HEMATOPOIETIC STEM CELLS AND THEIR DYNAMICS

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**Introduction**

Hematopoiesis is an amazingly complex system. Through the course of this chapter, we review the arguments supporting the idea that, similar to all other mammals, humans have HSC at the root of hematopoiesis. We concentrate on the consequences of this view. Let us take humans as an example: At any time, roughly 400 HSC actively contribute to hematopoiesis, each cell replicating, on average, once a year. At the other extreme of the hematopoietic tree, $3.5 \times 10^{11}$ cells are replaced every single day. The amazing scaling of sheer cell number and replication rates poses a challenging problem for anyone who envisions a mathematical description of this multi-scale system of cells which functions as a complex organ. In the following we summarize the salient biology of HSC and attempt to show how some simple scaling and dynamical considerations may shed light on the dynamics of hematopoiesis.

**Evidence for hematopoietic stem cells**

Hematopoiesis couples together in a complex hierarchical organization, many types of blood cells. The various types of circulating blood cells have a finite lifespan and are continuously being replaced by new cells produced in the bone marrow. Under equilibrium conditions, cellular output from the bone marrow is of the order of $3.5 \times 10^{11}$ cells per day [1] in humans. Indeed, approximately 1% of the erythrocytes are replaced daily while the average time that neutrophils remain in the circulation is of the order of hours [2,3,4]. Bone marrow output increases in response to higher demands (e.g. bleeding or infection) or as a result of neoplastic transformation of hematopoiesis as in chronic myeloid leukemia (CML) [5] or polycythemia vera (PV) [6].
It is well known that the different types of blood cells originate at different stages of hematopoiesis, exhibiting a high level of interdependence, with a tree-like organization. At the root of blood formation are the hematopoietic stem cells (HSC) [7,8]. Nowadays, the existence of HSC is taken for granted since they make bone marrow transplantation possible, a procedure that is performed almost routinely in many major medical centers and has provided curative therapy for a variety of otherwise lethal genetic/metabolic or neoplastic disorders [9].

However, despite this unanimous recognition, isolation or direct observation of HSC has not been achieved to date. Indeed, the existence of hematopoietic stem cells is often inferred indirectly and almost always as an a posteriori event. For many years, the presence of these cells was demonstrated by marking with retroviral or lentiviral vectors [10,11,12] or studied from informative gene marking experiments such as X-linked genes (e.g. glucose-6-phosphate dehydrogenase (G6PD) [13] or chronic granulomatous disease (CGD)) [14]. The presence of HSCs was initially inferred from the observation that lethally irradiated mice died due to hematopoietic failure and this could be averted by transplantation of syngeneic bone marrow [7,15]. Infusion of different limiting numbers of bone marrow cells led to the formation of spleen colony forming units in mice, with the number of colonies being proportional to that of the injected cells [15]. Subsequent experiments showed that each spleen colony arose from a single cell and the progeny of each colony included cells from both myeloid as well as lymphoid lineages [16,17].

Although some investigators believe that hematopoietic stem cells can be isolated, others are of the view that a single hematopoietic stem cell may not be isolated in pure form for a variety of reasons. However, with the advent of fast, multicolor flow
cytometry, populations of long-term reconstituting cells have been isolated and shown to rescue irradiated mice and can be used for serial transplantation [18,19,20].

The cell surface expression profile of HSC is becoming increasingly well defined, although controversy here also exists. Essentially all investigators agree that human HSC are lineage negative (Lin− i.e. negative for CD4, 8, 10, 14, 15, 16, 19, 20), Thy1lo, c-kit+ and CD38− but there is no agreement on their CD34 expression: some believe that they are positive [21] while others have shown that CD34− cells isolated from the bone marrow can reconstitute lethally irradiated mice and give rise to CD34+ cells (Figure 1) [22,23,24,25]. Murine HSC are also Lin− (CD4, 8, B220, Gr-1, Mac-1), Thy1lo, c-kit+ Sca-1+. Transplantation of a single HSC (murine or human) in sublethally irradiated severe combined immune deficient (SCID) mice allows hematopoietic recovery and maintenance of hematopoiesis for the lifetime of the animal, confirming the long term self renewal and differentiation ability of these cells [21].

**Properties of hematopoietic stem cells**

A stem cell is operationally defined as a cell that has long term self-renewal capability and at the same time is able to give rise to progeny cells that differentiate along various possible lineages. Progenitor cells differ from HSC in that they have less self-renewal potential and a more restricted differentiation profile (Figure 1). In the case of HSC, the progeny are erythrocytes, platelets, various types of granulocytes (neutrophils, eosinophils and basophils), monocytes/macrophages, lymphocytes (T and B), natural killer (NK) cells and mast cells. The two daughter cells that arise from mitosis of a single HSC can have completely different fates, suggesting that ‘decisions’ along the path of differentiation can occur within one replication event and widely interpreted as being due
to a stochastic process [15,26,27]. Limiting dilution studies and competitive repopulation experiments in murine models suggest that perhaps a single stem cell may be enough to maintain hematopoiesis in the mouse [28], illustrating the long term self-renewal and differentiation ability of these cells. Moreover, the progeny of these cells can be used for serial transplantation up to 4 or 5 times, confirming the long term self-renewal properties of the cells [29]. Studies in larger mammals such as cats and non-human primate show that hematopoiesis is a polyclonal process: in other words different stem cells are contributing to blood formation at any time [30,31]. HSC can be operationally divided into an active and a reserve pool. Cells in the active pool contribute to hematopoiesis and are associated with the endosteal surface of bone [32]. There is evidence that cells selected to contribute to hematopoiesis, can do so for a very long time, perhaps the lifetime of the individual [33]

**Sources of hematopoietic stem cells**

(i) *Harvesting*

For many years, the main source of HSC has been the bone marrow itself. Harvesting of the bone marrow in humans required a surgical procedure with general anesthesia. For safe stem cell transplantation in humans, a minimum of $2 \times 10^8$ mononuclear cells per kilogram is required. The cells are harvested by multiple aspirations from both posterior iliac crests. Many of the cells collected are not stem cells but more committed progenitors that engraft rapidly and start producing mature cells.

(ii) *Mobilization*

With the recognition that both hematopoietic stem and progenitor cells circulate in the blood at low levels and that their numbers increase after chemotherapy and/or
cytokine therapy (e.g. cyclophosphamide and granulocyte-colony stimulating factor, G-CSF, respectively), most centers now collect these cells by apheresis after therapy with G-CSF (a process known as mobilization). HSC mobilization with G-CSF is through an indirect mechanism: the cytokine stimulates cells of the myelomonocytic lineage to release proteolytic enzymes such as elastase, cathepsin G, proteinase 3 and gelatinase (MMP-9) that disrupt cell adhesion molecule interactions such as VCAM-1-α4β1, c-kit receptor with surface expressed c-kit [34] and perhaps most importantly alters the gradient of stromal cell derived factor 1 (SDF-1, also known as CXCL12) between the bone marrow and the blood (higher in the latter) inducing the cells to emigrate to the vascular compartment [35]. The current view is that SDF-1 is the main anchor of HSC to their endosteal niche (see below). There are additional cytokines that mobilize HSC into the circulation including GM-CSF, IL-3 and stem cell factor (SCF) but their exact mechanisms of action are not so clear [36]. The optimal time for HSC collection can be determined by serial monitoring of the circulating CD34+ cell count. The main advantage of transplantation with peripheral blood stem/progenitor cells is faster hematopoietic reconstitution and therefore a shorter interval of neutropenia and thrombocytopenia, therefore minimizing the risk of fatal infections or hemorrhage. In addition, the donor (especially in the case of allogeneic transplantation) avoids a surgical procedure with its associated risk and the pain due to the multiple aspiration sites. However, it must be pointed out that for specific diseases, bone marrow is still the preferred source of stem cells compared to apheresis collections of mobilized progenitors.

