]. They have in common that the leukemic cell bulk exerts pressure on all immature hematopoietic cell types, since all immature cell types require either space or cytokines. In these models hematopoietic stem cells survive longer than all other cell types if there is a shortage of resources. To evaluate whether this observation is realistic, we consider literature reports of patients with bone marrow metastases.

Metastases in the bone marrow consume resources and impair healthy blood cell formation. If HSC are most robust to a shortage of resources, as observed in our models, blood cell formation will be restored after treatment of the metastases. This is the case, since, due to the high robustness of HSC to environmental pressure, the marrow still contains sufficient stem and early progenitor cells to restore the hematopoietic system and the impaired blood cell counts observed before treatment are caused by shortages of late progenitors and precursors. Indeed, this feature of the models is in line with clinical case reports. Bone marrow metastasis can lead to a severe impairment of healthy blood cell formation, however, shortly after treatment of the primary cancer blood cell counts recover [7, 96, 112, 213] which indicates that HSC and early progenitors have survived in the presence of the metastases, whereas

more mature progenitors have been drastically reduced due to spatial restrictions. When the metastases are eradicated by therapy stem and early progenitor cells drive the recovery of the hematopoietic system.

Malignant diseases of the blood forming system have been modeled by many authors. Examples include [20, 39, 41, 59, 75, 139, 145, 159, 160, 165, 171–173, 220] (different types of leukemias) and [11, 12, 142, 149–151, 176] (myeloproliferative neoplasms).

5.3 Models of the stem cell niche

HSC require the so-called stem cell niche to maintain their function. Animal models suggest that leukemic cells interfere with the hematopoietic stem cell niche and thus impair blood cell formation [30, 43, 94]. Whether leukemic cells interact with the hematopoietic stem cell niche in the human bone marrow is difficult assess experimentally. A combination of clinical data and mathematical modeling supports the hypotheses that human AML stem cells impair the niche. For a subclass of AML (so-called ALDH rare AML) it is possible to reliably separate hematopoietic stem and progenitor cells from leukemic cells [89]. This allows us to observe the time evolution of leukemic blasts and HSC in humans. Clinical trial observations in ALDH rare AML patients suggest that the decline of HSC occurs before the relapse-related increase of blasts [215].

Based on this observation we developed a mathematical model of the stem cell niche which is based on the following assumptions, see Fig. 8:

- HSC and LSC require a stem cell niche to maintain their stemness
- HSC and LSC compete for spaces in the same stem cell niche
- Upon division one stem cell gives rise to two stem cells. One of the two progeny cells occupies the niche of the parent cell and remains a stem cell.
- The other daughter cell attempts to occupy a niche space. During this process LSC can dislodge HSC from the niche and vice versa. If the daughter cell is successful in occupying a niche, it remains a stem cell, otherwise it differentiates into a progenitor. Cells that have been dislodged from the niche differentiate into progenitors.
- Progenitor cells proliferate and can give rise to progenitors or precursors. After a maximum number of divisions, progenitors differentiate into precursors.
- Precursor cells proliferate and can give rise to precursors or mature cells. After a maximum number of divisions, precursors differentiate into mature cells.
- Healthy cell proliferation rates and the maximal number of divisions healthy progenitors and precursors can perform before further differentiation are regulated by a feedback signal.

