New Insights from Studies of Clonal Hematopoiesis
Christopher J. Gibson and David P. Steensma

Abstract
Clonal hematopoiesis (CH) describes an asymptomatic expansion of blood cells descended from a single hematopoietic stem cell. Recent studies have shown that CH increases in frequency with aging and is often driven by somatic mutations in genes that are recurrently mutated in hematologic malignancies. When CH is associated with a mutation in a leukemia-associated gene at a variant allele frequency of 0.02 or greater, it is termed "clonal hematopoiesis of indeterminate potential" (CHIP). CHIP has a 0.5% to 1% risk per year of progression to hematologic neoplasm, and increases both all-cause mortality and the risk of myocardial infarction and ischemic stroke due to a proinflammatory interaction between clonally derived leukocytes and vascular endothelium. CH frequently emerges in the context of immune-mediated marrow failure syndromes such as aplastic anemia, whereas CH emerging after cytotoxic cancer therapy is strongly associated with subsequent development of a therapy-related myeloid neoplasm, especially if a TP53 mutation is present. However, risk factors for developing CH other than aging, marrow failure, and cytotoxic radiotherapy or chemotherapy are poorly defined. In this review, we discuss the epidemiology, molecular mechanisms, and clinical consequences of this common and clinically important biological state.

Introduction
The transformation of a normal cell into a malignant cell requires sequential acquisition of multiple genetic alterations over the course of many cell divisions (1–4). Many tissues harbor precancerous populations of cells that have acquired only a subset of alterations necessary for full malignant transformation. In clinical practice, screening for these precursor lesions [e.g., atypical nevi (5), adenomatous colon polyps (6), dysplastic cervical lesions (7)], and preemptive resection to prevent progression to an invasive neoplasm, is an important component of preventive care.

In the blood, where precursor lesions do not present as a visible process such as a polyp or nodule, investigators have instead focused on detecting evidence of oligoclonality as a proxy for premalignant potential. In the 1990s, X-chromosome inactivation patterns in blood cells were found to be skewed ≥3:1 in 40% of women over age 60, but such skewing was rare in newborn girls, consistent with the hypothesis that genetic events capable of driving clonal expansion of hematopoietic cells occur stochastically and accumulate over time (8).

In 2012, sequencing of blood from older women with skewed X-inactivation ratios showed that approximately 5% of such women harbored somatic mutations in TET2, a gene encoding an epigenetic modifier that is recurrently mutated in several hematologic malignancies (HM; ref. 9). Two other studies in 2012 showed that some adults harbor mosaic chromosomal copy-number changes in their blood, similarly indicative of somatic clonal expansion (10, 11). However, the strongest evidence that clonal hematopoiesis (CH) is prevalent in healthy aging populations came in late 2014 with the publication of three high-profile articles (12–14). Features of these and other important recent CH studies are summarized in Table 1.

Describing CH
The three 2014 studies took advantage of a deep understanding of genomics to identify CH using existing sets of next-generation sequencing data from completed studies directed at answering other questions. Two of those studies were exome sequencing efforts aimed at identifying germline risk variants for diabetes (13) and schizophrenia (14), whereas a third included control blood samples paired with exome sequencing of solid tumors performed for The Cancer Genome Atlas (12). Each study included thousands of subjects. The core genomics concepts underlying these studies are central to understanding CH and are summarized in Figs. 1 and 2. A central point of these figures is that, because the sequencing methodology and depth vary between studies of CH, the types of clones discovered, and the reported rates of CH, vary as well, making it difficult to directly compare many of the studies to one another.

Because a number of research groups around the world have published influential studies in this area of biology in rapid succession, a variety of terms have been applied with differing degrees of stringency to a spectrum of clonal hematopoietic states, which has created an inconsistent lexicon that may cause confusion. For clarity, in this review, we will use the following terminology:

Clonal hematopoiesis (CH): refers generally to any clonal expansion of hematopoietic cells, detected by any method. CH alone is not known to carry any clinical or prognostic implication.

