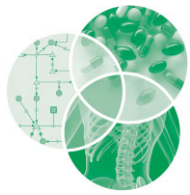
Focus Article



Modeling the architecture and dynamics of hematopoiesis

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Hematopoiesis is a multistep process that results in the production of a variety of blood cells with different morphologies and diverse functions. All of these cells have their origin in hematopoietic stem cells (HSCs) 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 a model of hematopoiesis based on stochastic cell behavior. Multiple compartments are introduced to keep track of each cell division process and increasing differentiation. Despite its simplicity, the model is able to account for the salient features of hematopoiesis and is compatible with considerable and independent experimental data. The model is applicable to hematopoiesis across mammals and can be used to understand the dynamics of various disorders both in humans and in animal models. © 2009 John Wiley & Sons, Inc.

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INTRODUCTION

Blood is composed of a variety of cells suspended in a fluid medium known as plasma. With the exception of lymphocytes, circulating blood cells have a finite lifespan. The average lifetime of an erythrocyte (red blood cell) is 120 days, neutrophils stay in the circulation for about 12 h while platelets survive in the circulation for approximately 14 days. 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] The physiological process responsible for blood cell formation is known as hematopoiesis. In mammals, hematopoiesis occurs in active areas of the bone marrow known as red marrow. At the root of hematopoiesis are the hematopoietic stem cells (HSCs) that are operationally defined by their combined property of self-renewal and an ability to give rise to all the various differentiated cells present in blood.[1–5] Several investigators have developed models that capture this process at various levels of complexity.[6–16] Many of these models were developed with specific conditions in mind such as cyclic hematopoiesis[17] or hematopoietic reconstitution after stem cell transplantation. These models rely on differential equations, sometimes with delay to accommodate the diverse dynamics of hematopoietic disorders, but all models, to some extent 'compartmentalize' hematopoiesis based on known physiology. These models have provided many novel insights into the control of hematopoiesis under normal conditions[18–21] as well as the pathophysiology of cyclic hematopoiesis and optimization of therapy with growth factors.[8,14,22,23] Although differential equations are excellent for modeling large populations, it is becoming clearer that the pool of active HSC and the most primitive progenitors are small and so stochastic effects can be important. Moreover, it is now increasingly accepted that the behavior of individual cells throughout hematopoiesis is stochastic in nature,[24,25] hence we opted to develop a model that accounts for the stochastic behavior of individual cells. In the following, we discuss our approach to modeling this process for human and other mammalian species[26]

Understanding the architecture and dynamics of hematopoiesis requires knowledge of HSC dynamics, although this is by no means sufficient. Hence, we start by considering the number of HSCs that are contributing to blood formation, the rate of replication of these cells, and how this pool of cells changes during human

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growth from birth to adult life. Subsequently, we discuss a model that connects HSC dynamics and 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.[27] This environment also relates the cells together in space imposing a functional architecture to the process. Hence, hematopoietic cells together with the cues emanating from the rest of the body (e.g., erythropoietin, androgens, glucocorticoids, colony stimulating factors, etc.) engage in a dynamic process that is able to rapidly respond, as a whole, to the various demands for cellular output.

When things go wrong, we face bone marrow disorders, which can be classified into either 'failure syndromes' where one or more blood cell lineages are reduced or absent or 'myeloproliferation syndromes' where one or more cell lineages are produced in excess. Rarely, disorders characterized by either hematopoietic failure or myeloproliferation exhibit oscillatory behavior whereby the circulating levels of various cell lineages vary in a rhythmic pattern as a consequence of inherited (congenital) or acquired defects or as a result of therapy.[28] We briefly discuss some applications of our approach to understand 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 dynamics in hematopoiesis ranging from excess cell production, to neutral drift of mutant clones to periodic and sustained cycling as a result of feedback mechanisms.

HEMATOPOIETIC STEM CELLS

Active and reserve HSC

HSCs were initially inferred by the experimental observation that limiting dilutions of bone marrow cells can be used to reconstitute hematopoiesis in syngeneic mice that had received myeloablative radiation therapy.[3] In principle, one HSC can rescue a lethally irradiated mouse and reconstitute hematopoiesis for the remaining lifetime of the animal, providing ultimate proof of the self-renewal and differentiation capability of these cells.[29,30] The reader should note that hematopoiesis is not necessarily restricted to the bone marrow and can occur in other organs including the spleen in some animals. Moreover, during ontogeny, the yolk sac, liver, and spleen are also major sites of hematopoiesis.

Evidence from other mammals, including humans, point to a total number of HSC which is small—studies on the frequency of bone marrow reconstituting activity in various mammals suggest that the number of HSC (active and reserve) may not exceed a total between 11,000 and 22,000 cells.[31–34] HSCs 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.[35] The current view is that cells in the active compartment are those which occupy the stem cell niches, dividing 'asymmetrically' so that one daughter cell remains in the HSC compartment, that is, remains a stem cell (self-renewal) while the other starts the path toward differentiation (Figure 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.[36] 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 observe.[37] These observations have important implications on the evolution of mutant clones in the active HSC compartment.[38–40] In particular, under normal conditions one expects the active pool of HSC not to change in size.

