blood® Review Series

MYELODYSPLASTIC SYNDROMES IN THE AGE OF GENOMIC MEDICINE

Causes and consequences of clonal hematopoiesis

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Clonal hematopoiesis (CH) is described as the outsized contribution of expanded clones of hematopoietic stem and progenitor cells (HSPCs) to blood cell production. The prevalence of CH increases dramatically with age. CH can be caused by somatic mutations in individual genes or by gains and/or losses of larger chromosomal segments. CH is a premalignant state; the somatic mutations detected in CH are the initiating mutations for hematologic malignancies, and CH is a strong predictor of the development of blood cancers. Moreover, CH is associated with nonmalignant disorders and increased overall mortality. The somatic mutations that drive clonal expansion of HSPCs can alter the function of terminally differentiated blood cells, including the release of elevated levels of inflammatory cytokines. These cytokines may then contribute to a broad range of inflammatory disorders that increase in prevalence with age. Specific somatic mutations in the peripheral blood in coordination with blood count parameters can powerfully predict the development of hematologic malignancies and overall mortality in CH. In this review, we summarize the current understanding of CH nosology and origins. We provide an overview of available tools for risk stratification and discuss management strategies for patients with CH presenting to hematology clinics.

Introduction

The acquisition of somatic mutations in healthy human cells is ubiquitous, increases with age, and underlies the development of cancer and nonmalignant disease.[1,2] Somatic mutagenesis of stem cells leads to genetically divergent clonal populations of cells and decreased stem cell pool diversity.[3] Although anatomical constraints challenge our ability to fully capture the genetic diversity in solid organs, circulating blood cells are easily sampled and represent the genetic diversity of the entire hematopoietic system. The study of clonal hematopoiesis (CH; clonally expanded hematopoietic stem and progenitor cells [HSPCs] with acquired selective growth advantage)[4-6] has allowed for an in-depth assessment of mechanisms underlying somatic genetic variation and related malignant and nonmalignant outcomes. Early descriptions of CH came from observations of biased X chromosome inactivation[7,8] and the clonal origins of chronic myelogenous leukemia.[9] In the decades since these discoveries, CH has gained relevance as a hematologic malignancy precursor, analogous to monoclonal gammopathy of uncertain significance and monoclonal B-cell lymphocytosis (MBL), and established precursor conditions for plasma cell and lymphoid malignancies.[10] CH investigations provide insights into the mechanisms and kinetics of leukemogenesis and the role of environmental factors in the selection and maintenance of cancer-causing HSPC clones. CH is associated with significant clinical outcomes, including an increased risk of hematologic cancers,[11-13] increased all-cause mortality,[4] and various nonmalignant conditions.[14] We review CH nosology, relative fitness, and associated clinical outcomes, concluding our review with a discussion of the management of patients with CH.

CH nosology

Myeloid CH: CHIP, CCUS, and M-mCA

Among the various types of CH (Figure 1), 2 entities have been formally defined by the detection of acquired pathogenic variants in ≥1 genes known for or suspected of promoting clonal expansion of HSPCs.[15-17] CH of indeterminate potential (CHIP) refers specifically to CH possessing somatic mutations in leukemia driver genes at a variant allele fraction (VAF) of ≥2% in the absence of diagnosed blood disorder or cytopenia. Clonal cytopenia of undetermined significance (CCUS) describes CHIP in the presence of persistent, unexplained cytopenia in which dysplastic features of myelodysplastic syndrome (MDS) are absent.[16,17] CHIP and CCUS are detected in 10% to 20% of individuals aged >70 years[4-6,15] and are rare in individuals aged <50 years.[5] The epigenetic modifiers *DNMT3A*, *ASXL1*, and *TET2* are mutated in 87% of CHIP/CCUS cases. Mutations in *JAK2*, *TP53*, *SF3B1*, and *SRSF2* are commonly observed in the remaining cases.[4,5,12] Although the genetic drivers overlap, the genetics of CH is largely distinguishable from that of overt malignancy.[6,15,18]

Somatic copy number abnormalities occur in ~2% of individuals aged >70 years in the absence of any other manifestation of hematologic malignancy.[19,20] These mosaic chromosomal alterations (mCAs) are another subtype of CH and include

