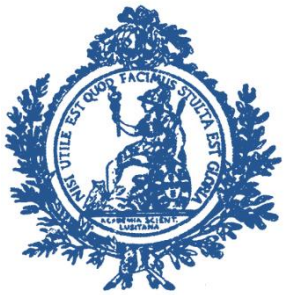
Jorge M. Pacheco * David Dingli

DARWIN AND THE SOMATIC EVOLUTION OF CANCER



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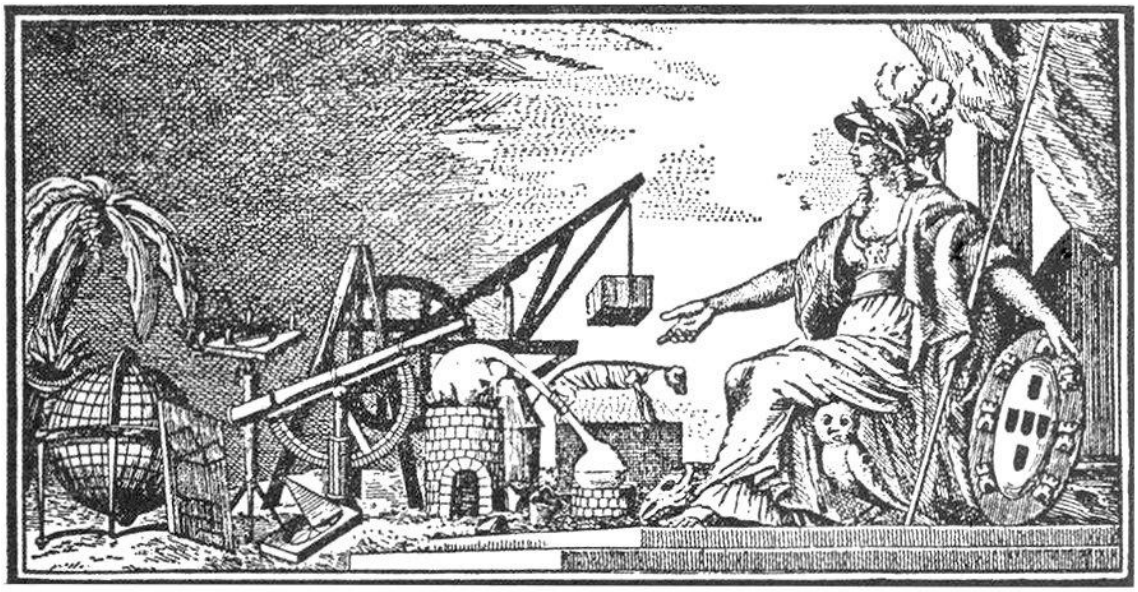

DARWIN AND THE SOMATIC EVOLUTION OF CANCER

Jorge M. Pacheco * David Dingli

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 event and increasing differentiation. The mathematical model is not only able to account for well-known features of hematopoiesis but it is also applicable to hematopoiesis across mammals and can be used to understand the dynamics of various disorders both in humans and in animal models. The fact that these dynamical processes are evolutionary conserved in nature brings cancer dynamics into contact with Darwinian evolution, providing a new perspective of looking at cancer progression and cure.

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 life-time of an erythrocyte (red blood cell) in humans is 120 days, neutrophils stay in the circulation for about 12 hours, 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]. 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 chronic myeloid leukemia (CML) [2] or polycythemia vera (PV) [3]. At the root of blood formation are the hematopoietic stem cells (HSC) that reside in the bone marrow [4,5]. The presence of these cells was initially inferred from bone marrow reconstitution experiments after total body irradiation in mice[4]. HSC make bone marrow transplantation possible, a procedure that has provided curative therapy for a variety of otherwise lethal genetic/metabolic or neoplastic disorders[6]. Several investigators have developed models that capture this process at various levels of complexity [7,8,9,10,11,12,13,14,15,16,17]. Many of these models were developed with specific conditions in mind such as cyclic hematopoiesis [18] or hematopoietic reconstitution after stem cell transplantation. These models typically 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 the known physiology of the process. 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 [19,20]. Hence we shall employ here a stochastic model of hematopoietic cells and discuss our approach to modeling this process for human and other mammalian species [21].