Scaling of the active HSC pool

It is clear that hematopoietic output is very different across species: what murine hematopoiesis produces in its *lifetime* (~2 years) is similar to the total marrow output of a human in a *day*.[41] As far as is known, hematopoiesis emerged only once in evolution. Therefore, it makes sense to consider that this process has been adapted to accommodate different demands imposed by larger body mass. One approach to tackle this conundrum is allometry, a discipline that compares various anthropomorphic characteristics.[42,43] This approach can be justified from the following observations: (1) basal metabolic rate (B) scales allometrically with mass (M) over 27 orders of magnitude ($B \sim M^{3/4}$),[44] (2) the purpose of erythrocytes is to transport oxygen, a major substrate for energy production, and (3) HSC by definition should be equally represented in the circulation by their progeny. The erythrocyte output from hematopoiesis can be determined from the total circulating reticulocyte count and therefore, if an allometric relationship between total circulating reticulocytes and adult mass can be determined

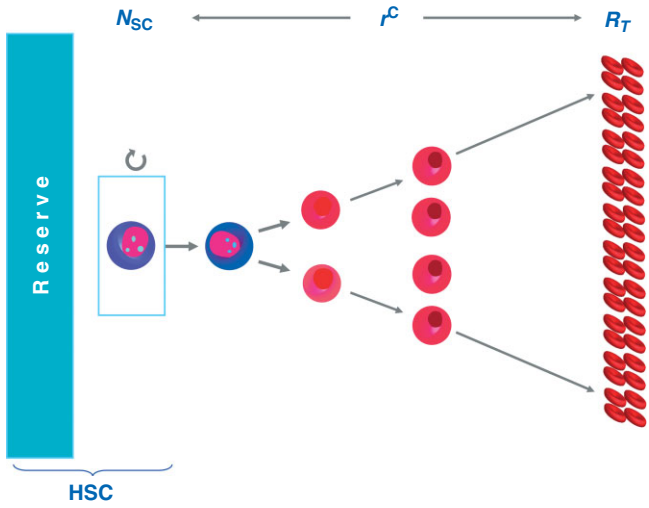

FIGURE 1 | Hematopoietic stem cells are divided into an active pool and a quiescent reserve. All active HSCs are equally represented in the circulation. Consequently, if the number of reticulocytes (R_T) present in the blood scales with the mass of the adult species, then the number of active HSC (N_{SC}) should scale in the same way with mass. The progeny of HSC grow exponentially (r^C) leading to normal cellular output from hematopoiesis.

(Figure 1), one expects from (3) that the same relationship should hold between mass and N_{SC} (see Ref 45). Using data for over 40 mammalian species, we determined that $N_{SC}(M) = N_0\, M^{3/4}$. Using this relationship together with the known number of active HSC in safari cats (≥ 40)[37] under physiological conditions, we can determine N_{SC} for any mammalian species based on its adult mass. This relationship leads to an estimate of $N_{SC} \approx 400$ for an average human adult,[45] a prediction that is very close to what has been deduced from informative studies in patients with chronic granulomatous disease.[46] The relationship also predicts that one HSC is enough to maintain hematopoiesis in the mouse, an estimate that also has experimental validation.[29,30] Finally, the model is supported by the observation that perhaps 13 HSCs are active soon after bone marrow transplantation in the cat, which means that ~116 would be productive in humans. This estimate is also in agreement with experimental data.[47] Therefore, we conclude that N_{SC} increases with mass allometrically. Interestingly, this relationship leads to N_{SC}~10,000 for the African elephant ($M = 4500$ kg), close to the lower estimate of total HSC.[31,45] If the total number of HSC is conserved, then smaller mammals have a higher reserve of HSC than larger ones. This might explain in part why murine HSC can be used so efficiently for serial transplantation.

A similar analysis of the relationship between circulating reticulocytes and mass during human ontogenic growth suggests that N_{SC} scales linearly with mass. At birth, $N_{SC} \approx 20$–40, and the pool increases with mass to reach adult size.[48]

The other major consideration in HSC dynamics is the rate of cell replication across species. Given that the species-specific basal metabolic rate also follows an allometric relationship with mass, it should come as no surprise that HSC replication also follows the same relation. Indeed HSC replication in the cat, mouse, and human scales with mass as $R \sim M^{-1/4}$.[45] In other words, the cell rate of replication decreases with increasing mass. Indeed, murine HSC divide once every 7 weeks while in humans they divide approximately once per year. Data based on serial telomere shortening are compatible with these estimates.[49]

Implications on HSC dynamics

Since HSCs are long lived cells, they are at risk of acquiring mutations. The risk of mutation is related to their rate of replication, the number of cells, and the lifetime of the animal.[50] Mutations in these cells can have serious implications leading to various tumors (e.g., CML,[51] polycythemia vera,[52] and myelofibrosis with myeloid metaplasia) or acquired marrow failure syndromes (e.g., PNH[53]). Given the relatively small size of the active HSC pool in humans and their slow rate of replication, it can be shown that stochastic effects may exert important effects on the evolutionary history of mutant clones.[39] 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.[54–58]

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 approximately once per year. This implies that the process scales across 9 orders of magnitude with respect to amplification, whereas cell replication rates vary from once a year to more than once a day.