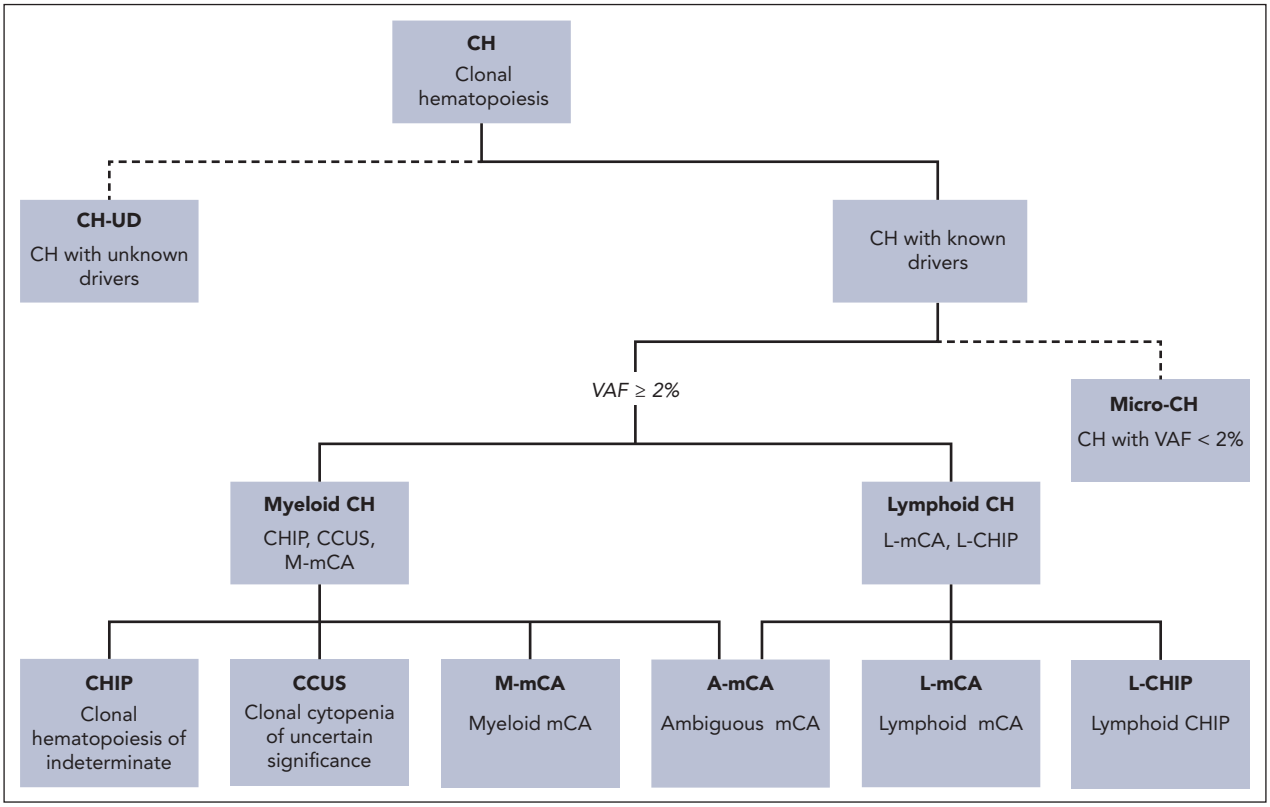

Figure 1. Nosology of CH. CH is the expansion of a clonal population of HSPCs. Many instances of CH are caused by unknown drivers and can be referred to as CHUD (dotted branch). When molecular drivers of CH are known, they can be further subclassified as initiating events of myeloid malignances (myeloid CH) or lymphoid malignancies (lymphoid CH). Myeloid CH is classified as CH caused by somatic mutations in myeloid malignancy-driver genes at VAF ≥ 2%. The term CHIP is used in the absence of cytopenias, and CCUS is used when cytopenias are present. Mosaic chromosomal alterations (mCAs) can also be drivers of myeloid CH (m-MCA). Lymphoid CH is subdivided into CH caused by mutations in drivers of lymphoid malignancy with a VAF ≥ 2% (L-CHIP) or mCA that reflect chromosomal abnormalities driving lymphoid malignancies (L-mCA). Ever-improving sensitivity of next-generation sequencing has led to an increasing identification of low-abundance (VAF < 2%) clones, which some have referred to as micro-CH. The clinical significance of these low-abundance clones remains to be fully elucidated.

amplifications, deletions, and copy-neutral loss of heterozygosity.[21-25] Like CHIP/CCUS, mCAs accumulate with age and are associated with subsequent hematologic malignancy.[12,21-23] Niroula et al used the differential frequency of mCAs detected in individuals with prevalent hematologic malignancy to identify mCAs associated with a near-exclusive risk of myeloid malignancy (myeloid mCA [M-mCA]) and those associated with both myeloid and lymphoid malignancies (ambiguous mCA [A-mCA]).[12] As expected, M-mCAs and A-mCAs occurred in the chromosomal loci of known drivers of myeloid malignancy.[12] Although mCAs may be observed without comutations,[21] co-occurrence of M-mCAs and mutations in candidate drivers occur in a minority of CH cases,[12,21] mimicking the genetic interactions of chromosomal alterations and somatic mutations observed in overt myeloid malignancy.[26,27]

