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MATHEMATICAL MODELS OF STEM CELL BEHAVIOR (M KOHANDEL AND M PRZEDBORSKI, SECTION EDITORS)

Understanding Normal and Pathological Hematopoietic Stem Cell Biology Using Mathematical Modelling

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Abstract

Purpose of Review Hematopoietic stem cells (HSCs) produce all blood cells via a tightly controlled production system. Disruptions to control mechanisms can induce serious disorders, including leukemias. In this review, we provide an overview of how mathematical modelling has contributed to our understanding of normal and pathological HSC biology.

Recent Findings Through the increased availability of a variety of experimental and clinical data, new approaches to mathematically modelling HSCs have revealed how clonality is regulated in the hematopoietic system over time, how increasingly clonal hematopoietic and leukemic stem cell populations contribute to the development of acute myeloid leukemia, and the mechanisms and kinetics of HSC regulation.

Summary Mathematical modelling is a complementary tool to quantitatively explore HSC and hematopoietic regulation. Studies combining experimental, clinical, and theoretical approaches have deepened our understanding of HSC biology and aid future investigations to reveal the mechanisms of HSC maintenance and production.

Keywords Hematopoietic stem cells · Mathematical models · Acute myeloid leukemia · Clonal hematopoiesis

Introduction

HSCs have been extensively studied since their discovery, and mathematical modelling has developed in step. In this review, we explore mathematical and computational models of HSC biology that have shed light on pathophysiological mechanisms and their impact on the development of leukemias and other HSC-related diseases. Readers may also be interested in other recent reviews whose focus is beyond the scope of this article [1–3].

The organization of this review is as follows. We first briefly survey the development of the G_0 model for the cell cycle and then turn to a discussion of stem cell involvement in acute myeloid leukemia, discussing both deterministic and stochastic models. We next turn to a discussion of models for clonal diversity within the context of clonal hematopoiesis and pre-leukemic dynamics and close with a brief consideration of recent insights into stem cell physiology using for example, lineage tracking and an examination of symmetric versus asymmetric division.

The Regulation of the HSC Cell Cycle

Little positive can be claimed for the development of the atomic bomb, but one by-product was the abundant availability of radioactive tracers after the Second World War ended. Cell biologists soon realized that these could be used to probe the processes involved in tissue development, maintenance, and repair. Inspired by a flood of data, they soon developed a conceptual framework for thinking about how cells replicated and formulated the notion of the cell cycle and its component phases, first the *S* phase during which DNA was

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replicated and M phase during which mitosis took place before cytokinesis. It was soon realized [4] that there was an intervening period between the cessation of S and commencement of M that became known as the G_2 phase and a period known as G_1 between cell birth and the commencement of S. The four phases, G_1, S, G_2, and M, constitute the proliferative phase P of the cell cycle, and cells were viewed as progressing through these stages in a sequential and orderly fashion. Thus was the state of knowledge in the early 1960s.

Experimental work on regenerating liver [5, 6] (see the review by Epifanova et al. [7]) led to the important concept of the G_0 phase of the cell cycle, also known as a resting phase, between mitosis (and cytokinesis) and G_1. This extended conceptual model of the cell cycle was formulated within a mathematical framework [8, 9] to analyze labelling data and has played a pivotal role in the development of our ideas about the regulation of stem cell development. This model was applied to the bone marrow stem cell population, speculating that cells differentiated out of the G_0 phase and that the cell population must be autoregulatory, so any loss of cells would eventually be recovered by the compensatory movement of cells from G_0 into the active proliferative phase.

Using the G_0 model of the cell cycle [8•], Mackey [10] attempted to understand the origin of cyclical neutropenia (see Mackey 2020 [11] for a full account). As shown in Fig. 1, the model is simply a variation of the model of Burns and Tannock [8•] in the following ways. It was assumed that cellular differentiation takes place out of the G_0 compartment at rate δ, there is a potential loss to death (apoptosis) of cells within the proliferative phase at rate γ, and finally, the rate of cellular re-entry from the G_0 compartment into the proliferative phase takes place at rate β that is a decreasing function of the total number N of G_0 phase cells:

$$\beta(N) = \beta_0 \frac{\theta^n}{N^n + \theta^n}, \tag{1}$$

where β_0 is the maximal rate of re-entry, θ denotes the half-maximal G_0 population, and $n > 0$ is the Hill coefficient.

The dynamics of the G_0 phase cell numbers are governed by the differential delay equation:

$$\frac{d}{dt}N(t) = -\delta N(t) - N(t)\beta(N(t)) + 2e^{-\gamma\tau}N(t-\tau)\beta(N(t-\tau)), \tag{2}$$

where τ is the duration of the proliferative phase P of the cell cycle. (The origin of the three terms on the right hand side is easily understood: the first two are, respectively, the loss from G_0 due to differentiation and loss due to re-entry into the proliferative phase. In the third term $N(t-\tau)\beta(N(t-\tau))$ represents the flux of cells leaving G_0 a time τ ago—and thus entering the proliferative phase. This is diminished by the factor $e^{-\gamma\tau}$ because of the random cell loss during the proliferative phase of the cell cycle, amplified by the factor of 2 due to mitosis and cytokinesis.) This equation has a rather astonishing array of solution behaviors [12], which makes it of interest to mathematicians [13–18] as well as modellers of hematological diseases [11]. For example, applying this model to data from mice, Mackey characterized key parameters of HSC kinetics, including rates of differentiation and re-entry into the pool and the number of effective divisions between the HSCs and terminally differentiated cells in mice [19].