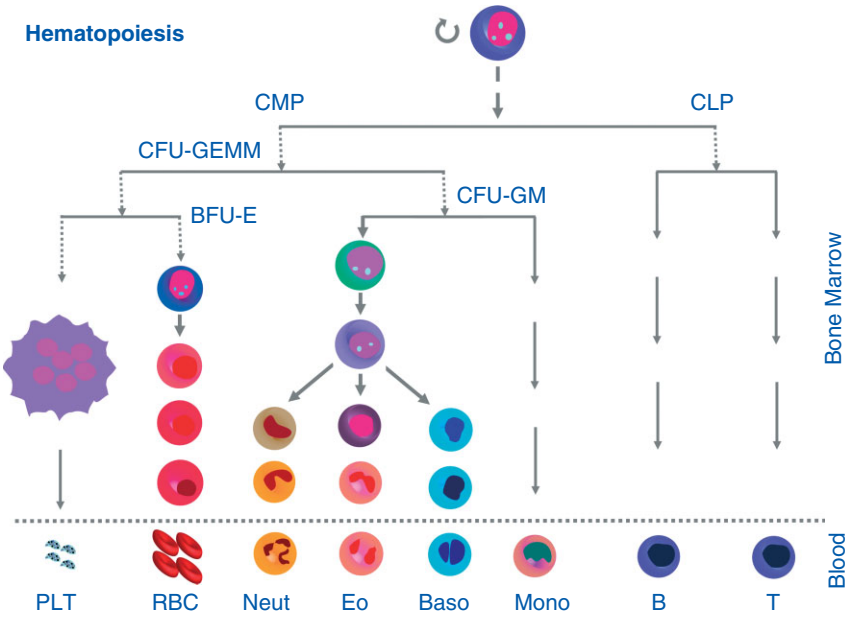

FIGURE 2 | Hematopoiesis has a tree-like structure with the hematopoietic stem cells at the root of the process. Each cell division gives rise to progeny cells that can retain the properties of their parent cell (self-renewal, probability $1 - \varepsilon$) or differentiate (probability ε). As the progeny 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).

Experimental work suggests that hematopoiesis is organized in a tree-like structure involving many steps associated with progressive cellular replication and differentiation (Figure 2). The HSCs lie at the root of the process which give rise to more committed progenitors. Interestingly, self-renewal is not a property restricted to HSC alone—more differentiated progenitors can also self-renew,[59] albeit 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—they must lie at the root of the hematopoietic tree.

A compartmental model of hematopoiesis

Understanding the architecture and dynamics of hematopoiesis requires a determination of: (1) the number of replication steps linking HSC to the cells found in the circulation,[1] (2) the rate at which the intermediate cells replicate, and (3) the probability that the cells differentiate versus self-renewal. Moreover, it is thought that cell fate is determined stochastically,[24,25] meaning that probabilistic considerations have to be accommodated. We have developed a model that captures many of the features of hematopoiesis based on the concept of compartmentalization (Figure 3(b)).[26] These compartments should not be considered as discrete anatomical structures but simply as a convenient accounting tool to keep track of the fate of each cell. The smallest compartment houses the active HSC, whereas the last (largest) compartment houses the cells that are leaving the bone marrow to enter the circulation.

For the purpose of the model, whenever a cell divides, both daughter cells have the same fate: they either differentiate or retain the properties of

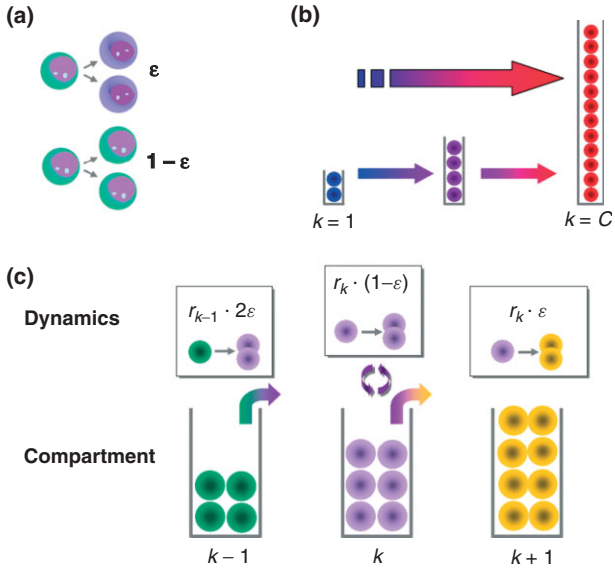

FIGURE 3 | The architecture and dynamics of hematopoiesis. (a) For the purpose of the model, cells divide symmetrically to give rise either to daughter cells that both differentiate (probability ε) or both self-renew (probability $1 - \varepsilon$). (b) The process linking HSC to the circulating blood is composed of many compartments that grow in size and with cells replicating at faster rates. C is the last compartment where cells are dividing. (c) Cell dynamics between adjacent compartments. On average, the size of each compartment is constant due to coupling of output from one compartment to replenish the 'loss' of cells from output into the next downstream compartment. Cellular output is linked to differentiation of the cells.