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 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 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 [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. 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 lineage is reduced or absent or "myeloproliferation syndromes" where one or more cell lineages are produced in excess. In particular, mutations in the stem cell compartment can give rise to the appearance of "new cell species", which compete with wild-type cells, very much in the same way, albeit at a nearly microscopic scale, that species compete for survival in macroscopic ecosystems. Cancer can then be modeled as an evolving eco-system, where Darwin's theory of natural selection plays a ubiquitous role. We briefly discuss some applications of our approach to understand the dynamics of a variety of disorders including chronic myeloid leukemia (CML) and paroxysmal nocturnal hemoglobinuria (PNH). We chose these disorders because in our view they illustrate different aspects of abnormal evolutionary dynamics in hematopoiesis.

Hematopoietic stem cells

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] suggest a number around 400, whereas experimental studies in patients who underwent bone marrow transplantation indicate that, after the procedure, hematopoiesis is maintained by ~111 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' so that one daughter cell remains in the HSC compartment that is, remains a stem cell (self-renewal) while the other starts the path towards 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 [27]. 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 [28]. These observations have important implications on the evolution of mutant clones in the active HSC compartment [29,30,31]. In particular, under normal conditions one expects the active pool of HSC not to change in size.

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 [32] 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, tree trunk thickness etc often scale with mass as $Y \sim Y_0 M^b$ where b is typically a multiple of ¼.

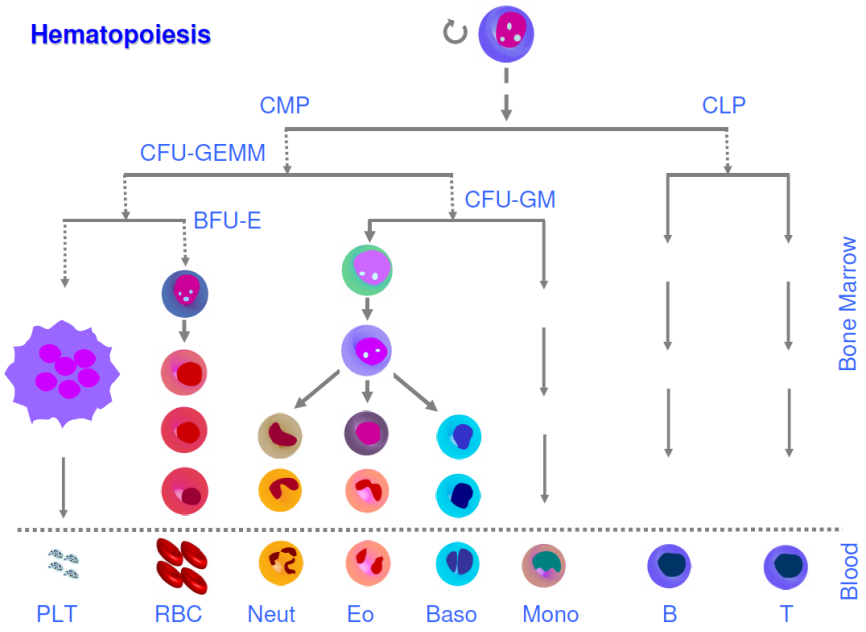

Figure 1. Hematopoietic tree. Hematopoiesis has a tree-like structure with the hematopoietic stem cells at the root of the process. Each cell division gives rise to progeny cells that can retain the properties of their parent cell (self-renewal, ↻) or differentiate, "*moving down*" the hematopoietic 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).

