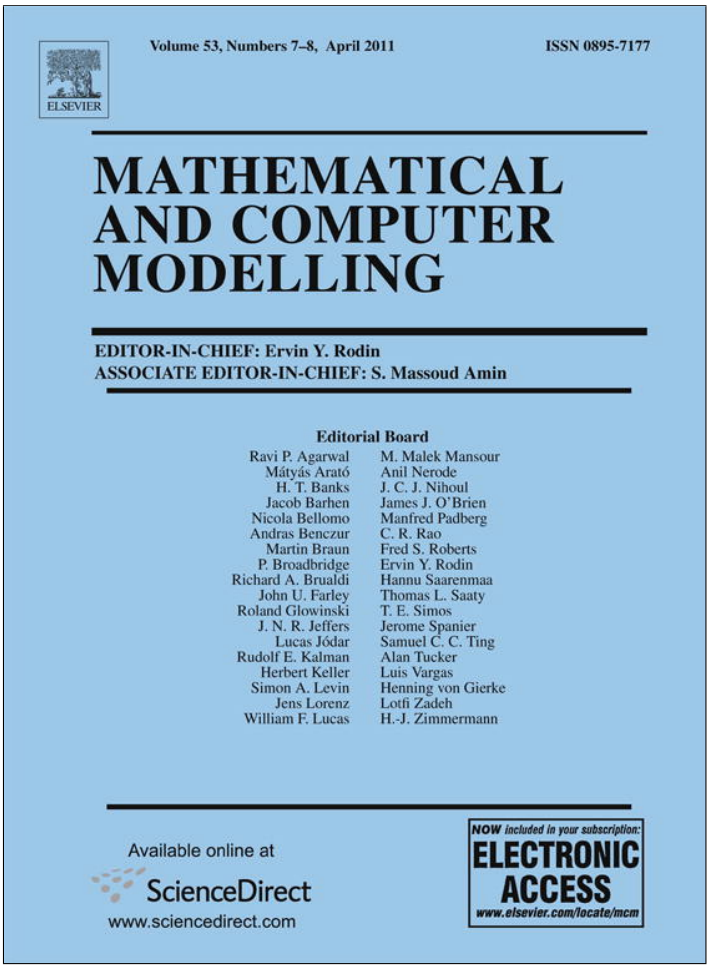

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Modelling hematopoiesis in health and disease

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ABSTRACT

Hematopoiesis is the process responsible for maintaining the number of circulating blood cells that are undergoing continuous turnover. At the root of this process are the hematopoietic stem cells (HSC), that replicate slowly to self-renew and give rise to progeny cells that proceed along the path of differentiation. The process is complex, with the cells responding to a wide variety of cytokines and growth factors. We discuss the mathematics of hematopoiesis based on stochastic cell behavior. Multiple compartments are introduced to keep track of each cell division process and increasing differentiation. The same mathematical model that describes normal hematopoiesis across mammals as a stable steady state of a hierarchical stochastic process is also used to understand the detailed dynamics of various disorders both in humans and in animal models. The microecology of the multitude of cell lineages that constitute what we call troubled hematopoiesis evolves in time under mutation and selection, the paradigmatic components of Darwinian evolution. Thus, the present approach provides a novel perspective for looking at cancer progression and cure.

1. Introduction

Blood is composed of a variety of cells suspended in a fluid medium known as plasma. Circulating blood cells have a finite lifespan and are continuously being replaced by new cells produced in a process known as hematopoiesis. During post-natal life, in humans, hematopoiesis is restricted to the bone marrow, but in other mammals, the spleen can also contribute to blood cell formation. The average lifetime of an erythrocyte (red blood cell) in humans is 120 days, neutrophils remain in circulation for about 12 h, while the average lifetime of a platelet is 14 days. These numbers apply to *Homo sapiens*, and change across mammals, reflecting the diverse requirements that different mammalian species exhibit. Being the basis of metabolic substrate and energy transportation to the entire organism (including heat distribution), blood is directly coupled to the organism's metabolic rate, as we shall discuss in detail below. In order to maintain relatively constant numbers of circulating blood cells, the total output from the bone marrow in a healthy adult human is of the order of 3.5×10^{11} cells per day [1]. This number is increased under conditions of higher demand (e.g. bleeding, infection or injury) or as a result of neoplastic transformation of hematopoietic cells as occurs in the chronic myeloid neoplasms such as chronic myeloid leukemia (CML) [2] or polycythemia vera (PV) [3].

The staggering cell numbers associated with hematopoiesis in humans suggest that modeling hematopoiesis by means of differential equations is more than appropriate. Indeed, such is the tradition in mathematical biology, despite the fact that already in the mid-1990s evidence was accumulating that hematopoiesis is stochastic in nature [4,5]. How can this be? Below we shall provide compelling arguments supporting this stochastic view of hematopoiesis (compared to deterministic

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models based on differential equations). Moreover, we shall also convey the message that stochastic effects in hematopoiesis, depending on the cellular site of origin, can have a measurable and sizable impact whose propagation, in more committed stages, proceeds in a deterministic fashion. We shall do so generally by addressing hematopoiesis across mammals and by investigating specific hematopoietic disorders in this light.

At the root of blood formation are the hematopoietic stem cells (HSC) that reside in the bone marrow [6,7]. The presence of these cells was initially inferred from now classic experiments on bone marrow reconstitution after total body irradiation in mice [6]. Bone marrow transplantation, by providing a new source of HSC, is a procedure that provides curative therapy for a variety of otherwise lethal genetic/metabolic or neoplastic disorders to thousands of patients every year [8]. Several investigators have developed models that capture this process at various levels of complexity [9–19]. Many of these models were developed with specific conditions in mind such as cyclic hematopoiesis [20] or hematopoietic reconstitution after stem cell transplantation. Such models, which typically resort to differential equations, sometimes including delay, almost invariably 'compartmentalize' hematopoiesis on the basis of those features of its known physiology which are considered most relevant. Unlike those models, our approach will be based on developing a stochastic framework of normal hematopoiesis. Like those models, ours will make use of a compartmentalized framework. Throughout our work, however, the number of compartments is constant and characteristic of the hematopoietic system that we argue is structurally identical across mammals [21], instead of changing from hematopoietic disorder to hematopoietic disorder. The rationale behind this assertion includes the following observations. (i) To our knowledge, hematopoiesis only emerged once in evolution and is similar across species. (ii) There is strong experimental evidence that human HSC can fully reconstitute hematopoiesis in immunodeficient mice. When trouble happens, its effect is to perturb (often in a fundamental way) this very same base model which accounts for normal hematopoiesis.