their parent (Figure 3(a)). Although our model does not allow for asymmetric division of individual cells, on a population level, asymmetric cell division can be readily accommodated. A detailed analysis of the impact of the symmetry of cell division on evolutionary dynamics of mutations has been presented elsewhere.[38] We consider the 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 $(k+1)$ and with probability $1-\varepsilon$ both daughter cells remain in the same compartment (self-renewal) (Figure 3(c)). The rate of replication of cells in compartment k is given by r_k, and the number of cells in any compartment remains constant (on average) because cells that are lost from one compartment as a result of differentiation are replaced by input from the upstream compartment $(k-1)$ (Figure 3(c)). These 'rules' apply to all compartments except the first that harbors the HSCs that self-renew and differentiate with equal probability to maintain their own population and provide cells to the next downstream compartment. In other words, $\varepsilon_0 = 0.5$. Finally, the output from the last compartment C reflects hematopoietic cell output.

In the absence of more specific data, we shall assume that ε is the same across all compartments (see below). In any given time step, compartment k loses on average $(2\varepsilon-1)N_k r_k$ cells: with probability ε the compartment loses $N_k r_k$ cells per unit time step, gaining new $N_k r_k$ cells with probability $(1-\varepsilon)$. This net loss is compensated by replenishment from compartment $k-1$, by an amount given, on average, by $2\varepsilon N_{k-1} r_{k-1}$ per unit time: with probabilityε, $2N_{k-1}r_{k-1}$ cells are exported from compartment $k-1$ into compartment k per unit time step. Under stationary conditions, it follows that

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

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

$$\frac{N_k}{N_{k-1}} = \gamma \equiv \frac{2\varepsilon}{2\varepsilon - 1}\frac{1}{r} \qquad (2)$$

To the extent that $\gamma > 1$, one obtains an exponential increase in the size of each compartment.[26]

Model calibration and parameter estimation

In order to be useful, this model requires determination of the relevant parameters. We utilized quantitative data for granulopoiesis because various stages of this process can be accurately determined from morphological evaluation of the bone marrow. In this process, $\approx 10^{10}$ myeloblasts give rise to $\approx 1.4 \times 10^{11}$ myelocytes, a process that requires four divisions.[60,61] This input together with Eq. (2) leads to $\gamma \approx 1.93$. Equation (2) can also be rearranged to estimate 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 $C \approx 31$.

HSCs on average replicate once per year[1,49] while the most committed granulocyte precursors can replicate up to five times per day.[62] 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.[62]

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 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.[26]

Testing 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 based on serial telomere shortening experiments.[1,49] Moreover, our model can determine the compartment where a mutant clone appeared based on the size of the circulating mutant cells. It can also determine the lifetime of such a clone based on its size. We utilized these features to test the model independently.

It is known that healthy adults have small clones of circulating neutrophils and erythrocytes that harbor mutations in the enzyme complex known as *PIG-A*.[63] Mutant cells can be detected using flow cytometry as a result of loss of specific cell surface proteins (e.g., CD55 and CD59).[53,64] This gene accumulates mutations at a normal rate[65] and since both neutrophils and erythrocytes lack these proteins, the mutation has to occur at the level of the CFU-GEMM (Figure 2). In healthy adults, the frequency of mutant cells is of the order of $11 \times 10^6 - 51 \times 10^6$ neutrophils.[63] One can assume that at any time, a single clone is responsible for these mutant cells.[40,66] This implies that the mutation had to occur in one of 20,000 to 100,000 CFU-GEMM cells. The model would place these cells within compartments 5–8 $(k = 5-8)$ and 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.[63]

HEMATOPOIESIS ACROSS MAMMALS AND APPLICATIONS

The architecture of hematopoiesis is conserved across mammals

Humans are the best-studied mammals, and the model presented can accommodate a variety of human disorders without any modification.[40,58,67,68] However, the model is not restricted to humans, and application of the model 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 species is characterized by its mass, with $N_{SC}(M) = N_0\, M^{3/4}$ ($N_0 \approx 15.9\ \text{kg}^{-3/4}$)[45] while the rate of HSC replication scales as $R(M) = R_0\, M^{-1/4}$($R_0 \approx 2.9\ \text{kg}^{1/4}\ \text{year}^{-1}$).[45] The size of each compartment (k) grows as $N(k) = N_{SC}(M)\gamma^k$ while the compartment-specific replication rate scales as $r(k) = R(M)r^k$.[26,69] Using these relationships, the marrow output and HSC replication rates in various mammals can be determined and compared with experimental observations.[69] A sample of model predictions for various mammalian species is provided in Table 1.[69] These predictions are similar to what has been observed experimentally and provide support for the concepts and assumptions used to generate this model of hematopoiesis.