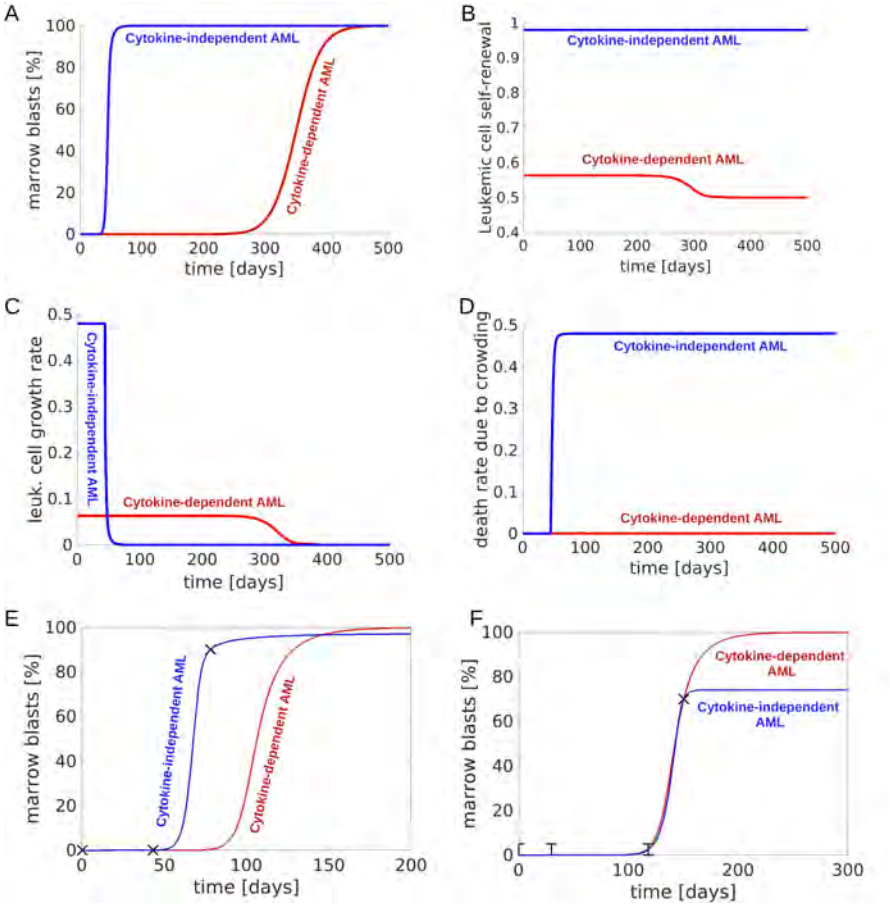

Fig. 7 Dynamics of the models of cytokine-dependent and cytokine-independent AML. (A) Cytokine-independent AML (blue curve) shows a faster disease progression compared to cytokine-dependent AML (red curve). Proliferation rates and maximal self-renewal probabilities of leukemic cells are the same for both simulated scenarios. (B) In cytokine-independent AML self-renewal is always maximal, since it is not down-regulated by the feedback signal (constitutive activation), in cytokine-dependent AML self-renewal is down-regulated by the feedback signal since in homeostasis the concentration of the feedback signal is low and it further decreases if blasts accumulate. (C) Net growth (gain due to proliferation minus loss due to death) of leukemic cells. In cytokine-independent leukemia, leukemic cell expansion is fast and abruptly decreases due to crowding-induced cell death. In cytokine-dependent AML the feedback signal reduces leukemic cell growth. (D) In cytokine-dependent AML cell death increases due to marrow crowding. This is not observed in cytokine-dependent AML since the feedback signal keeps the total cell number below a critical threshold. (E) Example for patient data (indicated in black) agreeing only with the model of cytokine-independent AML. (F) Example for patient data (indicated in black) agreeing with both models. Models from Sections 5.1 and 5.2 with $m = n = 2$. (A)-(D) are based on the following parameters for leukemic cells: $a^l_{1,max} = 0.98$, $p^l_1(t) \equiv p^l_1 = 0.5/day$, $k_c = 1.85 \cdot 10^{-9} kg$, $k_l = 0.25 \cdot k_c$, $d^l_n = 0.1/day$, $d_0 = 2 \cdot 10^{-9}/day$, $x^* = 4 \cdot 10^9/kg$ (corresponding to 2 times the homeostatic marrow cell count), cells are measured per kg of body weight. Data and parameters of healthy cells taken from [193]. (own figure)

For details and equations we refer the reader to [215]. Simulations suggest that the probability that LSC dislodge HSC has to be higher compared to the probability that HSC dislodge LSC to observe a clinically relevant growth of the LSC population. Unlike the models from Sections 5.1 and 5.2 this model can recapitulate the decline of healthy stem cells observed in ALDH rare AML before clinically overt relapse, see Fig. 9. Systematic comparison of different models, including models where HSC and LSC reside in separate niches, suggest that the decline of HSC before manifestation or relapse of the disease requires a competition on the level of stem cells [214]. Without direct competition between HSC and LSC the decline of HSC counts before onset of the relapse cannot be reproduced. This finding supports the view that LSC and HSC compete for the stem cell niche in human ALDH rare AML.

Dynamics in the stem cell niche play a role in different hematological diseases. Models for several aspects of the stem cell niche can be found in literature, see e.g., [16, 23, 90, 126, 127, 152].

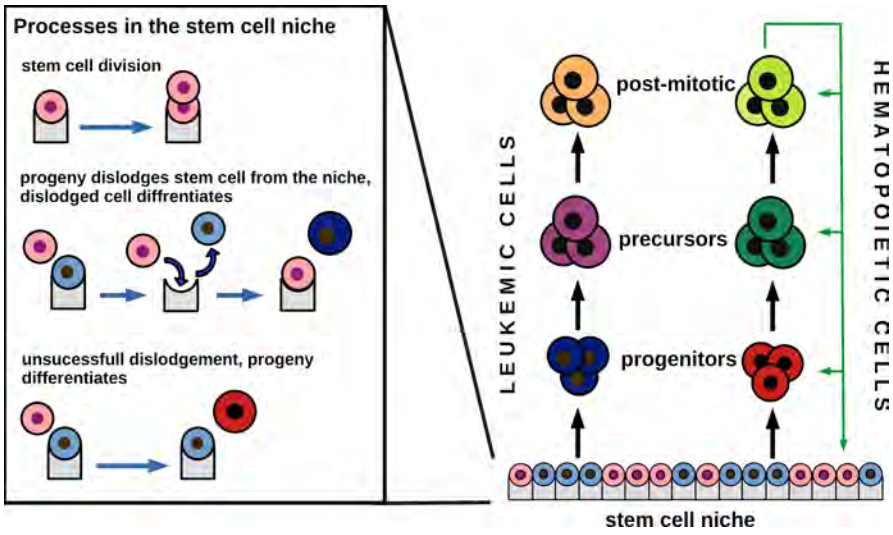

Fig. 8 Model of the stem cell niche. HSC and LSC compete for spaces in the stem cell niche. Progeny of LSC can dislodge HSC from the niche and vice versa, dislodged cells differentiate. The model accounts for stem, progenitor precursor and post-mitotic (i.e., blasts and mature) cells. Healthy cells are subjected to a feedback mechanism. (own figure)

5.4 Models of clonal evolution

A clone is composed of all cells that have identical genetic information, i.e., of all cells that carry the exact same set of genetic variants. Many hematological malig-

