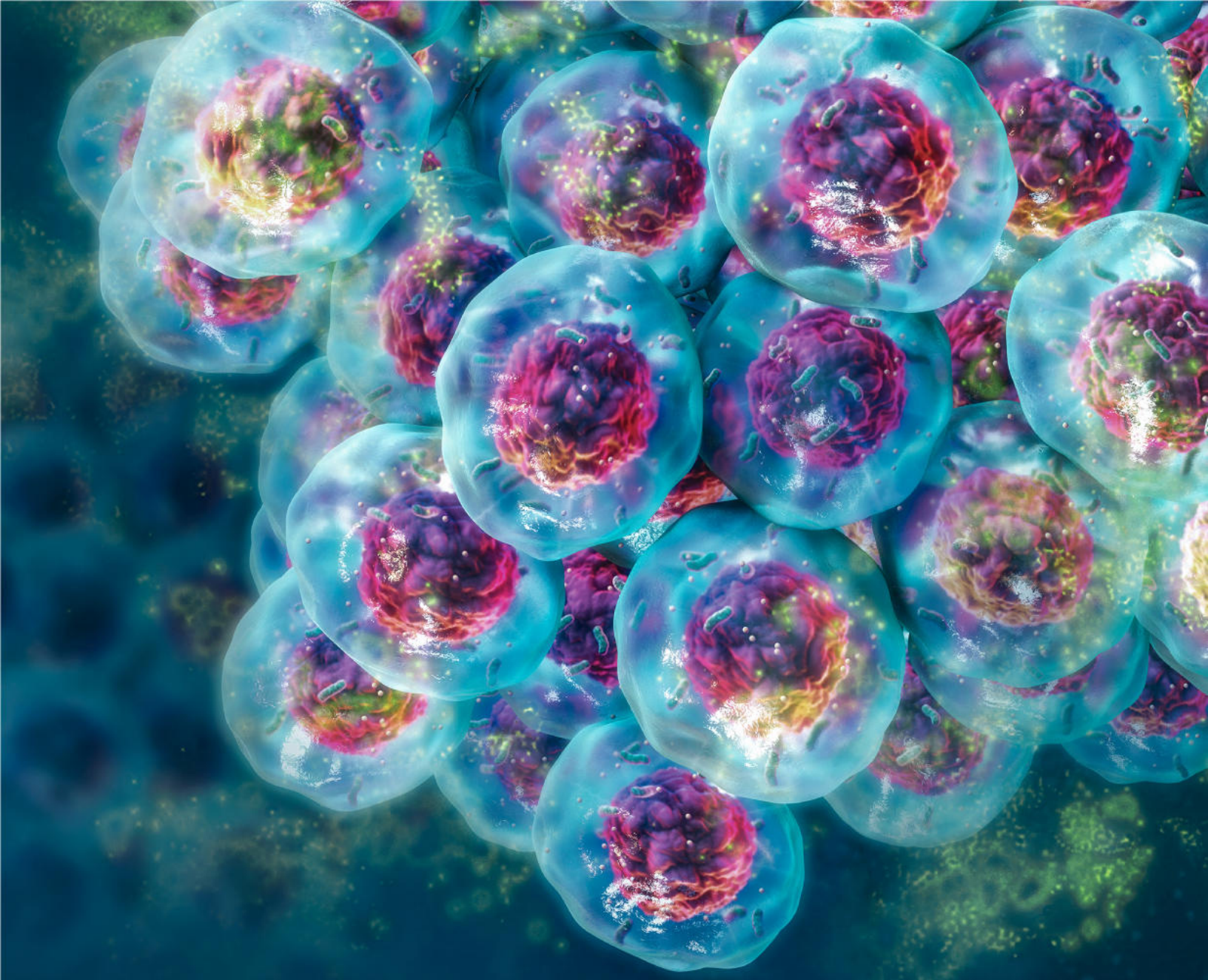

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Ontogenic Growth as the Root of Fundamental Differences Between Childhood and Adult Cancer

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Key Words. Acute lymphocytic leukemia • Acute myelogenous leukemia • Adult haematopoietic stem cells • Cancer stem cells • Hemopoietic stem cells • Self-renewal

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ABSTRACT

Cancer, the unregulated proliferation of cells, can occur at any age and may arise from almost all cell types. However, the incidence and types of cancer differ with age. Some cancers are predominantly observed in children, others are mostly restricted to older ages. Treatment strategies of some cancers are very successful and cure is common in childhood, while treatment of the same cancer type is much more challenging in adults. Here, we develop a stochastic model of stem cell proliferation that considers both tissue development and homeostasis and discuss the disturbance of such a system by mutations. Due to changes in population size, mutant fitness becomes context dependent and consequently the effects of mutations on the stem cell population can vary with age. We discuss different mutant phenotypes and show the age dependency of their expected abundances. Most importantly, fitness of particular mutations can change with age and advantageous mutations can become deleterious or vice versa. This perspective can explain unique properties of childhood disorders, for example, the frequently observed phenomenon of a self-limiting leukemia in newborns with trisomy 21, but also explains other puzzling observations such as the increased risk of leukemia in patients with bone marrow failure or chemotherapy induced myelodysplasia. STEM CELLS *2016;34:543–550*

SIGNIFICANCE STATEMENT

We use a mathematical model to show that the stage of development of a person from a newborn until they reach adult life has a major impact on the behavior of mutations within stem cells that can lead to cancer. We show how at different stages, various mutational patterns can provide a fitness advantage or even disadvantage depending on their context. We illustrate this with specific examples from acute leukemia in children and adults.

INTRODUCTION

Cancer is the result of complex interactions between multiple (epi)genetic mutations that enable cells to escape from the regulatory mechanisms that normally enforce cell cycle control [1]. Although tissues have evolved an architecture that suppresses the accumulation of multiple mutations, their accumulation is eventually an unavoidable consequence of cell replication which is necessary to maintain tissue homeostasis (due to the limited lifespan of most cells) and repair from injury [2]. Consequently, the risk of cancer increases with age [3]. The actual number of cancer initiating mutations varies between tissues from a few in most types of leukemia to perhaps up to 20 or 30 in some solid tumors [4]. The screening of tumors for the presence of "driver" oncogenes in a clinical setup is becoming increasingly feasible and in some cases treatment strategies can be personalized based on this approach [5]. However, individualized identification of driver mutations remains a challenging issue, in part due to the substantial genetic diversity in tumors found between and within patients [6, 7]. Most mutational hits are phenotypically silent and perhaps disturb very little if at all normal tissue homeostasis [8, 9]. Such mutations can lead to clonal expansion or they can lead to lineage extinction and thus increase or decrease the risk of additional mutations [10, 11].