Cell behavior across species

Interestingly, the average lifespan (L) of mammals also tends to scale allometrically with mass as $L(M) = L_0\, M^{1/4}$ ($L_0 \approx 8.6\ \text{kg}^{-1/4}$ year).[50] 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 similar across all mammalian species.[69] This result supports the Hayflick hypothesis which suggests that the number of divisions a cell can undergo is fixed,[70] while providing a theoretical foundation for the suggestion by Shepherd et al.[71] These arguments suggesting that HSC replication rate is dictated by the metabolic rate of the host species are supported by experimental evidence. A single human HSC can rescue a lethally irradiated mouse with reconstitution of hematopoiesis.[29,30] If HSC had an 'intrinsic' (or built in) rate of replication (~1/ year in the human body), it would not be possible for the mouse to survive such an experiment because it will take a very long time for hematopoiesis to recover. Rather, the murine environment and metabolism imposes a replication rate that is characteristic of the species in which the cell is hosted.

Clinical applications

The utility of a model depends on its ability to be applied to as wide a variety of conditions as possible. In this respect, the proposed model has been used to understand the dynamics of CML[67] in the presence and absence of therapy, CN in humans[68] and the grey collie[72] and the clonal origin and evolution of multiple clones in PNH.[40,58,66] Owing to space restrictions, we discuss these diseases and their modeling briefly here 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.

Chronic Myeloid Leukemia

CML is the best-studied human tumor and is characterized by the *bcr-abl* oncoprotein.[51] The disease starts in a HSC and is characterized by 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 (1) the number of leukemic stem cells driving the disease, (2) the phenotypic effect of *bcr-abl* expression on CML cells, (3) the fraction of CML cells responding to therapy, and (4) the effect of imatinib on the leukemic cells.[67] Our model naturally fits the 'two-slope' curve of the decline in *bcr-abl* with time due to therapy.[73,74] This observation is due to the architecture and dynamics of hematopoiesis and CML itself. The model suggests that the number of leukemic stem cells driving CML is small and between one and eight cells.[67] Interestingly, the effect of *bcr-abl* is to enhance the self-renewal of CML progenitors ($\varepsilon_{CML} < \varepsilon_0$) 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. These model predictions have experimental validation.[75,76] Imatinib reduces the fitness of mutant cells compared with normal ones, enabling the latter to take over

TABLE 1 | Hematopoiesis Across Mammals (Details 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	$10^{8.91}$	$10^{10.6}$	$10^{10.72}$	$10^{10.93}$	$10^{11.05}$	$10^{11.71}$

hematopoiesis ($\varepsilon_{IMAT} > \varepsilon_0$). Moreover, at any time, perhaps 5% of the cells are reversibly responding to therapy.[67] The implications of stochastic dynamics on CML therapy are currently being evaluated.

Cyclic Neutropenia

CN is a rare congenital or acquired disorder characterized by stable cycling of neutrophils (and often other circulating cell lineages). Neutrophil counts can reach dangerously low levels (<500/ μL), increasing the risk of serious infections.[7–9] Many patients with CN have mutations in neutrophil elastase (*ELA2*), an enzyme that is only expressed in cells of the neutrophil and monocyte lineage starting with myeloblasts and monoblasts but not in earlier compartments.[77] The mechanism of cycling in this disease is unclear. We proposed that expression of the mutant *ELA2* 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.[68] Assuming that adjacent cell compartments are related to each other with a feedback mechanism, we derived a function that links the compartment where *ELA2* is initially expressed with the frequency of the cycles observed. As a result we could determine the last compartment where cell behavior is normal due to a lack of *ELA2* expression. We found that for the most common cycling frequency (21 days), cells in compartment 18 are not expressing *ELA2*, which coincides with the CFU-GM pool: cells further downstream express *ELA2* and have a reduced self-renewal, leading to a reduction in granulocyte output. The body senses this reduction and responds by increasing G-CSF production which increases self-renewal of progenitors and cells further downstream leading to an increase in granulocyte output. Cycling persists since neutrophils themselves scavenge G-CSF, 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.[68]

The grey collie is a spontaneous animal model of CN but the cycling frequency is 14 days.[9] We scaled our model of hematopoiesis to the grey collie based on its mass and could determine the period of oscillations in the same animal. Interestingly, our model correctly predicted the cycling frequency in the grey collie, assuming that the phenotypic effect of the molecular defect in the dog is similar to that in humans[72] providing further support for the robustness and applicability of the present framework.