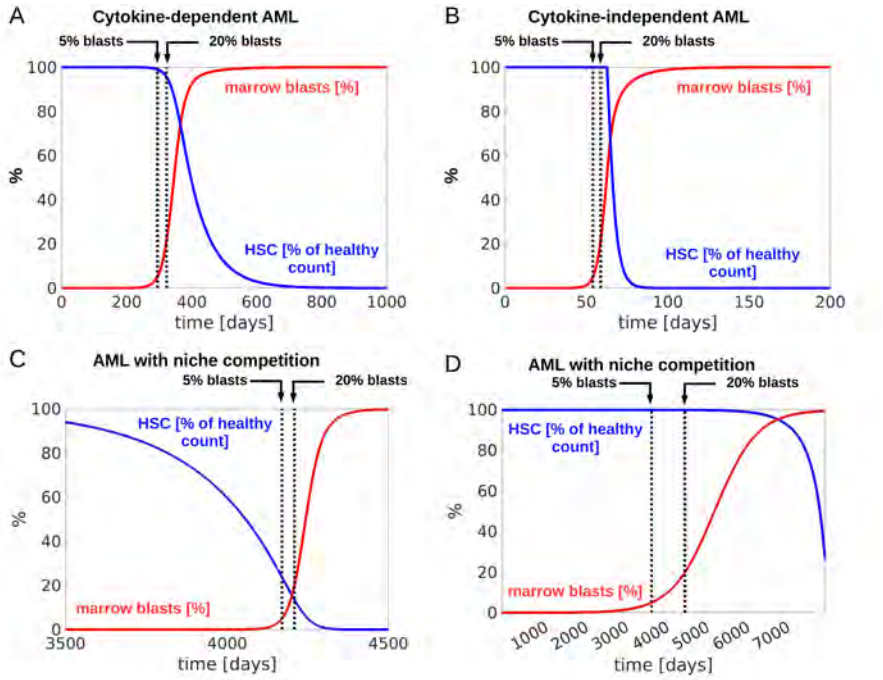

Fig. 9 Time evolution of HSC and blasts in different models of AML. (A) In cytokine-dependent AML no significant decrease of HSC counts is observed at disease onset or relapse. Disease onset is defined as 20% marrow blasts, relapse as 5%. (B) In cytokine-independent AML no significant decrease of HSC counts is observed at disease onset or relapse. The model of the stem cell niche can reproduce the observation that in some patients HSC counts are decreased at diagnosis and even decrease before overt relapse (C) whereas in other patients they are not significantly decreased (D). Models and parameters: (A) Model from Section 5.1 with $n = m = 3$, healthy cell parameters as in [190], $a^l_{1,max} = 0.99$, $a^l_{2,max} = 0.8$, $p^l_1(t) \equiv p^l_1 = 0.35/day$, $p^l_2(t) \equiv p^l_2 = 0.45/day$, $d^l_m = 2.3/day$. (B) Model from Section 5.2 with $n = m = 3$, healthy cell parameters as in [190], $a^l_{1,max} = 0.99$, $a^l_{2,max} = 0.8$, $p^l_1(t) \equiv p^l_1 = 0.35/day$, $p^l_2(t) \equiv p^l_2 = 0.45/day$, x^* equal to twice the marrow cell count in healthy homeostasis, $d_0 = 2.3/x^*/day$, $d^l_m = 2.3/day$. (C)-(D) Model and parameters from [201], in (C) the probability of HSC dislodgement equals 1, in (D) the probability of HSC dislodgement equals 0.95, other leukemic cell parameters are identical to healthy cell parameters. (own figure)

nancies have a complex clonal architecture [13, 54, 64, 218]. This means that the malignant cell bulk is composed of multiple clones, the sizes of which evolve over time. This observation is of high clinical relevance, since primary manifestation and relapse of the disease are often triggered by different clones. The more different clones exist, the higher is the probability that at least one is resistant to therapy.

Clonal evolution is shaped by many factors. Properties of cells such as proliferation or self-renewal and the presence or absence of treatment are two examples. Using sequencing technologies it is possible to measure or to reconstruct the clonal

composition of leukemic cells at diagnosis and at relapse [169, 199]. However, in many cases it remains an open question how the detected mutations impact on kinetic cell properties. Different mutations may lead to similar phenotypes and the impact of known mutations may also depend on the presence of new, yet un-characterized hits. Furthermore, kinetic cell properties may also depend on metabolic or epigenetic states [97, 202]. Clonal dynamics are significantly shaped by kinetic cell properties and two clones with similar kinetic parameters may show similar dynamics, even if the specific phenotypes are triggered by different mutations. We, therefore, consider a model on the level of cell phenotype (proliferation and self-renewal).

To study how proliferation and self-renewal impact on clonal selection, we consider multi-clonal versions of the models from Sections 5.1 and 5.2 which have been described in [189]. The model is illustrated in Fig. 10. For simplicity we do not consider multiple progenitor and precursor stages and only distinguish between mitotic cells (sum of stem, progenitor and precursor cells) and post-mitotic cells (i.e., mature cells and post-mitotic blasts). The model accounts for healthy cells and n leukemic clones. The number of mitotic cells of clone i per kg of body weight at time t is denoted by $l_1^i(t)$, the number of post-mitotic cells as $l_2^i(t)$. Proliferation rate and maximal self-renewal fraction as p_l^i and a_l^i. The respective amounts of healthy cells are denoted by $c_1(t)$ and $c_2(t)$. In analogy to Section 5.1 we obtain the following system of ordinary differential equations describing the clonal competition.

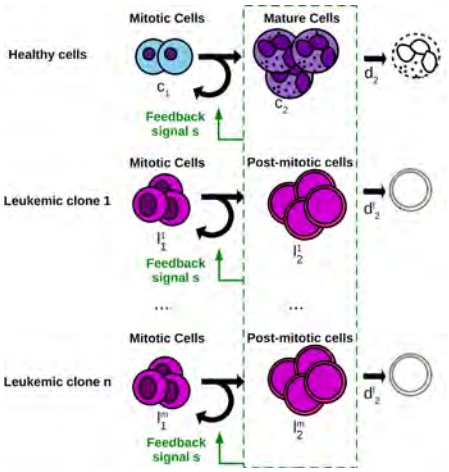

Fig. 10 Model of clonal competition. The model describes the competition of healthy cells and multiple leukemic clones. For simplicity we distinguish only between mitotic and post-mitotic cells. The model does not account for new mutations, it only considers the competition of a fixed number of clones.