Usually, cancer development is described on the background of healthy tissue homeostasis with continuous cell turnover under a steady state, for example, an approximately constant number of active tissue specific stem cells [12–14]. Although this may be reasonable in adults, it likely neglects important aspects of tissue homeostasis during childhood and adolescence, when tissues are growing and require different rates of cell replenishment at

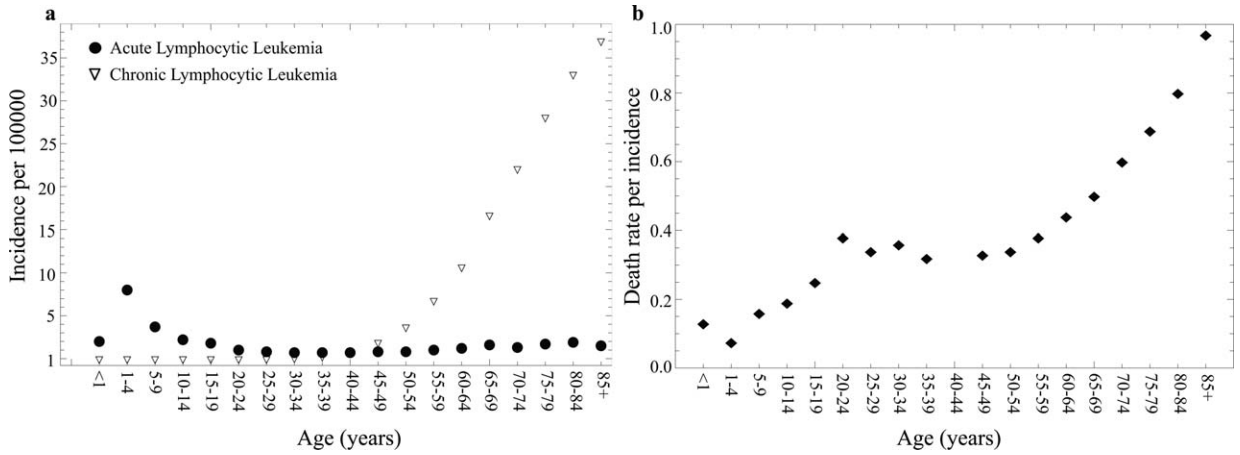

Figure 1. Incidence and death rates of leukemia with age. **(Left)**: Incidence of acute lymphocytic leukemia (ALL, black dots) and chronic lymphocytic leukemia (CLL, black triangle). ALL usually is driven by a hyper-proliferating clone and often requires immediate treatment at diagnosis. CLL develops slower and often presents with a high clonal load in the bone marrow. Such chronic leukemias occur in older persons, while ALL is frequent in children, very rare in middle-aged adults and increases in incidence in older persons (<65). **(Right)**: Death rate per leukemia incidence (pooled data of acute and chronic lymphocyte as well as acute and chronic myeloid leukemia). The risk of death for children with a diagnosis of leukemia is comparably low (7.5% at age 1-4 years), plateaus in middle-aged adults ($\sim$35% between 20 and 55) and increases significantly in older persons (<55). The data was taken from [21].

different ages to accommodate this natural phenomenon. Allometric scaling arguments suggest that there should be different stem cell pool sizes as a function of age, but also between species [12, 15]. Age, also leaves its mark by being associated with differences in stem cell proliferation—a phenomenon that is observed both in mice [16] and in humans, for example, by accelerated telomere shortening in hematopoietic cells during childhood through adolescence as a consequence of stem cell pool expansion [17, 18].

An expansion of the stem cell population immediately has implications for the impact of oncogenic mutations. Selection pressure and mutant cell fitness would be expected to vary at different developmental stages within a tissue, since the impact of stochastic effects would depend on the size of the population, being more pronounced in unregulated populations of smaller size. The effect of a mutation naturally becomes context dependent in such a population of changing size. This also implies that there may be differences not only on the risk of the development but also on the progression of specific cancers at different developmental stages. Indeed, incidence curves of some cancers, for example, acute lymphoblastic leukemia (ALL) in children, do not follow the trend that multistage theory would predict [19]. Furthermore, treatment outcome for some cancers are superior in children compared with adults and actual cure is frequent (up to 90% probability of cure in ALL in children), however, this is also critically dependent on the age at diagnosis [20] (Fig. 1). Finally, some childhood disorders present with unique properties with the most striking example being transient leukemia or transient myeloproliferative neoplasm (TL), a disorder that may be present in up to 30% of children born with trisomy 21.

In most cases, TL resolves without specific therapy, but in a few cases, it progresses into acute megakaryoblastic leukemia (AMKL), sometimes years after the myeloproliferative clone had become undetectable.

In the following, we propose a minimal model of a stochastic system of cell dynamics under physiologic conditions (homeostasis) that takes into account the role of expansion of the stem cell pool during childhood and adolescence, until a state of quasi-constant population size is reached in adult life. We then discuss the dynamics of various types of mutants with different phenotypes and show how the same mutation can affect tissue homeostasis differently depending on the stage of development (or growth). This framework offers potential explanations for numerous clinical observations including the phenomenon of transient leukemia in newborns with trisomy 21. The model also suggests that there may be surprising similarities between some childhood disorders and bone marrow failure conditions in adults.

Materials and Methods

We propose a minimal model of stochastic stem cell dynamics that combines an expansion phase of the stem cell pool during development and a quasi-constant stem cell population in adulthood. We discuss basic properties of healthy homeostasis and investigate the disturbance of such a system by mutants. The dynamics of stem cells in a tissue is fully described by three different types of stem cell divisions: (a) symmetric self-renewal (resulting in two stem cells), (b) asymmetric self-renewal (resulting in a stem cell and a progenitor cell), (c) symmetric differentiation (resulting in two progenitor cells) (Fig. 2). In our model, at any given time, we pick a stem cell at random to proliferate. This stem cell undergoes one of the three possible patterns of divisions (1)–(3) with probabilities p_r (symmetric self renewal), p_a (asymmetric self renewal), and p_d (symmetric differentiation), respectively. As the outcome of a stem cell proliferation is random with respect to these probabilities, the number of stem cells can change.