The last source of HSC is umbilical cord blood that is harvested soon after delivery of the neonate. Cord blood is enriched with hematopoietic stem and progenitor
cells and can be used for transplantation in the pediatric population, especially if the recipient mass is less than 25 kilograms [37]. As experience with the procedure has expanded, multiple cord blood donors have been pooled and used to reconstitute hematopoiesis in adult recipients [38]. In general bone marrow reconstitution and hematopoietic recovery are slower after cord blood transplantation compared to autologous or allogeneic peripheral blood/bone marrow transplantation [39,40].

The stem cell niche

It has been next to impossible to expand HSC in culture and demonstrate persistence of both self-renewal and multilineage commitment. This observation suggests that a HSC can only function properly when it resides in an appropriate microenvironment, now known as the stem cell niche, a concept initially proposed by Schofield in 1978 [41]. Consequently, the definition of a stem-cell becomes a functional one. One can even venture further and state that a cell is not a stem cell anymore when it is outside the niche since the latter seems to determine the fate of the HSC: cell cycle status, replication rate and symmetry of division/differentiation [42,43,44]. At present the exact nature of the growth factors that regulate HSC behavior is not clear and it is possible that these requirements change as the host animal ages. However, insightful experiments from transgenic mice are starting to shed important light on the nature of the stem cell niche.

The intimate association between hematopoiesis and bone led to the discovery of the role of osteoblasts in HSC niche formation [32,45]. Mice with a conditional knockout of bone morphogenic protein (BMP) receptor 1A have ectopic trabecular like bone along their long bones and concomitantly have an increase in the number of HSC [46] –
Transgenic mice with osteoblasts that constitutively express parathyroid hormone and parathyroid hormone related receptors experience an increase in osteoblast numbers as well as in HSC [47]. The high calcium concentration present in the osteoblast niche is essential for homing and retention of HSC to this niche and for this purpose, HSC express the calcium-sensing receptor that enables them to respond to the calcium signal [48]. Osteopontin, produced by osteoblasts in response to various stimuli and secreted into the extracellular matrix is another important regulator of the number of HSC. Low levels of osteopontin lead to an increase in the number of HSC, suggesting that it is a negative regulator of stem cell number [49].

More recently, a second niche, in relation to endothelial cells, has been identified and called the vascular niche [50,51]. HSC interact with endothelial cells via signaling lymphocyte activation molecule (SLAM) receptors on sinusoidal cells both in the bone marrow as well as the spleen [52]. The current view is that perhaps the osteoblastic niche provides a quiescent environment for HSC maintenance while the vascular niche provides the environment required for differentiation and mobilization. From an ontological perspective, the intimate relationship between HSC, osteoblasts and vascular endothelial cells has been rationalized on the basis of the close embryological origins of these cells. HSC and vascular endothelial cells are both derived from hemangioblasts [52] while the hematopoietic system is derived from the mesoderm around the aorta while osteoblasts originate from the surrounding mesenchyme [53].

The microenvironment in the stem cell niche is composed of cell-to-cell interactions via cell-surface molecule expression, chemokines and growth factors. This orchestra determines the fate of the cell(s) within the niche. The list of molecular
mediators that regulate the HSC is increasing and includes angiopoietin-1/Tie-2, SDF-1 (CXCL12)/CXCR4, very late antigen 4 (VLA-4), leukocyte function antigen 1 (LFA-1), FGF-4, VEGF, SCF/c-kit, Wnt, N-cadherin and indirectly G-CSF, GM-CSF, FLT-ligand and possibly many others [54, 55, 56]. The latest discovery has been the role of the sympathetic nervous system on stem cell function. Reticular cells, present in the bone marrow stroma, appear to respond to noradrenaline by expressing the β3 adrenergic receptor [57]. Adrenergic stimulation of reticular cells leads to dephosphorylation of the transcription factor Sp-1 that leads to destabilization of the factor and a reduction in CXCL12 production. Lower concentrations of CXCL12 allow HSC to enter the circulation. As a result of this interaction of the sympathetic nervous system with HSC, the number of circulating HSC varies with the circadian rhythm, with the peak in HSC seen during maximal lighting, while their number falls during the dark part of the cycle [57].

**How many stem cells?**

Although most of biology is a descriptive science, quantitation is essential, since ultimately, number and dynamics can tell us what is possible. Indeed, for many situations, an understanding of the phenotype requires a dynamic description. The desire to understand the details of hematopoiesis and how it responds to varying demands and the origin and evolution of various hematopoietic disorders make the determination of the number of hematopoietic stem cells important. A few years ago, it was proposed that the total number of hematopoietic stem cells is conserved across mammals and may be between 11,000 and 22,000 cells [58]. Perhaps this suggestion may appear unusual, but there is experimental evidence from mice, rats and cats that can accommodate such a
proposition [30,59,60,61]. Theoretical arguments based on this hypothesis have been used to address the largest land mammals – elephants [61]. If we assume that these observations/inferences are true, they beg several questions including (i) why is the number of HSC conserved?, (ii) are mammals born with the full complement of HSC?, (iii) what are the reasons and evolutionary mechanisms that have selected or imposed such a constraint on this pool of cells?, (iv) are there similar constraints on other tissue specific stem cells? There is no doubt that the demands on hematopoiesis are different across mammals [30]. One can show that the total marrow output during the lifetime of a mouse is roughly the same as an adult human produces in a day [30,62,63]. Therefore, we need to tackle the issue of how many stem cells actively contribute to hematopoiesis in a given adult mammal. Until now, no unambiguous answer has been provided in the laboratory. It has been proposed that hematopoiesis is maintained by a subset of ‘active’ HSC and supported by a quiescent ‘reserve’ that may be called upon to contribute depending on circumstances [64]. With such a hypothesis, perhaps the number of ‘active’ stem cells may differ across mammals without requiring the total number of cells to change.