Lymphoid CH: L-CHIP and L-mCA

Lymphoid-CHIP (L-CHIP) refers to CHIP with mutations in genetic drivers of lymphoid malignancies.[12] Like myeloid CHIP/CCUS, the prevalence of L-CHIP increases with age though it is less common than its myeloid counterpart. There is a more balanced distribution of genotypes in L-CHIP, with some L-CHIP mutations, such as *NOTCH1*,[12] also recurrently observed in the lymphoid malignancy precursor state, MBL.[28] Chromosomal aberrations that drive lymphoid malignancy (lymphoid mCAs [L-mCAs]) are the most commonly observed mCAs. As with M-mCAs, in some cases, L-mCAs reveal biallelic targeting, involving the chromosomal loci of L-CHIP genes.[12]

CHUD

A substantial fraction of CH lacks detectable mutations or chromosomal alterations affecting known driver genes.[5,29,30] CH with unknown drivers (CHUD) may represent outgrowth of hematopoietic clones caused by acquired variants in genes that are yet to be characterized, noncoding or epigenetic alterations, or genetic drift of stem cell populations.[31,32] Emergence of CHUD may be observed within the first 2 decades of life, with expansion rates similar to those of CH with known drivers.[33] CHUD is also associated with increased risk of hematologic malignancy and greater all-cause mortality though this risk of adverse outcomes in CHUD is likely lower than the risk associated with CH with known molecular drivers.[5,29,34] However, dramatic instances of CHUD without malignancy have also been reported.[35]

Micro-CH

The lower limit for VAF that defines CHIP/CCUS[16] is based on sequencing error rates in early studies.[4,15,34] Contemporary targeted error–corrected sequencing detects less abundant clones, remarkably demonstrating ubiquity of CH with mutations in *DNMT3A* and *TET2* in adults aged >50 years.[36] The term micro-CH has been proposed for these low-abundance

clones (VAF < 2%).[37] In 1 study investigating the prevalence of 15 different hot spot mutations associated with acute myeloid leukemia (AML), including mutations in *IDH1/2*, *FLT3*, and *DNMT3A*-R882, ultradeep sequencing identified CH with VAF as low as 0.8% in individuals aged < 60 years.[6]

Contextualizing CH: origins and fitness

CH origins and dynamics

Somatic mutations in HSCs that cause CH can be acquired early in life.[38-40] Because mutations steadily accumulate with age, they can be considered as a molecular clock to map the long-term dynamics of both healthy and premalignant HSCs.[30,39,41] For most individuals, somatic events do not involve known driver genes.[30,39] Sequencing for both driver and passenger mutations has been used to derive Bayesian logistic growth models that reconstruct hematopoietic lineage hierarchies using input from sequencing of serial blood samples[33] or individually isolated HSC colonies.[39] More recently, Weinstock et al introduced passenger-approximated clonal expansion rate, an approach that uses the acquisition rate of passenger mutations identified through whole genome sequencing to estimate the relative fitness of CH clones using material from a single blood draw.[42]

CH origins and subsequent dynamics are genotype specific. For instance, decelerated growth is observed for some clones,[33,43] and spontaneous attrition of less fit CH clones over one's life span is probable. Relative to other genotypes of CH, *DNMT3A*-mutant clones are less likely to display clonal growth.[43] *DNMT3A* clones likely have faster clonal outgrowth at younger ages, followed by deceleration in older ages. This decelerated clonal expansion with age is further suggested by the fact that *TET2*-mutated CH becomes the most prevalent CH driver in older adults.[33] Splicing factor–mutated CH emerges at later ages,[6,33] with greater growth rate and relative fitness than those of other common CH genotypes.[44] CH clones that reach the VAF threshold for CHIP/CCUS (VAF ≥ 2%) are more commonly linked to malignant and nonmalignant outcomes.[43] The clinical consequences of micro-CH are less certain but may also be driver gene specific and best estimated based on change in clone size over time.[44]

Clonal fitness and context-dependent selection

Clone fitness is context dependent. Intrinsic and extrinsic selection pressures influence genotype-dependent clonal expansion or attrition (Figure 2). Chemotherapy and radiation are clear selection pressures with major influence on clonal dominance and ascendency.[45-50] Cytotoxic chemotherapy exposure is associated with an increased rate of *TP53*- and *PPM1D*-mutant CH, particularly in patients treated with platinum-based chemotherapy and radiation.[46,47,49] Deep sequencing before and after chemotherapy shows that CHIP, micro-CH, and mCAs driving therapy-related myeloid malignancies may precede chemotherapy exposure.[25,47,48] Importantly, this finding suggests that rather than directly causing CH through DNA damage, chemotherapy exposure, in many cases, drives selection and transformation of pre-existing CH clones.[47,51] Clonal outgrowth is faster for *TP53*, *PPM1D*, and *CHEK2* than for other CH mutations in the presence of chemotherapy and/or radiation.[49,51] Although the strength of association is moderate, there is also a clear causal relationship between tobacco exposure and *ASXL1*-mutated CH suggesting more broadly that toxin-related cellular stresses at least partially contribute to the development and expansion of CH in a genotype-specific manner.[52,53]