The stem cell population size remains approximately constant, if $p_r = p_d$, increases if $p_r > p_d$ and decreases if $p_r < p_d$. For an expanding stem cell population that transitions into a state of quasi constant population size (adult life), the ranking of these probabilities must vary with the number of stem cells: for example, stem cells have an increased probability of symmetric self-renewal when the stem cell population is small

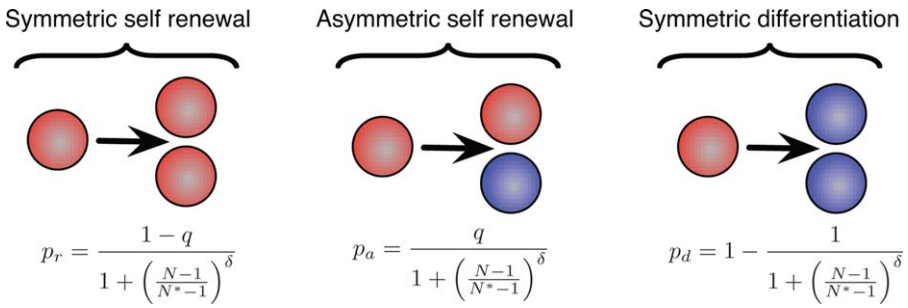

Figure 2. Schematic representation of stem cell division patterns and corresponding probabilities. Stem cell proliferation results in two additional stem cells with probability p_r (symmetric self renewal), one stem cell and one progenitor cell with probability p_a (asymmetric self renewal), or differentiates into two progenitor cells with probability p_d (symmetric differentiation). We implement a feedback of the stem cell population size N on these probabilities to allow an initial growth of the stem cell pool towards an equilibrium that is reached when $p_r=p_d$, see also Fig. 3. If $q=0$ (no asymmetric divisions), the population fluctuates around N^*. More general, for any value of q, the equilibrium stem cell population size is $\sqrt[\delta]{1-q}(N-1)+1$. As our parameterization implies $p_d=0$ for $N=1$, the extinction probability of the undisturbed stem cell population is 0.

and growth is necessary. We can implement this in our stochastic model by a feedback from the stem cell population size N on the probability of symmetric self-renewal p_r to ensure a surplus of symmetric self-renewals compared with symmetric differentiations for small populations and vice versa for large populations. It seems quite likely that during cycles of chemotherapy, such changes in the stem cell pool population size also occur with therapy induced cell death followed by recovery due to an increase in symmetric self-renewal (see below). We adapt the framework of enzyme-kinetics and closely follow the idea of Michaelis-Menten kinetics [22] which is often used in similar context to introduce feedback and regulation on physiologic processes since many processes in physiology are regulated by negative feedback. Therefore, we assume for the three probabilities

$$p_r(N)=\frac{1-q}{1+((N-1)/(N^*-1))^\delta} \quad (1)$$

$$p_a(N)=\frac{q}{1+((N-1)/(N^*-1))^\delta} \quad (2)$$

$$p_d(N)=1-p_r(N)-p_a(N) \quad (3)$$

Here, N is the actual size of the stem cell pool and N^* represents the equilibrium stem cell population size for $q=0$, whereas the general case is given by the solution of $p_r=p_d$. The parameter δ represents the strength of the feedback signal on stem cell replication. If $\delta\to 0$, the probability of self-renewal and the probability of differentiation are independent of N and the population size follows a neutral random walk (stem cell divisions are completely random). If $\delta\to\infty$ these probabilities become step functions, where $p=1$ if $N<N^*$ and $p_r=0$ if $N>N^*$. In this limit, the population size would always be either N^*-1, N^*, or N^*+1 at equilibrium (stem cell divisions are deterministic). Thus, a small δ implies a broad stem cell population size distribution around N^*, whereas a large δ leads to narrow distributions. We expect an intermediate value of δ in realistic scenarios. As we have $p_d=0$ for $N=1$ and any $\delta>0$, the extinction probability of the undisturbed stem cell population is zero in the presence of feedback. In other words, our implementation avoids the extinction of a healthy undisturbed stem cell population by chance.

In addition, we introduce a probability q to account for the occurrence of asymmetric stem cell divisions. Asymmetric divisions do not change the size of the stem cell population. Thus, a perfectly asymmetrically dividing stem cell population would maintain its size and cannot be invaded by a mutant that is also restricted to asymmetric division. However, development and growth require the capacity of symmetric stem cell self-renewal. Asymmetric divisions allow us to scale time and adjust the required expansion of the stem cell pool accordingly, while at the same time ensuring continued output of differentiated cells.

Model Parameterization

This model can be applied to tissues in the body that are characterized by a hierarchy of cells that undergo turnover and are maintained by a stem cell population. However, to illustrate important aspects of the model and to compare those to clinical observations, we parameterize the model to the hematopoietic system. Exact information about many properties of human hematopoietic stem cells including the

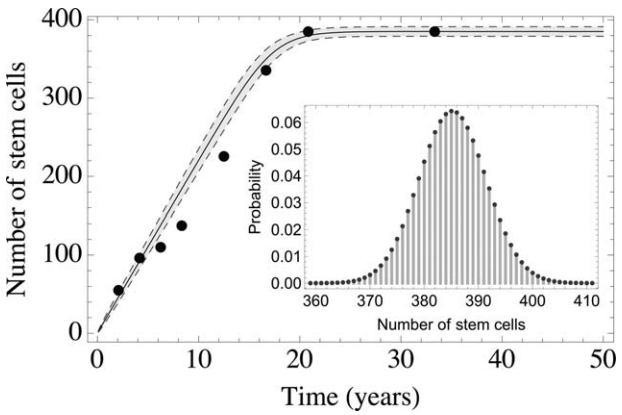

Figure 3. Stem cell pool expansion during adolescence. **(Main panel)**: Average stem cell pool expansion with age (line), corresponding standard deviation (dashed lines) and estimates of the stem cell pool based on allometric scaling (dots) [15]. The parameters of the underlying stochastic process are $r_w=1y-1$, $q=0.95$, $N^*=525$ and $\delta=10$. This resembles predominantly asymmetric stem cell divisions and a stem cell pool expansion due to rare symmetric self-renewal. Symmetric stem cell differentiation only becomes frequent in the equilibrium phase. **(Inner panel)**: Probability distribution of stem cell number at the equilibrium of the model.