Paroxysmal Nocturnal Hemoglobinuria

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.[53] 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 distinct mutations in *PIG-A*),[78] and despite the lack of specific disease-modifying therapy, a significant fraction of patients have resolution of the disorder.[79] The mechanism of clonal expansion is also unclear and a 'second hit' apart from *PIG-A* mutation 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 that (1) starting with a HSC that harbors a mutated *PIG-A*, it is most unlikely that a second independent mutation occurs in another HSC. Rather the second mutation will occur in a more committed cell such as a CFU-GEMM.[40,66] (2) 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.[65]

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.[58] Moreover, our predictions of average clone size approach to what has been reported in large, 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.[79] Therefore, our model can provide rational explanations for many of the observed dynamics in this disorder.

CONCLUSION

Hematopoiesis is a highly complex process that results from the interactions between many different types of cells and the exchange of chemical messages between them. The process is rich in dynamics and has 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. However, despite its complexity, the essential elements of blood cell formation can be captured by a model whereby cells divide and differentiate in a stochastic fashion. Although the model is 'coarse grained', its parameters are robust and seem to be applicable across mammals. The model is able to accommodate a variety of disorders including CML, cyclic hematopoiesis, and the clonal evolution and dynamics of PNH. Future work will build on this model so that it can be applied to an even wider range of hematopoietic disorders.

REFERENCES

1. Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, et al. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci U S A* 1994, 91:9857–9860.
2. Kondo M, Wagers AJ, Manz MG, Prohaska SS, Scherer DC, et al. Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Annu Rev Immunol* 2003, 21:759–806.
3. McCulloch EA, Till JE. Proliferation of hemopoietic colony-forming cells transplanted into irradiated mice. *Radiat Res* 1964, 22:383–397.
4. McCulloch EA, Till JE. Perspectives on the properties of stem cells. *Nat Med* 2005, 11:1026–1028.
5. Morrison SJ, Uchida N, Weissman IL. The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol* 1995, 11:35–71.
6. Mackey MC. Cell kinetic status of haematopoietic stem cells. *Cell Prolif* 2001, 34:71–83.
7. Haurie C, Dale DC, Mackey MC. Cyclical neutropenia and other periodic hematological disorders: a review of mechanisms and mathematical models. *Blood* 1998, 92:2629–2640.
8. Haurie C, Dale DC, Mackey MC. Occurrence of periodic oscillations in the differential blood counts of congenital, idiopathic, and cyclical neutropenic patients before and during treatment with G-CSF. *Exp Hematol* 1999, 27:401–409.
9. Haurie C, Dale DC, Rudnicki R, Mackey MC. Modeling complex neutrophil dynamics in the grey collie. *J Theor Biol* 2000, 204:505–519.
10. Haurie C, Person R, Dale DC, Mackey MC. Hematopoietic dynamics in grey collies. *Exp Hematol* 1999, 27:1139–1148.
11. Colijn C, Mackey MC. A mathematical model of hematopoiesis: II. Cyclical neutropenia. *J Theor Biol* 2005, 237:133–146.
12. Colijn C, Mackey MC. A mathematical model of hematopoiesis—I. Periodic chronic myelogenous leukemia. *J Theor Biol* 2005, 237:117–132.
13. Shochat E, Stemmer SM, Segel L. Human haematopoiesis in steady state and following intense perturbations. *Bull Math Biol* 2002, 64:861–886.
14. Schmitz S, Franke H, Loeffler M, Wichmann HE, Diehl V. Model analysis of the contrasting effects of GM-CSF and G-CSF treatment on peripheral blood neutrophils observed in three patients with childhood-onset cyclic neutropenia. *Br J Haematol* 1996, 95:616–625.
15. Schmitz S, Loeffler M, Jones JB, Lange RD, Wichmann HE. Synchrony of bone marrow proliferation and maturation as the origin of cyclic haemopoiesis. *Cell Tissue Kinet* 1990, 23:425–442.
16. Wichmann HE, Loeffler M, Herkenrath P, Gerhardts MD, Wesselborg C, et al. Mathematische modelle in der haematologie. *Klinische Wochenschrift (Springer)* 1983, 61:935–940.
17. Foley C, Mackey MC. Dynamic hematological disease: a review. *J Math Biol* 2009, 58:285–322.
18. Santillan M, Mahaffy JM, Belair J, Mackey MC. Regulation of platelet production: the normal response to perturbation and cyclical platelet disease. *J Theor Biol* 2000, 206:585–603.
19. Mahaffy JM, Belair J, Mackey MC. Hematopoietic model with moving boundary condition and state dependent delay: applications in erythropoiesis. *J Theor Biol* 1998, 190:135–146.
20. Mackey MC, Dormer P. Continuous maturation of proliferating erythroid precursors. *Cell Tissue Kinet* 1982, 15:381–392.