Understanding the architecture and dynamics of hematopoiesis requires knowledge of HSC dynamics, although this alone is not sufficient. Hence, we start by considering the number of HSC that are contributing to blood formation, the rate of replication of these cells and how this pool of cells changes during human growth from birth to adult life. Subsequently, we discuss a model that connects HSC dynamics to bone marrow output as seen in healthy adults. Given the similarities of hematopoiesis in mammalian species, we show how allometric principles can be used to unify the dynamics of hematopoiesis across mammals.

Hematopoietic cells divide and differentiate under the influence of the marrow microenvironment that provides the necessary cues in the form of cell-to-cell contacts, cytokines and growth factors [22]. This environment also relates the cells together in space imposing a functional architecture on the process. Hence, hematopoietic cells together with the cues emanating from the rest of the body (e.g. colony stimulating factors, erythropoietin, glucocorticoids, etc.) engage in a dynamic process that is able to rapidly respond, as a whole, to the varying demands for cellular output.

When trouble sets in, we face bone marrow disorders, which can be classified into either "failure syndromes" where one or more blood cell lineage is reduced or absent or "myeloproliferation syndromes" where one or more cell lineage is produced in excess. We will not consider disorders due to specific deficiencies of essential minerals or vitamins such as iron or folate respectively, but restrict ourselves to diseases that result from acquired somatic mutations in hematopoietic cells. In particular, mutations in the stem cell compartment can give rise to the appearance of "new cell species", which compete with wild-type cells, in very much the same way, albeit at a nearly microscopic scale, as species compete for survival in macroscopic ecosystems. Cancer can then be modeled as an evolving ecosystem, where Darwin's theory of natural selection plays a ubiquitous role. We briefly discuss some applications of our approach in understanding the dynamics of a variety of disorders including chronic myeloid leukemia (CML), cyclic neutropenia (CN) and paroxysmal nocturnal hemoglobinuria (PNH). We chose these disorders because in our view they illustrate different aspects of abnormal evolutionary dynamics in hematopoiesis.

2. Normal hematopoiesis

2.1. Hematopoietic stem cells (HSC)—active and reserve HSC

How many stem cells actively contribute to hematopoiesis in a given adult mammal? Until now, no unambiguous answer has been provided in the laboratory. Studies from patients with chronic granulomatous disease [23] (where hematopoiesis is actually normal) suggest a number around 400 cells, whereas experimental studies in patients who underwent bone marrow transplantation indicate that, after the procedure, hematopoiesis is maintained by $\sim$116 stem cells [24]. On the other hand, the demands on hematopoiesis are clearly different across mammals [25]: The total marrow output during the lifetime of a mouse is roughly the same as an adult human produces in a day. But what kind of HSC are we talking about? HSC are operationally divided into an 'active' pool (N_{SC}) where cells are dividing and contributing to hematopoiesis and a 'reserve compartment' in which the cells are inactive and may be called upon to divide as necessary [26]. The current view is that cells in the active compartment occupy specific stem cell niches, and divide 'asymmetrically' in such a way that one daughter cell remains in the HSC niche, that is, remains a stem cell (self-renewal), while the other starts the path towards differentiation (Fig. 1).

There is some evidence that once a HSC is selected to contribute to hematopoiesis, it can do so for a very long time, if not the lifetime of the mammal [31]. Indeed, it has been proposed that clonal succession in large mammals does not occur or perhaps occurs so slowly that it is very difficult to detect [32]. These observations have important implications for the

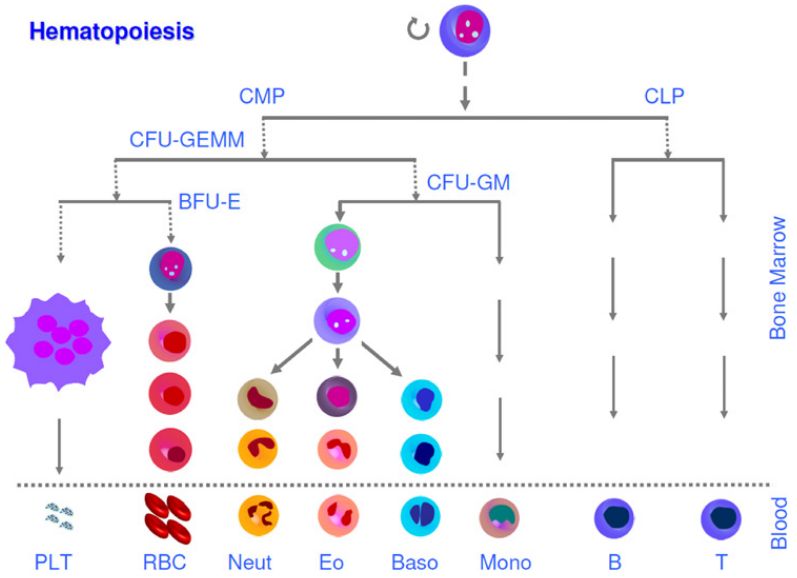


Fig. 1. *Hematopoietic tree*. Hematopoiesis has a tree-like structure with the hematopoietic stem cells at the root of the process. Each cell division gives rise to progeny cells that can retain the properties of their parent cell (self-renewal, ↻) or differentiate, "*moving down*" the hematopoietic tree. As the progeny move further away from the HSC, their pluripotent ability is increasingly restricted (CMP: common myeloid progenitor; CLP: common lymphoid progenitor; BFU-E: erythroid burst forming unit; CFU-GM: granulocyte–macrophage colony forming unit). Such a hierarchical organization is very effective in minimizing the risk of development of disorders due to acquired somatic mutations [27]. Bio-inspired paradigms constitute nowadays a rich source of development of new functional systems at the nanoscale and microscale [28–30].

evolution of mutant clones in the active HSC compartment [33–35]. In particular, under normal conditions one expects the active pool of HSC not to change in size.

2.2. Scaling of active HSC across mammals

In order to understand the current body of evidence, while providing a quantitative answer to the question posed at the start of this section, one may resort to allometric principles [36] combined with the above definitions of active and reserve HSC pools.