Sex differences in peripheral blood counts have been observed in population cohorts,[54] and male sex is associated with worse outcomes in myeloid malignancy.[55] In CH, mutations in splicing factors and *ASXL1* are more common in males with CHIP than in females, whereas *DNMT3A* mutations are enriched in females.[56-58]

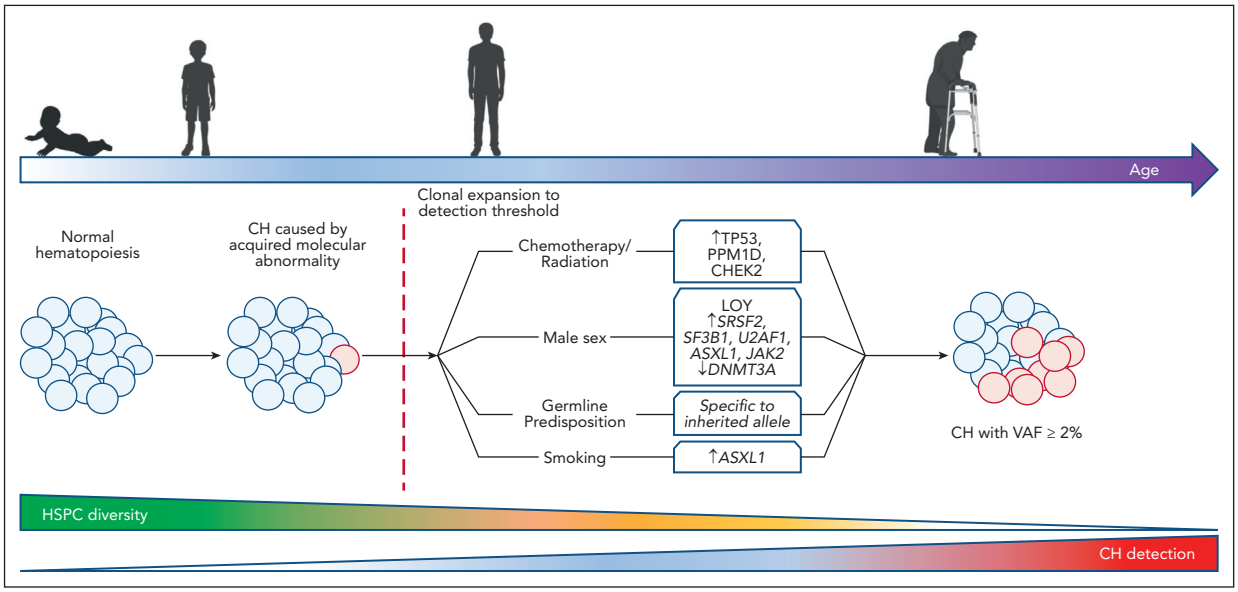

Figure 2. Context-dependent expansion of CH genotypes. Several lines of evidence suggest molecular abnormalities driving CH are acquired early in life. Detection of CH is rare for individuals aged <50 years. The ability to detect CH increases with age, corresponding to a decline in HSPC diversity. Male sex, inherited germ line predisposition, systemic inflammation, chemotherapy/radiation therapy exposure, and smoking each select for distinct CH genotypes.

The biological rationale for these observed sex differences in CH has not been fully resolved, but similar genotype-specific sex biases are observed in myeloid malignancies.

Although germ line and somatic mutations in inflammatory regulators are uncommon in CH and hematologic malignancy, inflammation may directly affect HSC proliferation and renewal and influence CH selection.[59] An extreme case is aplastic anemia, in which specific CH genotypes appear to have a competitive advantage in the setting of cytotoxic T-cell–mediated autoreactive HSC destruction.[60-63] In murine models, *Dnmt3a*-mutant HSCs outcompete healthy cells in the context of chronic infection and interferon gamma signaling,[64] and *Tet2* loss causes myeloproliferation in the presence of gut microbial stimuli.[65] In humans, expansion of CH clones may also be a consequence of inflammaging, the age-related chronic sterile low-grade systemic inflammation implicated in the pathogenesis of age-related chronic illnesses.[66,67] However, data demonstrating clonal outgrowth as a direct result of inflammation are lacking. Increased prevalence of CHIP has been documented in people with HIV infection[68] and autoimmune disease,[69,70] but the directionality of these associations is not clear. Although anti-inflammatory compounds may mitigate the risks of some inflammation-associated comorbidity in CHIP,[71] it is unclear whether targeting inflammatory signaling pathways will affect clonal fitness.

