Experimental Hematology

Experimental Hematology 2020;83:48–56

Clonal hematopoiesis as a model for premalignant changes during aging

David P. Steensma, and Benjamin L. Ebert

Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA

(Received 18 November 2019; revised 6 December 2019; accepted 7 December 2019)

Over the course of the human life span, somatic DNA mutations accumulate in healthy tissues. This process has been most clearly described in blood and bone marrow, esophagus, colon, and skin, but cumulative DNA damage likely affects all tissues of the body. Although most acquired genetic variants have no discernable functional consequences, some randomly occurring somatic mutations confer a relative fitness advantage on a single stem cell and its progeny compared with surrounding cells, which may lead to progressive expansion of a clone (i.e., a genetically identical group of cells). When these mutations occur in a cell with the capacity to self-renew and expand, the mutations persist, and such clonal expansion is a risk factor for further mutation acquisition and clonal evolution. Hematopoietic stem cells are a special case of clonal expansion because both the stem cells and their blood cell progeny circulate in large numbers, and these cells are not subject to some of the anatomical restrictions that characterize other tissues in which somatic mutations conferring a fitness advantage also occur. Therefore, clonally restricted hematopoiesis can have biological and clinical consequences that are distinct from clonal expansions in other tissues. Such consequences include not only clonal progression to overt myeloid neoplasia (or, less commonly, to lymphoid neoplasia) driven by acquisition of secondary mutations in the cells of the expanded clone, but also cardiovascular events and, most likely, other diseases that are influenced by aberrant function of mutant blood cells. A more detailed understanding of how clonal hematopoiesis arises and how clonal selection and expansion occur, as well as development of strategies to avert the clinical consequences associated with clonal hematopoiesis, may both improve public health and yield more general insights into the biology of aging.

One of the most unexpected and interesting developments in the biology of human aging in recent years has been the discovery of the ubiquity of somatic mosaicism in various tissues of older persons [1–4]. Clonal expansion in aging tissues is often driven by some of the same acquired mutations conferring relative fitness that have been previously associated with overt malignancy in those same tissues, yet most people with these mutations do not have malignancy and will never develop a neoplasm [4,5].

Because of the availability of large numbers of cells for serial assessments, blood is especially amenable to study when it comes to investigating somatic mosaicism, with next-generation sequencing platforms providing a method for rapid and sensitive detection of somatic mutations, either in a targeted fashion or via whole-exome or whole-genome approaches. As a result, understanding of the prevalence, pathobiology, and clinical associations of clonal expansion of hematopoietic cells is advancing rapidly, and this has served as a model for parallel processes in other tissues [6–8].

While terminology related to this quickly evolving field is in flux, the term *clonal hematopoiesis* has been the most widely applied shorthand for the state in which one hematopoietic stem cell or progenitor has acquired a somatic mutation that confer an advantage relative to neighboring cells, and then contributes an outsized proportion of blood cell production in comparison to representation among surviving hematopoietic stem cells. This biological state (not a disease, per se) has emerged as a risk factor for both hematological neoplasia and other health problems, including

Offprint requests to: David P. Steensma, Dana-Farber Cancer Institute, D2037, 450 Brookline Ave, Boston, MA 02215; E-mail: david_steensma@dfci.harvard.edu

0301-472X/
https://doi.org/10.1016/j.exphem.2019.12.001

cardiovascular events [9]. In many cases of acute leukemia, in contrast, expansion of clonal cells also occurs, but these cells fail to differentiate and merely inhibit hematopoiesis, and residual blood cell production largely depends on remaining healthy nonclonal cells [10].

One of two specific genes—*DNMT3A* and *TET2*, encoding proteins both of which have roles in DNA methylation—is somatically mutated in most instances of clonal hematopoiesis (Figure 1) [6–8]. These mutations can be detected at a DNA variant allele frequency (VAF; i.e., proportion of abnormal DNA sequencing reads relative to total reads) of at least 2% in the blood of more than 10% of people by age 70 years. As these mutations are heterozygous and loss of heterozygosity is uncommon with these particular alleles, a 2% VAF means that 4% of blood cells are derived from a single clone—a dramatic expansion, given that adult humans have 50,000–200,000 hematopoietic stem cells, so that a neutral somatic variant would be observed at a VAF on the order of 1×10^{-5}. In some cases of clonal hematopoiesis, VAFs as high as 40%–50% are observed, which means that in such cases, a single somatically altered stem cell is giving rise to the majority of the affected person's circulating blood cells [11].

Clonal hematopoiesis predisposes to overt cancer diagnosable using conventional World Health Organization (WHO) clinicopathological criteria [12], similar to somatic expansions in other tissues, but is also associated with increased all-cause mortality and a variety of clinical phenotypes [13]. Another gene, *TP53*, is less commonly mutated than *DNMT3A* or *TET2*, but is a strong predictor of clonal evolution, especially in the presence of cytotoxic chemotherapy or radiotherapy that results in suppression of normal hematopoiesis at the expense of clonal selection of a chemo- and radioresistant *TP53* mutant clone [14–16].