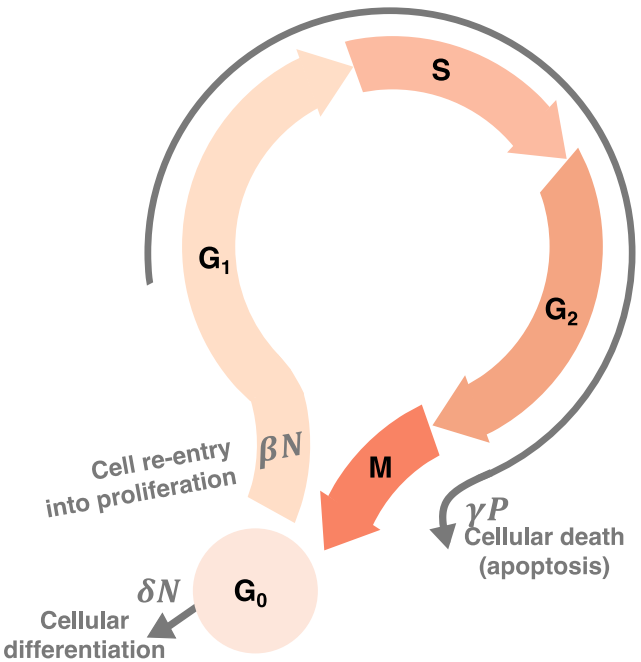

Fig. 1 The amended Burns and Tannock cell cycle model. The duration of the proliferative phase P is τ, and δ is the rate of cellular differentiation, while γ is the rate of apoptosis in the proliferative phase. β is the rate of cellular re-entry from the G_0 phase (population number N) into the proliferative phase and was assumed to have the form given in Eq. (1)

Pathology in Hematopoietic Stem Cells: Acute Myeloid Leukemia

The establishment of the Burns and Tannock G_0 model provided a framework for investigations into both normal and pathological HSC dynamics. Indeed, theoretical work has developed in step with recent experimental advances into perturbed hematopoiesis. Acute myeloid leukemia (AML), an aggressive blood cancer that accounted for 62% of the leukemia-related deaths in the USA in 2019 [20], is characterized by a rapid clonal expansion in the blood and bone marrow of myeloid stem cell progenitors with truncated differentiation. The cause for this perturbation is rooted in a genetic disruption of an HSC or its progenitor that occurs either by the acquisition of de novo mutations, prior HSCs disorders (like myelodysplastic syndromes or myeloproliferative neoplasms), or DNA damage through toxic exposures including

chemotherapy and radiation [20–22]. Improvements in supportive care and in allogenic stem cell transplantation strategies have recently increased the 5-year survival rate of younger patients [20]. However, the standard therapy for AML, which primarily targets and kills leukemic blast cells, remains largely unchanged [20, 23]. While AML occurs in all age groups, it is most prevalent in people over 65 years old, and current treatment strategies do not meaningfully improve their survival [20]. Therefore, the development of novel therapeutic strategies for AML is needed. For this, a better understanding of pathological hematopoiesis and the effects of mutations on HSC differentiation and proliferation is required. This section will discuss of the use of mathematical modelling to understand blood production mechanisms during AML.

Deterministic Models

Myelodysplastic syndromes refer to the group of clonal disorders characterized by ineffective hematopoiesis resulting in cytopenia. These diseases progress slowly and carry high risks of developing into secondary acute myeloid leukemia. Understanding the interactions between clonal expansion caused by aberrant HSCs and normal hematopoiesis in myelodysplastic syndromes may give insight into the development of AML.

Walenda et al. [21] developed a system of ordinary differential equations to describe the self-renewal and proliferation of clones and the flux of normal and malignant cells through different stages of differentiation: from hematopoietic stem cells to mature cells and from leukemic stem cells (LSCs) to dysplastic precursors. Leukemic stem cells are relatively rare HSCs harboring mutations that seed downstream leukemic cells [24]. The authors [21] examined the dynamics of disease development initiated by a single LSC clone by simulating the number of cells in each compartment (Fig. 2). By varying parameter values in the model, they showed that a high self-renewal rate in LSCs is essential for the development of myelodysplastic syndromes. This suggests that the increase in self-renewal is a hallmark of myelodysplastic syndromes and a possible factor for AML development. Thus, downregulating the self-renewal rate of malignant stem cells might be an efficient strategy to incorporate in the treatment of myelodysplastic syndromes.

Other groups of clonal hematopoietic stem cell malignancies, myeloproliferative neoplasms, are characterized by a relatively slow, but uncontrolled, proliferation of blood cells in the myeloid lineage. Most patients with myeloproliferative neoplasms carry a specific mutation (*JAK*2 V617F) that makes the HSCs more sensitive to growth factors [25]. Additionally, myeloproliferative neoplasms increase the probability of developing more aggressive secondary blood cancers, such as acute and chronic myeloid leukemias, and the *JAK*2 V617F mutation was found, in rare cases, to occur de novo in AML [25, 26].

The Cancitis model consists of a system of ordinary differential equations that describes the proliferation of HSCs and malignant stem cells into mature normal and malignant blood cells [27]. In this system, self-renewal rates of both normal and leukemic stem cells are regulated by the number of dead cells through inflammation. The model reflects the idea that cancer development is caused by changes in the frequency of stem cell division and that regulatory feedback allows stem cells in the bone marrow niche to further divide into blood cells when needed.

To understand how AML progresses from myeloproliferative neoplasms, Sajid et al. [22•] analyzed the Cancitis model [27] and explored the coupling between inflammation and myeloproliferative neoplasms (Fig. 3). Four steady states (labelled trivial, hematopoietic, malignant, and coexistence) were found, and treatment was studied with the objective of pushing the system from a malignant steady state to a stable coexistence or hematopoietic steady state. Through bifurcation analysis, the authors found two relevant parameters that altered the stability of the steady states and improved prognosis when decreased: the reproduction ratio of HSCs over LSCs and the ratio of inhibition of the hematopoietic relative to leukemic cells. Further simulations of the *JAK*2 V617F allele burden with respect to these two key parameter values support these results. The analysis of Andersen et al. [27] also suggests that a therapeutic target for myeloproliferative neoplasms may be the reduction of the self-renewal rate of malignant stem cells, reinforcing the hypothesis that diseases caused by clonal expansion of precursor cells from the myeloid lineage can be treated by controlling the differentiation of LSCs.