Germ line predisposition to CH

Inherited bone marrow failure syndromes (IBMFSs) are characterized by ineffective hematopoiesis and an increased risk of MDS/AML. In the absence of phenotypic bone marrow failure, germ line mutations in *CEBPA*, *DDX41*, *GATA2*, *RUNX1*, and *SAMD9/9L* are associated with familial predisposition to MDS/AML.[72] CH is detected at increased rates in IBMFSs and many familial predisposition syndromes, highlighting a relationship between germ line variants and selective pressure for the acquisition of specific somatic mutations (Figure 2).[72] Somatic compensation can lead to CH in IBMFSs, involving the acquisition of molecular abnormalities that augment expression of the unmutated allele or otherwise bypass the deleterious effects of the mutated allele.[72] In Schwachman Diamond Syndrome, CH is characterized by recurrent mCA (isocromosome 7q and deletions at 20q) or inactivating mutations in *EIF6*,[73] which functionally correct inherited defects in translational efficiency and aberrant p53 activation. In severe congenital neutropenia, *CSF3R*-mutant CH is a mechanism of compensatory myeloid hyperproliferation that predicts leukemic transformation.[74] CH may also arise as a form of somatic reversion, correcting or removing the germ line molecular defect. This is the most commonly observed cause of CH in telomeropathies, in which in addition to CH caused by skewed X chromosome inactivation, mCAs, and CHUD,[75] acquired mutations in the promoter of the *TERT* allele possessing the inherited germ line variant are observed.[76] Somatic compensation has also been observed in Fanconi anemia and in patients with germ line *SAMD9/9L* (reviewed previously[72]). Specific patterns of CHIP, such as *GATA2* deficiency, may be observed in other rare but high-penetrance inherited alleles.[77]

Genome-wide association studies have identified a set of common germ line variants that predispose to CH. Both rare and common variants have been identified, with rare variants having higher penetrance for CH and common variants having less penetrance.[78] An 8-bp intronic deletion in *TERT* was associated with a 1.37-fold increase in CH driver mutations, suggesting telomere maintenance may play a role in predisposition to CH.[29] More common coding variants in *TERT* associate with *JAK2*-mutant myeloproliferative neoplasms but not with *JAK2*-mutant CH: a discrepancy that may indicate a short or nonexistent CH phase when *JAK2* is mutated.[79] A genome-wide association study performed to identify germ line variants influencing clonal expansion rates determined by passenger-approximated clonal expansion rate identified a germ line variant in the core promoter of *TCL1A* associated with slowed growth of *TET2*-mutant CH and significantly reduced odds of CHIP involving mutations in *TET2*, *ASXL1*, *SF3B1*, and *SRSF2* relative to *DNMT3A*.[42] This *TCL1A* variant is also associated with mosaic loss of chromosome Y.[80] Functional studies identified an aberrant expression of *TCL1A* in CHIP-mutant HSCs that drives clonal expansion.[42] This same *TCL1A* variant was separately associated with a modest increase in *DNMT3A*-mutant CHIP but not other genotypes,[81] consistent with the observation that *TCL1A*-mediated reduction in fitness for certain HSC clones specifically increases the likelihood of *DNMT3A* clones.[42] Other germ line variants including a frameshift variant in *CHEK2* that confers a 2.2-fold increased risk of CHIP[78] and an intergenic variant near *TET2* observed in people of African ancestry that confers a 2.4-fold increased risk of CHIP have been observed.[81] These findings underscore the need to study CH and germ line susceptibility to CH in cohorts of individuals from diverse genetic ancestral backgrounds.

CH-associated malignant outcomes

Hematologic malignancy

Molecular drivers of myeloid CH are common initiators of myeloid malignancy, and CHIP, CCUS, and M-mCA are associated with an increased risk of subsequent myeloid malignancy (Figure 3).[4,5,11,29,36] Although the relative risk of myeloid malignancy is increased, the absolute risk remains small, with an estimated rate of malignant transformation from ~0.5% to 1% per year in CHIP/CCUS.[4,82]

The risk of transformation to myeloid malignancy is determined by the presence of specific molecular and hematologic features. CHIP/CCUS caused by a single mutation in *DNMT3A* has the lowest risk of progression compared with all other CHIP/CCUS genotypes.[83] In contrast, CHIP/CCUS with mutations in splicing factor genes, *TP53*, *IDH1*, *IDH2*, and *RUNX1* have the highest risk of evolution to myeloid neoplasia.[11,13,49,83] Clone size, as indicated by VAF[4,11,12] and clonal composition, including the number of molecular abnormalities,[21,83-85] are also prognostic. Large-scale longitudinal analyses of micro-CH with serial sequencing are needed to clarify the significance of low-abundance clones. The clonal hematopoiesis risk score (CHRS) was developed as a clinical tool to estimate the risk of progression to myeloid malignancy in CHIP/CCUS based on the presence of molecular and hematologic features.[83] CHRS characterizes CHIP/CCUS into high-, intermediate-, and low-risk groups based on the presence or absence of 8 features: mutations in high-risk genes (splicing factors *JAK2*, *TP53*, *IDH1*, *IDH2*, *RUNX1*, or *FLT3*); single mutation in *DNMT3A*; VAF ≥20%; presence of ≥2 mutations; mean corpuscular volume