One approach that may help us to understand this potential conundrum, is to resort to the field of allometry [65] combined with the above definitions of active and reserve HSC pools. In biological systems, many observables (Y) that are related to nutrient transport, such as metabolic rate and tree trunk thickness, scale with mass as $Y \sim Y_0 M^b$ where $b$ is typically a multiple of $\frac{1}{4}$. There are competing explanations for the origin of these exponents and this issue is by no means resolved [66,67,68]. In order to apply these principles with respect to HSC and hematopoiesis, some general observations
are in order: (i) hematopoiesis has appeared only once during evolution (as seen by the similarities between the process across mammals, enabling detailed studies in the murine model and applied to other species), (ii) while hematopoiesis is distributed across various bones, it is functionally coupled by the circulation and so HSC effectively function collectively as a single hematopoietic organ, and (iii) from the definition of the HSC, every active HSC is equally represented in the circulation of a given mammal. With these premises, we were able to provide an allometric estimate of the size of the active stem cell pool in mammals ($N_{SC}$) as a function of their mass [69]. The simplest marker of cellular marrow output is the total number of circulating reticulocytes ($R_T$) that can be estimated in many mammals from knowledge of their circulating blood volume, the red cell concentration and the percentage of reticulocytes present in circulation as a function of erythrocytes. Taking into account the variable maturation rate of reticulocytes across species, we could determine that $R_T$ scales with the mass of the adult mammal as $R_T \sim M^{3/4}$ [69]. From (iii) above, we conclude that $N_{SC} \sim M^{3/4}$. Therefore, if $N_{SC}$ and the mass of any adult mammal are known, $N_{SC}$ for any other adult species can be determined based on premise (i) above. Using this relationship and the estimated number of active HSC in Safari cats, allowed us to calculate that in humans $N_{SC} \approx 400$, in agreement with experimental observations derived from patients with chronic granulomatous disease [14]. The same relationship suggests that after bone marrow transplantation, a typical adult human has $N_{SC} \approx 116$, which is again in excellent agreement with experimental data [70]. This model also predicts that a number of the order of 1 HSC can maintain hematopoiesis in a mouse for its lifetime [69], a prediction
that is supported by experimental observations [28]. Consequently, the smallest mammal, a shrew with a mass of 3 gram would also require 1 or very few HSC to maintain hematopoiesis. 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. Therefore, the size of the HSC pool based on allometry falls within the limit proposed by Abkowitz et al [14]. However, our estimates are based on the number of cells that are *active* in blood formation and hence represented in the circulation. The remaining cells are inactive and we consider them to constitute a reserve pool of cells. If the number of HSC is conserved, then one should consider the sum of these two pools, compatible with the hypothesis proposed by Kay[64]. Interestingly, the species specific HSC replication rate (*B*), naturally also follows an allometric relationship with adult mass: in any given species, HSC replicate at a rate which decreases with increasing mass (*B* ~ $M^{-1/4}$) [69].

### Expansion of the HSC during human ontogeny

Several hereditary/congenital HSC and non-HSC disorders are amenable to gene therapy approaches. Although individually these disorders are rare, they make the HSC compartment an important therapeutic target (e.g. X-SCID and retroviral vector therapy, [71]. Thus, it is essential to determine the size of the active HSC pool in newborns and how the active pool increases over time to reach adult levels. A small active HSC pool requires highly efficient cell transduction if the genetic/metabolic defect is to be corrected successfully. In order to gain some insight on the size of this pool during human ontogeny, we have applied the previously discussed allometric scaling that relates the reticulocyte count with mass to data derived during normal human growth. We found that
the active HSC pool scales linearly with mass during human ontogeny. Perhaps the most relevant observation was that the number of active HSC in newborn babies is quite small, in the range of ~20 [72], making them a difficult target for gene therapy. It is at present unclear whether babies are born already equipped with the full pool of HSC or whether their population expands in time to reach the estimated adult size.

**Stem cell aging / clonal succession**

Aging is a natural process that is experienced by every living organism and associated with a slow but continuous modification in phenotype and physiology. The process is poorly understood and sometimes conflicting hypotheses have been proposed in an attempt to explain it. Given the importance of stem cells in tissue maintenance and their long term contribution to tissue homeostasis and repair, it is understandable that considerable effort has been expended to understand how stem cell number and function change as a function of age. For ethical reasons and experimental convenience, most of the work has been done in murine models. Intriguingly, in most common strains of mice, the number of HSC seems to increase with age [73]. It has been reported that in older mice there are higher numbers of circulating HSC and progenitors and mobilization is easier in older species [74]. However, the data on HSC mobilization as a function of age in humans shows that the number of HSC mobilized and obtained in collections decreases as a function of age when healthy, allogenic donors are considered [75].

Competitive repopulation assays show that HSC taken from older mice have a reduced potential for hematopoietic reconstitution, and in time the cells experience a progressive bias in their ability to give rise to progeny cells: HSC taken from young mice preferentially give rise to cells of the lymphoid lineage while HSC derived from older
mice seem to be skewed towards the myeloid lineage. These behaviors appear to be inherited and related to epigenetic changes in the cells that accumulate with age. However, the changes in number and phenotype are not solely cell autonomous. There is increasing evidence that the stem cell niche function also changes with age and exhibit differences in signaling patterns that may have an impact on HSC behavior. This has been best described in the case of *Drosophila* germ-line stem cells. Moreover, calorie restriction, that is known to increase longevity in animal models, also ‘improves’ HSC function [76]. Thus, HSC harvested from calorie restricted aged mice, exhibit improved function even when transplanted in non-calorie restricted mice.

The various mechanisms postulated to influence the aging process and its speed of development include mutations in genomic DNA via reactive oxygen species, ultraviolet irradiation and the intrinsic error rate of the DNA replication machinery [77]. Although HSC have DNA repair mechanisms as well as checkpoint controls, these are not perfect and mutations accumulate that could contribute both to the process of aging as well as carcinogenesis. The DNA replication machinery is unable to replicate the ends of chromosomes and as a result, ~50 – 70 bases of DNA are lost with each replication cycle from the ends of each double helix. Chromosomes have a specialized complex of nucleoproteins called telomeres that protect the ends of DNA and serve as an internal clock for the number of divisions a given cell has undergone. If telomeres lose a critical part of their length, the cell activates pathways that lead to cell death. HSC have some ability to maintain their telomeres by expression of telomerase, the enzyme complex that utilizes an RNA template to extend the nucleotide repeats present in telomeres. However, it is well known that even HSC experience serial shortening of their telomeres [1] leading
to functional decline. The importance of telomere maintenance in HSC is further supported by the syndrome of dyskeratosis congenita where the main cause of death is bone marrow failure due to loss of HSC function [78]. These diseases are characterized by defects in telomere maintenance mechanisms. The most recent studies suggest that the serial accumulation of DNA damage is the main determinant of HSC senescence [79,80].