More recently, Bangsgaard et al. [26] expanded on Sajid's model [22•] by including multiple aberrant clones to understand oscillations in patient cell counts and therapeutic resistance. They allowed normal and malignant stem cells to mutate into more aggressive LSCs in two ways: HSCs into different LSCs (parallel mutations) or HSCs into a single LSC line wherein further mutations increase its fitness (sequential mutations). Simulations using this model show that when one clone is significantly less aggressive than the second, the less aggressive clone can be effectively targeted by the immune response or treatment. However, this reduction in a single clone removes the inhibitory effect on the second and allows for growth in leukemic cells [25] typical of cancer resistance [28, 29]. Since the pool of LSCs can be highly heterogeneous in leukemia, Bansgaard et al. further studied at least three malignant clones with varying levels of inhibition. In agreement with clinical data, they observed oscillations in LSC numbers following fast targeted treatment. Hence, they concluded that if the fatal growth of one aberrant clone is quickly prevented, an initial drop in aberrant stem cell numbers is observed, rapidly followed by similar oscillations in LSCs occurring at higher

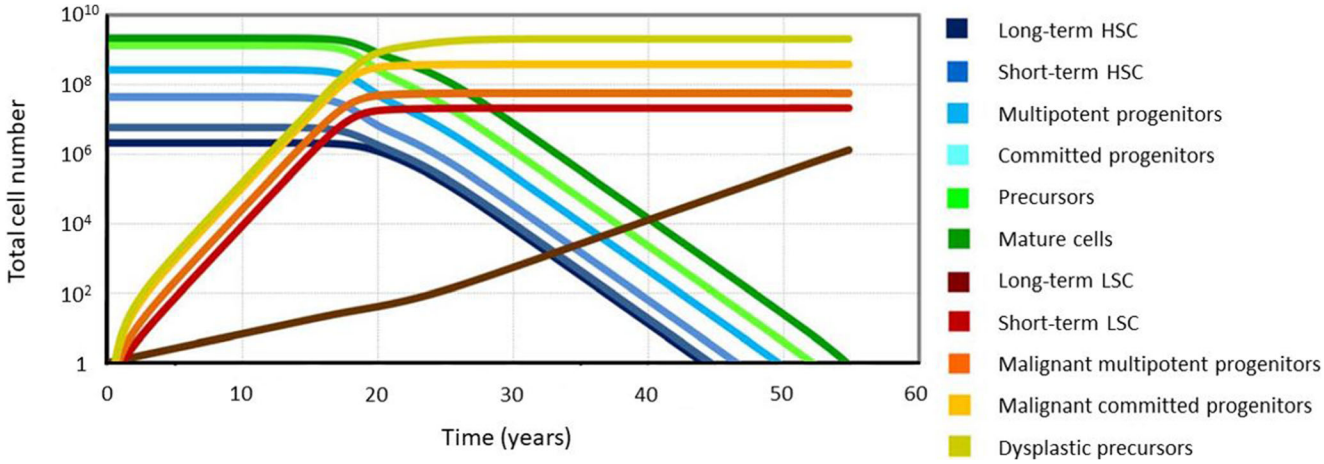

Fig. 2 Simulated dynamics of the development of myelodysplastic syndrome. An initial leukemic stem cell (long-term LSC, dark red line) expands over time due to a higher self-renewal rate than normal hematopoietic stem cells (long-term HSC, dark blue line). Both long-term HSCs and LSCs go on to produce cells lower in the hematopoietic hierarchy (first differentiating into short-term HSCs and LSCs, respectively, and then into multipotent progenitors, committed progenitors, precursors (or progenitors), and finally mature cells; progeny of LSCs are indicated by malignant and dysplastic). When cell numbers in the bone marrow reach a threshold, a feedback signal is triggered, and the self-renewal of all stem cells decreases. The leukemic cells finish by outcompeting the normal hematopoietic cells. Adapted from Walenda et al. [21] under Creative Commons CC-BY

concentrations due to the resistance mechanisms mentioned above. The ability of the model to reproduce clinical data provides strong evidence of the possible, more complex dynamics driving the evolution of myeloproliferative neoplasms into secondary and more aggressive leukemias like AML.

To understand the dependency of AML on cytokine signalling, Stiehl et al. [30••] designed two models of leukemia and their interactions with healthy hematopoietic cells, one cytokine-dependent (leukemic cell expansion depends on cytokines for expansion) and the other independent of the influence of signalling. In both models, the dynamics of each cell type are described by their proliferation rate, fraction of self-renewal, and rate of death. For healthy and leukemic cells, cytokines were assumed to determine the fraction of self-