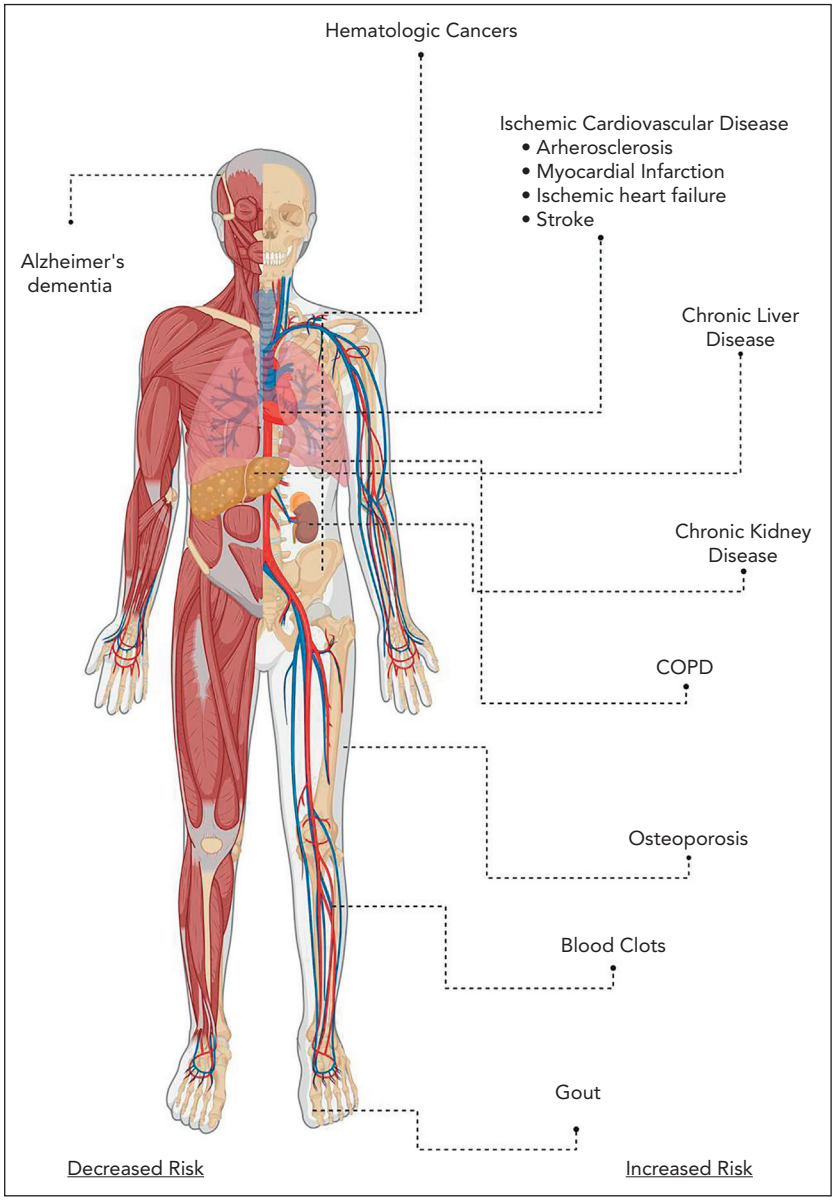

Figure 3. Malignant and nonmalignant consequences of CH. COPD, chronic obstructive pulmonary disease.

≥100 femtoliters; red cell distribution width ≥ 15%; presence of CCUS vs CHIP; and age ≥ 65 years (Figure 4).[83] Features not included in the CHRS may also have predictive value. MN-predict, a recently published risk algorithm, combines serum chemistries and body mass index in addition to molecular features to predict individual risk of specific myeloid neoplasms.[86] Additionally, cytogenetic abnormalities may initiate myeloid malignancy, and mCAs independently predict malignant transformation in CHIP/CCUS.[21,83] Molecular evaluation of CH that considers mCAs in conjunction with somatic mutations may be beneficial.[12,21,83]

Chronic lymphocytic leukemia (CLL) is often initiated by copy number abnormalities, and L-mCAs including trisomy 12, deletion of 13q, and copy-neutral loss of heterozygosity at the *NOTCH1* locus are associated with subsequent CLL or small lymphocytic lymphoma.[12] Niroula et al also demonstrated a 20.5-fold increase in the risk of CLL for patients with L-CHIP relative to that of controls. The clinical significance of L-CHIP or L-mCA relative to MBL is unclear. Although CHIP/CCUS more commonly precedes myeloid malignancy, lymphoid malignancy is also possible.[4,12] That *DNMT3A* mutations are detectable in multipotent HSCs and lymphocytes[87] suggests certain CH genotypes occurring in common hematopoietic progenitors could drive myeloid or lymphoid malignancy outcomes.