This review summarizes some of the recent developments in clonal hematopoiesis as an exemplar of premalignant changes during human aging. Clonal hematopoiesis must be understood not as an isolated phenomenon but within the context of somatic mutation acquisition in all tissues throughout the human life span. The extent to which clonal states are also a risk factor for various nonneoplastic disorders or altered patterns of response to specific therapies for hematological and other diseases is an area of active research.

A brief history and evolution of terminology related to clonal hematopoiesis

The term *clone* was first used in an agricultural context in 1903 by botanists Herbert Webber and Orator Fuller Cook to describe asexual propagation of plants by transplant or cuttings [17]. In 1912, plant geneticist George Harrison Shull proposed expanding the term to animals; tadpoles were the first nonplant organisms cloned in 1952. By the 1970s, use of the term *clone* had expanded to describe any group of genetically

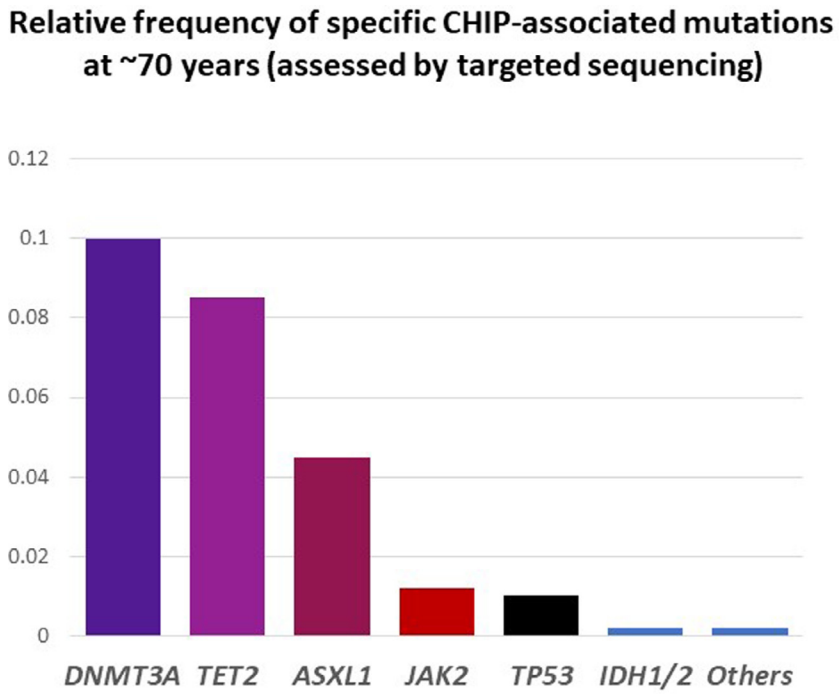

Figure 1. The most common mutations in clonal hematopoiesis of indeterminate potential (CHIP) include *DNMT3A* and *TET2*, followed by *ASXL1, JAK2,* and *TP53*. *IDH1/2* mutations are less common. Dozens of other mutations occur, but these are relatively rare.

identical cells or nucleic acid strands, not just whole organisms [18].

From the 1990s onward, study of serial mutation acquisition in blood cells paralleled analysis of clonality [19]. Analysis of nonrandom X chromosome inactivation in women with informative markers had been used by Philip Fialkow and others since the 1960s to demonstrate clonality in myeloid neoplasms (e.g., that myeloproliferative neoplasms are clonal), and skewed X inactivation had been found to be associated with aging in apparently healthy women [20–22]. In 2012, Lambert Busque and his colleagues detected mutations in *TET2*, a gene that had just been linked to myeloid neoplasia in 2009, in 5% of older women with skewed X chromosome inactivation, but did not find mutations in any younger women or older women with balanced X-chromosome inactivation [23]. In the 1990s, apparently healthy individuals had been noted to have very low levels of BCL translocations that are commonly associated with lymphoma detectable in the blood in the absence of a lymphoid neoplasm, or *BCR-ABL* fusions associated with chronic myeloid leukemia but without leukemia—albeit only transiently and at very low levels (<1% VAF), unlike the more stable and higher allelic burden (>5% VAF) *TET2* mutations [24,25].

Also in 2012, two series reported a high prevalence of large structural variations of chromosomes (>1% in the general population older than age 50), and such somatic mosaicism was associated both with all-cause mortality and also the development of hematologic malignancies [26,27]. In 2014, three groups reported data from a series of whole-exome sequencing studies that had been performed for other purposes, such as to look for predisposing germline alleles to schizophrenia, diabetes, and other prevalent conditions [6–8]. Because blood-derived DNA had been used for these experiments, the analyses were re-purposed to study clonal hematopoiesis collectively in more than 30,000 individuals. This led to the finding that clonal hematopoiesis is common with aging and that clonal hematopoiesis results from a relatively restricted set of genes, including genes commonly associated with hematologic malignancies [28].