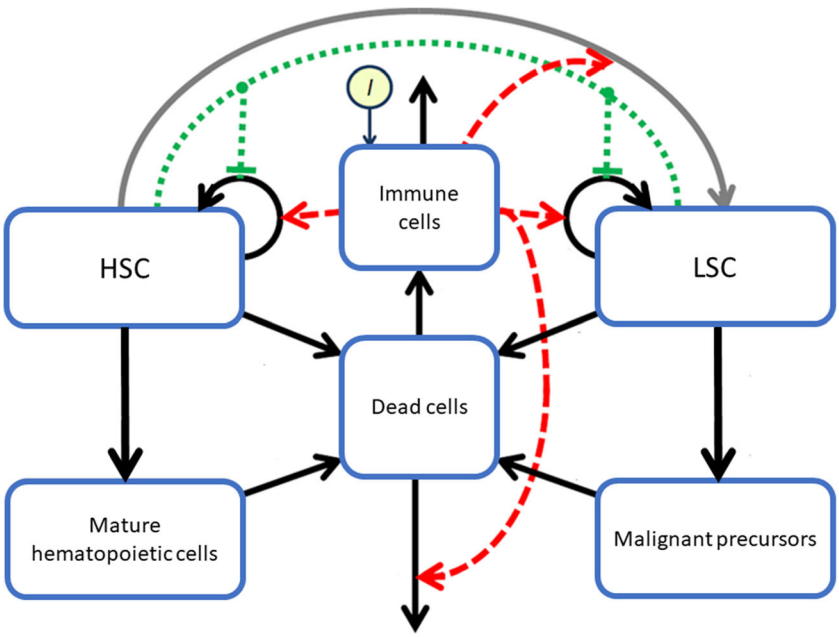

Fig. 3. Flow diagram of the Cancitis model. The Cancitis model considers six compartments: normal hematopoietic stem cells and their mature progeny, malignant leukemic stem cells and their mature progeny, dead cells, and immune cells. Exogenous inflammatory stimuli activate the immune cells (I), which then promote the mutation of HSCs, the self-renewal rates of both normal and leukemic stem cells, and the clearance of dead cells. Black arrows represent the rates of the flow in and out of the different cells compartments. Red dashed arrows symbolize the modulation of the immune response on the rates of the normal and perturbed hematopoiesis. Green dotted arrows denote the inhibitory effect caused by crowding in the bone marrow. Adapted from Andersen et al. [27] under Creative Commons CC-BY

renewing cells to differentiating cells. Integrating these two models with bone marrow aspirate data from patients allowed Stiehl et al. to develop criteria to distinguish cytokine-dependent and cytokine-independent AML, primarily related to the speed of relapse (earlier relapse suggests cytokine-independent AML). Further, they found that AML independent of cytokine signalling had worse overall survival. Given the difficulty in assessing whether a patient's AML is cytokine-dependent or cytokine-independent, the authors suggested evaluating the autonomous growth of leukemic cells in culture and/or surface receptor markers to determine the type of AML prior to treatment.

Stochastic Models

Jäkel et al. [31•] looked at the effects of each individual stem cell in addition to the dynamics of cell populations by extending the stochastic, compartmental model of Dingli et al. [32], which counts single cells at the different differentiation phases in hematopoiesis. Jäkel explicitly modelled erythrocytes, granulocytes, and thrombocytes and added a feedback mechanism with lineage-specific growth factors. Each compartment in the model represents a stage in the differentiation process from HSCs to mature circulating cells, with the HSC compartment size assured to remain constant through asymmetric cell division. At each time step, cells in each compartment can differentiate with a probability depending on their hematopoietic stage and the concentration of lineage-specific growth factors. They modelled the concentration of these growth factors as a deterministic feedback signal depending on the number of mature cells in each corresponding lineage. This model captured clonal expansion in AML by allowing for two kinds of mutations, the first giving bone marrow cells unlimited replicative capacity (giving rise to exponential growth of immature cells which are quickly eliminated in circulation) and the second blocking differentiation. The latter causes an exponential increase of immature cells at the level of the block, which ultimately slows down and drops to zero as the replicative capacity of the cells decreases. The main conclusion of Jäkel et al. [31•] is that the two mutations must be acquired in the same cell for AML to develop. As in Bangsgaard et al. [26], they suggest that tumor heterogeneity is an important factor in the development of AML.

There is evidence that the mode of stem cell division, i.e. asymmetric (one daughter cell remains the same type while the other differentiates) versus symmetric (two daughter cells have same type), may have a significant impact on cancer development [32–34]. In the context of AML, modelling predicts that leukemic stem cell proliferation and self-renewal rates are important for disease development. However, little is known about the mechanisms by which asymmetric and symmetric cell division is regulated in HSCs because HSC division occurs rarely and determining the mode of division is difficult.

Wu et al. [33] established a comparative study of asymmetric and symmetric cell division through stochastic stem cell models with mutations. They captured the dynamics of cell division with using the Fokker-Planck equation for the joint probability distribution of the number of normal and mutant stem cells at a given time. For asymmetric division, the number of stem cells in the population remains constant, so the joint probability distribution is simply the distribution of the number of normal stem cells. The authors found that the expected values for both the numbers of normal and mutant stem cells are identical for the two division modes, and symmetric division yields a larger variance of the total population size compared to the asymmetric division model. These results highlight the importance of stochasticity in defining different division patterns, since they observed the same deterministic dynamics in asymmetrical and symmetrical cell division.

HSC Clonal Diversity: Modelling to Understand Clonal Hematopoiesis and Pre-leukemic Dynamics

As highlighted in the previous section, AML and other leukemias develop through mutations. Modelling mutational evolution is therefore crucial to understanding these clonal diseases. Evidence of clonal evolution within the HSC compartment has been accruing since early studies tracing X chromosome inactivation, the process whereby X chromosome in the female embryo is progressively silenced [35]. More recently, reduced clonal diversity in the hematopoietic system has come to light through a number of associations between driver genes and diseases. Here, we outline modelling efforts to understand the dynamics of clonality in HSCs and its impact on hematopoietic biology.