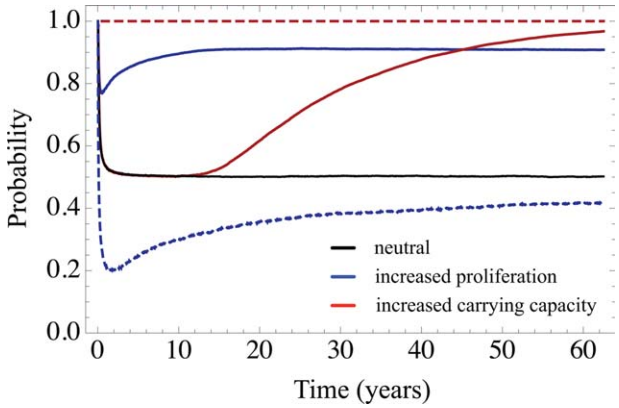

Figure 4. Context dependent mutant fitness. Probability for neutral mutants (black line), mutants with increased proliferation rate $r \rightarrow 1.5$ (blue lines), and mutants with higher carrying capacity $N \rightarrow 500$ (red lines) to constitute at least 50% of the population, starting from 50% mutants at time 0. The probability is recorded either during the growing phase (solid lines), or in equilibrium phase (dashed lines). Mutations that increase proliferation are advantageous in the growth phase, but disadvantageous in equilibrium. In contrast, mutations that increase the carrying capacity are neutral during growth, but advantageous in equilibrium.

number of active hematopoietic stem cells as well as the ratio of symmetric and asymmetric proliferations are not known precisely. However, different experimental as well as *in vivo* observations suggest a proliferation rate of approximately one division per stem cell per year [23]. Furthermore, allometric scaling between species suggests about 400 active hematopoietic stem cells in humans contribute to hematopoiesis and this population size is approximately reached at the age of 18 years [12, 15]. This last observation is also supported by patterns of accelerated nonlinear telomere shortening in hematopoietic cells during childhood and adolescence that transitions into a linear decrease in adults [18]. This pattern can be explained by the predominance of an asymmetrically dividing stem cell population that increases linearly in time due to occasional symmetric self-renewal of some cells [17]. We applied these constrains to our model and fitted it to the growth dynamics inferred via allometric scaling arguments from reticulocyte counts [12]. This leads to the parameter combination $q=0.95$, $\delta=10$, and, $N^*=525$, which accurately reproduces the observations (Fig. 3). However, the following observations and conclusions only require the general property of an initially expanding stem cell pool with a transition into a phase of quasi-constant population size and are independent of the exact parameterization.

Results

Neutral Mutations

The phenotype of a cell is defined by its proliferation parameters, in our model the proliferation rate r_w, the probability of asymmetric cell divisions q_w and the feedback strength δ_w. In the following the subscript w refers to wild type cells while m refers to mutant cells. A neutral mutation, however, is phenotypically silent and by definition, the proliferation parameters remain unchanged. If cell proliferation is regulated by feedback mechanisms, the fate of a mutation depends on the composition (size) of the stem cell pool and therefore the exact time at which it occurred. A neutral mutation that appears early in development has a low extinction probability and can reach high abundance despite no fitness advantage. The same mutation has a much higher extinction probability and is unlikely to grow enough to compose a significant fraction of the population during the equilibrium phase, when the cell population size has reached the maximum expected for an adult (e.g., *PIG-A* mutant cells leading to the phenotype of paroxysmal nocturnal hemoglobinuria) [2, 24–26] (Fig. 4). Thus the expansion phase is not only important for development, but also influences the standing clonal diversity in adulthood significantly and sets the stage for potential subsequent mutations (Fig. 5). We also note that these observations also provide an explanation for (a) the high frequency of *PIG-A* mutant cells in patients with hypoplastic/aplastic anemia and hypoplastic myelodysplastic syndromes [26–28] and (b) a mechanism for the perhaps paradoxically increased risk of acute leukemia in patients with aplastic anemia [29].

Context Dependent Mutant Fitness

The fate of a neutral mutation depends on the composition (size) of the stem cell population (Fig. 4). The same holds for non-neutral mutations. Depending on the exact phenotypic change, a mutation might be advantageous at one developmental stage and neutral or even disadvantageous during another stage of development. Our model captures three important changes in cell behavior. These are either changes in cell proliferation rate (corresponding to a change in r), feedback strength (corresponding to a change in δ), or proliferation patterns, for example, the ratio of symmetric and asymmetric cell divisions (corresponding to changes in N^* or q). Changes in proliferation rate r or designated stem cell pool size N^* have contrary effects. A mutation that increases the rate of proliferation has a fitness advantage early in life and is neutral during equilibrium (adulthood). During the stage when the stem cell pool is expanding, the probability of

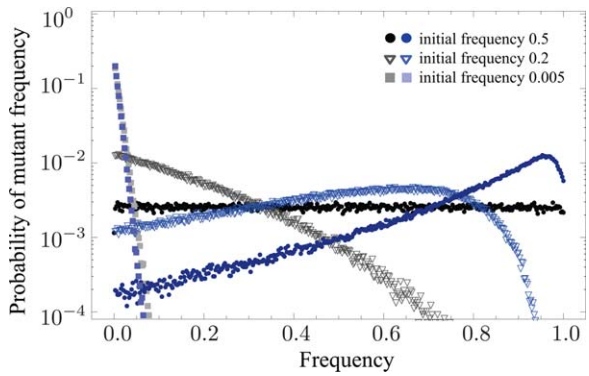

Figure 5. Stem cell pool composition in homeostasis. The probability of either a neutral mutant (grey) or a mutant with increased proliferation rate $r_m=1.5$ (blue) to constitute a certain frequency of the stem cell pool in homeostasis, if the mutant occurred early (initial frequency of 0.5, dots), at an intermediate (initial frequency 0.2, triangles), or late (initial frequency 0.005, squares) during the expansion of the stem cell pool. An early neutral mutation (black dots) undergoes a random walk and consequently constitutes any fraction of the stem cell pool with the same probability. An increased proliferation rate is a significant fitness advantage early (dark blue dots), but vanishes for mutants in late stages of development (light blue squares).

