



Published in final edited form as:

Semin Hematol. 2017 January ; 54(1): 43–50. doi:10.1053/j.seminhematol.2016.10.002.

Clonal Hematopoiesis

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Abstract

Cancer results from multistep pathogenesis, yet the pre-malignant states that precede the development of many hematologic malignancies have been difficult to identify. Recent genomic studies of blood DNA from tens of thousands of people have revealed the presence of remarkably common, age-associated somatic mutations in genes associated with hematologic malignancies. These somatic mutations drive the expansion from a single founding cell to a detectable hematopoietic clone. Owing to the admixed nature of blood that provides a sampling of blood cell production throughout the body, clonal hematopoiesis is a rare view into the biology of pre-malignancy and the direct effects of pre-cancerous lesions on organ dysfunction. Indeed, clonal hematopoiesis is associated not only with increased risk of hematologic malignancy, but also with cardiovascular disease and overall mortality. Here we review rapid advances in the genetic understanding of clonal hematopoiesis and nascent evidence implicating clonal hematopoiesis in malignant and non-malignant age-related disease.

Introduction

The identification of pre-malignant states, such as cervical dysplasia and colonic tubular adenomas, and early interventions to prevent malignancy are major achievements in public health and cancer biology. However, pre-cancerous states for some hematologic malignancies, especially those of myeloid lineages, have proved elusive. In the 1990s, experimental evidence for blood clonality among healthy women raised the possibility that clonal hematopoiesis is an early step in the multistep pathogenesis of hematologic malignancy[1]. More recently, large-scale studies enumerating the common mutations in hematologic malignancies have enabled the targeted search for the cellular and molecular basis of pre-cancerous lesions in hematopoiesis. Finally, over the past few years, a number of researchers using a diversity of genomic technologies have converged on the finding that clonal hematopoiesis is indeed common, age-related, and pre-malignant.

Adult stem cells are an important but imperfect defense against the accumulation of oncogenic mutations. Most mutations, even *bona fide* oncogenic drivers, either occur in

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post-mitotic cells, or are acquired and lost harmlessly through cellular differentiation and turnover[2,3]. Somatic genetic lesions in adult stem cells, by contrast, can accumulate and persist throughout life[4]. In aged people, the genetic diversity within the HSC compartment is significant: the human body harbors approximately 10,000–20,000 HSCs[5], and each HSC may acquire on the order of one exonic somatic mutation per decade[6]. Given a large enough population, every base pair in the genome will be mutated within at least one HSC. These mutations provide the substrate for clonal selection—the majority will have no effect on fitness, or will be deleterious and lead to clonal extinction. Rarely, mutations arise that drastically spur the expansion of the progeny of a single stem cell, leading to the state of clonal hematopoiesis.

Rapid advances in the biologic and clinical understanding of clonal hematopoiesis may reveal numerous opportunities to benefit patient care. For all that we currently know about the genetics of cancer, we may look back on the 2010s and find that we understood relatively little about the profound effects of somatic mutations and pre-malignant lesions on diverse disease states throughout the body. Here we review our current genetic understanding of clonal hematopoiesis and associations with malignant and non-malignant age-related disease.

Biological, Clinical, and Technologic Convergence on Clonal Hematopoiesis

Studies in healthy women provided the first evidence that hematopoiesis can become oligoclonal with increasing age. Clonal skewing, inferred from non-random X-allele inactivation, was observed in peripheral leukocytes in 20–25% percent of healthy women over 60 years of age[7,8], but much less frequently in younger women. X-inactivation-based assays were also used to demonstrate that hematologic malignancies are clonal diseases, even in the absence of a clone-defining cytogenetic abnormality[9,10], and clonal remissions implied the persistence of pre-leukemic clones in the multistep pathogenesis of AML[11].

This led to a proposed model of leukemogenesis in which driver somatic mutations were hypothesized to occur sequentially in HSCs[4]. In support of this model, pre-leukemic HSCs harboring initiating but not late mutations have been prospectively separated from people with hematologic malignancies in CLL[12,13], HCL[14], and AML[15–17]. Anecdotal examples of separate myeloid and lymphoid malignancies sharing a common *TET2*-mutant precursor underscore the multilineage potential of pre-malignant clones[18]. Populations of non-leukemic HSC from AML patients with *DNMT3A* and *TET2* mutations were found to carry high allelic burdens of these mutations and sometimes harbor multiple driver mutations, consistent with numerically significant and genetically complex expansions of pre-leukemic HSCs[15–17].

These two ideas, that clonal hematopoiesis may be common in advanced age, and that leukemia evolves from pre-leukemic HSCs, supported the idea that leukemogenic mutations could generate pre-malignant clones in healthy people. Direct genomic study of women with clonal hematopoiesis inferred from X-inactivation skewing revealed that a small fraction of clonal hematopoiesis was associated with recurrent somatic mutations in *TET2*[19],

solidifying a mechanistic foundation shared between clonal hematopoiesis and hematologic malignancy.