In these 2014 series, most mutations observed were in single genes and with a median VAF of almost 10% [7]. The three most common genes mutated were *DMMT3A, TET2,* and *ASXL1; JAK2* and *TP53* were the next most frequently mutated, and there was a long list of rarer mutations present in <1% of individuals with clonal hematopoiesis [29]. As whole-exome sequencing is relatively insensitive to small clones (Figure 2),

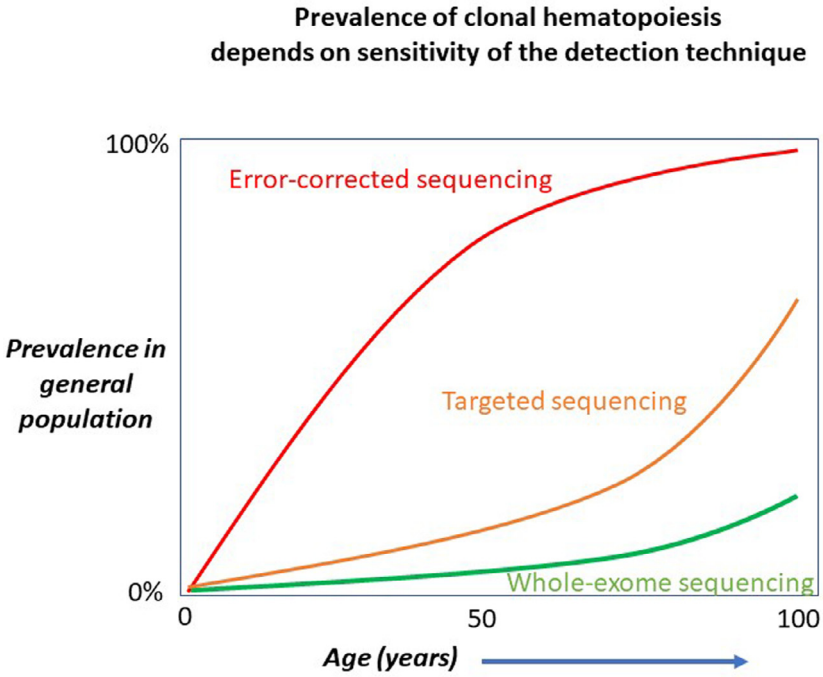

Figure 2. The prevalence of clonal hematopoiesis depends on the analytical sensitivity of the technique used to detect it. With very sensitive techniques, such as error-corrected methods that can detect populations with a variant allele frequency (VAF) of <0.01%, clonal hematopoiesis is almost universally observed by middle age. Whole-exome and whole-genome sequencing techniques typically have less depth of coverage and sensitivity, and may not detect clonal hematopoiesis below VAFs of 7%–10%. Targeted sequencing platforms with >100 × depth of coverage may be able to detect clonal hematopoiesis at the 1%–2% VAF level, and in this setting the prevalence of clonal hematopoiesis exceeds 10% by age 65–70 years of age. Adapted, with permission, from Jaiswal and Ebert [1].

subsequent studies were performed using error-corrected targeted sequencing with a much greater depth of coverage, and these studies demonstrated that clonal hematopoiesis with clones at <1% VAF is almost universal with aging [30,31]. However, it is unclear whether these tiny clones have clinical importance, as the larger clones detected in the whole-exome sequencing analyses do.

Clonal hematopoiesis with aging was also described without any known leukemia-associated driver mutation such as those listed previously, either because the current known catalogue of driver mutations is incomplete, because of mutations in the noncoding genome, or, more speculatively, because epigenetic changes without accompanying DNA mutation may also lead to clonal drift or expansion [6,32]. In addition, initiating clonal changes were detected in large data sets of acute myeloid leukemia (AML) patients years before the AML emerged [31,33,34].

Recognizing a need for a term to describe this biological phenomenon, in 2015 we proposed clonal hematopoiesis of indeterminate potential (CHIP) both to highlight the potential for this state to progress to a hematological neoplasm (~0.5% risk per year) and to distinguish this clonal state from WHO criteria-diagnosable disorders such as myelodysplastic syndromes (MDS), which remain defined by the presence of clinically meaningful cytopenias (by definition, absent in CHIP), dysplastic blood and marrow cell morphology, excess myeloblasts, or somatic chromosomal variants exclusive of several nonspecific alterations such as loss of the Y chromosome or trisomy 8 [35]. Our proposed CHIP definition required individuals to have a VAF of at least 2%, which reflected both technical limitations of next-generation sequencing platforms commonly used in clinical laboratories and the unclear prognostic significance of smaller clones detected only with error-corrected or other very sensitive sequencing techniques.

Aging-related clonal hematopoiesis (ARCH) is another term that has been used to describe an overlapping set of states and that does not require a specific VAF or the presence of a putative leukemia driver mutation [36]. Clonal hematopoiesis is a shorthand that is commonly used to describe all these states, although this usage is somewhat problematic both because overt hematologic malignancies are also defined by a clonally restricted hematopoietic process and because healthy hematopoiesis, in turn, is a consequence of the cumulative contribution of tens of thousands of different clones [37].