Despite HSC aging, serial transplantation experiments show that HSC can function for longer than the lifetime of the animal. Indeed, HSC have been transplanted serially up to 5 times without loss of repopulating ability in murine species [29]. One potential explanation for this observation is the unusual long telomeres possessed by murine chromosomes. Yet, calculations in humans would suggest that with replication rates of ~1/year, HSC can contribute to hematopoiesis for many years if not the lifetime of the mammal if only telomeres attrition was the only determinant of the duration of stem cell function [69]. There is evidence that once HSC start to contribute to hematopoiesis, they tend to do so for a long time [33]. The question then arises, whether there is clonal succession in the active HSC pool. In other words, is there a continuous shift in the use of stem cells that are active in blood formation or are the same cells utilized till exhaustion (including telomere attrition) and then stochastically replaced from HSC present within the reserve pool (clonal succession)? The answer to this question is not yet fully known but there is some evidence to support clonal succession in a feline model [13]. However, the strongest evidence for clonal succession in this model required observations of animals after stem cell transplantation when fluctuations in the cells contributing to hematopoiesis are expected. Indeed, even for cats under steady state conditions, it has been estimated that HSC clones can contribute to hematopoiesis for
more than 300 weeks, which is equivalent to at least half the lifetime of this mammal [13].

**Stochastic dynamics within the HSC pool**

The small number and slow replication rate of HSC in the active pool, can have important consequences on the evolutionary dynamics of mutations that arise within this cellular compartment. As discussed above, HSC tend to contribute to hematopoiesis for a long time (years in humans) and therefore mutations in these cells can be retained leading to a clonal population that may expand or decay over time. Serial accumulation of mutations makes these cells a dangerous target for the development of cancer. In the absence of immortal DNA strand co-segregation [81]. These cells have various mechanisms that reduce the appearance and rapid expansion of mutant cells including (i) the slow replication rate of these cells ([69,82], (ii) checkpoint control from a variety of tumor suppressor genes (REF), (iii) expression of plasma membrane carriers that pump out of the cells a broad spectrum of genotoxic agents (e.g. P-glycoprotein)[83]. However, mutations in HSC do occur and are responsible for a number of neoplastic and non-neoplastic disorders such as chronic myeloid leukemia (CML) [84] and paroxysmal nocturnal hemoglobinuria (PNH) [85] respectively. It would seem that the number of active HSC is determined by the availability of active niches that can house them, that in turn, must be correlated with the demands for hematopoietic cell output. HSC are under the influence of various signals from the cells forming the niche, the extracellular stroma, cytokines and growth factors (see above). The overall input from these various sources together with the transcriptome of the cell presumably define the behavior of the HSC and determining whether a given cell replicates, the symmetry of replication and the
commitment to differentiation of the progeny cells along specific pathway(s). Although the detailed molecular pathways that regulate stem cell behavior are incompletely understood, the HSC dynamics can be approximated by a Markov process. Such a coarse-grained approach is also in line with the experimental evidence that hematopoiesis may be a stochastic process [26,27]. Despite this stochastic behavior at the individual cell level, the number of terminally differentiated blood cells appears to be fairly constant. This observation is due to the sheer number of cells present at the end of the hematopoietic tree that ultimately masks the stochastic behavior of individual cells at all levels of hematopoiesis. As expected, the impact of this stochastic behavior is strongest closer to the root of the process i.e. at the stem and progenitor cell levels [62,86] since these are the smaller cell populations.

Under steady state conditions, it is thought that the number of HSC is constant and the population behaves in a homogenous way since the cells are chemically coupled despite being separated in space [87]. The evolutionary dynamics of mutated cells in such a population can be modeled by a simple stochastic birth-death process known as a Moran process [88] (Figure 2A). In each cycle of this process, one cell is chosen for reproduction and another is chosen for elimination so that the total population remains constant. The rate at which events take place is dictated by the replication rate of HSC and deduced from the allometric relations specific for each adult mammalian species. A normal cell that is chosen for reproduction can mutate with probability $\mu$. Experimental observations estimate that the normal mutation rate per gene is of the order of $\mu = 10^{-7}$ per replication [89]. Thus, when a normal stem cell is selected to reproduce, with probability $1 - \mu$, it gives rise to two normal daughter cells, while with probability $\mu$, the
mitotic event produces one normal cell and one mutated cell (Figure 2B). When a mutated HSC reproduces, it gives rise to more mutated cells since the probability of a mutant cell to revert to normal (wild-type) is essentially negligible. A mitotic event increases the number of HSC by one and, therefore, one cell from the whole pool is chosen at random for elimination. More precisely, the cell chosen for elimination is exported, and it initiates its journey of increasing specialization down the hematopoietic cascade. The probability that a cell is chosen for reproduction is proportional to its fitness, \( r \). Normal cells have fitness \( r = 1 \), cells with mutations that enhance their fitness have \( r > 1 \), while mutations that confer a fitness disadvantage have \( r < 1 \). Figure 2C illustrates the process of stem cell replication. Since normal HSC replicate approximately 1/year [69,82], then in one year approximately \( \sim 400 \) HSC divisions take place: this provides the time scale for our in silico evolutionary dynamics. By repeating the Moran process many times, probability distribution functions are obtained that illustrate the evolutionary histories of mutations in a population of virtual individuals [86].

The Moran process has two absorbing states (of course, if we wait an arbitrarily long time, ultimately all cells will be mutated): either the clone expands to take over the entire population or it is stochastically eliminated and goes extinct. During the finite lifetime of each individual organism, clonal invasion (i.e. complete replacement of the HSC by a mutant clone) is rarely achieved, and what is normally observed is an intermediate state. Moreover, it is often the case that the appearance of disease does not require that all the HSC are mutated (see below). A small population of mutated HSC (e.g. cancer stem cells, CSC) may be enough to induce disease, if not a lethal burden [86,90]. Interestingly, such modeling predicts that a mutant HSC clone can invade the
whole population (rarely), go extinct or persist at relatively ‘stable’ levels that may or may not cause disease (Figure 3). A major determinant of the outcome is the fitness advantage of the mutant cells compared to their normal counterparts. *In silico* studies of such a population of cells show that starting from one mutated HSC, the probability of invasion increases with higher fitness, while the chance of extinction falls down. However, even for a fitness advantage of 2 –which is a very high fitness advantage [91,92] - such a cell has a 50% probability of clonal extinction (for large populations). While it is clear that the fitness conferred by the mutation is an important parameter when describing the phenotype of cancer cells, it is easier to define “*r*” than to provide experimental estimates of its value, even for well defined mutations such as *bcr-abl*.

The diagnosis of various hematopoietic tumors, requires that a minimum fraction of cells in the bone marrow must be abnormal for the tumor to be clinically detectable or defined. These definitions are somewhat arbitrary and subject to revision. For example, at least 10% plasma cells have to be present for multiple myeloma to be diagnosed and 20% bone marrow blasts define acute leukemia [90]. If we take these thresholds into consideration, it can be shown that when the mutant clone expands and approaches say 20% of $N_{sc}$, stochastic clonal extinction becomes an increasingly rare event, although it is still possible [86]. The evolutionary trajectories of the mutant cells are described by probability distribution functions that are always ‘one humped’ functions of time [86,90]. The fitness advantage of the mutant cells has a major impact on the time required for the clone to either completely invade or reach the diagnostic threshold. As the fitness advantage increases, the peak of the distributions moves to the left (shorter time to reach threshold). The variance of the distributions also depends on the fitness associated with
the mutation. For a small fitness advantage, the distributions are very wide but, as the fitness advantage increases, they become narrower (smaller variance), because the process becomes more deterministic. Our results show that for a mutation with a low fitness advantage, the ‘average’ time to reach a diagnostic threshold or invasion (the mean of the corresponding distribution) can be of the order of the variance of the distribution, and as such lacks a precise meaning. In other words, these results suggest that each patient’s tumor is unique and has different dynamics even when arising from the same cell type. Of course, these observations relate to disorders where a single mutation could be enough to explain some aspect of the disease (e.g. the chronic phase of CML due to bcr-abl [93,94,95]). An important corollary of this model is that for a single gene mutation, knowledge of the time required for the development of the disease can give an estimate of the fitness advantage associated with that mutation. For example, if retinoblastoma were due to mutations in the Rb gene only, the fitness advantage of the mutation must be >1.7 to be compatible with the time frame in which the disease appears [86].