Clonal Hematopoiesis Harbors Hematologic Malignancy-Associated Mutations

Supported by these studies, several recent studies have queried genomic datasets for evidence of somatic mutations in blood DNA from tens of thousands of people, which have profoundly expanded our knowledge of the genetics and epidemiology of clonal hematopoiesis[20–24].

Single nucleotide polymorphism (SNP) microarray data from blood DNA has been mined to identify acquired copy number alterations (CNA) and uniparental disomy (aUPD) [25]. Two analyses of over 50,000 SNP microarrays detected age-associated increases in the frequency of mosaicism for large chromosomal abnormalities in the blood, approaching 2% of people by age 80, as well as a positive association between blood mosaicism and risk for subsequent hematologic malignancy[20,21]. Recurrent CNAs included deletions of 13q, 11q, and 17p, as well as trisomy 12, all characteristic findings in CLL[26]. Recurrent focal deletions involving *DNMT3A* and *TET2* were also observed, though only rarely[20].

Three studies analyzed existing blood DNA exome sequencing datasets from thousands of people without known hematologic neoplasms to identify somatic variants indicative of clonal hematopoiesis[22–24]. One study analyzed sequence data from 2,728 people enrolled in The Cancer Genome Atlas (TCGA) with one of 11 non-hematologic cancer types and no prior chemotherapy or radiation therapy [24]. This cohort provided the unique opportunity to compare sequence data from blood DNA to a patient-matched non-hematopoietic tissue control. The TCGA cohort is unlikely to be representative of the greater population, and may be enriched for genetic or environmental factors that influence the risk for solid tumors or clonal hematopoiesis. The two other studies analyzed cohorts that were unselected for hematologic parameters or malignancy and, importantly, included longitudinal health information. One of these studied 12,380 Swedish research participants with schizophrenia, bipolar disease, or no psychiatric diagnosis[23]. Finally, our study analyzed sequence data from 17,182 people enrolled either in the Jackson Heart Study or one of 22 type 2 diabetes (T2D) association studies[22].

Overall, these independent studies of separate datasets demonstrated remarkable agreement regarding the prevalence, age association, and spectrum of mutations characteristic of clonal hematopoiesis. Somatic mutations in blood are a rare finding in people under 40 (<1%), but rise with age, affecting 10–20% of people in their eighth decade (Figure 1A). Mutations in the most recurrently mutated genes mirrored the coding changes seen in these genes in malignancy[23]. The majority of mutations were C-to-T transitions, consistent with an age-related signature of mutations seen across many cancer types[22,27]. The majority of people with detectable mutations had a single mutation. In all studies, *DNMT3A* was the most commonly mutated gene, followed by *TET2* and *ASXL1*, and two thirds of the driver mutations causing clonal hematopoiesis occurred in just these three genes (Figure 1B). *TP53*, *JAK2*, *SF3B1*, *CBL*, *SRSF2*, *GNAS*, *PPM1D*, *BCOR*, and *BCORL1* were also

recurrently mutated in multiple studies, and a long tail of rare clonal hematopoiesis mutations is likely to be identified in larger datasets.

The size of the mutant clone detected in these studies is substantial; our study found a median clone size of ~18% of peripheral blood cells[22]., suggesting a dramatic expansion for clones which initiate from a single HSC. It is important to note that the observed median clone size is inversely proportional to the sensitivity of the method used to detect mutations. For example, a subsequent study using deep targeted sequencing for clonal hematopoiesis hotspot mutations (>1000x coverage), identified a median clone size of <0.03[28]. Although the lower limit for clinically important clone size is unknown, clone size is positively correlated with clinical significance, as is detailed below.

The prevalence of clonal hematopoiesis may be underestimated when analysis is limited to genes with known mutations in hematologic malignancies. An unbiased analysis revealed additional cases of clonal hematopoiesis with three or more putative somatic variants that were unlikely to be artifactual because they were age-related and associated with risk of leukemia and lymphoma[23]. Underestimation of the prevalence of clonal hematopoiesis may be due to the difficulty in detecting coding mutations in known cancer-associated genes poorly enriched by exome capture, mutations in genes not currently known to be cancer-associated, non-coding mutations, epigenetic dysregulation, CNAs, and other mechanisms.

Clonal Hematopoiesis is a Pre-Malignant State

The somatic mutations in clonal hematopoiesis persist for years and do not appear to resolve spontaneously, unlike previous studies that found that certain translocations can be transiently present in the blood of healthy individuals[29]. Follow-up analysis of 13 patients after 4–8 years demonstrated the persistence of all somatic mutations[22]. Analyses of a small number of people directly evaluated the evolution from clonal hematopoiesis to hematologic malignancy. In two cases of individuals with clonal hematopoiesis who subsequently developed AML, tumor specimens were obtained for genetic comparison. Somatic mutations and CNVs present in the blood sample were found at high allele fractions in the resultant leukemic clone, demonstrating the potential for clonal hematopoiesis to progress to acute leukemia[23].