Clonal hematopoiesis of indeterminate potential (CHIP): refers specifically to CH defined by recurrent mutations in driver
Yoshizato 2015 Analyzed 668 PB samples from 439 patients with aplastic anemia, including serial samples from 82 patients.

Steensma 2015 Defined "CHIP" as mutation in leukemic driver with VAF of >0.02 but not meeting WHO criteria for HM.

Jaiswal 2014 Analyzed whole-exome sequencing data from PB-derived DNA of 17,182 people, focusing on 160 genes recurrently mutated in HM. Clonal mutations were observed in 9.6% of people aged 70–79, 11.7% of people aged 80–89, and 18.4% of people 90 or older. A somatic mutation was associated with increased risk of developing HM (HR 1.1), increased all-cause mortality (HR 1.4), increased myocardial infarction (HR 2.0), and increased ischemic stroke (HR 2.6).

Genovese 2014 Analyzed whole-exome sequencing data from PB-derived DNA from 12,380 individuals; 10% over 65 years of age, but only 1% younger than 50, exhibited CH. CH was a strong risk factor for subsequent HM (HR 12) and death (HR 1.4).

Xie 2014 Analyzed blood-derived sequence data from 2,728 individuals from TCGA. PB cells of more than 2% of subjects (5–6 >70 years) contained mutations, often leukemia-associated genes.

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Young 2016 Used targeted error-corrected sequencing, which can detect clonal mutations as rare as 0.0003 VAF. Using this method, CH was found in 95% of PB samples from healthy 50–60-year-old participants in the Nurses’ Health Study.

Zink 2017 Performed whole-genome sequencing of 11,262 Icelanders and found 1,403 cases of CH, including almost all elderly persons studied. Mutations in recognized leukemia driver genes were evident in only a fraction of CH cases, but increased mortality was associated with CH regardless of whether a driver gene was present.

Gibson 2017 Whole-exome sequencing on pre- and post-ASCT samples from 12 patients who developed t-MN after autologous transplantation for Hodgkin lymphoma or non-Hodgkin lymphoma; targeted sequencing on cryopreserved aliquots of autologous stem cell products from 401 patients who underwent ASCT for non-Hodgkin lymphoma. Patients with CHIP had inferior overall survival compared with those without CHIP (10-year overall survival, 30.4% vs. 60.9%, respectively), including increased risk of death from t-MN and cardiovascular disease.

Gillis 2017 Among 13 cases of t-MN and 56 case–matched controls, CHIP at the time of the original tumor treatment was more common in cases and 38% of those with t-MN had preexisting TPS3 mutations.

Takahashi 2017 Among 14 cases of t-MN and 54 controls, CH was found at the time of the original tumor diagnosis in 71% of patients and 31% of controls.

Jaiswal 2017 Whole-exome sequencing to detect CHIP in PB cells in 4,726 participants with coronary heart disease and 3,529 controls. People with CHIP had a risk of myocardial infarction that was 4.0 times as great as in noncarriers. Mutations in DNMT3A, TET2, ASXL1, and JAK2 were each individually associated with non-Hodgkin lymphoma. Patients with CHIP had inferior overall survival compared with those without CHIP (10-year overall survival, 30.4% vs. 60.9%, respectively), including increased risk of death from t-MN and cardiovascular disease.

Coombs 2017 Performed deep-coverage targeted sequencing of paired tumor and blood samples from 8,810 individuals and identified CH in 25% of patients with cancer, including 4.5% with leukemia driver mutations (CH-PD). CH was associated with increased age, prior radiation therapy, and tobacco use. PPM1D and TP53 mutations were associated with prior exposure to chemotherapy, CH and CH-PD were associated with subsequent emergence of hematologic cancers, and CH-PD was associated with shorter survival.