In biological systems, many observables (Y) related typically to nutrient transportation, such as metabolic rate, the bronchial tree etc. often scale with mass as $Y \sim Y_0 M^b$ where b is typically a multiple of 1/4. Competing explanations for the origin of these exponents exist [37–39]. With respect to HSC and hematopoiesis, we note that (i) hematopoiesis has appeared only once during evolution (the similarities among the processes across mammals support this), (ii) although hematopoiesis is distributed across various bones, it is functionally coupled by the circulation and so HSC and hematopoiesis effectively function collectively as a single organ, and (iii) from the definition of the HSC, every active HSC is on average equally represented in the circulation of a given mammal. With these premises, we can provide an allometric estimate of the size of the active stem cell pool in mammals (N_{SC}) as a function of their mass [40]. A simple and reliably available marker of hematopoietic cell output is the total circulating reticulocyte count (R_T) that can be estimated for many mammals. Taking into account the variable maturation rate of reticulocytes across species, we determined that R_T scales with the mass of the adult mammal as $R_T \sim M^{3/4}$ [40]. Invoking premise (iii) from the above, we are led to conclude that $N_{SC} \sim M^{3/4}$. Therefore, if N_{SC} and the mass of *any* adult mammal are known, one can determine N_{SC} for *any other* adult mammalian species. Fortunately, this relationship has been determined for cats, allowing us to estimate that in humans $N_{SC} \approx 400$, in agreement with experimental observations [23]. The same relationship suggests that after bone marrow transplantation, a typical adult has $N_{SC} \approx 116$, again in excellent agreement with experimental data [24]. The scaling $N_{SC} \sim M^{3/4}$ enables us to explore the size of the hematopoietic stem cell pool of other mammalian species. Our model predicts that one HSC can maintain hematopoiesis in a mouse for its lifetime [40], a prediction that is supported by experimental observations [41,42]. Naturally, this lower limit extends to the smallest mammal, a shrew with a mass of 3 g. While there is no experimental validation of the active SC pool in elephants, our scaling predicts that an Asian Elephant (*Elephas maximus*, ∼4500 kg) has an active stem pool comprising ∼9600 cells. Naturally, the species specific HSC replication rate (B) also follows an allometric relationship with adult mass: HSC replicate at a rate which decreases with increasing mass ($B \sim M^{-1/4}$) [40]. Interestingly, it has been proposed that the total number of hematopoietic stem cells is conserved across mammals [43], the total number lying somewhere between 11,000 and 22,000 cells. Although there is (albeit limited) evidence that the number of HSC may be constant across mammals ranging from mice, rats to cats and humans and possibly elephants [25,44–46], this result apparently contrasts with the allometric considerations above. However, the estimates of $\sim 10^4$ stem cells do not distinguish between the active and the reserve pool. Our allometric estimates concern the active stem cell pool. Hence, it is gratifying that our estimates do not exceed the limits proposed even when extrapolated to the largest terrestrial mammals.

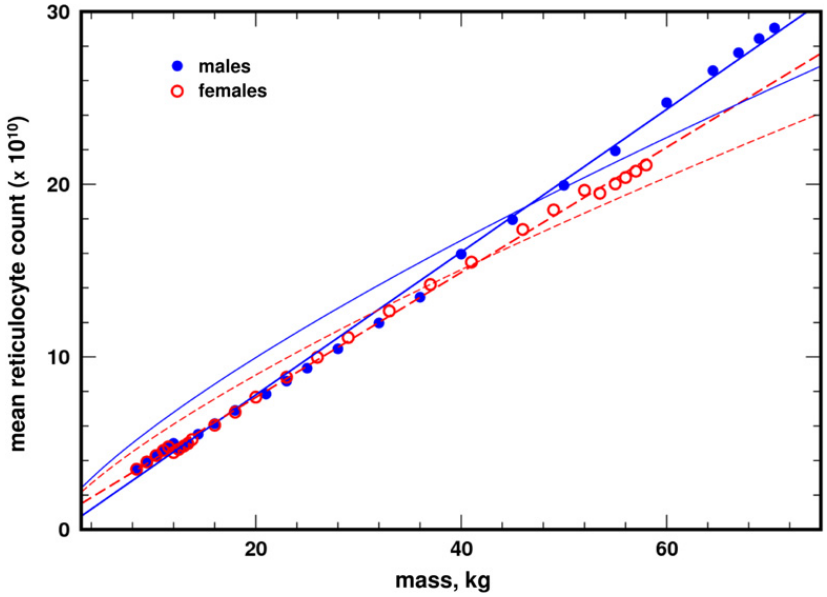


Fig. 2. *Scaling of the mean reticulocyte count (R_{TD}) with body mass during ontogenic growth in humans.* Data for females are shown with open circles (○), whereas data for males are shown with solid circles (●). The scaling of R_{TD} with mass is well approximated by a linear relation (straight dashed and solid lines, respectively), as opposed to a 3/4 scaling, illustrated by the curved lines (dashed and solid, respectively). The intra-species allometric relation with exponent 1 contrasts with the allometric exponent 3/4 obtained for inter-species scaling for mammals.

2.3. Scaling of active HSC during human ontogeny

So far we have discussed the size of the active stem cell pool in adult mammals. However, during their lifespan, species' requirements vary as well as the way in which they allocate (e.g., energy) resources. Consequently, one expects the number of active HSC to change during ontogeny. We have addressed this issue for the best studied species, namely humans, investigating the allometric scaling of human HSC during ontogenic growth [47]. We found that, similar to what happens with allometric scaling across adult species, the size of the active HSC follows the same scaling as the resting metabolic rate during growth in humans. For both quantities we found a linear scaling with mass [47], as shown in Fig. 2.

Once again, our findings suggest a common underlying organizational principle determining the linear scaling of both the stem cell pool and resting metabolic rate with mass during ontogenic growth within the human species, combined with a 3/4 scaling with adult mass across mammalian species. It is possible that such common principles remain valid for hematopoiesis in other mammalian species, although available data are too scarce to enable a proper answer to this question.

2.4. Implications of stochastic HSC dynamics

Since HSC are long lived cells, they are a population at risk of acquired mutation. The risk of acquiring mutations is related to the number of cells, their rate of replication, and the lifespan of the animal [27]. Mutations in these cells can have serious implications leading to various tumors (e.g. CML [48], polycythemia vera [3] and myelofibrosis with myeloid metaplasia) or acquired marrow failure syndromes (e.g. PNH [49]). Given the relatively small size of the active HSC pool in humans and their slow rate of replication, stochastic effects may exert very important effects on the evolutionary history of mutant clones (see, e.g., Ref. [34]). The implications of these dynamics include the possibility of clonal expansion (with neutral drift or due to a fitness advantage), clonal extinction or even stability, features that are compatible with various diseases [50–54].

2.5. From HSC to circulating blood cells: a compartmental model of hematopoiesis

The normal output from human adult hematopoiesis is 3.5×10^{11} cells per day [1]. However, the number of active HSC is ∼400 cells, each dividing ∼ once per year. This implies that the process scales across nine orders of magnitude with respect to amplification, while cell replication rates vary from once a year to more than once a day. Experimental work suggests that hematopoiesis is organized in a tree-like structure involving many steps associated with cellular replication and progressive differentiation (Fig. 1). At the root of the process lie the HSC that give rise to more committed progenitors. Interestingly, self-renewal is not a property restricted to HSC alone—more differentiated progenitors can also self-renew [55], although to a more limited extent, and they can only give rise to a more restricted repertoire of cells. In other words, 'stemness' is a matter of degree—only the cells that lie at the root of the hematopoietic tree can give rise to circulating blood cells of all types.