Abkowitz et al. [36] studied HSC kinetics by studying the mosaic pattern of the two X chromosomes in female safari cats. In safari cats, HSCs express one of either two genes (d or G glucose-6-phosphate dehydrogenase) but not both simultaneously. The expression of these genes can be used to track the relative expansion of hematopoietic clones. Abkowitz et al. [36] harvested bone marrow cells, transplanted them back into the animals, and observed excessive skew of one gene relative to the other within erythroid burst-forming and GM colony-forming units at both early (1 year) and longer (4–6 year) time points, indicating a selective growth advantage. The relative skew over time was driven by X chromosome genes impacting on HSC dynamics in vivo. From this finding, Catlin et al. [37] used X-chromosome inactivation to track HSC replication kinetics through the integration of human X-chromosome polymorphism data from 1416 women across two cohorts [37, 38] and a stochastic model tracking HSC

divisions over time. Their Markovian model consisted of two compartments (HSC and committed progenitors/differentiated cells). HSCs are born (to a maximal number of cells in the niche), die, and commit to the second compartment. After commitment, cells exit the hematopoietic compartment through death. Using the cohort data, the authors fit their model to estimate the rate of HSC replication and the steady-state size of the HSC compartment, a value particularly important for defining clonal dynamics within the blood (Fig. 4). Catlin's analyses suggest an HSC doubling time of once every 40 weeks and a compartment size of 11,000 cells.

With recent advances in sequencing technology, evidence has now solidified around the emergence of (non-)pathological clonal expansion within the HSC compartment and throughout the hematopoietic hierarchy. This expansion, called clonal hematopoiesis (CH) when associated with leukemias [40] or clonal hematopoiesis of indeterminate potential (CHIP) in the absence of hematological malignancies, arises through evolutionary and selective pressures present during normal hematopoiesis and aging [41]. In CH, common leukemia-associated genes are mutated, and the condition is frequently detected either prior to, or after, disease onset including myelodysplastic syndromes and AML. Whole genome sequencing in conjunction with predictive statistical models has shown the feasibility of using age-related clonal hematopoiesis to predict AML risk [42] in healthy individuals and the risk of AML relapse [43]. In CHIP, it is more common that only a single driver gene [44, 45] is mutated, and it may occur as a normal part of aging without any deleterious effects on an individual's health. The presence of oligoclonal blood in a 115-year-old woman in good health supports this curious finding [46].

To understand how evolutionary dynamics contribute to the development of CH over time, Watson et al. [47•] used a branching process model (Fig. 5B) with a number of publicly available sequencing studies to study the fitness advantage of specific single nucleotide variants (alteration of a single nucleotide in a given DNA sequence) and the evolutionary landscape of commonly mutated driver genes in CH.

Their model tracked cells capable of division along the hematopoietic hierarchy. The rates of death or terminal differentiation, changes in cell type, and asymmetric cell division were accounted for within each cell population. Mutations occurred from normal cells at a rate dependent of the cell type and the state of this cell (primarily its likelihood to divide).

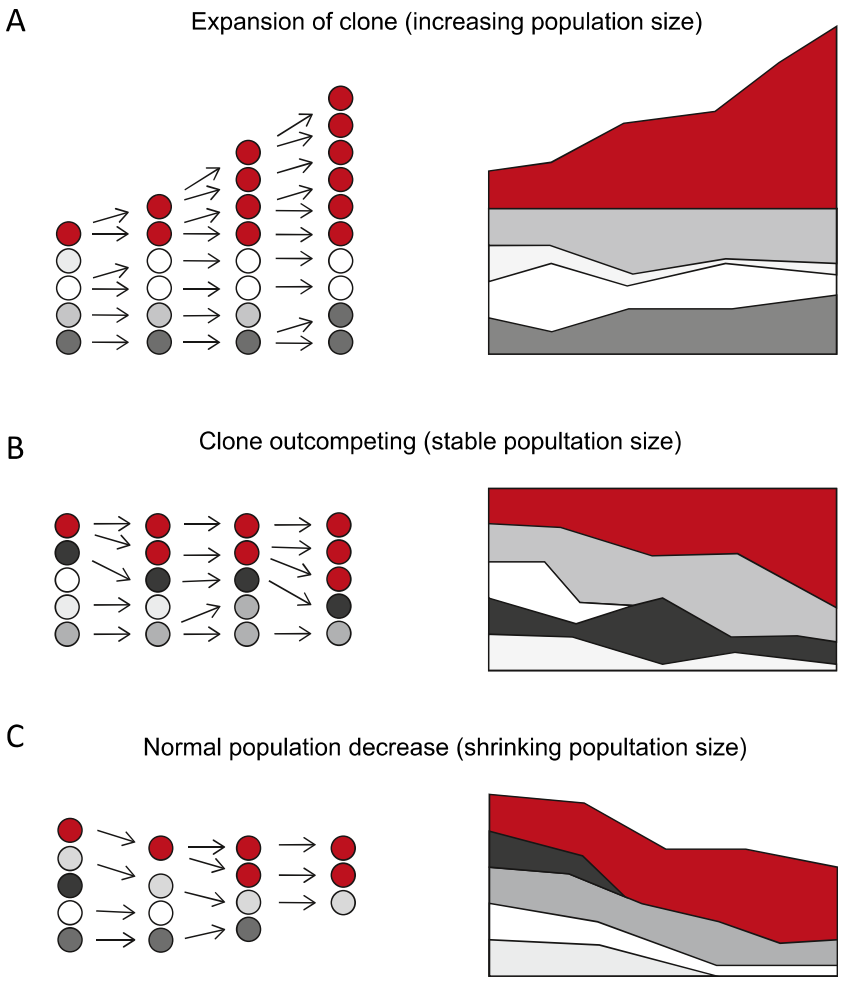

Fig. 4 Multiple scenarios of clonal population dynamics leading to clonal hematopoiesis. **A** One clone expands in size without affecting other clonal population dynamics, ending up relatively over-represented in the population. **B** Dominant clone expands through competition, reducing other clone sizes without changing the overall size of the population. **C** Dominant clone is resilient to total population size reduction. Reproduced from Lee-Six and Kent [39] under Creative Commons CC-BY