The concept of clonal expansion and tumor progression are well established features in cancer, but perhaps the idea of stochastic clonal extinction or latency are less obvious and one may think that these are artificial results of the model. However, there is clinical evidence for ‘spontaneous’ elimination of malignant clones, even though it is rare. Perhaps the most striking case is that of transient leukemia (TL) that often develops in children with Down’s syndrome [96,97]. Some studies suggest that perhaps up to 10% of children with Down’s syndrome may develop TL, usually within 5 days of birth [98]. This potentially lethal disease clearly affects an early progenitor cell in hematopoiesis (if
not the HSC) [96,98]; yet, in up to 85% of cases, the disease resolves with minimal or only supportive therapy [97]. Our model of stochastic dynamics accommodates this behavior. There are also reports of ‘spontaneous’ resolution of myelodysplastic syndromes as well as acute myeloid leukemia in patients [99] who did not receive any disease modifying therapy. Finally, the model suggests that malignant clones can experience latency and stability: in other words they do not change in size appreciably over long periods of time (including years). The best evidence for this is provided by a group of patients with essential thrombocythemia that were untreated. In this cohort, the size of the JAK2$^{V617F}$ clone was determined serially and shown to be stable over several years [100], again compatible with our model of stochastic HSC dynamics.

**Symmetry of stem cell replication and fitness**

One of the *sine qua non* properties of stem cells is self renewal. The fate of the daughter cells defines the symmetry of division: ‘symmetric’ division gives rise to two cells that have the same fate (both cells either differentiate or retain stem cell properties), but whenever the daughter cells have different fates, the division is considered to be ‘asymmetric’ (Figure 4A) [101]. In principle, asymmetric division should be enough to maintain tissues by simultaneously keeping the HSC population constant and feeding downstream compartments. However, this would not allow HSC expansion that is necessary during ontogeny of the hematopoietic (and other) systems or in response to injury or bone marrow transplantation. There is now good experimental evidence from DNA methylation patterns that all 3 types of SC division as depicted in Figure 4A occur, depending on the demands imposed on the SC pool [33,102] by the host.
The determinants of the (a)symmetry of HSC replication are not completely understood, although the interactions of the HSC with the stem cell niche and signals via the Notch, Hedgehog and Wnt pathways (including β-catenin) are known to be quite important [103,104,105]. Recent work in model organisms such as Drosophila is starting to decipher the potential impact of mutations on the (a)symmetry of stem cell replication. Some of the genes that have been recently shown to determine cell division fate include partner of inscuteable (PINS) [106], lethal giant larvae (LGL)[107], HUGL-1 and adenomatous polyposis coli (APC). Mutations in PINS, LGL and HUGL-1 result in the formation of tumor like tissue in these animals.

In order to understand the impact of mutations that alter the symmetry of HSC replication, we have developed the model illustrated in Figure 4 [108]. The fate of HSC daughter cells after a single HSC replication is determined by parameters $p$ and $q$ that define the probability of (a)symmetric cell replication. A normal HSC divides asymmetrically with probability $p_n$, and with probability $q_n$, the cell divides symmetrically to give rise to 2 differentiated cells. Finally, with probability $1 - p_n - q_n$, the cell divides symmetrically to give rise to two HSC (self renewal). Mutations confer to the cell a relative fitness $r$ and cells are chosen for reproduction according to their fitness (Figure 2C). The dynamics resemble a Moran process such that we impose the condition that the total cell population must remain constant. In order for this condition to be fulfilled, cells may be forced to divide symmetrically to replenish the pool if a cell is lost [108]. Under such a model scenario, what would be the impact of a mutation that increases the probability of self-renewal compared to that of a normal HSC (i.e. $p_c + q_c < p_n + q_n$)? One can show that such mutated cells will take over the
compartment, as they possess a selective advantage (even for $r = 1$). Mutations such as these, will enable the mutant to invade the population and impart an effective ‘reproductive fitness’ advantage to the cells. Naturally, if the mutation also gives a selective advantage whereby the mutant cells are chosen more often for reproduction, the average time needed for fixation will be significantly reduced [108]. Therefore, mutations that increase the self-renewal capability of cells may be an early event in cancer. Indeed, these mutations favor the mutated population to outgrow in number the normal cell population, thereby increasing the population of cells at risk, with the possibility to accumulate additional mutations and progression. This is perhaps one of the reasons why there is so much interest in the transformation of normal stem cells to cancer stem cells.

Because of their location at the root of the hematopoietic tree, HSC would be expected to be the only ones exhibiting long-term self renewal capability, as progenitor cells can self-renew but for a limited time [62]. However, it has been shown that acute myeloid leukemia can arise in progenitor cells such as CFU-GEMM that re-acquire stem cell-like properties and can drive the tumor. Indeed, there is good evidence for this both in the case of some de novo acute myeloid leukemia as well as the blast crisis that often accompanies chronic myeloid leukemia [109,110]. Our modeling suggests that for a tumor to arise due to mutations in a progenitor cells, such a mutant cell must acquire the potential for long-term self-renewal at an early step in its path to cancer, otherwise it will be eliminated relatively rapidly down the hematopoietic cascade [111,112].