Understanding the architecture and dynamics of hematopoiesis requires a determination of (i) the number of replication steps linking HSC to the cells found in the circulation [1], (ii) the rate at which the intermediate cells replicate, and (iii) the probability that the cells differentiate versus self-renewal. Moreover, it is thought that individual cell fate at each step of the process is determined stochastically [4,5], meaning that probabilistic considerations have to be accommodated. We have

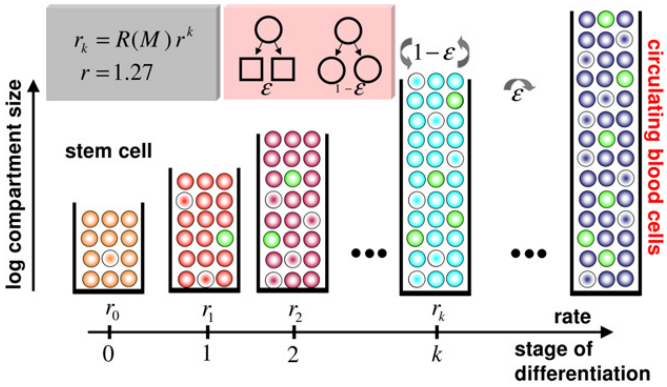


Fig. 3. *Compartmental model of stochastic hematopoiesis.* Compartments represent different stages of cell differentiation. Normal cells (different colors) are represented with bright centers. Mutations may give rise to new cell lineages, often identified with cancer cells, here represented with dark centers. In each compartment, cells differentiate with probability ε, thereby "moving" into the subsequent compartment, whereas with the complementary probability they remain in the same compartment, contributing to self-renewal. Mutated cells may exhibit a different value of ε (see Section 3). As cells move into downstream compartments, their replication rate r_k increases following the exponential relation depicted in the grey box, where $R(M)$, the replication rate of stem cells, is discussed for different mammals in Section 2.6. The compartmental model of hematopoiesis allows one to identify how many stages of cell replication/differentiation link HSC and circulating bone cells, how long a cell remains in each compartment, how often it divides in each compartment, etc. (See the main text for details.)

Table 1
Hematopoiesis across mammals. Here we tabulate some hematopoiesis-related properties of mammalian species derived from the allometric scaling relations discussed in the main text. M: average mass of mammalian species (in kg); N_{SC}: the size of the active stem cell pool, satisfying $N_{SC} = 15.9 \times M^{3/4}$; $R(M)^{-1}$: rate of replication of HSC, in week^{-1} and BM output: daily bone marrow output. (Additional details are provided in Ref. [55].)

Property	Mus musculus	Felix catus	Macaca mulatta	Canis familiaris	Papio sp	Homo sapiens
M (kg)	0.025	4.0	6.5	12.5	18.0	70.0
N_{SC}	1	45	65	105	139	385
$R(M)^{-1}$	7	25	29	34	37	52
BM output	$10^{8.91}$	$10^{10.6}$	$10^{10.72}$	$10^{10.93}$	$10^{11.05}$	$10^{11.71}$

developed a model that captures many of the features of hematopoiesis, based on the concept of compartmentalization (Fig. 3) [21]. It is important to emphasize that these compartments should be considered not as discrete anatomical structures but as more like a tool for keeping track of the replication stage of each cell. The smallest compartment houses the active HSC, while the largest (last) compartment represents the cells that are leaving the bone marrow to enter the circulation.

For the purpose of the model and in the absence of detailed experimental data on asymmetric cell division, we consider that whenever a cell divides, the two daughter cells have the same fate: they either differentiate or retain the properties of their parent (Fig. 3). Although our model does not explicitly allow for asymmetric division of an individual cell, at a population level, asymmetric cell division can be readily accommodated. The importance of the symmetry of cell division for the evolutionary dynamics of mutations has been presented elsewhere [33].

Let us consider population dynamics in a compartment k that harbors N_k cells. Whenever a cell is chosen to divide, with probability ε both daughter cells differentiate and move to the next downstream compartment $(k+1)$ while with probability $1-\varepsilon$, both daughter cells remain in the same compartment (self-renewal; Fig. 3). Cells in compartment k replicate at a rate $r_k = R(M)r^k$ (where $R(M)$ is the replication rate of the stem cells – see Table 1 – and $r = 1.27$ – see Section 2.5.1) and the number of cells in any compartment remains constant (on average) since cells that are lost from one compartment due to differentiation are replaced by input from the next upstream compartment $(k-1$; Fig. 3).

These 'rules' apply to all compartments except the first that harbors the HSC since these cells undergo self-renewal and differentiation with equal probability to maintain their own population and provide cells to the next downstream compartment. In other words, $\varepsilon_0 = 0.5$ and every cell division at the HSC level is asymmetric. Finally, the output from the last compartment C reflects hematopoietic cell output. In the absence of more specific data, we consider that ε is the same across all compartments (see below). This assumption can be relaxed as additional data become available. However, given the lack of quantitative estimates for this parameter during hematopoietic cell differentiation, we consider the simplest possible case, which will account for an average behavior. In any given time step, compartment k loses on average $(2\varepsilon - 1) \cdot N_k \cdot r_k$ cells: with probability ε the compartment loses $N_k \cdot r_k$ cells per unit time step, and gains $N_k \cdot r_k$ new cells with probability $(1-\varepsilon)$. Since $\varepsilon > 0.5$, this represents a net loss of cells that is compensated by replenishment from compartment $k-1$, by an amount that on average is given by $2\varepsilon \cdot N_{k-1} \cdot r_{k-1}$ per unit time: with probability ε, $2N_{k-1} \cdot r_{k-1}$ cells are exported from compartment $k-1$ into compartment k per unit time step. Under stationary conditions, it follows that

$$2\varepsilon N_{k-1} r_{k-1} = (2\varepsilon - 1) N_k r_k. \tag{1}$$

If we assume further that $r_k/r_{k-1} = r$, Eq. (1) can be rearranged to yield

$$\frac{N_k}{N_{k-1}} = \gamma \equiv \frac{2\varepsilon}{2\varepsilon - 1} \cdot \frac{1}{r}. \tag{2}$$

As long as $\gamma > 1$, one obtains an exponential increase in the size of each compartment [21].

In order to determine the model parameters, we utilized quantitative data for granulopoiesis since various stages of this process can be accurately determined from morphologic evaluation of the bone marrow. It has been determined that $\approx 10^{10}$ myeloblasts give rise to $\approx 1.4 \times 10^{11}$ myelocytes, a process that requires four divisions [56,57]. This input together with Eq. (2) leads to $\gamma \approx 1.93$. Eq. (2) can be rearranged to estimate also the minimum number C of compartments that separate the HSC from the most mature cells. Since we know $N_0 = N_{SC}$ and N_C we get $C \approx 31$.