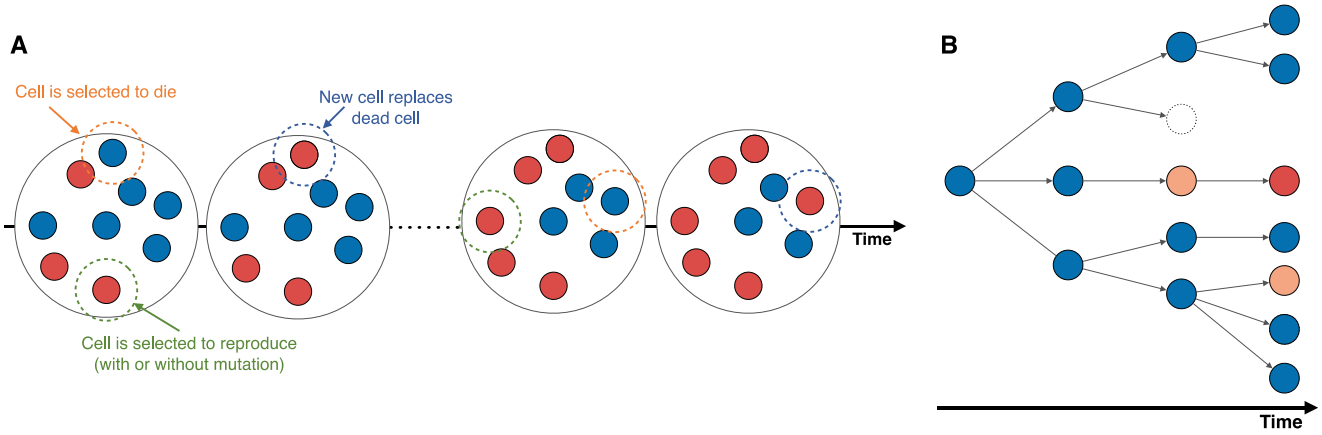

Fig. 5 Schematic overview of stochastic modelling approaches. **A** In an alternative Moran model, a cell within the (fixed size) population is randomly selected to die, and another cell is randomly selected to reproduce based on its fitness relative to the population. **B** Unlike the Moran model, the population size grows over time in a branching process. Reproduced with permission from Craig et al. [29]

Interestingly, they also accounted for the rare conversion of multipotent progenitors back into HSCs. For this, they tracked the number of divisions undergone by multipotent progenitors and assigned a progressively reducing probability of reversion with increases in this number. In contrast to the predictions of Catlin et al. [37], their analysis found an average of 13 HSC divisions per year (or every 28 days versus the once every 40 weeks from the inactivated X chromosome study). As highlighted earlier, the kinetics of HSC division have an important influence on a variety of diseases (i.e., AML) and their therapeutic outcomes, given that this value has a direct influence on the rate of self-renewal of individual cells. Watson et al. further predicted that with sufficiently sensitive and deep sequencing (detection of variant allele frequencies of 0.01% vs the typical 2% used for CH diagnosis), clonal hematopoiesis will be detectable in virtually all people over 50 years old and even in young adults (Fig. 6).

Using a Moran model of the HSC dynamics in conjunction with experimental data in an atherosclerotic mouse model and human data, Heyde et al. [48] recently established that downstream demands on the HSC pool (namely the increased inflammation that is associated with atherosclerosis-associated factors) create a vicious cycle that increases clonal hematopoiesis. This study suggests that clonal hematopoiesis is related to atherosclerosis not only through specific driver genes that increase inflammation, but also from elevated production demands in the HSC pool brought on by the increased number of leukocytes characteristic of atherosclerosis-associated factors.

Park et al. also [49] studied HSC evolutionary dynamics using a mathematical model in conjunction with in vivo data to assess how competition within HSC niches contributes to declines in clonal frequencies and the development of CHIP. In addition to parameterization from literature sources, Park et al. sampled bone marrow in both young and old mice to quantify the clonal composition and competition within an aging hematopoietic system. In contrast to Catlin et al. [37], Watson et al. [47•], and Heyde et al. [48], here the authors constructed a deterministic system that modelled the interactions between healthy and mutant HSCs and their production of mature myeloid and lymphoid dynamics. To represent the cytokine paradigm, wherein production into specific lineages is increased as the number of terminally differentiated cells is reduced (and vice versa) [50], two exponential feedback functions dependent on the number of myeloid and lymphoid cells (derived from both healthy and mutant HSCs) were coupled to HSC dynamics. Hematopoietic dynamics during aging were modelled as subject to heightened competition between healthy and mutant cells through increases in cell death rates and niche degradation. Competition was shown to impact greatly on the output of HSCs, affecting growth rates of both healthy and mutant HSCs and altering the relative proportion of each in the bone marrow. This imbalance was further aggravated by deteriorating bone marrow conditions during aging, a finding supported by evaluating the proportions of lymphoid and myeloid cells in aging mice. Overall, Park et al. [49] provide evidence that low levels of clonal competition can induce significant decreases in the number of HSCs given deteriorating environmental conditions, highlighting how the bone marrow microenvironment impacts on CHIP.