CH and HSCT

In hematopoietic stem cell transplantation (HSCT), CH detected in the donor or recipient can influence outcomes. Patients with lymphoma and myeloma with CHIP at the time of autologous transplantation had inferior survival and increased risk of therapy-related myeloid malignancy.[88,89] In keeping with this, an investigation of 81 individuals undergoing autologous

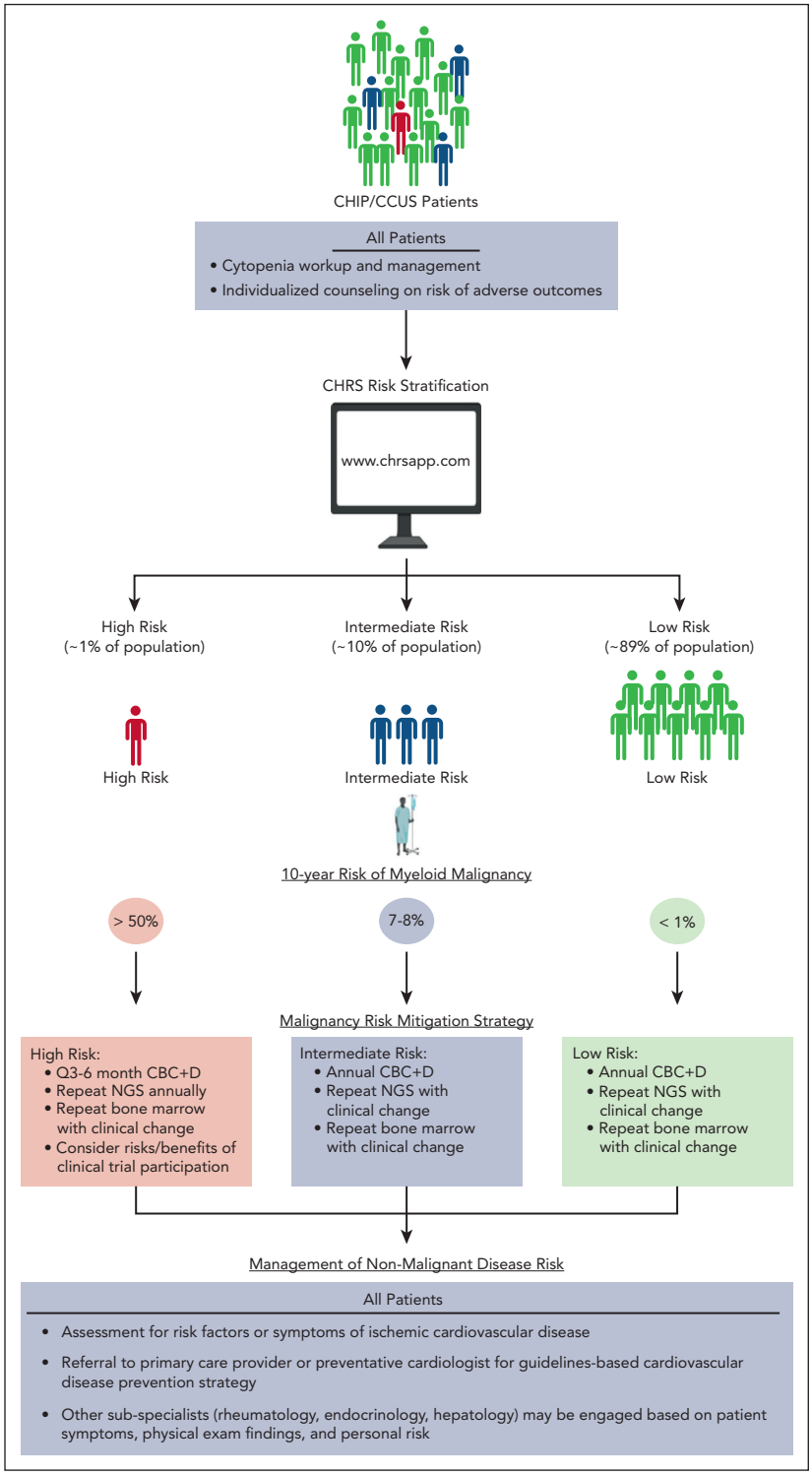

Figure 4. Current management strategies for CH. Patients with CHIP/CCUS are typically identified incidentally as routine screening for CHIP/CCUS is not currently recommended outside of the context of a well-designed clinical research study. We advise the use of the CHRS to risk stratify patients with CHIP/CCUS into high-, intermediate-, and low-risk groups. All patients with cytopenia should be offered a bone marrow biopsy and cytogenetic profile to rule out underlying MDS. Patients at high risk may be followed up every 3 to 6 months depending on cytopenia burden and the rate of clinical change. Bone marrow biopsies and NGS should be repeated for clinical changes that may indicate progression. These patients are most likely to derive benefit from therapeutic intervention clinical trials designed to prevent malignant transformation and, if interested, may be considered for these studies. Less frequent monitoring is indicated for patients at intermediate and low risk. Bone marrow biopsies should not be performed outside of initial workup of cytopenia or to investigate clinical changes that may be indicative of progression. These patients are statistically unlikely to derive benefit from therapeutic clinical trials designed to prevent malignant transformation, and these patients should not be routinely considered as candidates for these studies. All patients may derive benefit from healthy lifestyle modifications such as smoking cessation, reduction of visceral fat burden, and exercise. Patients with CVD risk factors may derive benefit from preventive cardiology evaluation and/or enrollment on clinical trials to prevent CVD outcomes. A complete review of symptoms should be performed on all patients with CCUS to evaluate for systemic immune and autoinflammatory disease, including VEXAS syndrome. CBC + D, complete blood count with differential; NGS, next-generation sequencing.

stem cell transplantation noted that in most individuals, CHIP mutations detected after transplantation were present in pre-transplant specimen, albeit as less abundant micro-CH clones. Preferential expansion of clones with CHIP-causing mutations in this milieu may suggest a reconstitution advantage for mutant HSCs.[90]

In allogeneic HSCT, donor-derived CHIP engrafts in recipients,[91] but the consequences are genotype dependent.[92] An increased risk of donor-derived leukemias is observed for *TP53*-mutant or splicing factor gene–mutant donor CH.[93] The reduced risk of relapse and higher rates of graft-versus-host disease observed for *DNMT3A*-mutant donor CH suggests

increased graft-versus-leukemia effect driven by *DNMT3A*-mutant donor–derived T cells.[93,94]

CH-associated nonmalignant outcomes

iCVD