21. Belair J, Mackey MC, Mahaffy JM. Age-structured and two-delay models for erythropoiesis. *Math Biosci* 1995, 128:317–346.
22. Mackey MC, Aprikyan AA, Dale DC. The rate of apoptosis in post mitotic neutrophil precursors of normal and neutropenic humans. *Cell Prolif* 2003, 36:27–34.
23. Foley C, Mackey MC. Mathematical model for G-CSF administration after chemotherapy. *J Theor Biol* 2009, 257:27–44.
24. Abkowitz JL, Catlin SN, Guttorp P. Evidence that hematopoiesis may be a stochastic process *in vivo*. *Nat Med* 1996, 2:190–197.
25. Gordon MY, Blackett NM. Route to repopulation—a unification of the stochastic model and separation of stem-cell subpopulations. *Leukemia* 1994, 8:1068–1072, discussions 1072–1063.
26. Dingli D, Traulsen A, Pacheco JM. Compartmental architecture and dynamics of hematopoiesis. *PLoS ONE* 2007, 2(:):e345.
27. Quesenberry PJ, Kittler E, Lowry P, Crittenden R. Stromal regulation of hemopoiesis and the role of subliminal growth factor production. *Leukemia* 1993, 7(suppl 2):S3–S4.
28. Steensma DP, Harrison CN, Tefferi A. Hydroxyurea-associated platelet count oscillations in polycythemia vera: a report of four new cases and a review. *Leuk Lymphoma* 2001, 42:1243–1253.
29. Spangrude GJ, Smith L, Uchida N, Ikuta K, Heimfeld S, et al. Mouse hematopoietic stem cells. *Blood* 1991, 78:1395–1402.
30. Lemischka IR, Raulet DH, Mulligan RC. Developmental potential and dynamic behavior of hematopoietic stem cells. *Cell* 1986, 45:917–927.
31. Abkowitz JL, Catlin SN, McCallie MT, Guttorp P. Evidence that the number of hematopoietic stem cells per animal is conserved in mammals. *Blood* 2002, 100:2665–2667.
32. Gordon MY, Lewis JL, Marley SB. Of mice and men . . . and elephants. *Blood* 2002, 100:4679–4680.
33. McCarthy KF. Population size and radiosensitivity of murine hematopoietic endogenous long-term repopulating cells. *Blood* 1997, 89:834–841.
34. McCarthy KF. Marrow frequency of rat long-term repopulating cells: evidence that marrow hematopoietic stem cell concentration may be inversely proportional to species body weight. *Blood* 2003, 101:3431–3435.
35. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 1978, 4:7–25.
36. McKenzie JL, Gan OI, Doedens M, Wang JC, Dick JE. Individual stem cells with highly variable proliferation and self-renewal properties comprise the human hematopoietic stem cell compartment. *Nat Immunol* 2006, 7:1225–1233.
37. Abkowitz JL, Linenberger ML, Newton MA, Shelton GH, Ott RL, et al. Evidence for the maintenance of hematopoiesis in a large animal by the sequential activation of stem-cell clones. *Proc Natl Acad Sci U S A* 1990, 87:9062–9066.
38. Dingli D, Traulsen A, Michor F. (A)symmetric stem cell replication and cancer. *PLoS Comput Biol* 2007,, 3(:):e53.
39. Dingli D, Traulsen A, Pacheco JM. Stochastic dynamics of hematopoietic tumor stem cells. *Cell Cycle* 2007, 6:441–446.
40. Traulsen A, Pacheco JM, Dingli D. On the origin of multiple mutant clones in paroxysmal nocturnal hemoglobinuria. *Stem Cells* 2007, 25:3081–3084.
41. Abkowitz JL, Persik MT, Shelton GH, Ott RL, Kiklevich JV, et al. Behavior of hematopoietic stem cells in a large animal. *Proc Natl Acad Sci U S A* 1995, 92:2031–2035.
42. Huxley JS. *Problems of relative growth*. New York: Dial Press; 1932.
43. Thompson DW, Bonner JT. *On growth and form*. New York: Cambridge University Press; 1992.
44. West GB, Woodruff WH, Brown JH. Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. *Proc Natl Acad Sci U S A* 2002, 99(suppl 1):2473–2478.
45. Dingli D, Pacheco JM. Allometric scaling of the active hematopoietic stem cell pool across mammals. *PLoS ONE* 2006, 1(:):e2.
46. Buescher ES, Alling DW, Gallin JI. Use of an X-linked human neutrophil marker to estimate timing of lyonization and size of the dividing stem cell pool. *J Clin Invest* 1985, 76:1581–1584.
47. Nash R, Storb R, Neiman P. Polyclonal reconstitution of human marrow after allogeneic bone marrow transplantation. *Blood* 1988, 72:2031–2037.
48. Dingli D, Pacheco JM. Ontogenic growth of the haemopoietic stem cell pool in humans. *Proc Biol Sci* 2007, 274:2497–2501.
49. Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, et al. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *J Exp Med* 1999, 190:157–167.
50. Lopes JV, Pacheco JM, Dingli D. Acquired hematopoietic stem cell disorders and mammalian size. *Blood* 2007, 110:4137–4139.
51. Goldman JM. Chronic myeloid leukemia—still a few questions. *Exp Hematol* 2004, 32:2–10.
52. Tefferi A. Polycythemia vera: a comprehensive review and clinical recommendations. *Mayo Clin Proc* 2003, 78:174–194.