Table 1. Summary of major studies of CH

<table>
<thead>
<tr>
<th>First author and year of publication</th>
<th>Major finding</th>
<th>Citations as of February 1, 2018*</th>
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<tbody>
<tr>
<td>Busque 2012</td>
<td>Exome sequencing of three older women with CH identified somatic TET2 mutations. Focused sequencing then identified TET2 mutations in 10 of 182 older women with skewed X-inactivation.</td>
<td>243</td>
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<td>Jacobs 2012</td>
<td>Analysis of chromosomal abnormalities in 3,717 cancer cases and 26,136 cancer-free controls from 13 GWAS.</td>
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<td>Laurie 2012</td>
<td>Using blood SNP microarray data from over 50,000 GWAS subjects, detectable clonal mosaicism was found to be rare (&lt;0.5%) before age 50 but increases to 3% in the elderly.</td>
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Abbreviations: ASCT, autologous stem cell transplant; GWAS, genome-wide association studies; HM, hematologic malignancy; HR, hazard ratio; Mb, megabases; PB, peripheral blood; SNP, single-nucleotide polymorphism; TCGA, The Cancer Genome Atlas; t-MN, therapy-related myeloid neoplasm; VAF, variant allele fraction; WHO, World Health Organization.

*All studies mentioned above are included in this publication’s references. Citation assessment using Thomson Web of Science Citation Index; more recent articles have not yet had to have large numbers of citations.

genes associated with HM, detected at a variant allele fraction (VAF) of at least 0.02 in adults with no known history of HM (15). This definition reflects accumulating evidence that adverse clinical consequences of CH are affected both by the biology of the specific genetic mutations present in the clone and by the size of the clone, and that these consequences are most likely to develop in patients with CH meeting CHIP criteria. We prefer "CHIP" to another commonly used term, "age-related CH" (ARCH; ref. 16). First, "indeterminate potential" emphasizes the possible clinical implications of CH meeting the specific criteria for CHIP. Second, although aging is strongly associated with increased incidence of CH, CHIP sometimes occurs in younger people as well. (An analogous example would be referring to "age-related atherosclerosis" or "age-related dementia.") Finally, all types of CH—including CHIP—increase in prevalence with advancing age, so it is not clear which specific groups "ARCH" should refer to.

In certain cases, it may also be useful to refer to "CH with known drivers" (of HM) or "CH with unknown drivers." CHIP is a subset of CH with known drivers, but it is possible to detect CH with
known drivers that does not meet the specific criteria for CHIP—for example, small hematopoietic clones marked by DNMT3A and TET2 mutations occurring at VAFs lower than 0.02, which are very common in middle-age adults (17). CH with unknown drivers, on the other hand, is defined by an accumulation of mutations not recurrent in large data sets (14, 18). CH without a driver mutation might in some cases arise as a result of waning hematopoietic stem cell (HSC) fitness, such that by old age an individual’s hematopoiesis is derived from fewer individual HSCs and thus has become more oligoclonal simply by attrition.

An important part of the terminology surrounding CH is the emerging body of evidence that its clinical implications are context dependent. For example, CH emerging following cytotoxic therapy for a solid tumor likely has a different biology and distinct clinical implications compared with CH emerging in the setting of aplastic anemia (AA; refs. 19–22). Patients with cancer and CH deserve unique attention, since the definition of CHIP specifically excludes those with one subgroup of cancers, HM. In a patient with HM, it cannot usually be concluded from bulk sequencing at a single time point whether a specific mutation is part of a malignant clone or subclone, or instead represents a distinct clonal process unrelated to the malignancy (23).

**Epidemiology and Characteristics of CH**

At clone sizes detectable by whole-exome sequencing, the incidence of CH increases substantially with advancing age (12–14, 18–20, 24). CH is uncommon in young individuals, detectable in <1% of those younger than age 40 years. However, CH and CHIP may be enriched in certain patient subgroups. For example, CHIP is present in about 2% of patients with myocardial infarctions (MI) occurring before age 50 (25). Conversely, CHIP is present in 10% to 15% of individuals age 70 or older, and CH with unknown drivers is even more common in these older populations.
common (>30% in the 70+ age group, with frequency depending on the study and method of analysis; refs. 14, 18).