Consistent with the hypothesis that the presence of initiating hematologic neoplasm-associated mutations is a pre-malignant state, clonal hematopoiesis was found to be associated with an increased risk of subsequent hematologic malignancy. Two of the original exome sequencing studies included health outcomes after DNA sampling. In >11,000 Swedish people with 2 to 7 years of national registry health outcomes follow-up data, clonal hematopoiesis was associated with a significantly increased risk of subsequent hematologic malignancy (hazard ratio (HR) 12.9 in multivariate analysis) [23]. In >3,000 people enrolled in the Jackson Heart Study and the Multiethnic Cohort with a median of 8 years of longitudinal follow-up, the risk of subsequent hematologic malignancy was increased among all people with clonal hematopoiesis (HR 11.1) and especially among the subset of people with a variant allele fraction of >0.1 (HR 49). As hematologic malignancies are rare in the

general populations, the risk of progression to overt malignancy is approximately 0.5–1% per year[22].

Based on the elevated risk of hematologic malignancy and the demonstration of clonal progression, clonal hematopoiesis is a pre-malignant state. Clonal hematopoiesis is similar to other pre-malignant hematopoietic clonal disorders such as MGUS and MBL with respect to age association, and risk of progression to frank malignancy[30,31]. In contrast to MGUS and MBL, the spectrum of subsequent hematologic malignancies after CHIP is broad, including myeloid, lymphoid, and plasma cell neoplasms, consistent with the likely origin of pre-malignant clones in multipotent hematopoietic stem cells.

The cellular and molecular ontogeny of lymphoma is currently under investigation[32]. Pre-lymphoma uncommitted progenitors have been identified in unique clinical situations[33], and some model of lymphomagenesis implicate HSCs[34]. Mutations in *TP53* are common across hematologic malignancies, whereas other mutations, for example in *JAK2*, have oncogenic effects in specific lineage contexts, therefore the risk of progression to lymphoma is likely conferred by a subset of mutations in clonal hematopoiesis.

Somatic Mutations and Clonal Expansion

The classical view of cancer genetics proposed two broad categories of genes involved in tumorigenesis: oncogenes and tumor suppressors. Landmark studies from tumor-causing viruses and human translocations led to the discovery of oncogenes which typically resulted in either abnormal signaling cascades in the mutated cells[35,36] or else aberrantly prevented apoptosis[37]. Tumor suppressors, which acted opposite to oncogenes, were first proposed to explain the action of inherited cancer genes[38], and were later shown to primarily function as checkpoints in cell cycle arrest following insults to the cell's genome[39].

However, the genomics of clonal hematopoiesis, and myelodysplastic syndrome in general[40], have not fit neatly into the classical models of cancer genetics. Oncogenic mutations analogous to those described above do occur in clonal hematopoiesis (*JAK2*, *CBL*, *GNAS*, and *GNBI*), but together account for less than 10% of clonal hematopoiesis[22]. Similarly, classical tumor suppressor mutations occur in *TP53*, but are in only a few percent of clonal hematopoiesis cases.

The vast majority (approximately 80%) of mutations in clonal hematopoiesis affect genes broadly involved in epigenetic regulation or RNA splicing with less clear roles in clonal dynamics. DNMT3A and TET2 are core regulators of DNA methylation. DNMT3A is a DNA methyltransferase responsible for de novo generation of 5mC[41], and TET2 is a methylcytosine dioxygenase that converts 5mC to 5-hydroxymethylcytosine (5hmC), an initial step in DNA demethylation[42]. Deletions, loss-of-function point mutations, frameshift mutations, and splice site mutations are characteristic for both genes[41,43], as well as the dominant negative R882 mutations in *DNMT3A*[44]. ASXL1 is a regulatory component of the Polycomb repressive deubiquitinase complex (PR-DUB) that mediates post-translational histone modification-based transcriptional repression[45,46]. The majority

of *ASXL1* mutations truncate C terminal regulatory domains[47], but it remains controversial whether these truncating mutations are gain-of-function events, or loss-of-function[48]. Hotspot missense mutations in the 3' spliceosome component subunits *SRSF2*, *SF3B1*, and *U2AF1* each lead to unique and characteristic alterations in 3' splice site selection[49]. The clinical and biochemical features of mutations in these genes in myeloid malignancies have been reviewed extensively elsewhere[41,42,50–52].

Murine models of loss of *TET2* and *DNMT3A* have recapitulated the competitive advantage at the stem cell level inferred from human studies [18,43,53,54]. Surprisingly, *Srsf2* mutation or deletion, heterozygous *Sf3b1* mutation or deletion, and transgenic expression of mutant *U2af1* resulted in impaired self-renewal[55–58], suggesting that the specific splicing event(s) that leads to clonal dominance may not be conserved between mouse and man.

How mutations in these genes lead to a clonal advantage for HSCs has been the subject of much study. We propose three models that may explain the preponderance of epigenetic and splicing gene mutations in clonal hematopoiesis (Figure 2).