Competing explanations for the origin of these exponents exist [33,34,35]. With respect to HSC and hematopoiesis, we note that (i) hematopoiesis has appeared only once during evolution (the similarities between the process across mammals support this), (ii) although hematopoiesis is distributed across various bones, it is functionally coupled by the circulation and so HSC effectively function collectively, and (iii) from the definition of the HSC, every active HSC is equally represented in the circulation of a given mammal. Under 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 [36]. The best marker of cellular marrow output is the total circulating reticulocyte count (R_T) that can be estimated in many mammals from knowledge of the blood volume, the red cell count and the percentage of reticulocytes present in the circulation. 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 $R_T \sim M^{3/4}$ [36]. From (iii) 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 1 HSC can maintain hematopoiesis in a mouse for its lifetime [36], a prediction that is supported by experimental observations [37,38]. Naturally, this lower limit extends to the smallest mammal, a shrew with a mass of 3 gram. On the other hand, 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 ($B \sim M^{-1/4}$) [36]. Interestingly, it has been proposed that the total number of hematopoietic stem cells is conserved across mammals [39], the total number lying somewhere between 11000 and 22000. 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 [25,40,41,42], this result apparently contrasts with the allometric considerations above. However, the estimates of ~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.

Implications on HSC dynamics

Since HSC are long lived cells, they are a population at risk of acquired mutation. The risk of mutation is related to their rate of replication, the number of cells and the lifetime of the animal [43]. Mutations in these cells can have serious implications leading to various tumors (e.g. CML[44], polycythemia vera [3] and myelofibrosis with myeloid metaplasia) or acquired marrow failure syndromes (e.g. PNH [45]). 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 [30]. 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 [46,47,48,49,50].

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 9 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 progressive cellular replication and differentiation (Figure 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 [51], 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 – only the cells that lie at the root of the hematopoietic tree can give rise to all types of circulating blood cells.

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 is determined stochastically [19,20], 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 2) [21]. 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, while 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 their parent (Figure 2). 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 [29]. 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 2).

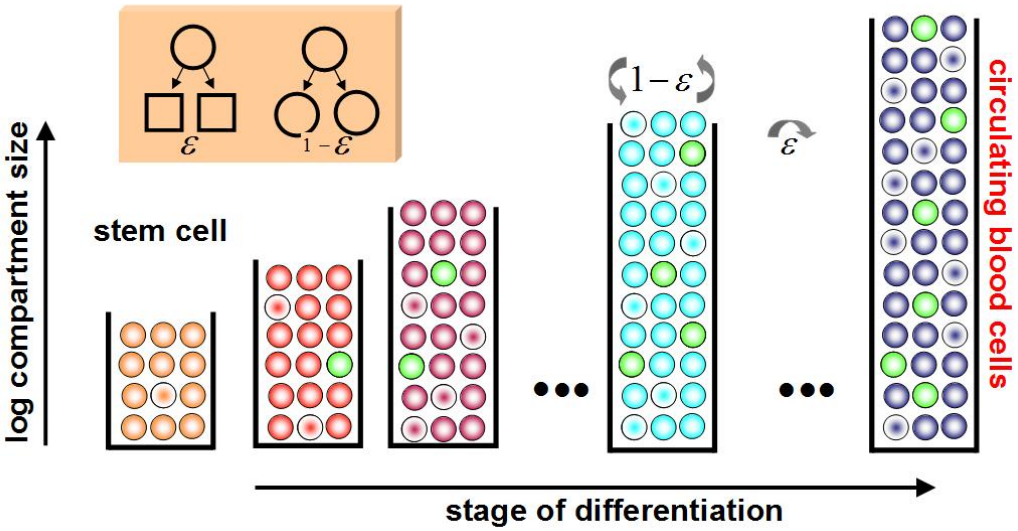

Figure 2. Compartmental model of stochastic hematopoiesis. Compartments represent different stages of cell differentiation. Normal cells (different colors) are represented with bright centers. Mutations may give rise to new cell lineages, often identified with cancer cells, here represented with dark 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. The compartmental model of hematopoiesis allows

one to identify how many stages of cell differentiation mediate between HSC and circulating bone cells, how long does a cell remain in each compartment, how often it divides in each compartment, etc. (see main text for details).