Cell of origin

CH was originally presumed to originate from HSCs, because it was reasoned that such a large clonal expansion must originate in a multipotent cell with capacity for self-renewal. However, since the 2014 studies that led to the definition of CHIP were performed on bulk sequencing of blood, detected genetic alterations might also have occurred in shorter lived or more differentiated precursor cells. More recently, though, lineage sorting and sequencing of leukocyte subsets in patients with CHIP have shown that the driver mutations can be found either across multiple lineages (myeloid, T, and B; refs. 17, 26, 27) or in the Lin−CD34−CD38− cell fraction (28). Mutations are durable and persist across multiple time points, also supporting the model of a long-lived, canonical HSC as the usual cell of origin for CH (17, 26–28).

Common mechanisms of mutation

Most CH-associated mutations likely occur stochastically over the course of an HSC’s lifespan, and the accumulation of mutations over time is responsible for the strong association between increasing age and emergence of clonal expansion. In CH, 55% to 65% of observed point mutations are C>T transitions—mutations that occur as a consequence of passive deamination of cytosine to uracil with misrepair back to thymidine, which constitute the dominant type of point mutation in most aging tissues (12, 13, 19, 20).

Epidemiologic features

CHIP is more common in men than women (13), which comports with an increased risk of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) in men compared with women. In addition, a slightly lower prevalence of CHIP has been reported among individuals of Hispanic ancestry compared with those of other ethnic backgrounds, corresponding to a lower published incidence of myeloid neoplasms other than acute promyelocytic leukemia among patients of Hispanic background (13).

The identification of ethnic associations with CHIP raises the possibility that there are inherited factors associated with its development. These could predispose to CHIP either by...
increasing the chance of developing a mutation, or by contributing either cell-intrinsic or cell-extrinsic contexts that reinforce selective advantages conferred by specific mutations. The best evidence so far for a germline risk is the identification of an 8-bp intronic deletion in TERT associated with a 37% increased risk of developing CH without known drivers (18). The mechanism by which the variant increases the risk of CH is not clear; although TERT encodes a component of the telomerase complex, this variant is not associated with decreased telomere length.

With respect to environmental exposures, cigarette smoking has a clear association with both CH and CHIP (13, 14, 20). The mechanism of this association may reflect accelerated mutagenesis driven by exposure to cigarette smoke. One recent study of CHIP in patients with solid tumors showed that, among current or former smokers, the point mutations defining CHIP were enriched for C>A substitutions, a mutation signature associated with smoking but only in those who had not received prior cytotoxic therapy (20).

**Characteristic distribution of mutations**

The mutations that define subsets of CH with known drivers occur in a characteristic set of genes at relative frequencies that are remarkably preserved across studies of unselected populations. DNMT3A is invariably the most commonly mutated gene, followed by TET2 and ASXL1. Other recurrently mutated genes include JAK2, PPM1D, TP53, SF3B1, SRSF2, CBL, and GNB1 (Table 2; refs. 12–14). Each gene displays a distinct mutational pattern that reflects the functional impact of the mutations. The mechanism by which these genes drive clonal expansion when mutated may differ depending on the gene. Although beyond the scope of this review, the molecular consequences of these mutations have been described in detail elsewhere. In particular, DNMT3A (29), TET2 (30), and ASXL1 (31) are all involved in epigenetic regulation of gene expression, and pathogenic mutations in these genes may confer subtle but pleiotropic effects that improve self-renewal capabilities in affected HSCs.