$$\left.\begin{aligned}\frac{d}{dt}c_1(t) &= (2a_1 s(t) - 1)p_1 c_1(t)\\ \frac{d}{dt}c_2(t) &= 2(1 - a_1 s(t))p_1 c_1(t) - d_2 c_2(t)\end{aligned}\right\}\text{healthy} \tag{37}$$

$$\left.\begin{aligned}\frac{d}{dt}l_1^1(t) &= (2a_l^1 s(t) - 1)p_l^1 l_1^1(t)\\ \frac{d}{dt}l_2^1(t) &= 2(1 - a_l^1 s(t))p_l^1 l_1^1(t) - d_l l_2^1(t)\end{aligned}\right\}\text{leukemic clone 1} \tag{38}$$

$$\dots$$

$$\left.\begin{aligned}\frac{d}{dt}l_1^n(t) &= (2a_l^n s(t) - 1)p_l^n l_1^n(t)\\ \frac{d}{dt}l_2^n(t) &= 2(1 - a_l^n s(t))p_l^n l_1^n(t) - d_l l_2^n(t)\end{aligned}\right\}\text{leukemic clone } n \tag{39}$$

$$s(t) = \frac{1}{1 + k\left(c_2(t) + \sum_{i=1}^{n} l_2^i(t)\right)} \tag{40}$$

For simplicity we assume that the death rate d_l and feedback parameter k are identical for all clones. Furthermore we assume that proliferation rates do not depend on the cytokine feedback. These simplifications have only minor impact on the observed selection dynamics [189]. An analogous model can be derived based on the assumptions from Section 5.2. This type of model neglects new mutations, instead it studies how leukemic cell properties (proliferation and self-renewal) shape clonal selection.

The model exhibits complex dynamics. Clones which dominate during the early phases of the disease decline over time whereas others dominate during late phases of the disease, Fig. 11. This illustrates that even in absence of new mutations the abundance of the different leukemic clones can considerably change over time.

Virtual Patient, Virtual Trial, Digital Twin

The term "virtual patient" has been used in many different contexts without clear definition [35, 111]. A virtual patient can be seen as a computer simulation of a patient or of specific aspects of a patient. Virtual patients can be used to study disease dynamics and clinical interventions. Simulations of large groups of virtual patients which represent inter-individual variability are referred to as *virtual (clinical) trial* or *in silio trial* [115, 210]. A *digital clone* or *digital twin* is a digital representation of a specific real patient. A digital twin can be used for the personalized planning of medical interventions and personalized prediction of disease dynamics [44, 82, 100, 211, 227]. A *personalized model* is a model which has been adapted and calibrated to recapitulate the dynamics observed in a specific patient. Personalized models can be used for similar purposes as digital twins, however, they are usually more focused on a specific aspect.

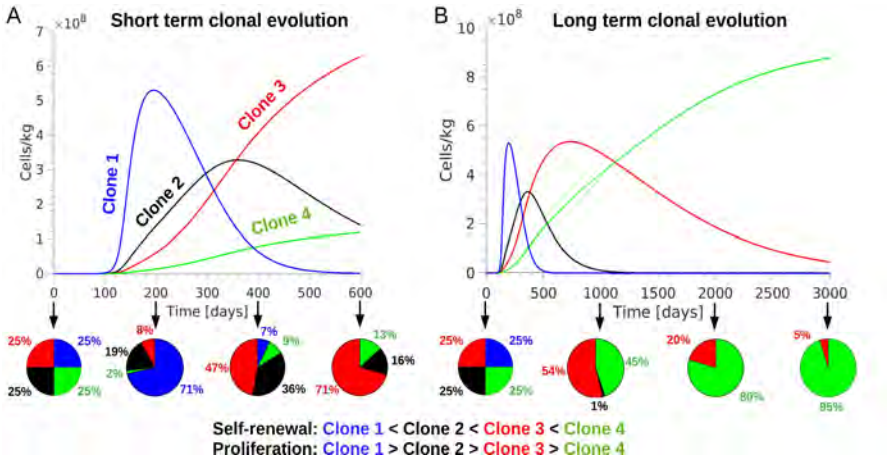

Fig. 11 Example simulation of clonal selection. Dynamics of short term (A) and long-term (B) clonal selection in the model from Section 5.4. Clones dominating short-term dynamics are driven to extinction for longer times. The model neglects new mutations. The observed heterogeneity emerges only from selection. At the beginning of the simulation healthy cells are in equilibrium and each clone consists of one cell per kg. of body weight. Parameters: $a_1^l = 0.9686$, $a_2^l = 0.9816$, $a_3^l = 0.9880$, $a_4^l = 0.9899$, $p_1^l = 1.19bay$, $p_2^l = 0.95/day$, $p_3^l = 0.84/day$, $p_4^l = 0.77/day$, $k = 1.75 \cdot 10^{-9} kg$, $d^l = 0.5/day$ healthy cell parameters as in [189]. (own figure, based on [189])

Systematic simulations for a large group of virtual patients each of which harbors many different clones with random proliferation rates and self-renewal probabilities allow to study how leukemic cell properties shape clonal selection [189]. The simulations reveal that at the time point when mature cell counts or marrow blasts assume pathological values 90%-95% of the total leukemic cell mass are derived from 5 or less clones in most virtual patients. This observation is in line with clinical data [54, 64] and suggests that clonal competition limits the number of large clones.

Since many of the classical chemotherapeutic agents preferentially act on fast dividing cells, it is important to understand how leukemic cell properties, i.e., proliferation and self-renewal, evolve during the course of the disease. Simulations of the model suggest that the dominating clones at the time of disease manifestation are characterized by high proliferation rates and high fractions of self-renewal. This is intuitive since this specific parameter combination results in an efficient clonal growth rate.