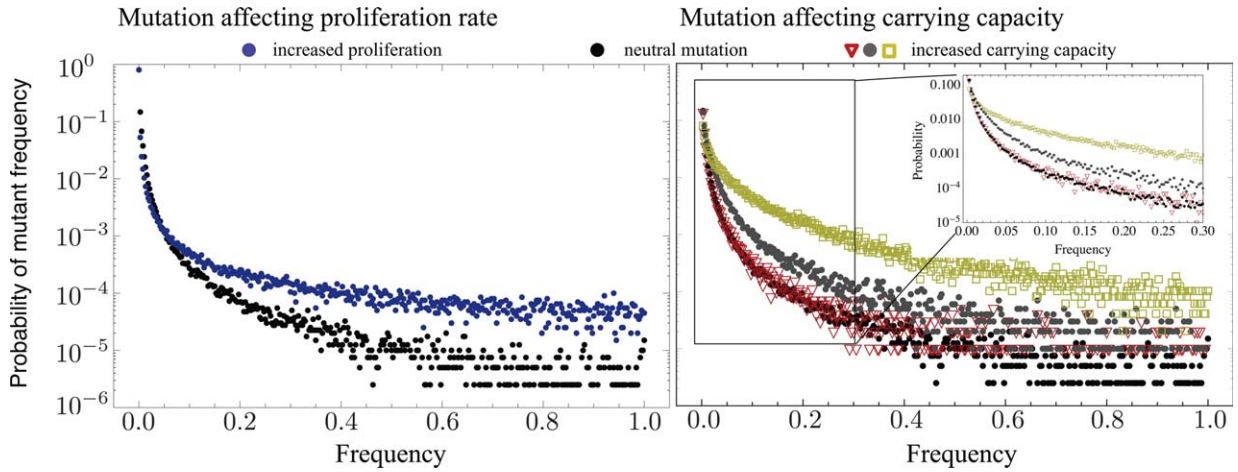

Figure 6. Expected fraction of mutants with different phenotypes in homeostasis. Left: The probability of a neutral mutation (black dots) or a mutation with increased proliferation rate $r_m \rightarrow 2$ (blue dots) to constitute a certain fraction of the stem cell pool in homeostasis, if mutants randomly occur at any time during adolescence. Right: Here the mutation increases the carrying capacity $N \rightarrow 725$ (effectively the transition probabilities are skewed to symmetric self renewal in homeostasis). The relative contribution of such a mutation to the stem cell composition depends on a person's age. The distribution follows a neutral mutation early in homeostasis (20 years, red traingels), becomes more frequent in intermediate ages (40 years, grey dots) and is likely to constitute a considerable fraction of the stem cell pool late in homeostasis (60 years, yellow squares). At different ages, mutants with different phenotypic properties are expected, potentially explaining differences in treatment outcome at young and old ages.

symmetric self-renewal is increased and thus a higher proliferation rate also increases the probability for that mutant clone to expand. An example of this scenario is ALL in early childhood that is characterized by rearrangement of the mixed lineage leukemia (MLL) gene. This mutation may be enough to drive this highly malignant disease that arises in utero and likely increases the rate of proliferation of mutant cells so that they take over hematopoiesis and leading to the highly lethal phenotype characteristic of this disease [30–32]. This advantage is lost in the case of a constant population (during homeostasis), as the probability of symmetric self-renewal and symmetric differentiation is equal. In such a case, the reproductive capacity of a cell with higher proliferation rate equals the reproductive rate of a healthy wild type stem cell [2]. Only cell turnover rates differ, and increased cell proliferation implies faster clonal extinction.

The effect is reversed for a mutation that increases the carrying capacity N^*. Such a mutation would appear neutral during the phase of stem cell pool expansion but gains a fitness advantage in homeostasis (Figs. 5, 6). Thus, the relative frequencies of such mutations (which alter the sensitivity of the cells to feedback regulation) are expected to increase with age and consequently are expected to play a more prominent role in older compared with younger individuals. This also suggests that we expect different phenotypic properties of cancers during life. A mutation that occurs once the HSC pool has reached its standing size in adults only expands to a significant fraction, if it is associated with an increased stem cell self-renewal potential. However, such a mutation might require many years to expand and reach a considerable fraction. One therefore expect cancers in adults to be more complex compared with childhood cancers, not only because stem cells had more time and cell divisions to accumulate mutations but also because the different milieus require more complex phenotypic changes for mutations to lead to the full tumor (e.g., leukemia) phenotype. Recent studies in childhood ALL support our hypothesis since MLL driven leukemia appears to have a considerably smaller number of mutations compared with adult onset acute leukemia [32, 33]. The most common mutation in pediatric ALL is *TEL-AML*1 that also arises in utero [34, 35]. However, studies show that this mutation is not enough to lead to the full malignant phenotype and additional mutagenic events leading to subsequent acute leukemia are required [36]. Our modeling suggests that neutral or near neutral mutations can expand to reach a substantial fraction of the HSC pool if they occur early during development. However, since additional mutations are needed for *TEL-AML1* to lead to disease, this explains why most children who at birth carry this mutation do not go on to develop ALL.

Transient Amplifying Leukemia

So far we only investigated changes in single traits, for example, the proliferation rate or the carrying capacity of a stem cell population. Of course, phenotypic changes in mutant cells can be more complex and potentially multiple cell properties can be influenced by one or a combination of several mutations simultaneously. As an illustrating example, we discuss the unique properties of transient amplifying leukemia, in particular how certain dynamic properties of the disease might be understood in terms of context dependent mutant fitness. Transient leukemia occurs in up to 30% of infants with Down syndrome (also known as trisomy 21). Recent studies show that a mutation in exon 2 of the gene coding for the transcription factor *GATA1* is involved. However, which additional factors on chromosome 21 cooperate with *GATA1* leading to disease is currently unknown [37]. The disease arises in utero and can cause fetal demise, such that the actual incidence may be even higher. The current standard of care is careful observation and in most patients transient amplifying leukemia resolves spontaneously in the first months of life. However, a fraction of patients progress to AMKL usually within the first four years of life that is derived by clonal evolution from the transient leukemia clone [38].