**Stem cell pool, longevity and cancer**

There is increasing evidence that like normal blood cells, hematopoietic tumors are also driven by cancer stem cells (CSC) [113]. Presumably CSC arise from HSC by
mutations, although not all CSC necessarily result from the malignant transformation of normal stem cells. Hence, HSC are potentially a double edged sword: a small and slowly replicating pool will minimize the risk of mutations, but at the same time facilitate the expansion of neutral or disadvantageous mutations. Mutations in HSC can lead to cancer, giving rise to tumors such as the myeloproliferative disorders (e.g. CML and polycythemia vera) [6,84]. Normally, cells are at highest risk of acquiring mutations during DNA replication. As already discussed, the rate of replication of the HSC decreases with the mass of a given species as $B_e = B_0 M^{-1/4}$ [69]. This implies that in larger mammals, the HSC divide more slowly and should acquire mutations due to replicative errors at a slower rate. On the other hand, the size of the active stem cell pool increases with mass ($N_{SC} \sim M^{3/4}$) and so the population of cells at risk is higher. It is also clear that a tumor clone is relevant and can cause disease only if it expands during the lifespan of the mammal. Interestingly, the expected lifetime ($L_E$) of a mammal also scales allometrically (increases) with mass ($L_E = L_0 M^{1/4}, L_0 = 8.6$) [114,115]. Finally, the probability of reaching a given fraction of mutated cells decreases as the size of the active stem cell pool expands. To explore the impact of these potentially conflicting variables on the development of CSC-derived disorders, we performed simulations of HSC dynamics taking these contrasting variables into consideration. For the purpose of our analysis, any species is defined by the average adult mass characteristic of that species and we estimated $N_{SC}$, $L_E$ and $B_e$ with the above definitions. The size of the species-specific active stem cell pool was assumed to be constant during the lifetime of a given mammal. We also considered that the mutation rate, $(10^{-7} \leq \mu \leq 10^{-5})$ is constant
across all mammals [77,89] given the similarity of the DNA replication machinery across eukaryotes and back mutations do not occur (very low probability event). A contamination threshold of 20% was assumed to be necessary to define disease in all species.

We illustrate some of the results in Figure 5, where we plot the probability of disease during the mammal’s lifetime as a function of both mass and fitness advantage of the mutant cell. The data suggest that larger mammals are better suited at resisting clonal expansion of mutations that are either neutral or confer only a marginal advantage. However, for mutations with a significant fitness advantage, the larger species are worse off compared to smaller mammals such as rodents. The reason behind these dynamics is that once such a mutation occurs, the probability of diagnosis does not necessarily decrease for larger mammals [115]. On the other hand, for any species, the expected number of mutations \( n_\mu \) within the HSC pool (i.e. the population at risk), is given by:

\[
n_\mu = \mu N_{SC} B_e L_E = n_0 M^{3/4}
\]

which favors smaller mammals [115]. The conclusion from all this is that the rate limiting or decisive step is the occurrence of the first mutation. As a consequence, larger mammals, with their larger active stem cell pool are protected from the expansion of neutral mutations but not from advantageous mutations. The results of these simulations are robust and remain true with respect to variations of the mutation rate and of the fraction of mutated cells necessary to cause disease within the HSC pool [115].

These in silico results are supported by clinical observations on humans and other animals. For example, a search of the mouse tumor database (http://www.nih.gov/science/models/mouse/resources/mtbdb.html) shows that the
spontaneous development of a myeloproliferative disorder (CMPD) has not been reported in this mammal. The incidence of CMPD is uncommon in dogs that share our environment and presumably are exposed to the same levels of background radiation as humans [116] (the only known external risk factor for the development of these diseases). Moreover, CMPD are quite uncommon in humans younger than 20 years of age (<10% of cases of CML occur in such people), again compatible with our model [90]. An unavoidable conclusion of our modeling is that the human species is a victim of its own success: while the average human lifespan provides the largest deviation from the predicted allometric lifespan (much longer than predicted), this comes at the price of higher risk of CMPD. Our modeling also naturally explains why the incidence of cancer tends to increase with longevity.

**Inherited and acquired hematopoietic stem cell disorders**

Given the importance of HSC, it is understandable that both genetic and acquired disorders that affect them are rare. However, inherited HSC disorders have provided considerable insights into the fundamental biology of a variety of basic cellular events (e.g. telomere maintenance and ribosomal biogenesis in the various forms of dyskeratosis congenita, and adenosine deaminase (ADA) or the common \( \gamma \) chain (\( \gamma_c \)) deficiency, characteristic of the most common subtypes of severe combined immune deficiency [117]. These inherited disorders generally manifest themselves in one of two ways: a marrow failure syndrome (single or multiple lineages) that may or may not be associated with an elevated risk of neoplasia or a profound immune deficiency. In general, these disorders can be cured by bone marrow transplantation if a suitable donor is available. Prototypic examples include dyskeratosis congenita (DC) which presents as an inherited
syndrome with bone marrow failure as the most important feature that threatens the life of the individual, while ADA or γc deficiency exhibit profound immunological defects and high susceptibility to infections. Acquired HSC disorders can also lead to bone marrow failure (aplastic anemia), neoplastic or ‘benign’ proliferation of mutant cells (e.g. chronic myeloid leukemia, CML and paroxysmal nocturnal hemoglobinuria, PNH). A partial list of inherited and acquired HSC disorders is presented in Table 1.

**Acquired hematopoietic stem cells disorders with CML and PNH as prototypes**

Chronic myeloid leukemia (CML) has been a paradigm setting neoplastic disorder for many reasons. It was the first tumor associated with a specific chromosomal abnormality (the Philadelphia chromosome (Ph), t(9,22)(p34;q11)) that subsequently was shown to bring the abl kinase proto-oncogene into the breakpoint cluster region (bcr) on chromosome 22, with the formation of a fusion product (bcr-abl) that leads to the uncontrolled activity of the abl kinase and therefore an oncoprotein. Expression of bcr-abl in HSC is associated with a phenotype compatible with the chronic phase of CML. Thus, it is thought that this single mutation may be enough to explain the chronic phase of the disease. However, in time, the mutant clone acquires additional mutations that induce progression to the accelerated phase and finally to the terminal blast crisis. CML is a true HSC disorder since the bcr-abl fusion gene is present in all types of blood cells including a small fraction of T and NK cells [118].

The identification of the abl kinase enabled the discovery of relatively specific inhibitors for this enzyme, initially with imatinib and now with more novel agents such as dasatinib and nilotinib that all bind reversibly with their target. This was another first in cancer therapy – the availability of a small molecule, inhibitor of a crucial pathway.
within cancer cells. Therapy with imatinib has changed the way CML is now treated and the majority of patients achieve excellent responses that can last many years. It is unusual for the blood counts not to return to normal (hematologic response) and many patients lose the signal of the Ph chromosome on marrow cytogenetics. A significant fraction of patients become negative for bcr-abl by quantitative real time polymerase chain reaction (Q-RT-PCR) assay, implying a minimum tumor reduction of 4 orders of magnitude. The first studies were performed in a mixed population of patients: those with recently diagnosed disease and others who had been treated with prior agents such as interferon and hydroxyurea. In these initial cohorts, imatinib failures were observed in time mainly due to the accumulation of mutations that led to drug resistance. Interestingly, the more recently diagnosed and treated patients have experienced fewer problems with resistance, perhaps in part because they were diagnosed at an earlier stage with a smaller tumor burden and so the population of cells at risk of acquired resistance and/or progression is reduced. Imatinib therapy is given continuously but only a fraction of CML cells are responding to the drug (~5%). However, the response kinetics are quite fast (usually within a couple of months). This is due to the mechanism of action of imatinib: while bcr-abl expression gives the CML cells (progenitors and precursors) a fitness advantage due to a higher probability of self-renewal, imatinib therapy reverses this fitness advantage to levels that are inferior to those of normal progenitor cells [119]. The result is that normal progenitors take over hematopoiesis quickly, despite the persistence of CML cells [120]. If therapy is stopped, the persistent progenitors ensure that relapse is rapid, usually within a matter of months [120,121].
One of the major outstanding questions in CML is whether therapy with these agents can cure the disease. There is no clear cut answer to this and tentative answers to this question have been based on a combination of disease biology and mathematical models. Some have suggested that imatinib can cure the disease [122] while others believe that it is unlikely that imatinib or any of the second generation tyrosine kinase inhibitors can eradicate the disease [120,121]. The reason behind the different predictions lies in the details of the models. While Roeder et al [122] that imatinib can kill CML stem cells depending on their cell cycle status, the other models do not make this assumption [120,121]. It has been shown experimentally that the majority of CML stem cells are quiescent and it is very difficult to stimulate them to enter the cell cycle [5]. There is experimental evidence that CML stem cells are independent of bcr-abl for their existence and therefore not sensitive to the drug (Holyoake). Moreover, CML stem cells express membrane pumps that belong to the ATP-binding cassette family that actively pump out imatinib and similar TKIs, providing further protection of the cells from these agents. In addition, the number of CML stem cells was recently estimated to be quite small in many patients [120]. This in itself presents a very steep hurdle for therapy since it is similar to the proverbial needle in a haystack problem. The answer to the question will only be answered in a definite way after extended follow-up of patients currently on the drug, although preliminary results seem to support models which do not require imatinib to kill stem cells [123,124].