To understand how therapy impacts on clonal selection we simulated classical cell-cycle dependent cytotoxic chemotherapy followed by bone marrow transplantation. The chemotherapy was implemented as a death rate that is proportional to the proliferation rate of the respective clone, i.e., the faster cells divide the faster they are eradicated by the therapy. The main idea of this approach is that the higher the proliferation rate of a clone, the more cells are actively cycling during administration

of the therapy and the higher the proportion of cells that are eliminated. For a more detailed model of chemotherapy see [20]. In the simulations we assume that clones consisting of less than one cell are permanently eradicated. An example simulation is depicted in Fig. 12 (A)

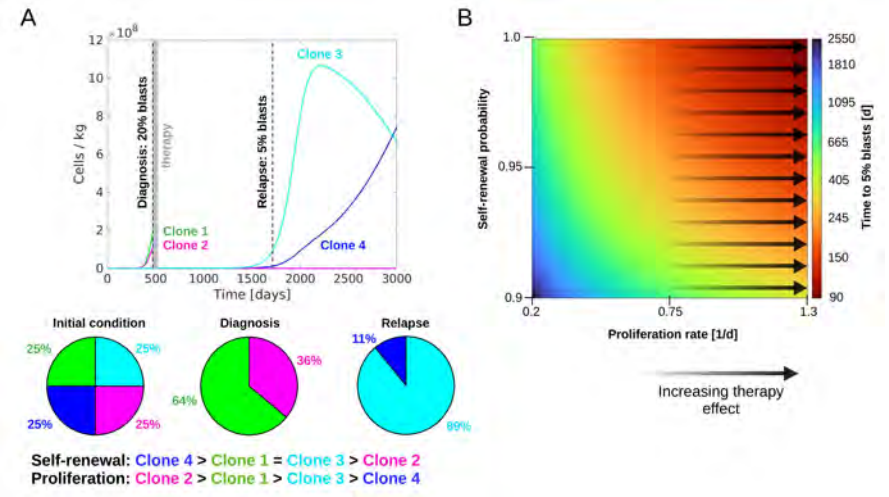

Fig. 12 Simulation of clonal competition in presence of chemotherapy. (A) Example simulation of clonal competition before and after treatment. The observed heterogeneity emerges solely from clonal selection, new mutations are not considered. The figure depicts the total cell count per clone. (B) Impact of cell properties on the speed of clonal expansion. The figure depicts how long it takes for a single leukemic clone to expand from an initial abundance of 1 cell per kg of body weight to 5% marrow blasts. For high self-renewal fraction and proliferation rate the expansion is fastest. In case of classic cytotoxic therapy acting on dividing cells clones with high proliferation rate have a selective disadvantage since they are preferentially eliminated by the therapy (indicated by black arrows). Under this selective pressure clones with small proliferation rates and high fractions of self-renewal expand fastest. This suggests that clones triggering relapse exhibit slow proliferation rates and high self-renewal fractions. Parameters: $a_l^1 = 0.9$, $a_l^2 = 0.897$, $a_l^3 = 0.9$, $a_4^l = 0.91$, $p_1^l = 1.2/day$, $p_2^l = 1.3/day$, $p_3^l = 0.3/day$, $p_4^l = 0.2/day$, $d^l = 0.5/day$, $k = 1.75 \cdot 10^{-9}$, therapy induced death rate: $100 \cdot p$, where p is the proliferation rate, healthy cell parameters as in [189]. (own figure)

Our therapy simulations are in line with the observation that in most patients relapse is triggered by different clones than primary manifestation. This observation supports the view that relapse can originate from clones which are already present at the time of diagnosis but below the detection limit. The expansion of these small clones is triggered by the selective pressure of chemotherapy and the eradication of large clones. This concept suggests that the appearance of 'new' clones at relapse not necessarily requires new mutations to occur during or after therapy.

Similarly as at the time of disease manifestation, the number of large leukemic clones at relapse is smaller than 5 in most simulations. The major clones at relapse are characterized by a small proliferation rate and a very high self-renewal. The first property is a direct consequence of the assumed mode of drug action. In the presence of classical cytotoxic drugs slow cell proliferation is an advantageous property since it leads to resistance. Among slowly proliferating clones those with high self-renewal grow faster compared to those with small self-renewal. In this sense, high self-renewal confers a growth advantage which supports positive selection in an environment acting against fast proliferation. The combination of slow proliferation and high self-renewal leads to therapy resistance and fast clonal expansion at the same time, see Fig. 12 (B). Furthermore, this combination of cell properties can explain the poor response to chemotherapy in relapsed AML patients.

Mathematical analysis of the models of AML clonal evolution implies that for long times exactly the clones with maximal stem cell self-renewal survive and all others are driven to extinction [33, 122]. Proliferation rate and self-renewal probability of progenitor and precursor cells impact only on transient dynamics but do not affect long-term selection. The clonal heterogeneity detected in AML patients suggests that clonal evolution occurs on a rather slow time scale, i.e., over years or decades [1]. In the mathematical sense, the clonal evolution detected in AML patients corresponds to transient dynamics of the system and may be very different from the clonal composition observed for times tending to infinity.

An extended version of this model taking into account random mutations allows us to reconstruct the phylogenetic tree of the clonal evolution in the virtual patients [194]. The extended model can reproduce clonal trees detected in clinical data. Also this model suggests that self-renewal increases during disease evolution and with the depth of the phylogenetic tree [194]. A murine adaptation of the model described in this section is considered in [187].

Models of clonal evolution have significantly contributed to our understanding of diseases of the blood forming system. For other examples, see [21, 57, 58, 71, 140, 207].

6 Towards Personalized Medicine

Personalized Medicine

The aim of *personalized medicine* is to tailor the management (or prevention) of a disease to the specific conditions of each individual patient [77, 137]. Multiple reasons could explain why patients suffering from "the same" disease show divergent clinical performance. Patient-related factors include genetic polymorphisms, comorbidities,

exposure to risk-factors, lifestyle and previous treatments. Examples for disease-related factors are specific genetic, epigenetic and metabolic traits of malignant cells or pathogeneic organisms. The aim of personalized medicine is to take these differences into account to better adapt treatment and prevention protocols to the patients' needs. Personalized medicine relies on multiple tools including omics technology, individual monitoring, medical imaging and targeted genetic analyses. Mathematical and computational modeling can contribute to personalized medicine by quantifying relevant disease parameters, optimizing treatment protocols and providing personalized predictions. The refined characterization of diseases using genetic and other technologies is also referred to as *precision medicine* [17].

Since mathematical models are helpful tools to understand and predict complex dynamics [9] they can substantially contribute to personalized medicine. Potential applications include treatment optimization, drug design, personalized prognostication of disease progression or prediction of therapy response [5, 6, 8, 31, 37, 95, 135, 136, 198, 200, 225, 226].