Clonal expansion in other tissues

Somatic mosaicism in hematopoietic tissues is just one example of clonal expansion associated with acquisition of somatic mutations, which most likely occurs in every tissue [5]. Although hematopoietic stem cells acquire about one exonic mutation every 10 years [10], the rate of acquisition of mutations in other tissues is less clear (but likely a similar order of magnitude, given that the common epithelial cancers are all most frequent beyond age 50).

For example, when esophageal tissue was sampled from nine normal donors and subjected to deep sequencing, age-associated acquisition of somatic mutations was observed in genes linked to esophageal malignancy, including *NOTCH1, NOTCH2, NOTCH3 ARID1A,* and even *TP53* [38]. Similarly, an RNA sequencing analysis of 29 normal tissues collected from 500 people as part of the Genotype-Tissue Expression (GTEx) project revealed clonal expansion in all tissues examined; skin, esophagus, and lung had the largest number of somatic mutations [5].

A recent detailed study of clonal architecture in the liver that included whole-genome sequencing of 482 microdissections of 100–500 hepatocytes from five normal and nine cirrhotic livers indicated a high incidence of clonal outgrowth in association with fibrosis and cirrhosis, suggesting that the tissue microenvironment may play an important role in initiation or expansion of emergent clones [39]. Experiments supporting this hypothesis with respect to clonal hematopoiesis are described in the section Clinical consequences of CHIP: Neoplasia.

A particularly interesting case is the brain. A dual-platform analysis of 102 genes in 173 adult human brain samples with >5,000-fold depth of sequencing revealed somatic mutations and macroscopic islands of mutated neurons in 50% of brains tested, and the most common mutations observed were in the same two genes that are most frequent in clonal hematopoiesis, *DNMT3A* and *TET2* [40]. Although detection of these specific mutations may be due to contamination of sequenced tissue by blood, the somatic mutations might also be in the microglia. Microglia are not derived from hematopoietic progenitors, but share some features with hematopoiesis-derived cells, including descent from the primitive yolk sac and a tissue scavenging role that resembles that of tissue macrophages.

Thus, it is clear that random accumulation of mutations over time that, in some cases, influences fitness of a certain cell and its progeny and contributes to clonal expansion is not at all unique to hematological cells. However, clonal expansion of hematopoietic tissue has distinct consequences because of the way blood cells circulate in large numbers and interact with other tissues, as described in the section Clinical consequences of CHIP: Other disorders [1].

We do not yet know the rate of acquisition of persistent somatic mutations or the prevalence of clonal hematopoiesis, or clonal expansion in other tissues, in any species besides humans. Clonal hematopoiesis

might be expected to occur in nonhuman primates because of similar hematopoiesis compared with humans and a long life span, but is thus far poorly studied [41]. Clonality testing in veterinary clinical practice is only in its infancy, and further developments are awaited [42].

Risk factors for development of clonal hematopoiesis: Germline and environment

The mechanisms by which clonal hematopoiesis arises may provide insight into why some individuals have clonal expansion earlier or to a greater degree than others. Among the mechanisms of somatic mutation acquisition, the most prevalent by far appears to be single-base-pair change as a result of the ubiquitous spontaneous deamination of methylated cytosine bases at CpG dinucleotides, resulting in a thymine substitution that may avoid DNA repair, rather than the more easily recognized uracil that is created by spontaneous deamination of unmethylated cytosine [43]. The consequence of this type of DNA misrepair is a stable thymine: adenine base pair in a daughter cell, rather than the parental cytosine:guanine paring. As a result, C-to-T transitions are by far the most common variant detected both in clonal hematopoiesis and in clonal expansion in other tissues. Replication errors by DNA polymerase, errors introduced as a result of misrepaired double-strand DNA breaks leading to small insertion-deletions and large chromosomal structural rearrangements also occur but are less common than single-base-pair changes.

Although clonal emergence with aging may be driven to some extent by chance/stochastic processes, several germline variants have been associated with an increased likelihood of clonal hematopoiesis. For instance, germline loss of the DNA glycosylase methyl-CpG binding domain 4 (MBD4), an enzyme that contributes to repair of DNA damage resulting from 5-methylcytosine deamination described above, not only increases the risk of clonal hematopoiesis and of MDS/AML, but also results in a more than 30-fold higher mutation burden (enriched for C-to-T transitions) in AML cells in affected individuals compared with AML generally [44]. MBD4 germline loss is rare, however, representing only 9 cases of among 10,683 TCGA database patients, and it may be that other more common but less highly penetrant germline DNA repair polymorphisms contribute to the risk for clonal hematopoiesis.