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) since cells that are lost from one compartment due to differentiation are replaced by input from the upstream compartment $(k-1)$ (Figure 2). 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, $\varepsilon_0 = 0.5$. 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 $(2\varepsilon-1)\cdot N_k \cdot r_k$ cells: with probability ε the compartment loses $N_k \cdot r_k$ cells per unit time step, and gaining new $N_k \cdot r_k$ cells with probability $(1-\varepsilon)$. This net loss 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

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

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

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

To the extent that $\gamma > 1$ 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. In this process, $\approx 10^{10}$ myeloblasts give rise to $\approx 1.4 \times 10^{11}$ myelocytes, a process that requires four divisions [52,53]. This input together with equation (2) leads to $\gamma \approx 1.93$. Equation (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 [1,54] while the most committed granulocyte precursors can replicate up to 5 times per day[55]. Hence $r \approx 1.27$ and, from equation (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 [55].

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 [21].

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 [1,54]. Moreover, our model can determine the compartment where a neutral mutant clone appeared based on the fraction of the circulating mutant cells. It can also determine the life-time 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 populations of circulating neutrophils and erythrocytes that lack expression of GPI linked proteins such as CD55 and CD59 [56]. These cells have total or partial deficiency of *PIG-A*, an enzyme subunit required for GPI biosynthesis. Mutant cells can be detected using flow cytometry due to loss of specific cell surface proteins (e.g. CD55 and CD59) [45,57]. The *PIG-A* gene accumulates mutations at a normal rate [58] and since both neutrophils and erythrocytes lack these proteins, the mutation has to occur at the level of the CFU-GEMM (Figure 1). In healthy adults, the frequency of mutant cells is of the order of $11-51/10^6$ neutrophils [56]. One can mathematically show that at any one time, it is more likely that a single founder clone is responsible for these mutant cells[31,59]. 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 to 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 [56].

Cell behavior across species

Humans are the best studied mammals and the model presented can accommodate a variety of human disorders without any modification [31,50,59,60,61]. 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 species is characterized by its mass, with $N_{SC}(M) = N_0 M^{3/4}$ ($N_0 \approx 15.9 kg^{-3/4}$) [36] while the rate of HSC replication scales as $R(M) = R_0 M^{-1/4}$ ($R_0 \approx 2.9 kg^{1/4} yr^{-1}$) [36]. The size of each compartment (k) grows as $N(k) = N_{SC}(M)\gamma^k$ while the compartment specific replication rate scales as $r(k) = R(M)r^k$ [21,62]. Using these relationships, the marrow output and HSC replication rates in various mammals can be determined and compared to experimental observations [62]. Model predictions for various mammalian species [62] 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 lifespan (L) of mammals (in the wild) also tends to scale allometrically with mass as $L(M) = L_0 M^{1/4}$ ($L_0 \approx 8.6 kg^{-1/4} yr$) [43]. 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 [62]. This result supports the Hayflick hypothesis which suggests that the number of divisions a cell can undergo is fixed [63], while providing a theoretical foundation for the suggestion by Shepherd et al [64]. These arguments which suggest that the HSC replication rate is dictated by the metabolic rate of the host species are also supported by experimental evidence. A single human HSC can rescue a lethally irradiated mouse with reconstitution of hematopoiesis [37,38]. 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 since 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.

The somatic evolution of hematopoietic stem cell disorders

So far we have been addressing normal hematopoiesis. However, the model should be capable of addressing also hematopoietic disorders, given the view of these as a stochastic competition

between different cell lineages, under mutation-selection dynamics, across the hematopoietic tree. In this context, we believe the model discussed so far is ideally suited to model hematopoietic disorders under this paradigm. As a result, the model has been used to understand the dynamics of chronic myeloid leukemia (CML) [61] in the presence and absence of therapy, and the clonal origin and evolution of multiple clones in paroxysmal nocturnal hemoglobinuria [31,50,59]. Here we shall briefly address the evolutionary dynamics of CML 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.