In this section we discuss two examples illustrating potential applications of mechanistic models in personalized medicine. Both examples combine mathematical modeling with clinical data and aim to predict the prognosis of AML patients. The first example is motivated by the question how self-renewal probabilities and proliferation rates of leukemic stem and non-stem cells impact on the course of the disease.

To approach this question we consider the model from Section 5.1 with $n = m = 3$ (i.e., the healthy cells are subdivided into stem cells, progenitor/precursor cells and mature cells; the leukemic cells are subdivided into leukemic stem cells, leukemic progenitor/precursor cells and post-mitotic blasts). We calibrate parameters of healthy cells using physiological bone marrow cell counts and dynamics after bone marrow transplantation. We run computer simulations to quantify how the speed of disease progression, quantified by the growth rate of blasts, depends on the leukemic cell parameters. For this purpose we choose homeostatic cell counts as initial condition for the healthy cells and a small number of leukemic stem cells, e.g., 1 LSC per kg of body weight, as initial condition for the malignant cells. We vary self-renewal probabilities and proliferation rates of leukemic stem and progenitor cells over the biologically plausible range and record how long it takes to observe the onset of the disease, e.g., defined by 20% of marrow blasts or a clinically manifest reduction of healthy mature cells.

The simulations suggest that the speed of disease progression is fastest if LSC proliferation and self-renewal approach the upper bound of the biologically plausible range. In the simulations self-renewal and proliferation of leukemic progenitors have only a negligible impact on the speed of disease progression, see Fig. 13.

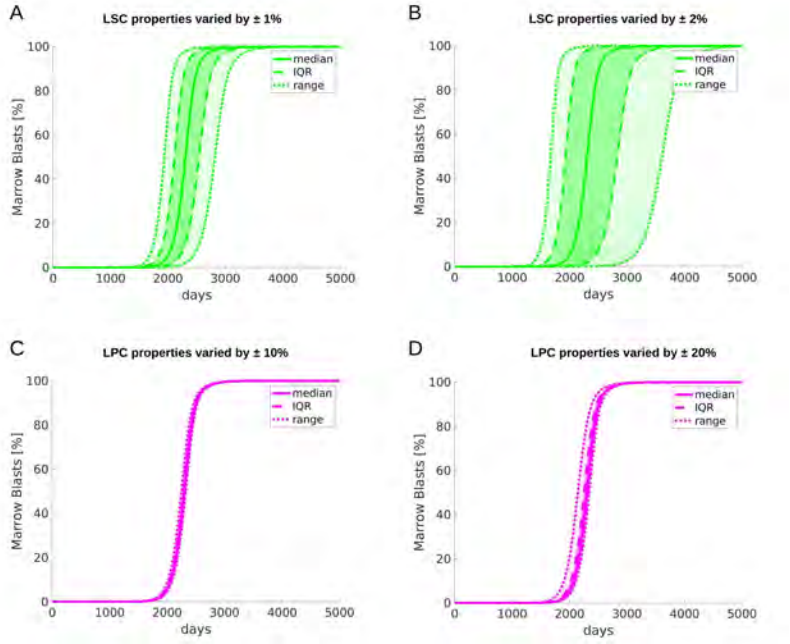

Fig. 13 Impact of leukemic cell properties on blast dynamics. Perturbation of LSC proliferation and self-renewal by ±1% (A) and ±2% (B) lead to considerable changes of marrow blast expansion kinetics. Perturbation of leukemic progenitor cell (LPC) proliferation and self-renewal by ±10% (C) and ±20% (D) have much less impact on growth dynamics of marrow blasts. This suggests that the growth dynamics of marrow blasts may provide information about LSC properties. Parameters: LSC: self-renewal under maximal stimulation 0.9, proliferation rate $0.15/day$; LPC: self-renewal under maximal stimulation 0.7, proliferation rate $0.45/day$; other parameters as in [190], perturbations using uniform distributions, IQR: inter quartile range. (own figure),

The latter observation suggests that the clinical course of the disease is mainly determined by the kinetic properties of LSC. Indeed, the generation of leukemic progenitor cells by LSC is the rate-limiting step of the blast production: Leukemic progenitors can divide only a limited number of times before they become post-mitotic and they divide on faster time scale compared to stem cells. Therefore, by propagating through the progenitor stage each single differentiating LSC is quickly transformed into an approximately constant number of post-mitotic blasts which accumulate in the marrow [190]. For this reason the number of differentiating LSC per unit of time the key determinant of blast production dynamics.

This observation suggests that it might be possible to extract LSC properties from clinical data. For this purpose we consider data from a cohort of AML patients with at least one relapse. For each patient we determine all possible combinations of LSC proliferation and self-renewal which can reproduce the clinically observed expansion of blasts between successful treatment (complete hematological remission) and

relapse.

Based on the estimated LSC properties it is possible to divide patients into two groups that significantly differ with respect to overall survival [190]. This example suggests that mathematical models can potentially provide insights into LSC dynamics and patient prognosis.

A major drawback of the discussed approach is that it requires data from the first relapse. A clinically useful risk-scoring, however, has to be available at the time of diagnosis. To be able to predict patient prognosis at the time of diagnosis, additional quantities have to be measured.

For this purpose we reconsider the model of the stem cell niche from Section 5.3. We ask whether the measurement of HSC and bone marrow blasts at the time of diagnosis may contain sufficient information about disease dynamics to parameterize a prognostic model. A clinical trial has provided evidence that low HSC counts at the time of diagnosis are related to poor overall and disease free survival [215].

To identify the main determinants of the speed of disease progression, we run model simulations varying cell parameters over biologically plausible ranges. In the simulations we record how long it takes to progress from one LSC per kg of body weight to 20% marrow blasts, which is a diagnostic criterion for AML. Model simulations suggest that the LSC proliferation rate and the HSC dislodgement probability are main determinants of the speed of disease progression [201].