HSC on average replicate once per year [1,58] while the most committed granulocyte precursors can replicate up to five times per day [59]. Hence $r \approx 1.27$ and, from Eq. (1), we finally obtain $\varepsilon \approx 0.84$. This means that while cells retain a limited self-renewal capability, most replication events lead to cell differentiation. This is compatible with observations based on pulse-chase experiments [59].

Our model parameter estimates are based on the well defined data for marrow output, but the size of the active HSC may be more model dependent. Hence, we determined the robustness of parameters r and ε over a range of values of HSC for the same daily marrow output. We found that these parameters changed by less than 4% when the active HSC was varied from 1 to 4000 cells, suggesting that these values are quite robust and 'characteristic' of hematopoiesis [21].

2.5.1. Calibrating the model

The model predicts that at least 31 divisions occur between HSC and circulating blood cells. This estimate is similar to what has been determined on the basis of serial telomere shortening experiments [1,58]. Moreover, our model can determine the compartment where a neutral mutant clone appeared on the basis of the fraction of circulating mutant cells. It can also determine the average lifetime of such a clone on the basis of its size. We utilized these features to test the model independently.

Essentially every healthy adult has small populations of circulating neutrophils and erythrocytes that lack the expression of GPI linked proteins such as CD55 and CD59 [60]. These cells have total or partial deficiency of *PIG-A*, an enzyme subunit required for GPI biosynthesis. The presence of such mutant cells can be detected using flow cytometry for these specific cell surface proteins (e.g. CD55 and CD59) [49,61]. The *PIG-A* gene accumulates mutations at a normal rate [62] and since both neutrophils and erythrocytes lack these proteins, the mutation has to occur at the level of the CFU-GEMM (Fig. 1). In healthy adults, the frequency of mutant cells is of the order of $11–51/10^6$ neutrophils [60]. One can mathematically show that at any one time, it is more likely that a single founder clone is responsible for these mutant cells than having two independent clones responsible for the neutrophil and erythrocyte lineages [35,63]. This implies that the mutation had to occur in one of 20,000 to 100,000 CFU-GEMM cells. The model places these cells within compartments 5 to 8 ($k = 5–8$) and we estimate that these clones will persist between 61 and 120 days (with the longer times for the smaller compartment). This prediction is in excellent agreement with the reported observations [60].

2.6. Cell behavior across species

Humans are the best studied mammals and the model presented can accommodate a variety of human disorders without any modification (see below) [35,54,63–65]. However, the model is not restricted to humans and application of this framework to other well studied mammals would strengthen its credibility. Given that hematopoiesis emerged only once in evolution, we assume that hematopoiesis in other mammals exhibits the same tree-like structure, which scales with mass in the same way as $N_{SC}(M)$. Consequently, each adult mammalian species is characterized by its mass, with $N_{SC}(M) = N_0 M^{3/4}$ ($N_0 \approx 15.9\ \mathrm{kg}^{-3/4}$) [40] while the rate of HSC replication scales as $R(M) = R_0 M^{-1/4}$ ($R_0 \approx 2.9\ \mathrm{kg}^{1/4}\mathrm{yr}^{-1}$) [40]. The size of each compartment (k) grows as $N(k) = N_{SC}(M)\gamma^k$ while the compartment specific replication rate scales as $r(k) = R(M)r^k$ [21,66]. Using these relationships, the hematopoietic output and HSC replication rates in various mammals can be determined and compared to experimental observations [66]. Our model predictions for various mammalian species [66] are in remarkable agreement with what has been observed experimentally and provide support for the concepts and assumptions used to generate this model of hematopoiesis. Interestingly, the average lifetime (L) of mammals (in the wild) also tends to scale allometrically with mass as $L(M) = L_0 M^{1/4}$ ($L_0 \approx 8.6\ \mathrm{kg}^{-1/4}\mathrm{yr}$) [27]. The total number of divisions (T) that a HSC undergoes depends on the replication rate of the cell and on the lifespan of that mammal. Therefore, $T \sim M^{-1/4} \cdot M^{1/4} \sim M^0$, meaning that the total number of divisions that a typical HSC undergoes is independent of mass and should be similar across all mammalian species [66].

This result is (i) compatible with the Hayflick hypothesis which proposes that the number of divisions that a cell can undergo is fixed [67], and (ii) provides a theoretical foundation for the suggestion by Shepherd et al. [68]. These arguments suggest that the HSC replication rate is dictated by the metabolic rate of the host species and are also supported by experimental evidence. Thus, a single human HSC can rescue a lethally irradiated mouse with reconstitution of hematopoiesis [41,42]. If HSC had an 'intrinsic' (or built in) rate of replication ($\sim$1/year in the human body), it would not be possible for the mouse to survive such an experiment since it will take a very long time for hematopoiesis to recover and