1. Mutations in these genes may lead to abnormal expression of genes involved in stem cell self-renewal. Because these genes act as transcriptional regulators, perturbation of their function is expected to lead to altered gene expression patterns. Might there be a shared set of genes that are dysregulated with each of these mutations? Intriguingly, models of *DNMT3A* and *ASXL1* loss-of-function both have abnormal expression in the *HOXA* gene cluster[59–61], a feature that is shared with MLL-rearranged AML[62]. Splicing factor mutations are also expected to affect gene expression levels in addition to splice isoforms. However very little commonality in dysregulated gene expression or splicing has been seen in the different spliceosome mutant mouse models, or between mouse and human samples[55–57].
2. Alternatively, mutations in diverse epigenetic regulators and splicing factors may disrupt the highly coordinated process of differentiation and lineage specification, increasing stem cell number via inefficient exit from this cell state. The specification of differentiated progeny from HSCs requires the commissioning and de-commissioning of thousands of enhancer regions[63] which involves coordinated changes in DNA methylation, histone marks and chromosomal architecture. It is possible that any mutation involved in the regulation of enhancer turnover leads to less efficient differentiation. Similarly, splicing mutations could cause inefficient differentiation by causing loss of expression of key lineage determining genes. Thus, a relative clonal expansion of mutant stem cells may occur in the absence of any proliferative or self-renewal advantage simply by increasing the number of cycles needed for a self-renewing cell to become a committed progenitor in response to instructive cues. In this model, a single unifying core gene expression pattern would not be predicted.
3. Mutations in these genes may lead to increased epigenetic or transcriptional heterogeneity, which allows for selection of particularly advantageous epigenetic states. Recent studies have revealed that tumors often have marked

transcriptional heterogeneity at the single cell level[64,65], as well as at the level of DNA methylation[66]. Such stochastic variation may also be associated with poorer prognosis[66], but thus far there is little evidence increased epigenetic or transcriptional heterogeneity promotes the selection of advantageous clones. Furthermore, while DNA methylation is a stably inherited mark, the stochastic changes conferred by histone enzyme or splicing factor mutations in any given cell is not expected to be heritable across cell divisions.

Selection of clones following marrow injury

Clonal hematopoiesis has been identified in specific clinical contexts wherein a subset of CHIP mutations appears to undergo positive selection. Aplastic anemia is an immune-mediated bone marrow failure disorder wherein autoreactive T cells suppress hematopoietic stem and/or progenitor cells. Clonal hematopoiesis has long been recognized as a common but surprising finding in aplastic anemia[67], and people with aplastic anemia are at markedly elevated risk for PNH, MDS, and AML. In comparison to aging, the autoimmune and inflammatory marrow environment of aplastic anemia may impose both shared and distinct selective pressures on HSCs. Multiple recent sequencing analyses have identified somatic mutations in aplastic anemia[68–71]. The largest of these studies (439 patients) identified somatic mutations in 47% of cases and somatic mutations associated with MDS or AML in approximately one third of cases[71]. In aggregate, these aplastic anemia mutations shared many features of age-related clonal hematopoiesis, including generally low allele fraction, an age-related mutation signature, and increasing prevalence with age. Like age-related clonal hematopoiesis, mutations in *DNMT3A* and *ASXL1* are common in aplastic anemia. These mutations, which likely confer increased self-renewal, may be selected for as aging or autoimmunity stimulates the turnover and replacement of HSCs. Mutations in these genes, together with *TP53*, *RUNX1*, and *CSMD1*, constituted a subset of aplastic anemia with “unfavorable” mutations associated with rising clones over time, poor response to immunosuppression, shortened overall survival, and increased risk of progression to MDS/AML. Surprisingly, not all age-related clonal hematopoiesis mutations are enriched in aplastic anemia. *TET2*, for example, is the second most commonly mutated gene in age-related clonal hematopoiesis, but *TET2* mutations are rare in aplastic anemia [68,71].

Mutations in *PIGA*, *BCOR*, and *BCORL1*, as well as uniparental disomy of 6p (6pUPD) constitute a separate group of somatic events seen in aplastic anemia but not age-related clonal hematopoiesis. The natural history of aplastic anemia with *PIGA/BCOR/BCORL1* mutations is unique, constituting a “favorable” mutation group with no age association, stable clone size, rare progression to myeloid neoplasm, increased response to immunosuppressive therapy, and improved overall survival[71]. The 6pUPD event is an immune evasive mechanism also seen in AML relapsed after haploidentical stem cell transplant, wherein the mismatched HLA haplotype is lost[72]. In aplastic anemia, a recurrent pattern of HLA alleles are lost, as the vast majority of cases of 6pUPD remove four specific HLA alleles that are overrepresented in people with aplastic anemia [73]. It is not known whether *PIGA*, *BCOR*, and/or *BCORL1* mutations also contribute to immune evasion. If so, these mutations could be expected to also confer clonal advantage in other clonal diseases limited by immune surveillance. *PIGA* is required for the biosynthesis of

glycosylphosphatidylinositol (GPI)-anchored proteins. CD55 and CD59 are two such GPI-anchored proteins whose absence explains the susceptibility to complement-mediated hemolysis in PNH. It has been hypothesized that *PIGA* mutations confer selective advantage in aplastic anemia by removing GPI-anchored proteins that contribute to immune recognition[74], and that autoreactive CD1d+ T cells recognize the GPI glycolipid itself[75]. The nuclear proteins BCOR and BCORL1 are both nuclear co-repressors that interact with histone deacetylases. *BCOR* and *BCORL1* vary in germline mutation phenotype, subcellular localization, additional interaction partners, and domain structure[76]. In addition to being common targets of somatic mutation in aplastic anemia, both genes are infrequently but recurrently mutated in AML[77,78], MDS[79], and age-related clonal hematopoiesis[22,24]. The function of *BCOR* and *BCORL1* mutations in leukemogenesis, clonal hematopoiesis, and immune evasion are all unknown.