The impact of LSC proliferation rate is straightforward since the faster LSC proliferate, the more progenitors and blasts are produced per unit of time. Simulations further indicate that for increasing LSC proliferation rates the impact of HSC dislodgement probability decreases. If HSC and LSC proliferate at similar rates, the HSC dislodgement probability has a non-linear impact on the speed of disease progression [201], see Fig. 14.

The biological rationale for this observation is as follows, see Fig. 15 (A)-(C): If the HSC dislodgement probability is small, most progeny of LSC do not acquire a niche space and differentiate. Therefore, the LSC population remains small. Since LSC are slowly dividing cells a small LSC population only gives rise to a small number of blasts per unit of time. This results in a slow disease progression, Fig. 15 (A). If the HSC dislodgement probability is high, practically all progeny of LSC acquire a niche space and remain stem cells. Therefore, no leukemic progenitors and no blasts are produced during the initial stages of the disease. If the time scale of HSC proliferation is similar to the time scale of LSC proliferation, the speed of disease progression is very slow. LSC rarely divide and practically no fast proliferating leukemic progenitors are produced. The high HSC dislodgement probability confines the time scale of disease progression to the time scale of LSC division: If LSC divide once to twice per year and their progeny are also LSC, the

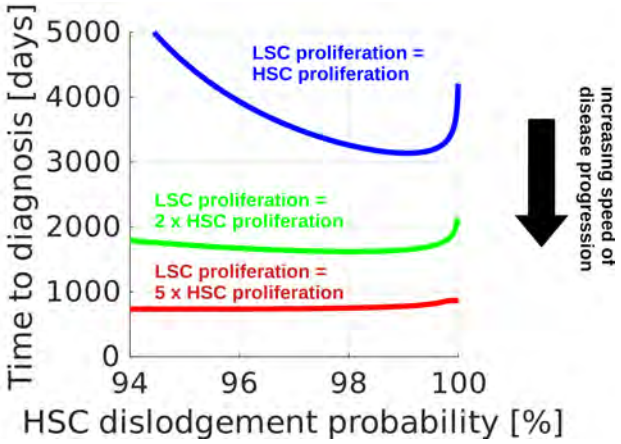

Fig. 14 Impact of LSC proliferation rate and HSC dislodgement probability on disease progression. The HSC dislodgement probability quantifies how probable it is that offspring originating from the division of LSC dislodge HSC from the niche. The higher the LSC proliferation rate the smaller the impact of HSC dislodgement probability on the speed of disease progression. If HSC and LSC proliferate at similar rates, the HSC dislodgement probability has a non-linear impact on the speed of disease progression. For low and high HSC dislodgement probabilities the disease progression is slow, for intermediate probabilities it is fast. The time to diagnosis in the simulations is defined as the time elapsing from an initial LSC count of one cell per kg of body weight to a system state with 20% marrow blasts. Parameters as in [201]. (own figure, adapted from [201])

amount of malignant cells increases slowly, Fig. 15 (C). Only the scenario with intermediate HSC dislodgement probabilities results in an efficient expansion of the LSC population and at the same time in an efficient production of fast proliferating leukemic progenitors. This scenario is related to a fast disease progression and a poor prognosis, Fig. 15 (B). In summary, according to the model the disease progression is fast if (i) LSC proliferate faster than HSC or (ii) LSC proliferation is similar to HSC proliferation and HSC dislodgement probability is not too high and not too small (which, for our parametrization of the model means around 97%) [201].

Inspired by these observations we propose a model-based risk stratification. We calibrate and fix parameters of healthy cells. We also fix the parameters of leukemic progenitors and precursors. We then check whether the model can reproduce the HSC and blast counts of a given patient at diagnosis assuming equal LSC and HSC proliferation rates. If this is possible we fit the HSC dislodgement probability and stratify patients based on the estimated HSC dislodgement probability. This allows a statistically significant sub-stratification of patients, Fig. 15 (D). If the diagnostic HSC and blast counts of a given patient can only be reproduced by the model assuming that LSC proliferate faster than HSC we fit the LSC proliferation rate. In this case patients are stratified based on the estimated LSC proliferation rate. Also this leads to a statistically significant risk-stratification [201]. It turns out that the model-based risk-stratification is an independent predictor in multivariate analysis [201]. This