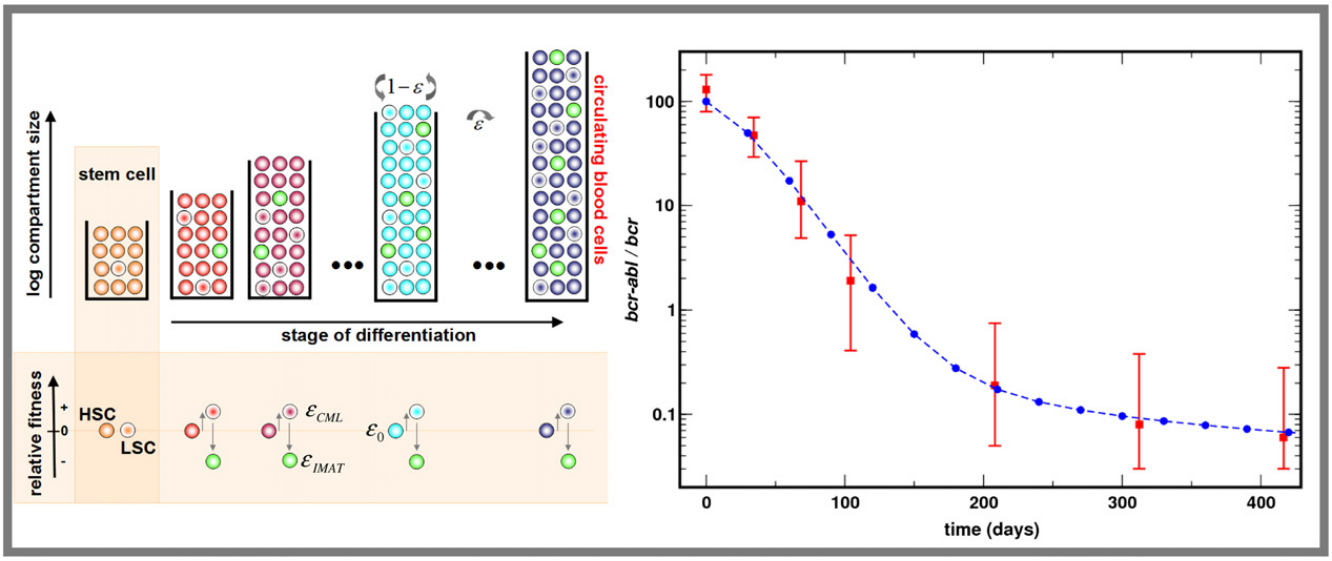


Fig. 4. *CML dynamics*. We maintain the notation of Fig. 3. In the lower left panel, we illustrate the relative fitness of each cell lineage in each compartment, which dictates the relative balance between the co-evolutionary dynamics of normal and cancer cells. Green cells represent cancer cells treated with imatinib, which, by changing their self-renewal probability ($\varepsilon_{\text{IMAT}} > \varepsilon_0$), effectively renders these cells disadvantageous with respect to normal (ε_0 and cancer $\varepsilon_{\text{CML}} < \varepsilon_0$) cells, thereby reducing their impact on the overall evolutionary dynamics of CML (see the main text for details). The right panel shows the impact of imatinib treatment on patients' disease burden as monitored via serial *BCR-ABL/BCR* estimates, leading to the typical two-slope curve characteristic of this process. Here a cohort of patients with early chronic phase CML were treated with imatinib for about a year. The mathematical model was fitted to the clinical data using a non-linear least squares fit and the parameters determined.

rescue the mouse. Therefore, one has to conclude that the murine environment and metabolism impose a replication rate that is characteristic of the species in which the cell is hosted.

3. Troubled hematopoiesis

So far we have addressed normal hematopoiesis. However, the model should be also capable of addressing hematopoietic disorders, given the view of these as a stochastic competition between different cell lineages, under mutation and selection across the hematopoietic tree. In this context, we believe that the model discussed so far is ideally suited for understanding hematopoietic disorders under this paradigm. As a result, the model has been used to understand the dynamics of chronic myeloid leukemia (CML) [65] in the presence and absence of therapy, cyclic neutropenia (CN) in humans [64], in the grey collie and generally across mammals [69], and the clonal origin and evolution of multiple clones in paroxysmal nocturnal hemoglobinuria (PNH) [35,54,63]. Here we shall briefly address the evolutionary dynamics of CML, CN and PNH and refer the readers to the appropriate references where the use of this model to understand such a diverse group of pathological states is discussed in depth.

3.1. Chronic myeloid leukemia (CML)

CML is perhaps the best understood human tumor and it is characterized by the expression of the *BCR-ABL* oncoprotein [48]. The disease starts in a HSC and is associated with myeloproliferation and a high risk of transformation to acute leukemia. The disease burden can be monitored by quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR) and targeted therapy in the form of tyrosine kinase inhibitors such as imatinib is available. We used serial *BCR-ABL* quantitation from patients treated with imatinib to determine (i) the number of leukemic stem cells driving the disease, (ii) the phenotypic effect of *BCR-ABL* expression on CML cells, (iii) the fraction of CML cells responding to therapy and (iv) the effect of imatinib on the leukemic cells [65]. Our model naturally fits the 'two-slope' curve of the decline in *BCR-ABL* with time due to therapy [70,71]—see Fig. 4. This observation is due to the architecture and dynamics of hematopoiesis and CML itself. The model suggests that a single leukemic stem cell is enough to drive CML [65]. Interestingly, the effect of *BCR-ABL* is to enhance the self-renewal of CML progenitors ($\varepsilon_{\text{CML}} < \varepsilon_0$) leading to both myeloproliferation and increased hematopoietic output but also implying that CML cells undergo a higher number of divisions before they appear in the circulation (Fig. 4).

These model predictions have experimental validation [72,73]. Therapy with imatinib reduces the fitness of mutant cells compared to normal ones, enabling the latter to take over hematopoiesis ($\varepsilon_{\text{IMAT}} > \varepsilon_0$). Moreover, at any given time, perhaps 5% of the cells are reversibly responding to therapy [65].

3.1.1. Stochastic CML dynamics

What is the impact of stochastic effects on CML? In humans its impact is sizable and detectable, as illustrated in Fig. 5.

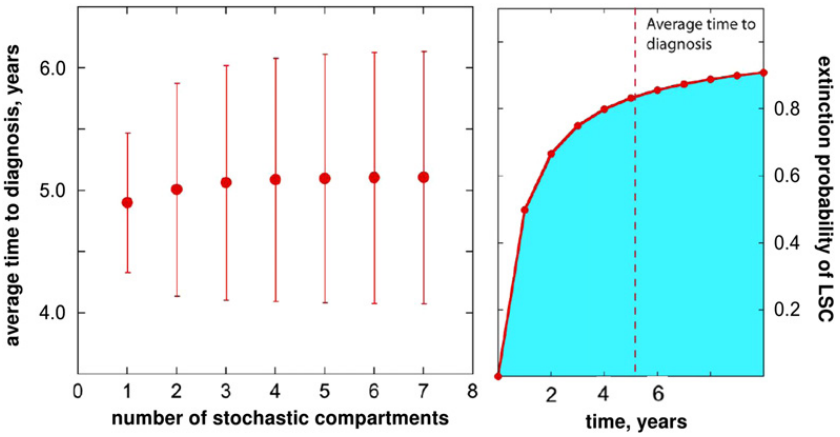


Fig. 5. *Stochastic effects on CML dynamics in humans*. In the left panel we show results for the average time (circles) required to reach diagnosis of CML starting from a single mutated stem cell in the HSC compartment. The average time has been computed using the full architecture of hematopoiesis, but treating stochastically a variable number of initial compartments, specified along the horizontal axis. The vertical bars provide the standard deviation of the results obtained by simulating one million virtual patients. The results indicate that treating more than the first four compartments stochastically may be redundant. In the right panel we plot the probability of stochastic extinction of leukemic stem cells (LSC) in the HSC pool as a function of time, showing that, in contrast to conventional expectations rooted on deterministic thinking, stochastic extinction of the clone is a very likely event, both before and after diagnosis occurs.