Chronic myeloid leukemia

CML is the best studied human tumor and is characterized by the *bcr-abl* oncoprotein [44]. 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 (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 [61].

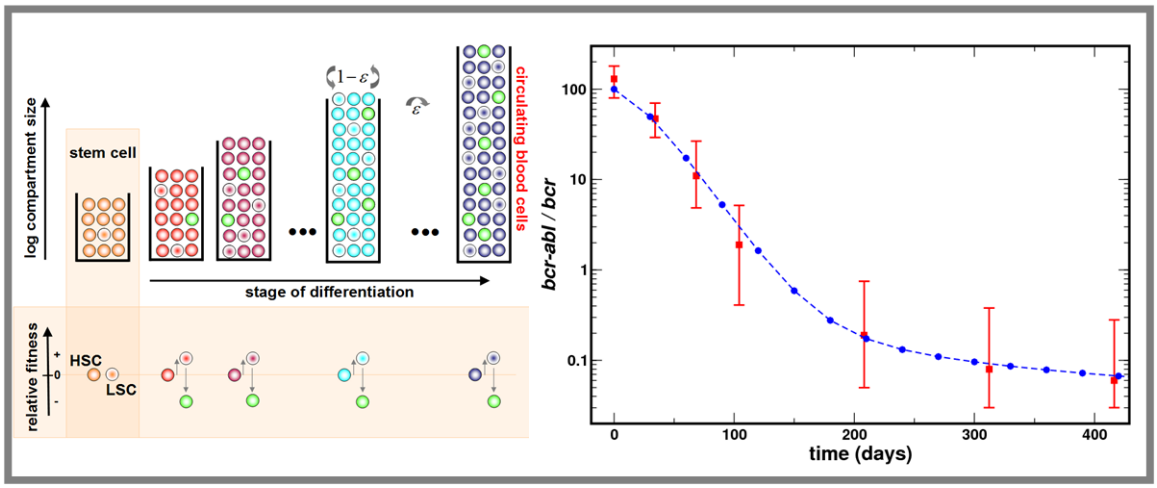

Figure 3. CML dynamics. We maintain the notation of Figure 2. In the lower left panel, we illustrate the relative fitness of each cell lineage in each compartment, which dictates the relative balance between the co-evolutionary dynamics of normal and cancer cells. Green cells represent cancer cells treated with imatinib, which, by changing their self renewal probability, effectively renders these cells disadvantageous with respect to normal (and cancer) cells, thereby reducing their impact in the overall evolutionary dynamics of CML (see main text for details). The right panel shows the impact of imatinib treatment on patients' disease burden as monitored by serial bcr-abl/bcr estimates, leading to the typical two-slope curve characteristic of this process.

Our model naturally fits the 'two-slope' curve of the decline in *bcr-abl* with time due to therapy [65,66] – see Figure 3. This observation is due to the architecture and dynamics of hematopoiesis and how it is disturbed by CML itself. The model suggests that a single leukemic stem cell may be enough to drive CML[61]. 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. This also implies that CML cells undergo a higher number of divisions before they appear in the circulation (Figure 3). These model predictions have experimental validation [67,68]. Imatinib reduces the fitness of mutant cells compared to normal ones, enabling the latter to take over hematopoiesis ($\varepsilon_{IMAT} > \varepsilon_0$). Moreover, at any time, perhaps 5% of the cells are reversibly responding to therapy [61]. The implications of stochastic dynamics on CML therapy are currently being evaluated. It is important to note that *bcr-abl* expression has no impact on the behavior of the leukemic stem cells. In other words, CML stem cell expansion can only grow by neutral drift, since they essentially do not 'feel' the effect of *bcr-abl* expression.

Stochastic effects on CML dynamics

What is the impact of stochastic effects on CML? In humans this impact is sizeable and detectable, as illustrated in Figure 4.