Chemotherapy is a potent selective pressure that alters the dynamics not only of leukemic and precursor clones, but also of unrelated clones potentially relevant to the emergence of therapy-related neoplasms. The persistence of leukemia-associated mutations in remission after induction chemotherapy is common and is associated with increased risk of relapse[80]. Therapy-related myeloid neoplasms (tMNs) are a feared outcome after chemotherapy and/or radiotherapy. Relative sparing and expansion of hematopoietic clones after cytotoxic therapy may be an intermediate step in the evolution of tMNs. In 5 out of 15 patients, non-leukemic, unrelated hematopoietic clones with mutations reminiscent of age-related clonal hematopoiesis rapidly expanded after induction chemotherapy for AML[81]. One study of paired analyses of *TP53*-mutant tMNs and antecedent hematopoietic cells identified low levels (0.003%–0.5% allele fraction) of the patient-matched *TP53* mutation years prior to the tMN. This finding, together with the finding in 22 t-AML genomes that the spectrum and number of mutations was similar to de novo AML, supports the idea that a pre-existing *TP53*-mutant clone is relatively spared after chemotherapy, increasing the likelihood of clonal evolution[82]. This study elegantly demonstrates that antecedents of tMN can precede and be selected by therapy. However, the likelihood that a pre-existing hematopoietic clone will evolve to tMN after chemotherapy, as well as the risk for tMN for people with or without clones undergoing chemotherapy are currently unknown. As larger *TP53*-mutant clones are not infrequently seen in age-related clonal hematopoiesis and aplastic anemia, it remains to be determined whether a *TP53*-mutant HSC has a clonal advantage in an intact marrow, or instead whether *TP53*-mutant HSC require an insult to the hematopoietic system equivalent to cytotoxic therapy for clonal expansion.

PPM1D, also known as WIP1, is a phosphatase whose expression is induced by p53 that functions to deactivate and degrade p53[83,84], thus forming a negative feedback loop attenuating p53 activation. Mutations in *PPM1D* in clonal hematopoiesis truncate the C terminus of the protein[23,24], resulting in a gain-of-function protein with enhanced suppression of p53[85,86]. Among people with solid tumors, prior chemotherapy is associated with *PPM1D*-mutant clonal hematopoiesis. Somatic mutations in *PPM1D* were found in blood cells from patients with breast cancer, ovarian cancer, and lung cancer[86–88]. Although it was initially unclear whether the *PPM1D* mutations were antecedent risk factors for malignancy, these mutations were subsequently shown to be highly associated with prior chemotherapy in a study of patients with ovarian cancer[89]. Moreover, *PPM1D*

mutations were associated with the number of prior chemotherapy regimens, and multiple *PPM1D* mutations were only seen among the most highly pre-treated cohort of patients with relapsed ovarian cancer[89], a finding that highlights the survival advantage conferred to cells with *PPM1D* mutations in this context. Interestingly, blood *TP53* mutations were not similarly enriched among the individuals that had prior chemotherapy.

Finally, it will be important to determine whether more mundane environmental insults, not just the extreme examples discussed above, are important factors in clonal expansion of mutant HSCs. For example, smoking was found to be associated with clonal hematopoiesis in the Swedish cohort[23], but it remains to be seen if this association is generalizable to other groups beyond schizophrenics. Excessive use of alcohol is associated with bone marrow suppression, but the reason for this association remains unclear. Impairment of alcohol metabolism was found to lead to HSC DNA damage and severe functional decline in a Fanconi anemia mouse model[90], providing a potential link between alcohol toxicity and HSC fitness. More detailed study of epidemiological factors associated with clonal hematopoiesis combined with testing in model systems will help determine if these or other lifestyle factors influence the frequency and rate of clonal expansion.

Clonal Hematopoiesis of Indeterminate Potential, A Provisional Diagnosis

In order to define the pre-malignant state of clonal hematopoiesis in clinical practice, and to distinguish it from MDS, we have proposed a working definition for the entity Clonal Hematopoiesis of Indeterminate Potential (CHIP) [91] (Table 1). Individuals with CHIP have a detectable clonal mutation or copy number alteration associated with hematologic neoplasia in the blood or bone marrow, and lack a known hematologic malignancy or defined clonal entity such as paroxysmal nocturnal hemoglobinuria (PNH), monoclonal B lymphocytosis (MBL), or monoclonal gammopathy of unknown significance (MGUS). The lower limit for allele fraction is provisionally set at 2%, a cutoff that enables the application of prevalence and risk association estimates from major studies to date[20–24], and is feasible with the resolution of current, standard clinical myeloid malignancy next generation sequencing(NGS) assays[92]. CHIP would be more prevalent with the inclusion of lower allele fractions[28], yet the clinical impact of small clones is currently unclear.