Incorporating stochastic dynamics in our compartmental model of hematopoiesis turns out to be a non-trivial procedure. The impact of stochastic fluctuations should be more pronounced in compartments with small populations since as a population grows, the effect of such fluctuations becomes less important. In other words, a mutant population in a small compartment will be much more sensitive to stochastic effects while such a mutation in large compartments will essentially follow deterministic dynamics. Therefore, one expects a transition in hematopoiesis where the impact of stochastic fluctuations can be well approximated by a deterministic model. Therefore, the life history of the clone downstream will follow deterministic dynamics. From a computational perspective, it is more efficient to simulate deterministic behavior and this will impart a considerable saving of computation time. To address this problem, we systematically determined the effect of including stochastic dynamics on an increasing number of early compartments downstream of the HSC on hematopoietic output. As a result we developed a hybrid model which allows us to decide how many of the 31 hematopoietic compartments are treated stochastically. The results are depicted in Fig. 5 and show that stochastic effects extend well beyond the HSC compartment. First of all, our results show that in the overwhelming majority of patients the leukemic stem cell (LSC) population undergoes extinction before the disease is diagnosed. Hence leukemic progenitors, susceptible to imatinib treatment, should be the natural target for CML treatment. The model independently predicts a profile of the response dynamics to treatment which closely matches data from clinical trials. It further predicts that early diagnosis together with administration of imatinib opens the path to CML eradication, leading to the wash out of the aberrant progenitor cells, ameliorating the patient's condition while lowering the risk of blast transformation and drug resistance. In summary, imatinib can actually cure CML, despite not hitting HSC. These results illustrate the relevance of stochastic effects on the dynamics of acquired HSC disorders but also demonstrate how evolutionary dynamics can offer new insights as regards fighting the "war on cancer".

3.1.2. CML across mammals

The number of early progenitors populating the first compartments is highly variable among mammalian species. This said, it is also clear from the previous discussion that this number is relatively small for all of them, suggesting that, once more, stochastic effects play a sizable role in CML dynamics across mammals. However, due to the rapid increase in the number of cells from compartment to compartment, stochastic effects may become negligible above a certain threshold compartment, rendering a simpler deterministic mathematical model more appropriate for describing cell dynamics from then on. The compartment defining the transition between stochastic and deterministic regimes will be named K. As the number of active HSC spans from ~ 1 (in mice) to almost 10^4 (in elephants) the value of K is expected to be highly variable and *decreases* with *increasing* adult mammalian mass. Below we shall employ the average time to diagnosis as a criterion for defining the limiting compartment K above which stochastic effects are no longer expected to play a sizable role.

For humans, this threshold is $K \approx 4$. Using this information we analyzed the stabilization of time to diagnosis in other species as the threshold value (defined by the number of compartments treated stochastically) was increased. As expected, the convergence of the time to diagnosis occurs for larger K values in smaller mammals, as shown in Fig. 6.

Naively one might expect that stabilization in time to diagnosis would occur in the compartment where a given total number of cells would be reached. This would render the determination of K trivial, since it would be enough to know the compartment K for which this bulk number of cells was reached, as a function of adult mammalian mass. However this argument does not hold, as the interactions between consecutive compartments lead to a much more sensitive hierarchical cell dynamics. This fact suggests that, as shown in Fig. 6, we use as a criterion to determine K the compartment value above

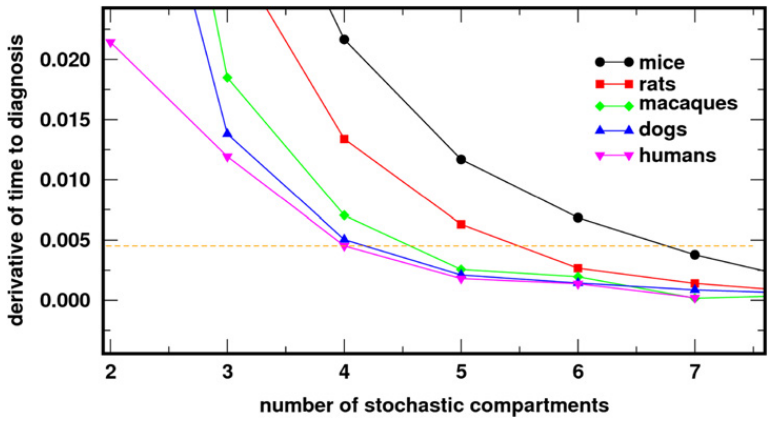


Fig. 6. *Stochastic effects on CML dynamics across mammals.* Each set of points shows results for the right derivative of the average time (circles) required to reach diagnosis of CML (with respect to the number of compartments treated stochastically). In all cases disease started from a single mutated stem cell in the HSC compartment. The average time has been computed using the full architecture of hematopoiesis, but treating stochastically a variable number of early compartments, specified along the horizontal axis. We used the result $K \approx 4$ deduced from Fig. 5 to define the common threshold line (horizontal dashed line) used to determine the critical compartment number K for different mammalian species. The figure indicates that the interaction between consecutive compartments renders the determination of the resulting K non-trivial, confirming, however, the intuition that smaller mammals exhibit larger K values.

which the derivative of time to diagnosis (versus the number of stochastic compartments) falls below a given threshold value. In our case, this threshold was chosen so that we re-obtain $K = 4$ for humans, as a result of the analysis of Fig. 5. Other criteria might be used, and certainly the answer is not unique, the answer depending on the observable at stake, although the general principle holds.

3.2. Cyclic neutropenia (CN)

CN is a rare congenital or acquired disorder characterized by stable cycling of neutrophils (and often other circulating cell lineages).

3.2.1. CN in humans

In humans, neutrophil counts can reach dangerously low levels ($<500/\mu$L), increasing the risk of serious infections [10–12]. A significant fraction of patients with CN have mutations in the gene coding for neutrophil elastase (*ELA*2), an enzyme that is only expressed in cells of the neutrophil and monocyte lineage starting with myeloblasts and monoblasts but not in earlier compartments [74]. The mechanism of cycling in this disease is unclear. We proposed that expression of the mutant *ELA*2 alters the self-renewal properties of CFU-GM and subsequent compartments by reducing their sensitivity to G-CSF, the major trophic factor for these cells [64]. Assuming that adjacent cell compartments are coupled to each other via a linear feedback mechanism (mediated precisely by G-CSF), we consider that depletion of cells from compartment j leads to an additional influx of cells from compartment $j-1$. As a result, we obtain a relationship between the compartment j, the probability of self-renewal (ε) and the replication rate of the cells in compartment j ($r_j = r_0 r^j$, where $r_0 = 1$ year^{-1} is the stem cell replication rate; see Section 2.5) that is defined by

$$\Omega_j = 4r_0^2 r^{2j-2}(2\varepsilon - 1)(2\varepsilon r + 1) > 0. \tag{3}$$

It can be shown that under defined conditions, oscillations in the number of cells within a given compartment will occur and persist without damping at a frequency that is given by

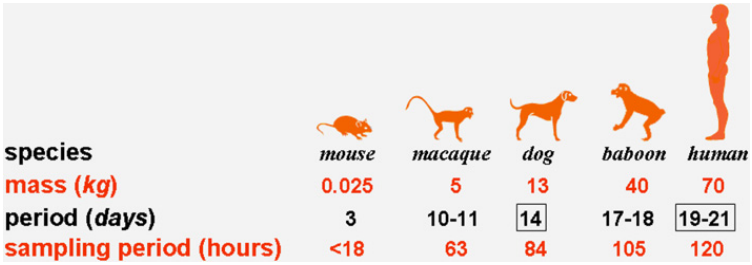
$$\nu = \frac{\sqrt{\Omega_j}}{4\pi}. \tag{4}$$