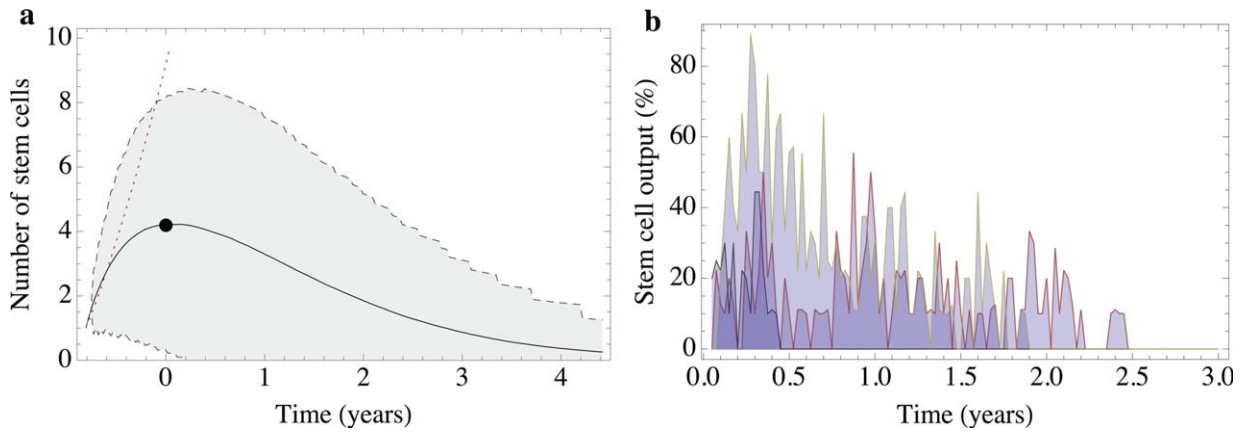

Figure 7. Model scenario for transient amplifying leukemia. **(Left)**: The mutational hit occurs early in embryonic development. The clonal load (black line) reaches its peak on average shortly after birth (black dot). The proliferation rate of the clone is increased and initially, the stem cell number is increased. However, the mutant clone is downregulated by the feedback mechanisms and gets slowly outcompeted once the healthy stem cell population reaches a considerable size. The contribution of the clone becomes negligible after approximately 1 year. However, total extinction of the clonal stem cell population requires about 4 years, and would be associated with a (decreasing) risk to accumulate further mutational hits that might lead to clonal evolution and acute leukemia. However, the variance (dashed line) of the mutant cell size is large. In some cases, the mutant might go extinct before birth; in others it might reach a dangerous abundance just by chance. **(Right)**: Three identical realizations of the same stochastic process (each shaded area of same intensity corresponds to a single realization). Shown is the relative contribution of the mutant stem cell population to haematopoiesis. The relative contribution decreases with age, but individual differences are large.

The existence of such a self-limiting leukemia that occurs with high frequency and exclusively in newborns but never in older adults with Down's syndrome suggests a context dependent mutant fitness. In our framework, such a phenotype might correspond to a mutation, that occurs very early in development (within the first hematopoietic stem cell doublings) and induces an increased proliferation rate $r_m=2r_w$ together with a reduced sensitivity to cell cycle regulating feedback $\delta_m=1/2$. Interestingly, our model derived from first principles makes such a prediction that is compatible with the phenotypic effect of *GATA1* mutations observed by [39]. The clone arises in the fetus and reaches its maximum size shortly after birth. Given the increased proliferation rate of the mutant cells, the clone contributes significantly to hematopoiesis initially and causes an overall expansion of the stem cell compartment (Fig. 7). This general pattern is supported by empirical observations [40, 41]. The reduced feedback strength δ_m increases the probability for symmetric differentiation of the clone early (or effectively reduces the equilibrium clonal stem cell number). Thus such a clone reaches its "intrinsic" carrying capacity where the probability of symmetric self-renewal equals the probability of symmetric differentiation ($p_r=p_d$) for much smaller stem cell pool sizes. Healthy stem cells continue to grow and create a milieu where the clonal stem cell population experiences permanent negative feedback ($p_r<p_d$). This eventually drives the clonal population to extinction and the disease would be self-limiting in the majority of cases. However, the variance of clonal size is high and individual differences can be large (Fig. 7). Therefore a small fraction of patients is at risk to acquire additional mutations that lead to AMKL that requires treatment. Interestingly, the contribution to hematopoiesis of the clone constantly decreases and clonal progeny might be undetectable before extinction. However, this remaining reservoir poses a risk of clonal evolution and can lead to the life threatening condition of AMKL in young children. Furthermore, because of the permanent negative feedback in a large stem cell pool, this particular mutation cannot reach high abundances in adults and thus it is only relevant as a disease in very young children.

Discussion

Currently, a major focus in cancer research relates to the detection and explanation of the vast mutational heterogeneity observed between patients with ostensibly similar tumors and the impact of this diversity on treatment response and evolution of resistance to therapy. Many of these studies make the implicit assumption that the same mutation induces the same phenotypic changes in cell proliferation properties and heterogeneity is a consequence of the underlying stochastic nature of the process. Undoubtedly, the stochasticity of cell proliferation and mutation accumulation is a major driving force of cancer evolution. However, as we have shown, the fitness of mutations can be context dependent and therefore its influence on tissue homeostasis may be different and depends on the age of the patient or the stage of the tumor. Thus, the relative contribution of a particular mutation to the composition of the stem cell pool becomes age dependent. Our conceptual model suggests that it is essential to take into account the developmental background of the tissue to properly define the effects of oncogenic mutations and is supported by recent studies comparing ALL in children versus adults [32, 33]. Theoretically, one would need to access the prevalence of particular mutations in large cohorts of healthy people at different defined age intervals, similar to Figure 6 to fully understand the impact of such mutations in the various contexts in which they can occur. Comparing those to the expected prevalence of neutral mutations could inform about the potentially differential phenotypic effects of particular mutations of interest. Unfortunately, currently this is not feasible due to technical limitations. However, advances in sequencing techniques might allow such studies in the near future.