The large cohorts in which CHIP was identified were partially but not exhaustively annotated for clinical characteristics. It is thus possible that some of the mutations identified in these unselected cohorts represent either clinical phenotypes or clinical contexts that were not adequately captured. For instance, JAK2 V617F mutations frequently drive polycythemia vera (PV) and essential thrombocythemia (ET) as monogenic lesions (32). Because PV and ET can be asymptomatic, some individuals with early or undiagnosed PV/ET may have been included in those cohorts.

Most individuals who have CHIP harbor only a single mutation (13, 14). The mutations found recurrently in CHIP may therefore be the only ones that can by themselves drive clonal expansion. As the number of individuals whose blood is analyzed by whole-exome or whole-genome sequencing climbs into the tens of thousands, the aggregation of data becomes analogous to an in vitro saturation mutagenesis experiment: All possible mutations occur at least once, and those that can drive clonal expansion in isolation do so. It may thus be possible to identify additional genes, not previously known to be involved in HM, that can drive clonal expansion in hematopoietic cells. However, such findings would need to be validated in functional experiments.

### Context Dependence: Mechanisms of Clonal Selection and Expansion

The features of CH described above apply to large, unselected populations of individuals. CH arising in specific clinical contexts has distinct characteristics.

**CH following cytotoxic therapy**

Exposure to chemotherapy or radiation acts as an evolutionary bottleneck that favors HSCs with intrinsic resistance to cytotoxic agents. This had previously been inferred from the fact that therapy-related AML and MDS (t-AML/MDS), defined as AML or MDS arising in patients with a prior history of exposure to cytotoxic therapy, are enriched for alterations affecting TP53 (33). More recently, studies of CHIP in cohorts of patients previously exposed to cytotoxic therapy have confirmed an enrichment of mutations in TP53 relative to their frequency in unselected cohorts (19, 20, 34, 35). This is true to an even greater degree with recurrent, gain-of-function mutations in PPM1D, a phosphatase that negatively regulates multiple components of the DNA damage response pathway (36). Although PPM1D mutations had not previously been described in HM, they have recently been shown to be specific for t-MDS/AML (37).

Most studies of CHIP in this context have assessed only a single time point after the exposure to cytotoxic therapy has occurred, raising the possibility that therapy is causing some of these DNA changes rather than selecting for mutations that already exist.

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**Table 2. Recurrently mutated genes in CHIP**

<table>
<thead>
<tr>
<th>Population and frequency</th>
<th>Mutated genes</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common in unselected populations</td>
<td>DNMT3A, TET2, ASXL1</td>
<td>Common MDS/leukemia-initiating mutations; all encode epigenetic/chromatin regulators that may give HSCs a growth or survival advantage in the marrow microenvironment. Associated with both a risk of progression to HM and vascular events.</td>
</tr>
<tr>
<td>Common in AA</td>
<td>PIGA, BCOR, BCORL1</td>
<td>Relatively benign/indolent mutations. Predict response to immunosuppressive therapy in AA.</td>
</tr>
<tr>
<td>Common after cytotoxic therapy</td>
<td>TP53, PPM1D</td>
<td>Strong risk factors for therapy-related myeloid neoplasia. Preexisting mutations that are selected for during cytoreductive therapy.</td>
</tr>
<tr>
<td>Less common</td>
<td>Splicing factor mutations (SF3B1, SRSF2, U2AF1, ZRSR2, LUC7L2), EZH2, RUNX1, JAK2, MPL, Ras pathway mutations (NRAS, KRAS, GNAS, GNB1, PTPN11), cohesin complex mutations (RAD21, STAG2, SMCC, SMCA, CTCF), CUN1, ATM, GATA2, WTI, ETV6, IDH1, IDH2, PHF6</td>
<td>Risk of neoplasia varies. Although some of these mutations (e.g., splicing factors) can clearly drive clonal expansion as sole lesions, others likely cannot. JAK2 and MPL may represent undiagnosed myeloproliferative neoplasia.</td>
</tr>
</tbody>
</table>
However, deep analysis of a small number of patients with t-AML for whom pretreatment samples were available identified the same TP53 mutations driving t-AML in the pretreatment samples (38). Moreover, CHIP in these cohorts still displays core features found in unselected populations, including a strong association with age and a predominance of C→T substitutions (19, 20), suggesting that the processes underlying the mutations themselves are similar regardless of context. On the whole, these data support a model in which CHIP usually—if not always—precedes cytotoxic therapy and expansion of preexisting CH is favored by the cytotoxic therapy.