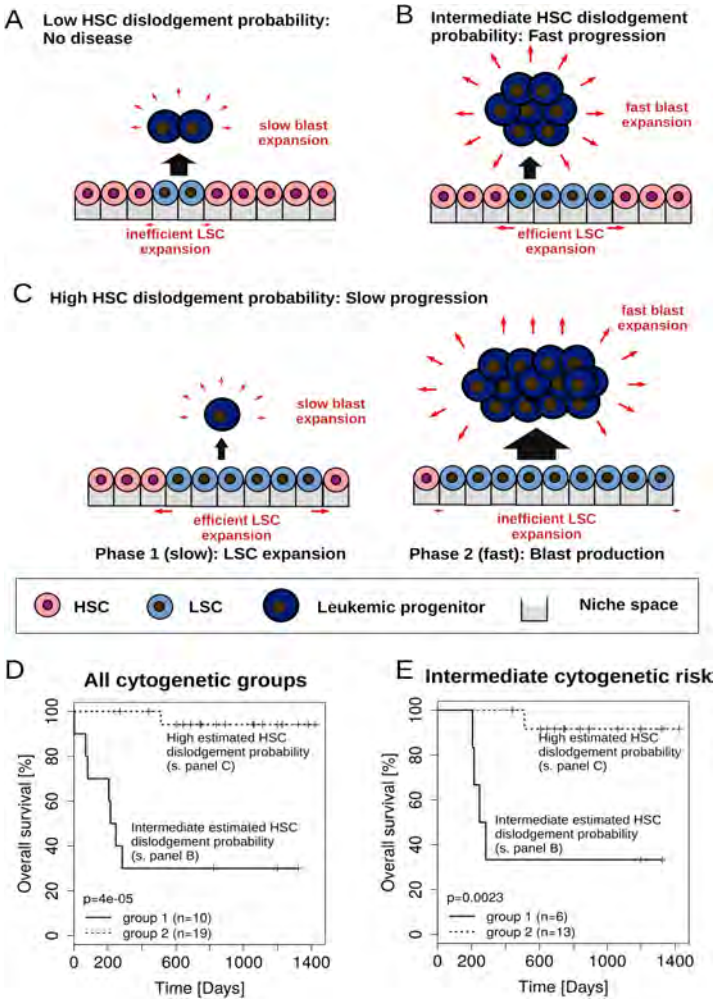

Fig. 15 Impact of HSC dislodgement probability of AML progression. If HSC and LSC proliferate at similar rates the probability with which LSC dislodge HSC impacts on the speed of disease progression. The expansion rates of leukemic blasts and stem cells are illustrated by red arrows (long arrow=fast expansion). (A) If HSC dislodgement probability is small, LSC expansion is slow since most progeny of LSC differentiate. Since a small number of LSC gives rise to a small amount of blasts per unit of time, the disease does not become manifest within life time. (C) If HSC dislodgement probability is very high, disease progression occurs in two phases. In the first phase practically all offspring of LSC are LSC, since they successfully dislodge HSC from the niche and maintain their stemness. Due to slow LSC division rates this leads to a slow invasion of the stem cell niche by LSC, which is asymptomatic for a long time. In the second phase when the number of HSC has drastically decreased, the number of LSC progeny that cannot dislodge HSC from the niche increases. The respective offspring differentiate into fast dividing leukemic progenitors. Since progenitors divide fast and the number of LSC is high this leads to a fast increase of blasts during the second phase of disease progression. Due to the long duration of the first phase, patient survival is good. (B) Intermediate values of HSC dislodgement lead to a balance between expansion of LSC and production of fast dividing leukemic progenitors. In this case disease progression is fast and survival is poor. (D) Parameterizing LSC properties based on HSC and blast counts at diagnosis patients can be assigned to two groups. One group corresponds to the scenario depicted in (B), the second group to the scenario depicted in (C). Both groups differ significantly with respect to overall survival. (E) Applying the model-based risk-stratification to patients with intermediate cytogenetic risk allows to further substratify these patients. This implies that the model-based risk-scoring provides information that is complementary to the clinically used cytogenetic stratification. (own figure, panels D and E based on [201])

implies that it provides additional information compared to the routinely used clinical stratification. Interestingly, the model-based risk-classification allows to further subdivide patients who belong to the same clinical risk-group, see Fig. 15 (E) and to improve the stratification proposed in [214], see [188]. These results suggest that the measurements of HSC and leukemic blasts at a single time point provide information about the speed of disease progression [188, 201].

It is important to note that the specific numerical values of the estimated parameters depend on the data *and* on the model. Applying a different model to the same data set results in different estimates. This makes it difficult to directly interpret the absolute values of the estimated quantities. Similarly, direct experimental measurements (if possible) may result in different numerical values compared to model-based estimates. However, applying the same model to all patients from a cohort allows to quantitatively compare the patients to each other and to rank them with respect to certain criteria. This is sufficient for many clinical applications. Many markers used in clinical practice have in common that their absolute values may be difficult to interpret (e.g., because they depend on the specific assay, method of measurement or condition of measurement). Nevertheless, when calibrated and applied to large cohort they provide important insights.

7 Discussion

Dynamics of the blood forming system result from the interaction of a plethora of processes which evolve on different time scales. Mathematical and computational models can help to understand and quantify the underlying biological mechanisms. Modeling renders biological concepts accessible to mathematical and computational tools, which allows to study the impact of specific sub-processes on a variable of interest. This helps to identify which aspects can be neglected and to simplify a model before it is applied to data. Especially if the available data is sparse such model reductions are important to extract information from the data.

In silico experiments can shed light on questions that cannot be targeted using *in vitro* or *in vivo* approaches. Dynamics of stem cells in the human bone marrow are one examples for this. Mathematical models can help to extract information from available data that is not accessible to intuitive approaches of visual inspection. The advantage of mathematical models is their ability to infer non-linear dynamics which can only be partially covered by straightforward scores.

Mathematical and computational models can help to quantify parameters that cannot be measured directly. This can be helpful in the context of personalized medicine or risk-scoring. Clearly, the specific parameter values extracted from a given data set depend on the model structure and other assumptions. For this reason they would differ from experimentally measured values, if these were available.

However, applying the same model to all patients of a cohort allows to rank patients with respect to certain properties (such as expected prognosis) and to relate the expected clinical course of a given individual to the clinical course of a reference group.

As most biomarkers used in clinical practice, the information extracted from patient data with the help of mathematical models has to be interpreted as a *surrogate* for biological processes. After careful calibration, such surrogates potentially provide useful and clinically relevant information. Since the clinical performance of a patient depends on a variety of potentially unknown factors, model-based predictions as well as clinical scores can be inaccurate. Taking potential drawbacks into account mathematical models are a powerful tool to predict risks or probabilities of certain events (e.g., the probability of disease progression, the probability of relapse, the probability of treatment failure) based on individual patient data, however, they usually cannot provide a deterministic prediction of individual fates.

Model-based risk-stratifications can be complementary to clinical approaches which are based on genetic data. For many diseases the spectrum of relevant mutations and their potential interactions and impacts on disease dynamics are not fully understood. Potentially unknown and therefore unassessed mutations may have unpredictable consequences for the fate of a patient. The same applies to non-genetic determinants such as epigentic or metabolic states. The model-based approach discussed here operates in the phenotypic space, i.e., it directly assesses cell properties such as proliferation and self-renewal. Therefore, it can provide additional information compared to genetic and cytological scores. The combination of omics technology, clinical risk-scoring and mechanistic modeling has great potentials to assess different aspects of the disease manifestation in a specific patient.

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