Integrated Approaches to Understanding HSC Kinetics and Biology

With improvements in viral barcoding, and sequencing and single-cell technologies, we are now more than ever able to derive new insights about HSC kinetics and biology through the combination of mechanistic mathematical models and novel biological experiments. These approaches in diverse animal models also help to reduce imprecision in estimates

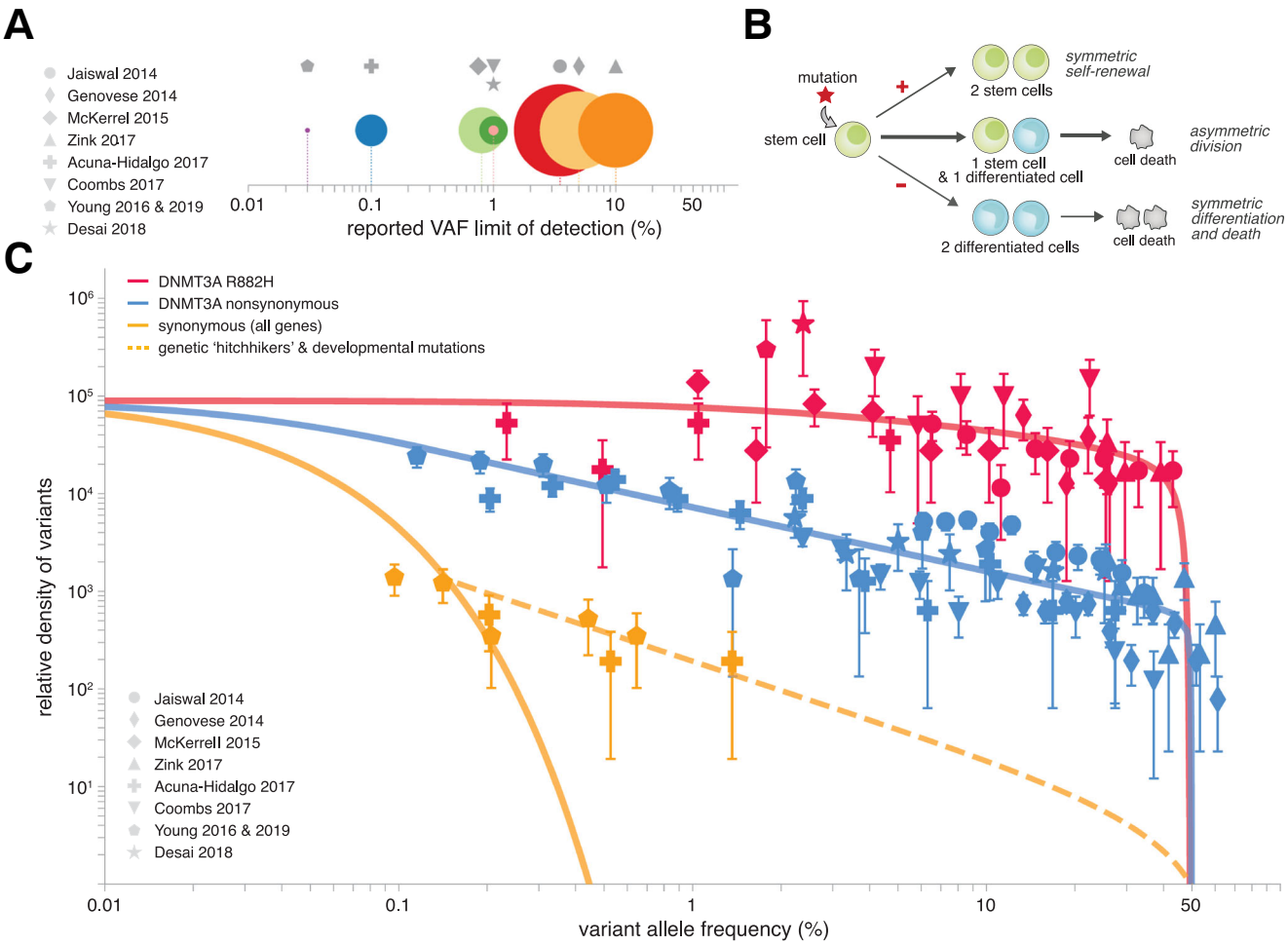

Fig. 6 Branching process modelling framework applied to the evolutionary dynamics of clonal hematopoiesis. **A** Number of participants (circle size) and variant allele frequency (VAF) from studies used in Watson et al. [46•] analyses. **B** Schematic overview of a branching model of HSC dynamics with driver mutations that cause imbalance towards self-renewal. Red plus sign: mutation causing increased rate of self-renewal; redinus sign: mutations cause decreased rates of differentiation or apoptosis. **C** Data points: variant allele frequency of mutant (DNMT3A) normalized by mutation rates; error bars: sampling noise; lines: predictions from branching model accounting for age distributions in original studies. Adapted from with permission from Watson et al. [46•]

that may arise due to differences in hematopoietic output between species [51].

Using a non-human primate model, Goyal et al. [52] tagged human stem and progenitor cells and tracked the lineage distribution of resulting clones. To follow differentiation biases over time, data from 10^6 to 10^7 mobilized and retransplanted lentivirally tracked human stem and progenitor cells were incorporated into a neutral model of hematopoiesis. (The neutral model assumes that mutated sequences or clones have no effect on fitness [53].) The Goyal et al. [52] model accounted for HSCs (unlabelled, due to not being mobilized, and transplanted, labelled cells) that were subsequently transiently amplified within the progenitor pool before release into circulation. The moments of the stochastic model were established by studying the generating function of the underlying probability distribution. In this way, Goyal et al. derived the sampled clone size distribution and compared it to data taken over 4–12 years. Using the steady-state clone size distribution, the authors inferred a variety of estimates for biologically uncertain parameters, including the proportion of asymmetric division in the HSC pool (small, in the range of 10^{-2}–10^{-1}). The proportion of asymmetric to symmetric division is key in pathological HSC biology, as other mathematical modelling work has suggested that symmetric division in stem cell populations may be protective against cancer [54]. Goyal et al. [52] also estimated the number of *active* HSC clones. (As mentioned above, the HSC pool is known to largely be quiescent (in G_0) at any given time [55].) Previous mathematical modelling by Dingli et al. estimated the active pool to be around 400 cells [56, 57]. Goyal's modelling indicated that between 10^3 and 10^5 HSCs are active in the primates they studied, which underlines the difficulty in obtaining precise estimates of HSC kinetics, given their relative scarcity and quiescence, and the

importance of integrating novel biological data with well-designed mathematical models.