In the context of our model, one can also understand the devastating effects of defects in sensing DNA damage or the ability to repair DNA as occurs in hereditary defects in BRCA or in xeroderma pigmentosum. Such conditions will not only permit the development of an abnormal number of mutations in cells at an early age but expansion of the stem cell pool in the relevant tissue (skin, breast, ovary, etc.) will facilitate the spread of such mutants that are well on the way to full malignant transformation and hence the high risk of malignancy in such conditions.

The framework of context dependent mutant fitness provides one possible explanation for differences in cancer prevalence and treatment response in children and adults. Early in development, one would expect mutations that increase cell proliferation rate to dominate. In homeostasis, mutations with skewed symmetric self-renewal properties are advantageous. Such mutants would slowly overtake the stem cell pool and out-compete healthy stem cells. Therefore, a larger cancer stem cell pool might drive leukemias in older ages. Classic treatment strategies like chemotherapy have to eradicate more cancer stem cells, imposing a higher risk of relapse or evolution of resistance. It on the other hand also explains childhood specific cancer types, for example, transient amplifying leukemia in newborns with trisomy 21. Particular stem cell phenotypes can invade an expanding stem cell population, but cannot reach high abundances in homeostasis. Thus, a comparison of disease progression in children and adults could provide additional insights, not only on disease development, but also on age specific organizational principles in stem cell driven tissues.

In addition to ALL, brain tumors are the second most common malignancy in children while they are relatively rare in adults. During early childhood there is significant growth experienced by the brain and our dynamic model can explain how mutations that occur early in development can expand either to reach fixation or to high enough levels to enable the sequential accumulation of additional mutations leading to disease in children that are otherwise rare in adults. We also note that TL is not the only neoplasm that undergoes spontaneous resolution in children—rarely, pediatric neuroblastoma also undergoes spontaneous cure, for reasons that remain unclear. It is possible that the dynamics of neuroblastoma in some children are similar to what we describe here for TL although this phenomenon is much less frequent than TL in children with trisomy 21. However, our modelling likely does not apply for malignancies that do not seem to be driven by malignant stem cells such as malignant melanoma or B cell ALL.

Our theoretical arguments follow closely what has been observed in empirical studies of TL and other hematopoietic stem cell disorders, such as aplastic anemia and PNH. They are also in line with the risk of leukemia after potentially curative (but mutagenic) chemotherapy. In TL, the median time to clearance of the circulating blasts is 58 days (range: 2–194 days) [42]. The risk of progression to AMKL is in the range of 16% [43] and the risk appears to decrease with age. Interestingly, the only parameter that seems to correlate with risk of progression to AMKL was the time for clearance of the circulating blasts. If this occurs quickly (a time shorter than the median of 58 days), the risk of AMKL progression was low, but if the blasts remained for longer, the risk was substantially higher. The median time to development of leukemia in various studies ranged from 9 to 38 months [42, 43]. Our results (Fig. 7A) show that the number of mutant HSC reaches a peak at birth and starts decreasing soon afterwards. By 36 months after birth, the number of mutant HSC is below 1 (extinct) in a substantial number of patients, explaining why AMKL develops relatively early in life in these patients. In addition, Figure 7B also illustrates the intrinsic stochasticity involved in the process that may in itself explain the variability in the duration of TL and the risk of progression to AMKL observed in this disorder.

It has also to be pointed out that TL has been rarely observed in children in the absence of phenotypic Down syndrome. In most of these cases, the child either was a mosaic for trisomy 21 or had trisomy 21 restricted to the blast cell population. However, more recently, infants with TL have been identified who have been studied at a molecular level and confirmed not to have Down syndrome. One child had a missense mutation in the initiation codon for *GATA1* that substituted valine for methionine that prevented translation of the protein [44]. We are also aware of two other patients with TL without mutations in *GATA1*. Although the causative mutation was not identified in one of these patients [45], the last case was associated with a germ line mutation in *BRAF* [46]. The importance of context (size) and the hematopoietic stem cell pool as depicted in our model, is also illustrated by the cumulative incidence of acute leukemia in children born with Fanconi anemia (FA), a rare form of inherited marrow failure syndrome. Children with FA have a 700-fold increased risk of developing AML compared with age matched controls [47]. However, the risk is not uniform in time—leukemic risk increases with age but then reaches a plateau by the age of 20 years [47] and reflects our predicted expansion of the hematopoietic stem cell pool during ontogeny and growth to adult life (Fig. 3)

These observations also shed light on the important clinical problem related to chemotherapy induced myelodysplastic syndromes and acute myeloid leukemia. Chemotherapy is not only mutagenic, but also stimulates stem cell replication while possibly reducing feedback control of the "static" HSC pool due to a reduction in HSC number. These two concomitant features may increase the probability that a mutant that arises grows and acquires a fitness advantage due to the smaller population of HSC present at that time. Similar reasoning could explain the rather paradoxical increase in risk of acute leukemia in patients with aplastic anemia. Here, presumably the loss of feedback due to the reduced number of HSC contributing to hematopoiesis, together with cytokine mediated stimulation for faster HSC replication can create an environment where any mutation that arises stochastically can expand leading to leukemic transformation. Our modeling therefore provides an interesting dynamic link between hematopoiesis in utero, through infancy and childhood as well as older individuals who develop cytopenias due to bone marrow failure for a variety of conditions. Similar arguments arising from tissue growth can explain the relatively high incidence of brain tumors in children compared with adults, and the high risk of malignancy in patients who harbor defects in DNA damage sensing or repair pathways.

Conclusion

We show that the fitness effect of a mutation can change at different developmental stages of a human. This

explains age specific patterns of childhood and adult cancers, as well as surprising phenomena like transient leukemias.