People with unexplained cytopenias are an important subgroup of CHIP. The provisional diagnosis of idiopathic cytopenia of undetermined significance (ICUS) describes a relatively common group of people with unexplained chronic cytopenias that do not meet the diagnostic criteria for MDS[93]. ICUS is a heterogeneous group that includes a significant clonal subset bearing CHIP/MDS-associated mutations, which are termed clonal cytopenias of undetermined significance (CCUS) [94]. People with CCUS are included in the diagnostic criteria of CHIP. The clinical outcome for CCUS versus MDS or CHIP without cytopenia is currently unclear.

We and others have argued against screening for CHIP among healthy adults, because the risk of progression to malignancy is low (0.5–1% per year), and there is currently no preventative therapy to target clones or retard progression[91]. However, people are identified with CHIP during molecular evaluations for other suspected hematologic

abnormalities, such as unexplained cytopenias. Clinical monitoring schemes have been proposed for people incidentally found to have CHIP, recommending interval differential blood counts to monitor for cytopenias or blasts[95]. Although it may be premature to define an optimal management algorithm, interval monitoring is reasonable given the low but continued risk of neoplastic evolution. In the future, the rationale for detecting and monitoring CHIP may also include risk modification of non-malignant sequelae of clonal hematopoiesis, which are discussed below.

CHIP and age-associated inflammatory disease: correlation or causation?

Pre-malignant states are generally assumed to have no impact on health in the absence of progression to frank cancer. Several reasons suggest that clonal mutations in HSCs might lead to organ dysfunction in the absence of malignancy. First, clonal selection and function are uncoupled: HSC with clonal advantage are not promoted based on the quality of their differentiated effector progeny, but instead at the level of stem cell self-renewal and survival[96]. Second, unlike solid tissues, hematopoiesis is not spatially constrained[97], thus an HSC clone may expand to be a large fraction of hematopoiesis. Third, HSCs give rise to the many differentiated cell lineages which compromise the immune and blood cell systems, in contrast to the stem cells in other tissues which typically produce only a limited repertoire of mature progeny. HSCs are probably exceeded in diversity of progeny only by embryonic stem cells. Thus, mutations in HSCs have the capability of influencing the function of many cell types. Finally, mature immune cells traffic to every organ and influence the pathophysiology of most disease states. Whereas a tubular adenoma will never impact gut absorption, a clone of dysfunctional HSC might generate millions of hyperactive B cells, tolerogenic antigen presenting cells, or mispolarized macrophages.

In our study[22], clonal hematopoiesis was associated with all-cause mortality (HR 1.4), attributable to cardiovascular disease and not to cancer. Clonal hematopoiesis was associated with all-cause mortality (HR 1.4) in a concurrent study[23]. Somatic mutations in blood were modestly associated with type 2 diabetes (T2D) in multivariate analyses (odds ratio 1.3, $p < 0.001$), whereas the association between mosaic large chromosomal anomalies and diabetes was pronounced (OR 5.3) [98], a difference that in part may be due to the identification only of relatively large clones in the latter study.

The most surprising and impactful risk association is the connection between clonal hematopoiesis and cardiovascular disease. In our multivariate analysis, people with clonal hematopoiesis were at higher risk of incident coronary heart disease (HR 2.0) and ischemic stroke (HR 2.6) [22]. Remarkably, this risk attributable to clonal hematopoiesis was comparable or greater in magnitude than the traditional atherosclerosis risk factors of total cholesterol >240 mg/dL, smoking status, and hypertension in our study. The association between somatic mosaicism and micro- and macrovascular complications of type 2 diabetes (T2D) (HR 5.1) further implicates clonal hematopoiesis with atherosclerotic disease[98]. Cardiovascular disease is the leading cause of mortality in the United States, and age-related clonal hematopoiesis appears to be a common and impactful risk factor with broad public health implications.

The associations between clonal hematopoiesis and cardiovascular disease may be due to unknown confounding variables, or they may be shared pathophysiologic consequences of aging. Alternatively, clonal hematopoiesis may have a causative role in cardiovascular disease, T2D, and potentially other age-related diseases. Macrophages are key regulators of inflammation, lipid biology, atheroma architecture, and insulin resistance[99,100]. Interestingly, restoration of impaired phagocytosis via anti-CD47 antibody treatment was recently shown to suppress atherosclerosis in mouse models[101]. Whether the somatic mutations conferring clonal advantage to hematopoietic clones alter the function of clonal macrophages and other effector cells is unknown. The finding that TET2 negatively regulates IL6 at the resolution of inflammation[102] suggests that the core genetic machinery of hematologic malignancies may have important roles in the cellular mediators of inflammatory disease.