An analysis of mosaic chromosomal alterations in the 151,202-participant UK Biobank revealed three loci at which germline variants were associated with *cis* chromosomal deletions or with loss of heterozygosity: *MPL* (encoding the thrombopoietin receptor) and two genes about which much less is known, *FRA10B* and *TM2D3-TARSL2* [45]. Another germline variant predisposing to aging-associated clonal hematopoiesis emerged from an analysis of 11,262 participants in the Iceland deCODE project, in which the presence of more than 20 mosaic somatic variants was used to classify patients as whole-genome sequencing "outliers" and therefore having clonal hematopoiesis, even in the absence of a mutation in a putative leukemia driver gene [32]. In the deCODE population, a germline intron 3 deletion of the gene encoding telomerase reverse transcriptase (*TERT*), important for telomere length maintenance, was found to predispose to clonal hematopoiesis ($p = 7.4 \times 10^{-12}$, with an odds ratio of 1.37)—yet, perhaps surprisingly, this variant was not associated with shorter telomeres in affected individuals. Finally, in another UK Biobank analysis, somatic loss of the Y chromosome in leukocytes was associated with more than 150 genetic variants (and with an increased likelihood of developing nonmyeloid cancers including prostate adenocarcinoma, germ cell tumors, and renal cell carcinoma), and this finding was validated in an additional population of 757,114 men of Japanese or European ancestry [46].

Further insight into germline predisposition to clonal hematopoiesis emerged from a German study of 500 allogeneic hematopoietic cell transplant-related donors aged 55 years or older [47]. Clonal hematopoiesis was present in 16% of donors, with a median VAF of 5.9%, and was associated with more chronic graft-versus-host disease and also a trend toward higher nonrelapse mortality—but lower neoplastic disease relapse, such that the net overall survival was unaffected by the clonal hematopoiesis state of the donor. Intriguingly, clonal hematopoiesis was present in 19.2% of related donors for recipients with myeloid malignancies compared with 6.3% for siblings with lymphoid neoplasms. Given the myeloid bias of clonal evolution in CHIP, this suggests a mutual germline predisposition to clonal states in both donor and recipient, with a possible contribution from a shared environment.

Clonal hematopoiesis is slightly less common in people of Hispanic ethnicity and is seen with increased frequency in males and in cigarette smokers [6,7]. The environment may also have an influence on the likelihood of acquiring clonal hematopoiesis. The effect of the environment on clonal outgrowth is also supported by a murine model in which Tet2 null mice (which at baseline have increased gut permeability to bacteria compared with wild-type mice) underwent either intestinal barrier disruption to increase bacterial translocation or administration of a toll-like receptor 2 agonist, mimicking systemic bacterial infection [48]. The mice treated in this way developed myeloproliferation with a severity that correlated with serum interleukin-6 levels. In contrast, Tet2 null mice that underwent intestinal detoxification or were raised in a germ-free environment mostly remained healthy. There are no data yet on the effect of the microbiome on clonal hematopoiesis in humans.

Risk factors for clonal expansion may differ from those contributing to clone origination. For instance, chemotherapy and radiotherapy suppress normal hematopoiesis to a greater extent than they do hematopoiesis from cells with clonal mutations of *TP53* or *PPM1D*, and patients with *TP53* mutant clonal hematopoiesis who undergo cytotoxic therapy are at greatly increased risk for therapy-related myeloid neoplasms [49]. The fact that in many cases clonal hematopoiesis is stable for years indicates that the expansion is being held in check, either because of a limitation on the microenvironmental niche that can be occupied by these cells until they acquire a stronger leukemic driver mutation or because of an endogenous immune response.

Clinical consequences of CHIP: Neoplasia

CHIP predisposes to neoplasia, mostly (but not exclusively) myeloid neoplasms [13]. This is thought to usually result from acquisition of a stronger driver mutation on a background CHIP clone. For instance, an illustrative case was described in 2018 in which a 45-year-old man had CHIP with three different truncating *TET2* mutations and a frame-shifting *ASXL1* mutation all at >20% VAF, then acquired an *RHOA* mutation and developed angioimmunoblastic T-cell lymphoma [11]. After successful treatment of the lymphoma, the following year the same patient acquired an *NPM1* mutation on the same CHIP background and developed AML.

There is overlap between clonal hematopoiesis and the concept of minimal residual disease. In a European cooperative group study of patients treated with intensive therapy for acute myeloid leukemia, for instance, treated patients in whom the only detectable mutation after achieving clinical–pathological complete remission was a "CHIP mutation" (*DNMT3A, TET2,* or *ASXL1*) had a low incidence of recurrent disease after 4 years, whereas most of those who still had detectable stronger leukemia drivers (e.g., *FLT3, IDH1/2, NPM1, RUNX1)* soon relapsed [50].

In the context of acquired aplastic anemia, the pathophysiology of which is usually a T cell–directed immune attack against hematopoietic stem cells, clonal mutations frequently emerge, again likely because of the relative fitness of cells bearing those mutations compared with neighboring cells, albeit in a markedly abnormal marrow environment [51]. Mutations in *PIGA, BCORL1,* or *BCOR* are associated with a response to anti–T-cell immunotherapy and a better prognosis, whereas other clonal mutations such as *DNMT3A* and Ras pathway mutations are associated with nonresponse to immunotherapy and with a greater likelihood of eventual clonal progression to MDS or another state.