**CH in AA**

Patients with aplastic anemia (AA) frequently develop CH with known drivers and can be broadly separated into two groups (21, 22). One group of patients, which tends to be older, is characterized by mutations in some genes recurrently mutated in CHIP, including DNMT3A and ASXL1, and has an inferior prognosis compared with other patients with AA, including an elevated risk of progression to MDS and AML. The other group, which tends to be younger, is characterized by mutations in genes that are not recurrently mutated in any other subsets of CH, including PIGA, BCOR, and BCORL1; these patients tend to respond well to immunosuppressive therapy.

These two groups of patients with AA present a powerful illustration of the interaction between driver mutations and biological context in CH. DNMT3A and ASXL1 mutations likely drive clonal expansion in this context, as they do elsewhere, but the relative rarity of other mutations common in CHIP, such as those in TET2 and JAK2, suggests that not all mutations are equally advantageous in the context of AA. Mutations in PIGA, BCOR, and BCORL1, which are not seen in unselected populations, must confer an advantage unique to AA, such as the promotion of immune escape from autoreactive T cells.

**CH in other contexts**

CH has been explored in a more limited fashion in other contexts. In the process of allogeneic HSCT transplantation (HSCT), undetected CHIP can be transferred from donors and engraft in recipients (26). Such “donor-engrafted CHIP” has been associated with the development of cytopenias in HSCT recipients, and may be a risk factor for donor-derived leukemia. The extensive modulation of the immune system that occurs during HSCT raises the question of whether features of immune surveillance (or other cell-extrinsic features) have a role in constraining aberrant clonal expansion under normal homeostatic conditions.

An important unanswered question is whether even putatively unselected studies of CHIP are actually reflective of contextual influences. For example, TET2 mutant HSCs have recently been shown to be preferentially selected for by proinflammatory environments (e.g., those containing TGFα) in vitro (39). Given the limitations of phenotypic annotation, it is impossible to know whether individuals with CHIP have, on average, "more" inflammation (however, this might be quantified) than those without CHIP. Furthermore, given the evidence discussed below that CHIP-associated mutations appear to actually drive proinflammatory signaling, it is also impossible to know—although not unanswerable—which came first, inflammation or CHIP.

**Clinical Consequences of CH**

In every study that has assessed survival, individuals with CH have been shown to have increased mortality compared with age-matched controls (13, 18–20). The degree of risk conferred depends on the study population and methodology. In patients who have CH with unknown drivers, the mortality risk is clear but small, with hazard ratios around 1.2 (18). The risk associated with CHIP, on the other hand, appears to be higher, with hazard ratios of 1.5 to 2 after adjustment for age (13, 18). This difference may reflect the biology underlying these different subsets of CH, because individuals with CHIP may suffer additional clinical consequences as direct effects of the specific mutations driving their clones.

**Risk of HM**

Because patients with CH have, by definition, clonal populations of blood cells—and, in many cases, these clones harbor mutations known to be involved in the pathogenesis of HM—it is not surprising that HM is one of the principal clinical consequences of CH. Although the relative risk of HM conferred by CH is substantial—between 4- and 15-fold (13, 14, 18)—the absolute risk is small. In unselected cohorts, the absolute risk of developing HM is 0.5% to 1% per year in patients with CHIP (13), a risk similar to the risk of developing myeloma in patients with monoclonal gammapathy of uncertain significance (40). The absolute risk of HM is two- to three-fold higher in other subsets of patients; for instance, in patients with non-Hodgkin lymphoma (NHL) who undergo autologous stem cell transplant (ASCT), the risk of t-MDS/AML is higher for patients with CHIP than those without (7.4% vs. 1.7% at 5 years following ASCT), equating to an annual risk of 1.5% (19).