A number of additional clinical associations have been made with clonal hematopoiesis. A modest increase in the risk for non-hematologic cancer was observed in people with chromosomal abnormalities in one study[21], and was not significant in another. One male-specific somatic event, loss of the Y chromosome in blood cells, is reported to be associated with shorter survival[103], increased risk of non-hematologic cancer[103], smoking[104], and Alzheimer's disease[105].

Conclusions

Clonal mutations in genes that are recurrently mutated in hematologic malignancies, are commonly detected in the blood of otherwise healthy aging people. Rapid progress in our understanding of clonal hematopoiesis raises questions spanning from stem cell biology to medicine. While model systems affirm a self-renewal advantage for HSCs with *Dnmt3a* and *Tet2* mutations, the mechanisms of clonal expansion are poorly understood for these and most other CHIP mutations. It also remains to be seen how niche dynamics interact with somatically mutated HSCs. With the expansion of HSCs, do HSC niches expand or are mutated HSCs selected to be less reliant on niche factors? While clonal advantage may be due to altered balance of self-renewal versus differentiation, competition for limited trophic signals, as seen in germinal center B cells, is another potential basis for the competitive advantage of mutant cells. Ultimately, understanding the mechanisms of clonal advantage conferred by somatic mutations is essential to the design of targeted interventions.

Clonal hematopoiesis is a novel risk factor for hematologic malignancy and atherosclerotic disease that is common among aged people. Many further insights will come from expansion cohorts, such as mutation-specific risk associations, and associations between CHIP and additional age-related diseases. There is much to be learned from investigation beyond the exome, as noncoding mutations and abnormal epigenetic states may be important drivers of clonal hematopoiesis.

Detection of CHIP in routine clinical practice has implications for both refined risk assessment and, potentially, for interventions that modify disease risk. If clones can be therapeutically suppressed, the role for broad screening for CHIP may become unequivocal. Clonal hematopoiesis, now more fully illuminated by genomic techniques, is an opportunity

for early interventions. With continued basic and translational study, hematologists may spend less time managing malignant catastrophes and more time preventing them.

Acknowledgements

This work was supported by grants from the NIH (R01 HL082945, R24 DK099808), the Department of Defense, the Edward P. Evans Foundation, and the Leukemia and Lymphoma Society. SJ is supported by the Training Program in Molecular Hematology T32 training grant (Brigham and Women's Hospital) and the Burroughs Wellcome Fund Career Award for Medical Scientists.

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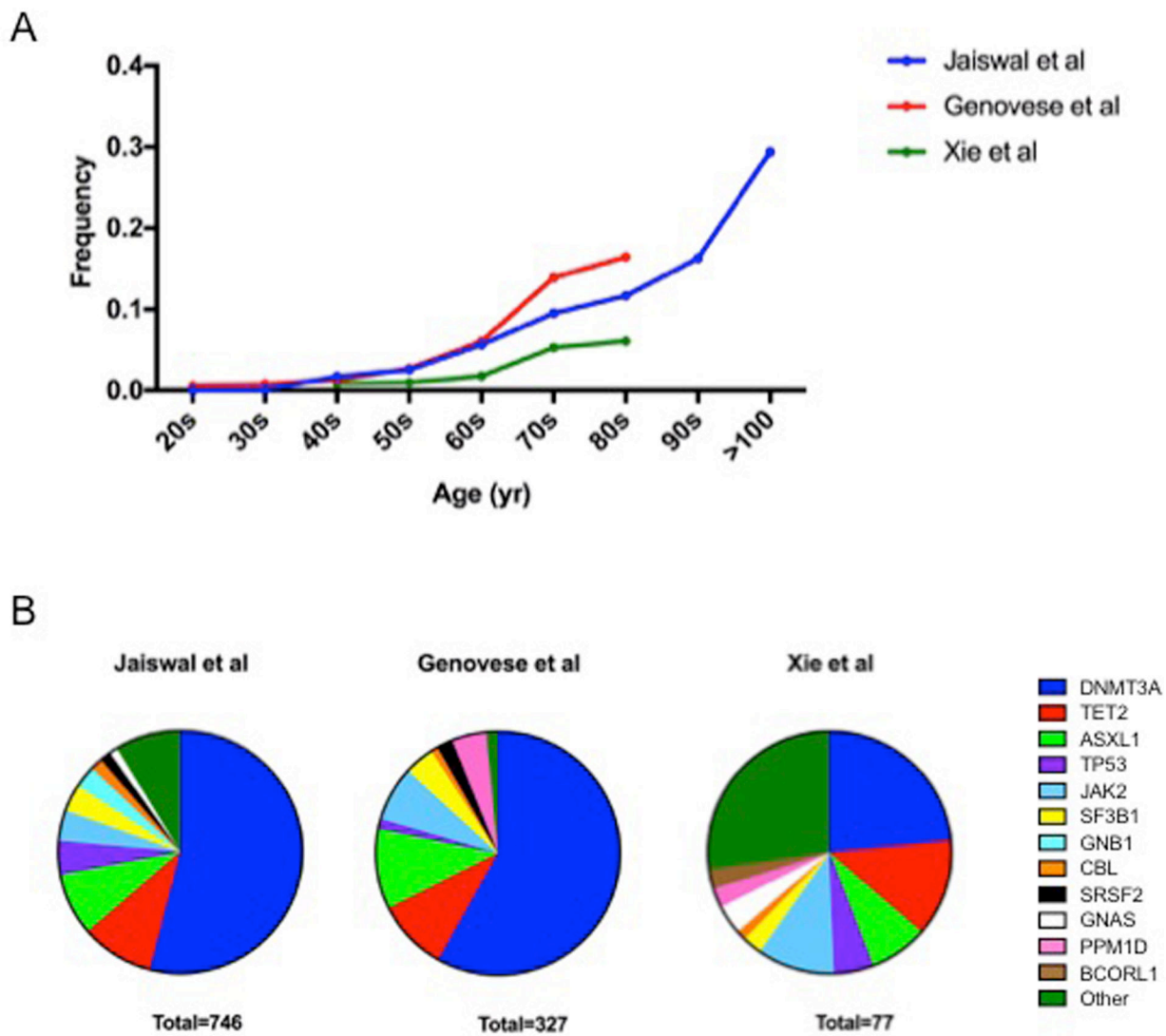