After stem cell transplantation, donor CHIP (i.e., a mutant clone derived from the allogeneic donor that engrafts in the recipient) contributes to cytopenias, and may also result in donor-derived leukemia [52]. After autologous hematopoietic cell transplant, too, clonal hematopoiesis is associated with adverse outcomes [53,54].

Clonal hematopoiesis can cause diagnostic classification confusion in patients with cytopenias and a nondiagnostic bone marrow morphology. The high frequency of CHIP in the general older population means that some patients may have cytopenias and mild morphologic changes due to reactive nonclonal cause together with an unrelated clonal process, which may cause uncertainty about the relative contribution of the clonal process to the cytopenia [55]. Given the large number of factors other than clonal hematopoiesis that can contribute to cytopenias, it may be difficult for clinicians to thoroughly rule out a concomitant nonclonal process in a patient who has both unexplained cytopenias and clonal hematopoiesis.

Individuals with unexplained cytopenias who have clonal mutations in genes associated with CHIP and MDS, however, are at increased risk for subsequent progression, with mutations in spliceosome genes and those with multiple mutations at high VAF (>20%) at greatest risk. Among patients with unexplained cytopenias, one multicenter analysis indicated that a coexistent clonal mutation resulted in a hazard ratio of 13 for progression to WHO-diagnosable MDS or another myeloid malignancy, compared with patients with idiopathic cytopenias and no mutation [56].

Clinical consequences of CHIP: Other disorders

Individuals with CHIP also have an increased risk of a cardiovascular event [57–59]. This risk (hazard ratio ~1.8 compared with controls without clonal hematopoiesis) is of a similar order of magnitude as well-established modifiable cardiovascular risk factors including cigarette smoking, hypertension, and hyperlipidemia. The pathophysiology of this risk has been extensively reviewed elsewhere [57,58,60], but briefly, murine models indicate that clonally derived monocytes and macrophages infiltrate atherosclerotic plaques and set up a pro-inflammatory reaction that recruits more pro-inflammatory macrophages, injures the endothelium, and accelerates atherogenesis. This process can be blocked either in experimental models with NLRP3 inflammasome inhibition or clinically with anti-cytokine therapy [59,61]. In a randomized, multicenter placebo-controlled clinical trial of more than 10,000 patients who had experienced myocardial infarction and still had an elevated C-reactive protein, treatment with canakinumab, an antibody against interleukin-1β, reduced recurrent cardiovascular events compared with placebo, and the greatest relative risk reduction with canakinumab therapy was seen in those

patients who had CHIP, especially *TET2* mutant clonal hematopoiesis [62,63].

Cardiovascular outcomes beyond atherosclerosis also appear to be influenced by CHIP. For instance, in one analysis of CHIP-associated JAK2 mutations, the risk of venous thrombosis was markedly increased compared with the risk in those without JAK2 clonal hematopoiesis [64]. Congestive heart failure outcomes are also worse among patients with CHIP, probably because of the locally altered myocardial remodeling driven by pro-inflammatory clonal macrophages [65].

In one study of 83 geriatric patients, CHIP was associated with increased frailty and increased levels of interleukin-6, tumor necrosis factor α and interleukin-8, though whether this association represents cause-and-effect or merely coexistence of different markers of accelerated aging is unclear [66]. One exceptional responder to chimeric antigen receptor T-cell therapy resulted from disruption of a copy of *TET2* in one of the engineered T cells, accompanied by cell expansion [67]. At present, it is not clear whether clonal hematopoiesis is associated with clinical conditions other than neoplasia and cardiovascular events [68].

The high frequency with which patients are found to have CHIP during genetic analysis of non-myeloid neoplasms [14,29], including germline testing of hereditary breast and other cancer syndromes, has prompted creation of clinics dedicated to patients with clonal hematopoiesis and other hematologic malignancy precursor conditions [69]. Our institute started a dedicated hematological neoplasm precursor condition clinic in 2019 to counsel such patients, who are understandably often quite anxious when they learn they have a mutation that can predispose to leukemia but is more likely to contribute to a myocardial infarction or stroke. In the future, it is essential to design interventions both to try to eliminate emergent clones and to try to reduce the cardiovascular and other consequences of clonally derived cells.

Key unanswered questions

Despite the rapid progress in this field, a number of important questions remain about clonal hematopoiesis and, by extension, about somatic variation in other tissues. In addition to the range of disease states associated with clonal hematopoiesis, the reason for long-term stability of clones in some patients and the drivers of both clonal emergence and clonal evolution are incompletely understood. The relative risk of different CHIP mutations for both cardiovascular disease and clonal progression is also not well understood, and it is unclear whether clonal expansion can result purely from epigenetic changes without somatic DNA changes at either the chromosome or single-nucleotide levels. In addition, clinical consequences of clonal expansion in other tissues beyond predisposal to neoplasia risk are almost entirely unknown.