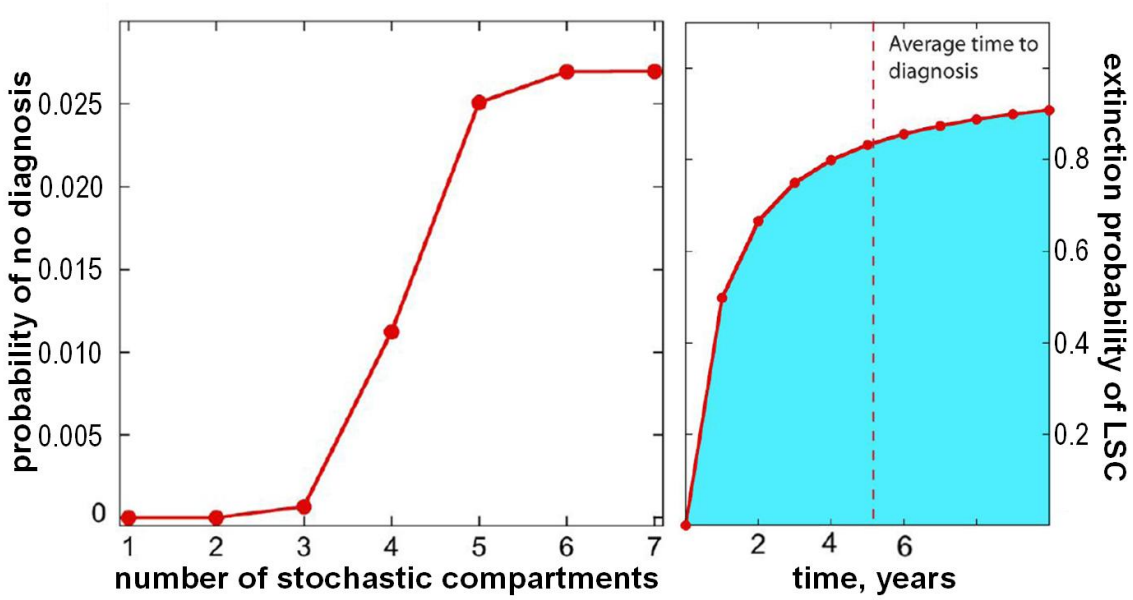

Figure 4. Stochastic effects on CML dynamics. On the left panel we show results for probability that CML is *not* diagnosed despite the disease being spawned by the appearance of a mutated stem cell in the HSC compartment. This probability has been computed by simulating one million virtual patients and computing the frequency of diagnosis. These simulations have been carried out treating stochastically a variable number of the smaller compartments, as indicated in the horizontal axes. The results indicate that treating more than the first 7 compartments stochastically may be redundant. On the right panel we plot the probability of stochastic extinction of leukemic stem cells in the HSC pool as a function of time, showing that, unlike 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. As a result we developed a hybrid model which allows us to decide how many of the 32 hematopoietic compartments should be treated stochastically. The results depicted in Figure 4 show that the stochastic effects extend well beyond the HSC compartment. First of all, our results show that in the overwhelming majority of patients the LSC population undergoes extinction before disease diagnosis (since the dynamics at the level of these cells follow a neutral Moran process). However, this is not the case for leukemic progenitors that have a fitness advantage in the absence of therapy. They are susceptible to imatinib treatment, and are 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 towards fighting the "war on cancer".

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 (GPI) [45]. 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*) [69], and despite the lack of specific, disease modifying therapy, a significant fraction of patients have resolution of the disorder [70]. 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 (i) 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 [31,50,59]. (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 [58].

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 [50]. Moreover, our predictions of average clone size approach those that have 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 [70]. Therefore, our model can provide rational explanations for many of the observed dynamics in this disorder.

Discussion

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 physiologic and pathologic 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 chronic myeloid leukemia, cyclic hematopoiesis and the clonal evolution and dynamics of paroxysmal nocturnal hemoglobinuria. Future work will build on this model so that it can be applied to an even wider range of hematopoietic disorders.

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