Figure 1:
 (A) Prevalence of clonal hematopoiesis per decade in three recent studies. (B) Recurrent mutations identified from exome sequence data from three recent studies.

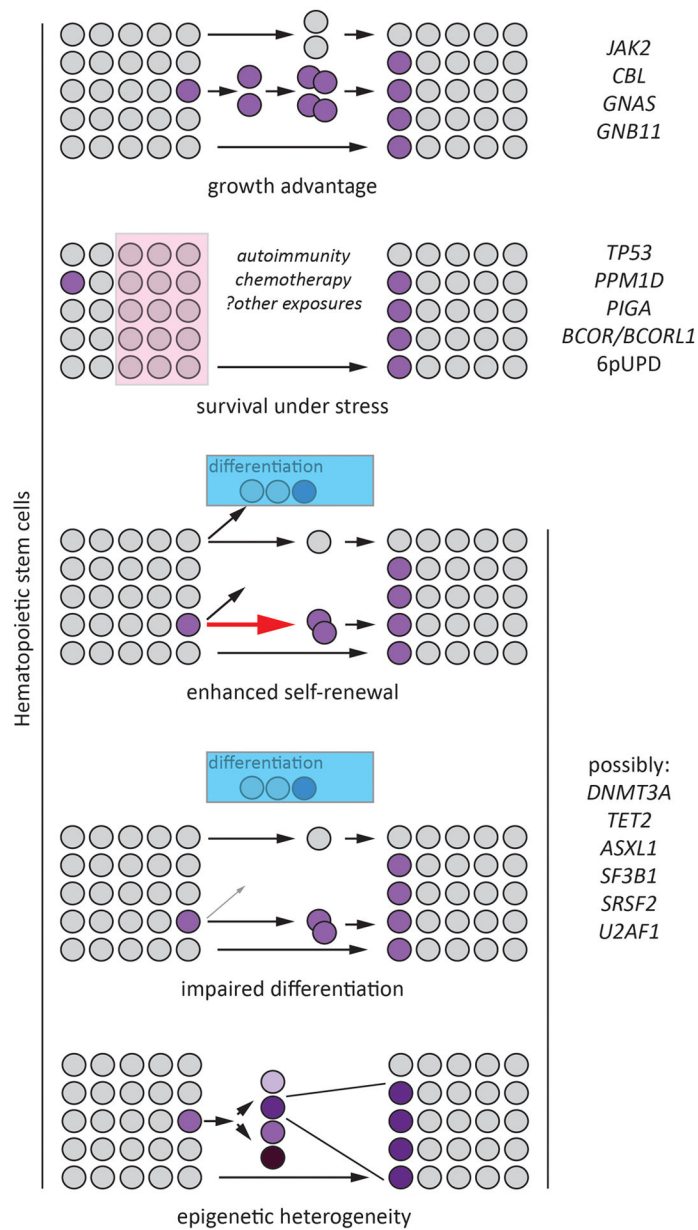


Figure 2: Mechanisms of clonal advantage in HSCs. Red box represents injury and cell death. Enhanced self-renewal and impaired differentiation may have similar HSC dynamics but differences in gene expression that primarily enforce self-renewal or impair differentiation, respectively.

Table 1:

Clinical criteria for idiopathic cytopenia of undetermined significance (ICUS), clonal hematopoiesis of indeterminate potential (CHIP), and myelodysplastic syndrome (MDS). Clonal cytopenia of undetermined significance (CCUS). Adapted from[91].

	Non-clonal ICUS	CHIP		Lower Risk MDS	Higher Risk MDS
Clonality	-	+	+	+	+
Dysplasia	-	-	-	+	+
Cytopenia	+	-	+ (CCUS)	+	+
BM Blast %	<5%	<5%	<5%	<5%	<19%
Overall Risk	Very Low	Very Low	Low (?)	Low	High

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