Conclusions

Clonal hematopoiesis is just one example of clonal expansion caused by somatic mutation acquisition in aging tissue, but it is a clonal expansion with special consequences including pro-inflammatory interactions with nonhematopoietic cells, as blood cells circulate and are not subject to the same anatomical restrictions that characterize clonal expansion in other tissues, which in contrast would be expected to have only more local effects. Given the high frequency at which clonal hematopoiesis is observed and the magnitude of cardiovascular risk associated with clonal hematopoiesis, the possibility of using anti-inflammatory approaches to prevent primary or secondary cardiovascular events and improve patient outcomes is attractive from a public health standpoint. Many investigative groups are actively searching for additional clinical associations.

Conflict of interest disclosure

DPS is on the data safety monitoring committee or has done consulting related to clinical trials at Celgene, H3 Biosciences, Janssen, Onconova, Otsuka, and Acceleron. BLE has received research funding from Celgene and Deerfield. He has received consulting fees from GRAIL, and he serves on the scientific advisory boards for and holds equity in Skyhawk Therapeutics and Exo Therapeutics.

Acknowledgments

DPS and BLE are supported by the Edward P. Evans Foundation and by National Institutes of Health (NIH) SPORE Grant P50CA206963. BLE is a Howard Hughes investigator and is supported by the Deerfield Foundation and by NIH Grants P01CA066996 and R01HL082945. DPS is also supported by the James & Lois Champy Fund.

References

1. Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. Science. 2019;366.
2. Hoang ML, Kinde I, Tomasetti C, et al. Genome-wide quantification of rare somatic mutations in normal human tissues using massively parallel sequencing. Proc Natl Acad Sci U S A. 2016;113:9846–9851.
3. Blokzijl F, de Ligt J, Jager M, et al. Tissue-specific mutation accumulation in human adult stem cells during life. Nature. 2016;538:260–264.
4. Martincorena I. Somatic mutation and clonal expansions in human tissues. Genome Med. 2019;11:35.
5. Yizhak K, Aguet F, Kim J, et al. RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. Science. 2019;364.
6. Genovese G, Kähler AK, Rose SA, et al. Clonal hematopoiesis and cancer risk in blood derived DNA sequence. N Engl J Med. 2014;371:2477–2487.

7. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488–2498.
8. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20. 1472−148.
9. Luis TC, Wilkinson AC, Beerman I, Jaiswal S, Shlush LI. Biological implications of clonal hematopoiesis. Exp Hematol. 2019;77:1–5.
10. Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell. 2012;150:264–278.
11. Tiacci E, Venanzi A, Ascani S, et al. High-Risk Clonal hematopoiesis as the origin of AITL and NPM1-mutated AML. N Engl J Med. 2018;379:981–984.
12. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–2405.
13. Bowman RL, Busque L, Levine RL. Clonal hematopoiesis and evolution to hematopoietic malignancies. Cell Stem Cell. 2018;22:157–170.
14. Coombs CC, Zehir A, Devlin SM, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell Stem Cell. 2017;21:374–382.e4.
15. Takahashi K, Wang F, Kantarjian H, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. Lancet Oncol. 2017;18:100–111.
16. Gillis NK, Ball M, Zhang Q, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. Lancet Oncol. 2017;18:112–121.
17. Webber HJ. New horticultural and agricultural terms. Science. 1903;18:501–503.
18. Steensma DP. The origin and evolution of the term "clone". Leuk Res. 2017;57:97–101.
19. Busque L, Mio R, Mattioli J, et al. Nonrandom X-inactivation patterns in normal females: lyonization ratios vary with age. Blood. 1996;88:59–65.
20. Fey MF, Liechti-Gallati S, von Rohr A, et al. Clonality and X-inactivation patterns in hematopoietic cell populations detected by the highly informative M27 beta DNA probe. Blood. 1994;83:931–938.
21. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem-cell and probable clonal origin of the disease. N Engl J Med. 1976;295:913–916.
22. Barr RD, Fialkow PJ. Clonal origin of chronic myelocytic leukemia. N Engl J Med. 1973;289:307–309.
23. Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat Genet. 2012;44:1179–1181.
24. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. Proc Natl Acad Sci U S A. 1994;91:8910–8914.
25. Biernaux C, Loos M, Sels A, Huez G, Stryckmans P. Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. Blood. 1995;86:3118–3122.
26. Laurie CC, Laurie CA, Rice K, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat Genet. 2012;44:642–650.
27. Jacobs KB, Yeager M, Zhou W, et al. Detectable clonal mosaicism and its relationship to aging and cancer. Nat Genet. 2012;44:651–658.
28. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374:2209–2221.
29. Hu Y, Ulrich BC, Supplee J, et al. False-positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res. 2018;24:4437–4443.
30. McKerrell T, Park N, Moreno T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. Cell Rep. 2015;10:1239–1245.
31. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nat Commun. 2016;7:12484.
32. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood. 2017;130:742–752.
33. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559:400–404.
34. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506:328–333.
35. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16.
36. Shlush LI. Age-related clonal hematopoiesis. Blood. 2018;131:496–504.
37. Gibson CJ, Steensma DP. New insights from studies of clonal hematopoiesis. Clin Cancer Res. 2018;24:4633–4642.
38. Martincorena I, Fowler JC, Wabik A, et al. Somatic mutant clones colonize the human esophagus with age. Science. 2018;362:911–917.
39. Brunner SF, Roberts ND, Wylie LA, et al. Somatic mutations and clonal dynamics in healthy and cirrhotic human liver. Nature. 2019;574:538–542.
40. Keogh MJ, Wei W, Aryaman J, et al. High prevalence of focal and multi-focal somatic genetic variants in the human brain. Nat Commun. 2018;9:4257.
41. Yu KR, Espinoza DA, Wu C, et al. The impact of aging on primate hematopoiesis as interrogated by clonal tracking. Blood. 2018;131:1195–1205.
42. Keller SM, Vernau W, Moore PF. Clonality testing in veterinary medicine: a review with diagnostic guidelines. Vet Pathol. 2016;53:711–725.
43. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature. 2013;500:415–421.
44. Sanders MA, Chew E, Flensburg C, et al. MBD4 guards against methylation damage and germ line deficiency predisposes to clonal hematopoiesis and early onset AML. Blood. 2018;132:1526–1534.
45. Loh PR, Genovese G, Handsaker RE, et al. Insights into clonal haematopoiesis from 8,342 mosaic chromosomal alterations. Nature. 2018;559:350–355.
46. Thompson DJ, Genovese G, Halvardson J, et al. Genetic predisposition to mosaic Y chromosome loss in blood. Nature. 2019;575:652–657.
47. Frick M, Chan W, Arends CM, et al. Role of donor clonal hematopoiesis in allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2019;37:375–385.
48. Meisel M, Hinterleitner R, Pacis A, et al. Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. Nature. 2018;557:580–584.
49. Hsu JI, Dayaram T, Tovy A, et al. PPM1D mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. Cell Stem Cell. 2018;23:700–713.e6.
50. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. N Engl J Med. 2018;378:1189–1199.

51. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373:35–47.
52. Gibson CJ, Kennedy JA, Nikiforow S, et al. Donor-engrafted CHIP is common among stem cell transplant recipients with unexplained cytopenias. Blood. 2017;130:91–94.
53. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J Clin Oncol. 2017;35:1598–1605.
54. Mouhieddine TH, Park J, Redd R, et al. Immunomodulator maintenance post autologous stem cell transplant predicts better outcome in multiple myeloma patients with clonal hematopoiesis of indeterminate potential [Abstract 2954]. Cancer Res. 2018;78:2954.
55. Kwok B, Hall JM, Witte JS, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. 2015;126:2355–2361.
56. Malcovati L, Galli A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood. 2017;129:3371–3378.
57. Khetarpal SA, Qamar A, Bick AG, et al. Clonal hematopoiesis of indeterminate potential reshapes age-related CVD: JACC review topic of the week. J Am Coll Cardiol. 2019;74:578–586.
58. Libby P, Sidlow R, Lin AE, et al. Clonal hematopoiesis: crossroads of aging, cardiovascular disease, and cancer: JACC review topic of the week. J Am Coll Cardiol. 2019;74:567–577.
59. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med. 2017;377:111–121.
60. Jaiswal S, Libby P. Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease [Epub ahead of print] Nat Rev Cardiol. 2019.
61. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science. 2017;355:842–847.
62. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med. 2017;377:1119–1131.
63. Svensson EC, Madar A, Campbell CD, et al. TET2-driven clonal hematopoiesis predicts enhanced response to canakinumab in the CANTOS trial: an exploratory analysis [Abstract 15111]. Circulation. 2018;138. A15111-A.
64. Wolach O, Sellar RS, Martinod K, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci Transl Med. 2018;10.
65. Dorsheimer L, Assmus B, Rasper T, et al. Association of mutations contributing to clonal hematopoiesis with prognosis in chronic ischemic heart failure. JAMA Cardiol. 2019;4:25–33.
66. Cook EK, Izukawa T, Young S, et al. Comorbid and inflammatory characteristics of genetic subtypes of clonal hematopoiesis. Blood Adv. 2019;3:2482–2486.
67. Fraietta JA, Nobles CL, Sammons MA, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature. 2018;558:307–312.
68. Arends CM, Weiss M, Christen F, et al. Clonal hematopoiesis in patients with ANCA-associated vasculitis [Epub ahead of print] Haematologica. 2019.
69. Bolton KL, Gillis NK, Coombs CC, et al. Managing clonal hematopoiesis in patients with solid tumors. J Clin Oncol. 2019;37:7–11.