Although CHIP-associated mutations are most frequent in myeloid malignancies, some, such as those in DNMT3A, TET2, or SF3B1, are recurrently observed in lymphoid malignancies as well (41–43). Because CH arises from aberrations in HSCs, which give rise to the full spectrum of hematopoietic cells, it is theoretically possible that CH could predispose to any type of HM, and some early series suggested a substantial risk of lymphoid neoplasms associated with CHIP (13). This finding, however, has not as yet been confirmed in larger cohorts with longer follow-up. Moreover, in patients with NHL undergoing ASCT, the presence of CHIP elevates the risk of t-MDS/AML but does not impact the risk of lymphoma relapse (19).

The risk of progression to HM is not the same for all patients with CHIP. The difference in risk may be partially explained by differential malignant potential conferred by specific mutations. For example, DNMT3A mutations found in CHIP are commonly inactivating frameshift or nonsense mutations, whereas those found in AML are enriched for R882 hotspot mutations (44, 45). More evolved clones likely have a higher risk of malignant transformation: for instance, CHIP in patients with NHL undergoing ASCT is frequently characterized by more than 1 mutation per patient (30% of those with CHIP), and these patients had a particularly elevated risk of t-MDS/AML (16.5% vs. 4% for those with only 1 mutation at 5 years; ref. 19). However, it is not currently possible to predict with certainty which patients with CHIP will go on to develop HM.
Risk of cardiovascular disease

Although the association between CHIP and HM was expected, early studies of CHIP in large cohorts also showed a surprising association between CHIP and ischemic cardiovascular disease, including MI and ischemic stroke (13). The biology underlying this association was initially unclear, with some suspecting that it was merely an epiphenomenon appearing to link two age-associated processes.

However, more recent studies in both human cohorts and animal models have replicated these findings, suggesting that CHIP has a causal role in the development of cardiovascular disease of a similar magnitude to hypertension, and that this role is driven in large part by specific functional effects of the mutations found in CHIP (25). The degree of risk elevation is proportional to clone size, with clones at VAF >0.1 posing the greatest risk. In mouse models, transplantation of Tet2-null bone marrow, replicating the biochemical effects of Tet2 mutations found in people with CHIP, accelerates the development of atherosclerosis in mice homozygously null for the low-density lipoprotein (LDL) receptor (25, 46). This effect is
dose dependent, mirrored the effect of clone size seen in humans.

Loss of Tet2 appears to drive atherosclerosis, at least in part, by altering expression of inflammatory response genes in macrophages, cells known to be critically important in the development of atherosclerotic plaques. Under normal conditions, macrophages take up and process LDL cholesterol from the surrounding environment, but exposing Tet2-null macrophages to LDL instead stimulates a transcriptional program similar to that generated by exposure to bacterial endotoxin, with particular upregulation of CXC chemokines (25). This alteration has been hypothesized to lead to the further recruitment of monocytes and neutrophils to the arterial intima and NLRP3 inflammasome activation (46, 47).

Other clinical consequences

Because CHIP affects the function of hematopoietic cells, it is conceivable that CHIP may contribute to any disease process that intersects the hematopoietic system. This represents a potentially dizzying array of pathologies that could reflect effects on platelets (e.g., a risk of thrombosis or bleeding), erythrocytes (e.g., anemia), and, most extensively, effects on leukocytes, through alterations in immunity or inflammation. Furthermore, since certain subsets of immune cells such as macrophages and T cells are extensively represented in non-hematologic tissues (48–50), CHIP may exert effects on non-hematologic disease processes in unexpected ways. So far, most of these possibilities remain unexplored.