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Author Contributions

All authors conceived and analyzed the model. All authors wrote the manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
2 Werner B, Dingli D, Traulsen A. A deterministic model for the occurrence and dynamics of multiple mutations in hierarchically organized tissues. J Royal Soc Interf 2013;10:20130349.
3 P Armitage RD. The age distribution of cancer and a multi-stage theory of carcinogenesis. Br J Cancer 1954;8:1–12.
4 Alexandrov LB, Nik-Zainal S, Wedge DC et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–421.
5 Chin L, Andersen JN, Futreal PA. Cancer genomics: from discovery science to personalized medicine. Nat Med 2011;17:297–303.
6 Gerlinger M, Rowan AJ, Horswell S et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. New Engl J Med 2012;366:883–892.
7 Sottoriva A, Spiteri I, Piccirillo SGM et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci USA 2013;110:4009–4014.
8 Greenman C, Stephens P, Smith R et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153–158.
9 Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature 2009;458:719–724.
10 Werner B, Dingli D, Lenaerts T et al. Dynamics of mutant cells in hierarchical organized tissues. PLoS Comput Biol 2011;7:e1002290.
11 Bauer B, Siebert R, Traulsen A. Cancer initiation with epistatic interactions between driver and passenger mutations. J Theor Biol 2014;358:52–60.
12 Dingli D, Pacheco JM. Allometric scaling of the active hematopoietic stem cell pool across mammals. PLoS ONE 2006;1:e2.
13 Roeder I, Horn M, Glauche I et al. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. Nat Med 2006;12:1181–1184.
14 Tomasetti C, Vogelstein B. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 2015;347:78–81.
15 Dingli D, Pacheco JM. Ontogenic growth of the haemopoietic stem cell pool in humans. Proc Royal Soc B 2007;274:2497–2501.
16 Kiel MJ, He S, Ashkenazi R et al. Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. Nature 2007;449:238–242.
17 Rufer N, Brümmendorf TH, Kolvraa S et al. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J Exp Med 1999;190:157–167.
18 Daniali L, Benetos A, Susser E et al. Telomeres shorten at equivalent rates in somatic tissues of adults. Nat Commun 2013;4:1–6.
19 Ward E, DeSantis C, Robbins A et al. Childhood and adolescent cancer statistics. Cancer J Clin 2014;64:83–103.
20 Kersey JH. Fifty years of studies of the biology and therapy of childhood leukemia. Blood 1997;90:4243–4251.
21 Krapcho M, Neyman N, Aminou R et al. Seer Cancer Statistics Review, 1975-2011. 2014.
22 English BP, Min W, van Oijen AM et al. Ever-fluctuating single enzyme molecules: Michaelis-Menten equation revisited. Nat Chem Biol 2005;2:87–94.
23 Brümmendorf TH, Bruna A, Dragowska W et al. Asymmetric cell divisions sustain long-term hematopoiesis from single-sorted human fetal liver cells. J Exp Med 1998;188:1117–1124.
24 Traulsen A, Pacheco JM, Dingli D. On the origin of multiple mutant clones in paroxysmal nocturnal hemoglobinuria. Stem Cells 2007;25:3081–3084.
25 Dingli D, Traulsen A, Pacheco JM. Stochastic dynamics of hematopoietic tumor stem cells. Cell Cycle 2007;6:461–466.
26 Dingli D, Luzzatto L, Pacheco JM. Neutral evolution in paroxysmal nocturnal hemoglobinuria. Proc Natl Acad Sci USA 2008;105:18496–18500.
27 Nafa K, Bessler M, Deeg JH et al. New somatic mutation in the PIG-A gene emerges at relapse of paroxysmal nocturnal hemoglobinuria. Blood 1998;92:3422–3427.
28 Araten DJ. The mutation rate in PIG-A is normal in patients with paroxysmal nocturnal hemoglobinuria (PNH). Blood 2006;108:734–736.
29 Socié G, Mary J-Y, de Gramont A et al. Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. Lancet 1996;348:573–577.
30 Biondi A, Cimino G, Pieters R et al. eds. Biological and therapeutic aspects of infant leukemia. Blood 2000;96:1–10.
31 Greaves M. When one mutation is all it takes. Cancer Cell 2015;27:433–434.
32 Andersson AK, Ma J, Wang J et al. The landscape of somatic mutations in infant. Nat Genet 2015;47:330–337.
33 Greaves MF, Maia AT, Wiemels JL et al. Leukemia in twins: lessons in natural history. Blood 2003;102:2321–2333.
34 Wiemels JL, Cazzaniga G, Daniotti M et al. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet Oncol 2015;354:1499–1503.
35 Mori H, Colman SM, Xiao Z et al. Chromosome translocations and covert leukemicclones are generated during normal fetal development. Proc Natl Acad Sci USA 2002;99:8242–8247.
36 Hong D, Gupta R, Ancliff P et al. Initiating and cancer-propagation cells in TEL-AML1-associated childhood leukemia. Science 2008;319:336–339.
37 Wechsler J, Greene M, McDevitt MA et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet 2002;32:148–152.
38 Zuna J, Ford AM, Peham M et al. TEL deletion analysis supports a novel view of relapse in childhood acute lymphoblastic leukemia. Clin Cancer Res 2004;10:148–152.
39 Li Z, Godinho FJ, Klusmann J-H et al. Developmental stage–selective effect of somatically mutated leukemogenic transcription factor GATA1. Nat Genet 2005;37:613–619.
40 MacLean GA, Menne TF, Guo G et al. Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. Proc Natl Acad Sci USA 2012;109:17567–17572.
41 Roy A, Cowan G, Mead AJ et al. Perturbation of fetal liver hematopoietic stem and progenitor cell development by trisomy 21. Proc Natl Acad Sci USA 2012;109:17579–17584.
42 Massey GV, Zipursky A, Chang MN et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. Blood 2006;107:4606–4613.
43 Gamis AS, Alonzo TA, Gerbing RB et al. Natural history of transient myeloproliferative disorder clinically diagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. Blood 2011;118:6752–6759.
44 Bidet A, Dulucq S, Aladjidi N. Transient abnormal myelopoiesis (TAM) in a neonate without Down syndrome. Br J Haematol 2015;168:2–2.
45 Nakashima MO, Shetty S, Chicka M et al. Transient abnormal myelopoiesis of a newborn not associated with chromosome 21 abnormalities or GATA1mutations. Pediatr Blood Cancer 2014;62:353–355.
46 Sekiguchi K, Maeda T, Suenobu S-I et al. A transient myelodysplastic/myeloproliferative neoplasm in a patient with cardio-facio-cutaneous syndrome and a germline BRAF mutation. Am J Med Genet 2013;161:2600–2603.
47 Alter BP, Giri N, Savage SA et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. Br J Haematol 2010;150:179–188.