Paroxysmal nocturnal hemoglobinuria (PNH) is another acquired genetic disorder of HSC [85]. In this disease, red cells are unusually sensitive to complement attack due to complete or partial deficiency in membrane proteins that protect them from the
membrane attack complex of complement. These proteins include CD55, CD59 and CD46. A mutation in a single gene known as \textit{PIG-A}, (phosphatidylinositol glycan anchor biosynthesis, class A) a subunit of an enzyme required for GPI biosynthesis, leading to a block of N-acetylglcoaminyl phosphatidylinositol (GPI) biosynthesis that is required to anchor all these proteins to the plasma membrane of cells. Many mutations in \textit{PIG-A} have been described that alter its transcription and/or activity [85,125,126]. The most severe cases are associated with a complete deficiency of \textit{PIG-A} with no expression of these membrane proteins. Many patients with PNH have more than one distinct clone of GPI-anchored proteins which means that at least two cells \textit{independently} acquired different mutations in \textit{PIG-A}. The cellular origin of these mutations is important: mutations in the HSC are expected to persist for a long time while mutations in progenitor cells would be expected to be transient [62]. Analysis of this problem has to take into consideration the fact that one clone must initially appear and expand to induce the disease phenotype (normally 20% of the neutrophils must be deficient in GPI-linked proteins for the disease to be clinically significant). While the first clone is expanding, a second independent mutation has to appear to give rise to the second clone. A mathematical analysis of such a scenario shows that it is very unlikely that both mutations occur at the level of the HSC [111]. It is far more likely that one of the mutations occurs in the progenitor pool, giving rise to a smaller clone that also remains detectable for a shorter time interval. This is supported by clinical observation.

The second major issue with PNH is the identification of the mechanism for clonal expansion of \textit{PIG-A} mutated cells. The consensus that has pervaded the longest has been that the mutant cells must have a fitness advantage that allows them to take over
hematopoiesis. This concept was provided with some additional support by the observation that every healthy adult human has small numbers of cells with mutations in PIG-A, perhaps implying that mutations in PIG-A are not enough to explain the disease [127]. Two hypotheses have been proposed that could lead to this scenario: (i) either the cells have an intrinsic advantage perhaps due to a mutation in a second gene or (ii) an immune attack against normal HSC gives the mutant cells an indirect (cell extrinsic) proliferative advantage. Recently, some evidence in support of proposal (i) has been provided by the description of 2 patients with mutations in the HMGA2 gene that is often mutated in benign tumors [128]. It was proposed that aberrant expression of this gene will provide the necessary proliferative advantage to the mutant cells. However, dynamic considerations suggest that this is unlikely to explain clonal expansion in the majority of patients with this disease since this scenario requires that two independent mutations occur in the same cell: one to mutate PIG-A and the second to mutate HMGA2. Given that the mutation rate in PNH cells is normal (and therefore very low), it would be very difficult to observe this phenomenon, even more so if more than one clone of mutant cells is present [111,112]. It can be shown that the PIG-A mutated cells present in healthy adults have their origin in progenitor cells that are present in much higher numbers compared to the active HSC pool. This also explains why such clones tend to be transient[127]. There is indirect evidence in support of an immune attack in PNH, presumably directed against the normal HSC (REFS). However, the disease is not treatable with immunosuppressive therapy, implying that the immune attack hypothesis may not explain clonal expansion either. Hence, to date, a good explanation for clonal expansion in PNH is not available. However, we hope that with these two examples, we
have illustrated to the reader the importance of understanding the dynamic behavior of cells in order to make sense of data and generate plausible hypotheses based on mathematical models: Only a quantitative description of the dynamics of hematopoietic cells will allow us to determine what is possible or not in hematopoiesis.

**Future developments**

(i) Hematopoietic stem cell plasticity

The relative ease with which HSC can be harvested, fueled by the expectation of in vitro HSC expansion have raised hopes that HSC may be able to give rise to cells that belong to other tissues which may or may not be derived from other germ layers (e.g. epithelial cells, neurons, cardiomyocytes, hepatocytes etc). This process has been described as cell ‘plasticity’ or ‘transdifferentiation’ and considered a violation of one of the central dogmas of developmental biology [129]. There is evidence in nature that such a phenomenon can occur. The example that is most often cited in support of transdifferentiation is the formation of Barrett’s esophagus where in response to chronic acid exposure, the lower esophageal epithelium changes from squamous cells to columnar cells, similar to those that line the stomach. However, we hasten to note that this is an example of an epithelial cell changing to a different type of epithelial cell and there is no crossing of germ layers. Some investigators have reported that HSC can give rise to neural cells, hepatocytes, cardiomyocytes and others although skepticism remains. Some leaders in the field of HSC biology are quite pessimistic that HSC can behave in this fashion in a meaningful way, namely that significant number of HSC can differentiate in a specific tissue to regenerate it after injury [130,131]. It is possible that some of the confusion in the field could be due to semantics and in an attempt to rectify
this problem, clear definitions have been proposed [132]. One of the major issues is how should a transdifferentiated cell be defined. Is it enough for the cell to have the shape of surrounding cells, express one or a few tissue specific markers or perform some function(s) typical of the target tissue? Another important question has been whether the number of cells that exhibit plasticity should be taken into consideration: is one cell among millions enough to prove the point? Proponents of HSC plasticity argue that in such a case, it is the exception that proves the rule, while opponents claim that such a rare event may not have any biological relevance. However, recent rigorous experiments suggest that the promise of HSC transdifferentiation is limited and most of the (rare) instances where this was observed were due to fusion of the HSC with the surrounding cells of the injured organ. Perhaps it is the exchange of DNA that leads to change in cell fate rather that intrinsic plasticity of the cells.