Lee-Six et al. [58••] more recently estimated the total size of the active HSC pool by combining a Moran model and phylogenetics with primary bone marrow aspirates and serial blood sample data from a 59-year-old man. Whole genome sequencing was performed on stem, progenitor, and mononuclear cells, and granulocytes for assessment of clonality and phylogenetic analysis. Targeted deep sequencing was also performed on certain mutations, enabling the back assignment of these mutations onto the phylogenetic tree constructed from the whole genome sequencing. To estimate the number of HSCs that contribute to terminal granulocyte production, they designed a stochastic hematopoiesis model. As in the stochastic models previously described, Lee-Six [58••] used a Moran model, which was originally developed to study population genetics. This model can be viewed as a prospective analogue of the retrospective phylogenetics analysis they deployed. Moran models are stochastic frameworks of fixed size populations in which the allelic composition of a population is studied under neutral selection. Birth and death processes occur in succession at each time step, maintaining the overall pool size (Fig. 5A). Lee-Six et al. used a variation of this model wherein one individual is randomly chosen to die, and another (distinct) individual is chosen to reproduce in each generation, with one daughter inheriting the same label as its mother and the second daughter inheriting the same label as the cell chosen to die. This modification allowed the authors to track lifespan with respect to death/division.

Demonstrating the equivalency between their formulation of the Moran model and the Wright-Fisher model, Lee-Six et al. [58••] were able to take advantage of several computations specific to the latter model, in particular for estimating the size of the HSC pool and the lifespan of distinct lineages in their model and tree. Together, their framework for whole genome sequencing, targeted sequencing, phylogenetics, and predictive Moran/Wright-Fisher models led them to conclude that the average size of the active stem cell pool is relatively constant over an adult lifetime and around 100,000 cells. This number is quite different from that of Abkowitz et al. [59], and an order of magnitude larger than that of Goyal et al. [52], both of whose estimates fall within the credible intervals of Lee-Six's model predictions (see Fig. 3 in Ref. [58••]). If true, this larger active HSC pool size has important implications for CHIP and also the development of blood cancers like AML that expand from cells of origin in the HSCs.

In addition to fundamental insights about HSC pool size, mathematical models combined with novel experimental strategies can also help to clarify HSC biology including kinetics and differentiation trajectories. For example, Laurenti et al. [57••] sorted and expanded long- and short-term HSCs from donated cord blood samples to analyze cell cycle kinetics in immunodeficient mice. Data for the time for cell cycle emergence and completion of mitosis were analyzed, in part, by estimating parameters using an agent-based model to infer the time for G_0 exit of both long- and short-term HSCs, picking up from the Burns and Tannock model discussed earlier. Agent-based models consider cells independently and assign a set of rules that dictate agents' (cells') behaviors. Laurenti et al. [60••, 61] considered each cell to have its own cell cycle length and G_0 exit time, with each randomly assigned according to a normal distribution. Cells were then modelled to divide if they received a randomly assigned signal to divide that was longer than their G_0 exit time, giving birth to one cell of the same type as the mother and another of type determined at random (i.e., only asymmetric division was considered). The results of Laurenti et al. [60••] suggest that both G_0 exit and cell cycle times are independent in long- and short-term HSCs: long-term HSCs were found to have a 54.5-h delay between division signal and the beginning of division and to complete their cell cycles in 19.7 h, whereas short-term HSCs began dividing 48.7 h after signal reception and took 15.8 h to divide.

Busch et al. [62•] also investigated the kinetics of normal hematopoiesis, combining modelling with transplantation experiments by generating a mouse line to track hematopoiesis. Mice were irradiated and single-cell and competitively transplanted to study the frequencies of HSCs contributing to lymphoid and myeloid lineages by monitoring label emergence in downstream compartments and estimating the kinetics in a cell flux model of hematopoiesis. Using a flux model, Busch et al. [62•] estimated the rates of differentiation between compartments and net proliferation at steady state. The authors' results quantified key HSC kinetic characteristics that can be used in future modelling and transplantation experiments. In particular, Busch et al. [62•] concluded that short-term HSCs act as amplifying cells in a more long-term way than multipotent progenitors.

Together, these studies demonstrate new avenues for research into HSC biology that blur the lines between experiments, data, and modelling. In each, new behaviors and characteristics of HSCs were identified, with long-term consequences for our understanding of how HSC populations are maintained over a lifetime and how (sub-)clonal populations arise in the hematopoietic system from the top of the hierarchy.

Discussion

The hematopoietic stem cell system has been intensively investigated since the 1960s. In step, mathematical modelling has revealed important HSC mechanisms and characteristics, helping to define and refine experimental and clinical research questions and establish fundamental properties of HSC biology. More recent work has been performed in even closer

collaboration with experimentalists and clinicians in innovative studies that provide precise estimates for parameters that were previously difficult to establish.

There is an increasing recognition of the role of quantitative fields, particularly predictive modelling, in biology. The ability to assess and track genetic composition longitudinally will continue to improve our picture of HSC behavior. In parallel, to translate this data, newer, more intricate, and refined mathematical models have emerged, demonstrating that advances in one discipline spur developments in another. Thus, we expect mathematical modelling to continue to play an extensive role as we continue to improve our understanding of healthy and pathological HSC biology and further new experimental and clinical approaches and modalities.

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Declarations

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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