CHIP in the Clinic: Approach to Patients with CH

Because next-generation sequencing is now well integrated into clinical care, it has become relatively easy from a technical standpoint to identify patients with CHIP. (The identification of patients with other forms of CH, such as CH with unknown drivers, is less straightforward, since this requires use of sequencing technologies that are not employed in most clinical settings.) This has led to an emerging conversation about whether patients should be screened for CHIP and what steps should be taken if CHIP is found.

Our position is that screening for CHIP in asymptomatic populations cannot currently be justified. No clinical trials have yet been published showing that any specific intervention can mitigate the clinical risks associated with CHIP, and extrapolation from our experience treating MDS and AML suggests that eliminating mutant HSC clones with low-risk therapies is currently not feasible. At present then, the only clear effect of identifying CHIP in asymptomatic individuals would be to elevate anxiety in both those individuals and their physicians, with no compensating benefit.

It is possible, however, that this position could change in the future. First, CHIP has until now been conceived along lines similar to a neoplasm, with a focus on identifying the aberrant clone. However, our deepening understanding of CHIP suggests that there may be other points of leverage, including prevention of clonal expansion and mitigation of CHIP’s effects on inflammation. Trials of anti-inflammatory agents or aggressive lipid-lowering therapy, for example, could be undertaken in patients with CHIP. More refined studies may identify genetic, epigenetic, or transcriptional characteristics of patients with CHIP who are at particularly high risk of progression to an HM, such as those with CHIP arising after cytotoxic therapy, for whom more aggressive therapy may be justifiable. Finally, although targeted therapies do not currently exist for most of the genetic lesions found in CHIP, several such agents are in various phases of clinical development.

These comments apply only to asymptomatic patients with normal blood counts. Patients with cytopenias that remain unexplained after a thorough workup have a relatively high risk of having at least one somatic driver mutation of HM on sequencing of peripheral blood (51, 52). Finding such a mutation, in turn, confers a positive predictive value of >80% for an underlying myeloid neoplasm among patients with nondiagnostic bone marrow examinations. Sequencing results alone are currently not sufficient for diagnosis or treatment decisions. Thus, in patients with unexplained cytopenias who would be candidates for some form of treatment, we recommend appropriate targeted sequencing in addition to but not in place of bone marrow aspirate and biopsy.

Conclusions and Unanswered Questions

CH has rocketed to prominence among basic and translational hematologists and is also engaging the interest of cardiologists and vascular biologists. However, despite vast improvements in our understanding of CH, important questions remain, and we are far from improving clinical outcomes for those with CHIP.

A key question is whether CH is always pathologic. Because stem cells senesce with advancing age, progressive oligoclonality in every tissue is likely an inescapable endpoint of aging. An extreme example was a 115-year-old Dutch woman whose entire hematopoiesis was derived from two functional HSCs (53).

If clonality by attrition is inevitable, what about CH with known drivers? Given the recent finding that nearly 100% of individuals by age 60 harbor HSC clones with pathologic DNMT3A or TET2 mutations, CH with known drivers may also be inevitable (17). With an HSC pool of 10,000 to 15,000 cells (54), and a basal rate of one nonsynchronous exonic mutation per decade of life per HSC (55)—the current best estimates—it is a reasonable probability that every individual will have at least one HSC with a mutation that can drive clonal expansion by middle age (Fig. 3), but not everyone will develop a clonal population of cells large enough to qualify as CHIP. It may thus be time to focus not just on the mutations found in CH but also on understanding the processes that make these mutations advantageous, and on identifying the features, such as clone size and mutation type, that distinguish the dangerous clones from the mundane.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

D.P. Steensma is supported by the Edward P. Evans Foundation.

Received February 15, 2018; revised March 28, 2018; accepted April 23, 2018; published first April 27, 2018.

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References

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