(ii) Stem cell engineering

Hematopoietic stem cells are the most accessible stem cell pool available. Hence they are attractive targets for gene therapy approaches to potentially cure many inherited disorders (see Table 1). Recent advances in vector design, especially the introduction of retroviral and lentiviral vectors that can integrate in the genome of the target cell seem to hold great promise. One of the success stories in stem cell engineering has been the expression of the \( \gamma_c \) gene in HSC from patients with X-SCID. This vector transduced \(~40\%\) of the hematopoietic cells (with activation) and corrected the defect in a small fraction of cells that expanded in time [71,133]. Importantly, therapy was associated with an improvement in the lymphocyte profile in 9 out of 10 treated patients with sustained gene expression for over 3 years in some of the children. Unfortunately, 2 of the 10
children treated have developed T cell leukemia due to aberrant expression of the \textit{LMO2} gene \cite{134}, that is known to be associated with de novo acute leukemia in childhood. Subsequent work showed that the vector had integrated upstream of exon 1 of the gene in one patient and within the first intron in the second patient but in either case the gene was aberrantly expressed. This highlights the potential risk of insertional mutagenesis and the need to better understand the ‘selection’ of insertion sites by these viruses and perhaps more importantly improved vector design to limit the possibility of such events. In the vector used (MFG), gene expression was under the control of the viral long terminal repeat that must have an enhancer element which led to the high level expression of the proto-oncogene \cite{134}. Perhaps the use of transcriptionally targeted vectors will serve as a further check against the problem of insertional mutagenesis although this problem cannot be eliminated completely. This approach requires though the identification of critical gene regulatory elements where tissue specific transcription factors bind to enable the recruitment of the transcriptional machinery. To date, very few such regulatory elements have been identified, including those for the globin genes and immunoglobulins. In addition, for even higher safety, gene silencers may be included to restrict the promoter activity residing in the vector to that transgene of interest. Again the best example of a tissue specific gene silencer is that for the globin cluster. Recent work has shown the feasibility of using critical elements of these sequences to achieve tissue specific and regulated (balanced) gene expression for the correction of thalassemia syndromes in animal models \cite{135,136}. The incorporation of such elements in vectors is limited by the size restriction of the genomes that can be efficiently packaged into the viral capsid.
(iii) Ex vivo stem cell expansion

Expansion of HSC in vitro has been one of the holy grails of stem cell transplantation since it would open many possibilities for the manipulation of these cells (such as gene correction) and also expand the utility of cord blood derived HSC. However, to date, expansion of HSC has been next to impossible. The task at hand is not simple since what is required are the necessary conditions that allow the HSC to expand and retain both self-renewal and differentiation ability. One of the reasons behind the reported experimental failures has been the choice of cytokines and growth factors used to achieve this aim [137] but perhaps even more important are the provision of a more realistic stem cell niche environment. Recently, the generation of pluripotent stem cells from adult human fibroblasts by expression of Oct3/4, Sox2, Klf4 and c-Myc has been described [138]. We believe that it is only a question of time before the detailed molecular mechanisms behind HSC self-renewal and expansion are defined, allowing efficient expansion of this important pool of cells in vitro.

In conclusion, the biology of HSC and their dynamic behavior are being deciphered at a fast pace. A complete understanding of HSC behavior requires both a detailed molecular description as well as a consideration of the dynamic behavior of these cells. Conceptually, HSC behavior can be rationalized using allometric principles together with stochastic dynamics. The small size of the active HSC pool requires a probabilistic description of these cells and stochastic considerations can have a major impact on the dynamics of this cellular compartment. Dynamics also help us understand clinically relevant observations pertaining to various neoplastic and acquired genetic
disorders of hematopoietic stem cells. In principle, these considerations should apply to other (non-hematopoietic) stem cell compartments as well.

Acknowledgments

Financial support from Mayo Foundation (DD) and FCT-Portugal (JMP) is gratefully acknowledged.
Figure legends

Figure 1. Hematopoietic stem cells and multipotent progenitor cells. The immunophenotype of HSC and MPP is becoming increasingly defined. The two terms are often used interchangeably but they refer to distinct cell types. Only the HSC have the ability for long term reconstitution of hematopoiesis. There is increasing evidence that HSC are CD34 negative. Both cell types appear to be uncommitted to any lineage, hence the designation Lin−. This requires that the cells are negative for CD10, 14, 15, 16, 19 and 20.

Figure 2. Hematopoietic stem cell dynamics. (A) The number of active HSC is considered to be fairly constant in time in adults. (B) Whenever a HSC divides, it can mutate to give rise to one normal cell and a mutated cell. Mutated cells cannot revert back to normal (theoretically, this is a very low probability event). (C) Stem cells are chosen for reproduction at random but based on their fitness, r. Mutant cells with a higher fitness (r>1) are chosen more often for reproduction.

Figure 3. Stochastic dynamics within the stem cell pool. IN a Moran process, the total number of cells is kept constant by first selecting a cell for reproduction, with selection being proportional to fitness and then selecting a cell at random for elimination (differentiation). Such a process can lead to 3 outcomes due to the finite lifespan of mammals: the mutant clone can be eliminated (A), it can remain latent and not cause disease (B) or it can expand to generate a burden high enough to cause disease (C). Note that full replacement of the HSC with mutant cells is not required to cause disease.

Figure 4. Hematopoietic stem cell reproduction. The fate of the daughter cells that arise from HSC replication define the symmetry of the reproductive event. Whenever the
daughter cells have the same fate, the division is considered symmetric while when the
two daughter cells have a different fate, it is an asymmetric division. These fates can be
defined by probabilities and mutations can alter these probabilities and the outcome of
mutant HSC dynamics.

**Figure 5.** Stem cell reproduction, longevity and the risk of cancer. The number of active
HSC increases with mammalian mass while the rate at which these cells divide decreases
with mass. However, larger mammals live longer than smaller ones. This aspects are
conflicting but the results of simulations suggest that larger mammals are at higher risk of
acquired HSC disorders compared to smaller mammals. The population of cells at risk
and the longer life-span of larger mammals eliminate the beneficial effect of slower
replication. It appears that the onset of the first mutation is the rate limiting step in
carcinogenesis.
Table 1. Inherited and acquired hematopoietic stem cell disorders

<table>
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<th>Acquired</th>
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PNH: paroxysmal nocturnal hemoglobinuria, CAT: congenital amegakaryocytic thrombocytopenia, TAR: thrombocytopenia with absent radii, CML: chronic myeloid leukemia, SCID: severe combined immune deficiency

* These disorders may or may not be amenable to therapy with stem cell transplantation
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macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N
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patients with chronic phase CML who have achieved a complete cytogenetic
causing partial deficiency of GPI-linked surface proteins (PNH II) in patients with


Figure 1
Normal stem cell

Mutant stem cell

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<td>Mutant</td>
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\[ P_M(k) = \frac{rk}{rk + 1(N - k)} \]
\[ P_N(k) = 1 - P_M(k) \]
Figure 3
Figure 4
Figure 5