53. Luzzatto L, Bessler M, Rotoli B. Somatic mutations in paroxysmal nocturnal hemoglobinuria: a blessing in disguise? *Cell* 1997, 88:1–4.
54. Massey GV, Zipursky A, Chang MN, Doyle JJ, Nasim S, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. *Blood* 2006, 107:4606–4613.
55. Tricot G, Mecucci C, Van den Berghe H. Evolution of the myelodysplastic syndromes. *Br J Haematol* 1986, 63:609–614.
56. Zipursky A. Transient leukaemia—a benign form of leukaemia in newborn infants with trisomy 21. *Br J Haematol* 2003, 120:930–938.
57. Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. *Blood* 1998, 92:3362–3367.
58. Dingli D, Luzzatto L, Pacheco JM. Neutral evolution in paroxysmal nocturnal hemoglobinuria. *Proc Natl Acad Sci U S A* 2008, 105:18496–18500.
59. Marley SB, Lewis JL, Gordon MY. Progenitor cells divide symmetrically to generate new colony-forming cells and clonal heterogeneity. *Br J Haematol* 2003, 121:643–648.
60. Donohue DM, Reiff RH, Hanson ML, Betson Y, Finch CA. Quantitative measurement of the erythrocytic and granulocytic cells of the marrow and blood. *J Clin Invest* 1958, 37:1571–1576.
61. Finch CA, Harker LA, Cook JD. Kinetics of the formed elements of human blood. *Blood* 1977, 50:699–707.
62. Cronkite EP, Fliedner TM. Granulocytopoiesis. *N Engl J Med* 1964, 270:1347–1352.
63. Araten DJ, Nafa K, Pakdeesuwan K, Luzzatto L. Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria genotype and phenotype are present in normal individuals. *Proc Natl Acad Sci U S A* 1999, 96:5209–5214.
64. Parker C, Omine M, Richards S, Nishimura J, Bessler M, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. *Blood* 2005, 106:3699–3709.
65. Araten DJ, Luzzatto L. The mutation rate in PIG-A is normal in patients with paroxysmal nocturnal hemoglobinuria (PNH). *Blood* 2006, 108:734–736.
66. Dingli D, Pacheco JM, Traulsen A. Multiple mutant clones in blood rarely coexist. *Phys Rev E Stat Nonlin Soft Matter Phys* 2008, 77:021915.
67. Dingli D, Traulsen A, Pacheco JM. Chronic myeloid leukemia: origin, development, response to therapy and relapse. *Clin Leuk* 2008, 2:133–139.
68. Dingli D, Antal T, Traulsen A, Pacheco JM. Progenitor cell self-renewal and cyclic neutropenia. *Cell Prolif* 2009, 42(3):330–338.
69. Dingli D, Traulsen A, Pacheco JM. Dynamics of haemopoiesis across mammals. *Proc Biol Sci* 2008, 275:2389–2392.
70. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961, 25:585–621.
71. Shepherd BE, Kiem HP, Lansdorp PM, Dunbar CE, Aubert G, et al. Hematopoietic stem cell behavior in non-human primates. *Blood* 2007, 110:1806–1813.
72. Pacheco JM, Traulsen A, Antal T, Dingli D. Cyclic neutropenia in mammals. *Am J Hematol* 2008, 83:920–921.
73. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, et al. Dynamics of chronic myeloid leukaemia. *Nature* 2005, 435:1267–1270.
74. Roeder I, Horn M, Glauche I, Hochhaus A, Mueller MC, et al. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. *Nat Med* 2006, 12:1181–1184.
75. Brummendorf TH, Mak J, Sabo KM, Baerlocher GM, Dietz K, et al. Longitudinal studies of telomere length in feline blood cells: implications for hematopoietic stem cell turnover *in vivo*. *Exp Hematol* 2002, 30:1147–1152.
76. Marley SB, Deininger MW, Davidson RJ, Goldman JM, Gordon MY. The tyrosine kinase inhibitor STI571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. *Exp Hematol* 2000, 28:551–557.
77. Horwitz MS, Duan Z, Korkmaz B, Lee H-H, Mealiffe ME, et al. Neutrophil elastase in cyclic and severe congenital neutropenia. *Blood* 2007, 109:1817–1824.
78. Hillmen P, Hows JM, Luzzatto L. Two distinct patterns of glycosylphosphatidylinositol (GPI) linked protein deficiency in the red cells of patients with paroxysmal nocturnal haemoglobinuria. *Br J Haematol* 1992, 80:399–405.
79. Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural history of paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 1995, 333:1253–1258.