Therefore, Eq. (4) defines the relationship between the frequency of neutrophil oscillations and the compartment, j, where they start due to a mutation or other insult. Using these relations, we could determine the last compartment where cell behavior is normal due to a lack of *ELA*2 expression. We found that for the most common cycling frequency (21 days), cells up to compartment 18 are not expressing *ELA*2, which coincides with the CFU-GM pool: cells further downstream express *ELA2*and have reduced self-renewal, leading to a reduction in granulocyte output. The body senses this reduction and responds by increasing G-CSF production which enhances self-renewal of progenitors and cells further downstream leading to an increase in granulocyte output. Cycling persists since neutrophils themselves scavenge G-CSF, therefore reducing the signal responsible for their increased output. Interestingly, some patients with CN have a cycling period of ∼50 days, in which case the earliest compartment where the defect could arise is compartment 8 (CFU-GEMM), still downstream of the HSC pool [64].

3.2.2. CN across mammals

Apart from being a human disease, CN has been well documented in the grey collie. The grey collie is a spontaneous animal model of CN but the cycling frequency is 14 days [12]. Given our recent use of the compartmental model of hematopoiesis

Table 2
CN across mammals. The table provides the mass and cycling period for CN across mammals from mice to humans. Numbers provided are estimated from the model except those boxed, which coincide with numbers determined experimentally. Also provided are guides to the minimum interval between neutrophil measurements for reliably determining potential cycling behavior.



species	*mouse*	*macaque*	*dog*	*baboon*	*human*
mass (*kg*)	0.025	5	13	40	70
period (*days*)	3	10-11	14	17-18	19-21
sampling period (hours)	<18	63	84	105	120

to provide insights into the mechanism of cycling of this disorder, we scaled our model of hematopoiesis to the grey collie on the basis of its adult mass in order to determine the period of oscillations in this animal. As a result, our model predicts that the cycling period changes with adult mammalian mass as $T = T_0 M^{1/4}$. Interestingly, our model correctly predicted the cycling frequency in the grey collie, implicitly assuming that the *phenotypic effect* of the molecular defect in the dog is similarly restricted to cells along the granulocyte and monocyte differentiation pathway as in humans [69].

Besides providing further support for the robustness and applicability of the present framework, this result also provides a link between the cycling period and the cells where the mutated *ELA*2 is expressed. Assuming that the biological effect in CN is the same in dogs (i.e. the mutant gene is expressed initially downstream of the CFU-GM cells), and the observation that the structure of hematopoiesis is invariant across mammals, our allometric scaling techniques can be used to correctly predict the period of cycling in humans, the grey collie or, for that matter, other mammals from mice to elephants (Table 2). In particular, our results also provide important clues regarding laboratory experiments performed with the purpose of investigating the link between the cycling period and the cells where the mutated *ELA2* is expressed. Our results in Table 2 show that laboratory blood samples in mice must be taken at time intervals much smaller than those considered to date. Indeed, experiments on mice have sampled blood counts [75] with a periodicity of one week, which is unfortunate given the predicted cycling period of about three days for CN in mice, which renders these blood samples ineffective as regards assessing the presence or not of CN in *ELA*2 mutated mice. As an aside, we suggest that when cycling is suspected, it is more efficient to take randomly timed rather than regularly timed blood samples to maximize the yield from such experiments.

3.3. Paroxysmal nocturnal hemoglobinuria (PNH)

PNH is an acquired HSC disorder due to a mutation in the *PIG-A* gene leading to loss of many cell surface proteins that are anchored to the plasma membrane of cells via a phospholipid tag [49]. The disease has many fascinating features including the presence of more than one distinct mutant clone (i.e. the same patient has two or more different clones with independent mutations in *PIG-A*) [76], and despite the lack of specific, disease modifying therapy, a significant fraction of patients have resolution of the disorder [77]. The mechanism of clonal expansion is also unclear and a 'second hit' apart from the *PIG-A* mutation (that gives the phenotype) has been considered necessary for clonal expansion since the *PIG-A* mutation itself does not give a fitness advantage to the cells. We utilized our model to show the following. (i) Starting with a HSC that harbors a mutated *PIG-A*, it is most unlikely that a second independent mutation in *PIG-A* occurs in another HSC. Rather the second mutation will occur in a more committed cell such as a CFU-GEMM [35,63]. (ii) Similarly, we could determine that a second mutation in a different gene that enables clonal expansion would be unlikely, given that the mutation rate in PNH cells is normal [62].

The major issue then is to explain how the PNH clone expands to produce disease. To answer this question, we used stochastic simulations within the active HSC pool to map out the incidence of a mutation in *PIG-A*, and how that mutant cell can expand into a clone. We showed that neutral drift alone can lead to clonal expansion of the mutant and this is sufficient to explain the known incidence of the disease in the United States [54]. Moreover, our predictions of average clone size approach what has been reported in large, independent population based studies. Finally, our model could predict the incidence of stochastic extinction of the clone—12% to 15% which is in excellent agreement with what Hillmen et al. reported [77]. Therefore, our model can provide rational explanations for many of the dynamics observed for this disorder.

4. Discussion

Hematopoiesis is a highly complex process that results from the interactions between cells of many different types and the exchange of chemical messages between them. The process exhibits a rich dynamics reflecting an underlying architecture that enables it to respond quickly to the various demands imposed by the body under both physiological and pathological conditions. Understanding these dynamics is essential if we want to make sense of a variety of pathological states that disturb this process. Despite its complexity, we argued here that the essential elements of blood cell formation can be captured by a model whereby cells divide and differentiate in a stochastic fashion. Such a 'coarse grained' view

of hematopoiesis provides parameters which are robust and seem applicable across mammals. However, the stochasticity of hematopoiesis is not solely due to variable determinants of cell fate, but becomes inescapable when one realizes that, despite the staggering cell numbers associated with circulating blood, at the root of hematopoiesis lie a small and deceptive (to date) number of hematopoietic stem cells. HSC dynamics is not only stochastic in nature but, as we have discussed here, the stochastic nature of HSC dynamics provides many hints towards a fundamental understanding of the nature of hematopoietic disorders. The stochastic nature of hematopoiesis, together with the hypothesis of a universal architecture of hematopoiesis combined with allometric scaling of HSC across mammals, allows one to provide a new perspective of such a complex and entangled system. We believe our results convey a fascinating message, and that this new perspective of hematopoiesis opens new avenues of research towards a better understanding of hematopoietic disorders and the devising of better means to overcome them.

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