CHIP/CCUS is causally linked to ischemic cardiovascular disease (iCVD), with the strongest associations observed for *TET2*- and *JAK2*-mutant CHIP/CCUS.[14] Animal studies clarified a causal role of CH in iCVD outcomes, demonstrating a clear acceleration of atherosclerotic plaque formation in mice that underwent transplantation with bone marrow with heterozygous or homozygous deficiency in *Tet2*.[14,95] These experiments also revealed inflammasome activation in *Tet2*-deficient mice, including increased expression of interleukin-1β (IL-1β), IL-6, and other proinflammatory molecules.[95] Data from human experiments link IL-6 receptor gene expression to reduced levels of the inflammatory marker, C-reactive protein, and decreased coronary artery disease.[96] Consistent with this finding, exploratory analysis of the canakinumab anti-inflammatory thrombosis outcomes study showed that the IL-1β inhibitor, canakinumab, reduced cardiovascular events among individuals with *TET2*-mutant CH.[71] In murine[97] and human data,[98] activation of the absent in melanoma (*AIM2*) inflammasome is noted in *JAK2* V617F macrophages. Together, these data point to anti-inflammatory compounds, particularly inflammasome inhibitors, as potential strategies to mitigate iCVD risk in CH. Among the other subtypes of CH, iCVD is also associated with the loss of chromosome Y[99,100] but not autosomal mCAs[24] or L-CHIP.[12]

The strength of the association between CHIP/CCUS and iCVD varies depending on the cohort examined. In cohorts enriched for individuals with CVD risk factors, CHIP/CCUS is associated up to approximately twofold increased risk of coronary artery disease, myocardial infarction, and ischemic stroke,[14] a similar level of risk conferred by smoking, hyperlipidemia, and diabetes.[14,95] In the UK Biobank healthier cohort, the association with iCVD was mild[101] or absent[58,78] when CHIP/CCUS genotypes were evaluated as a composite, and stronger associations were observed when *JAK2*- and *TET2*-mutant CHIP/CCUS were analyzed separately.[101] Individuals in the UK Biobank with established atherosclerotic CVD and CHIP/CCUS were also noted to have a higher rate of secondary ischemic events,[102] consistent with data reporting enhanced mortality in ischemic heart failure in patients with CHIP.[103] Clone size, a strong predictor of myeloid malignancy risk, is also prognostic of iCVD outcomes in CHIP/CCUS, such that individuals with VAF ≥ 10% had a greater likelihood of developing incident iCVD than those with smaller clones.[14]

Other age-related inflammatory diseases

Other age-related inflammatory diseases have been associated with CHIP, including chronic liver disease,[104] chronic kidney disease (CKD),[105-107] gout,[108] chronic obstructive pulmonary disease,[109] and osteoporosis (Figure 3).[110] Of these associations, the most robust observation is that CHIP with a VAF ≥ 10% doubles the risk of chronic liver disease, nonalcoholic hepatic steatosis, and cirrhosis.[104] This is consistent with the observation of a higher prevalence of nonalcoholic hepatic steatosis among individuals who have MDS/CMML than in controls without hematologic malignancy.[111] Like iCVD, the association between chronic liver disease and CHIP is powerfully genotype dependent, with a 17.6-fold increase in chronic liver disease for *JAK2*-mutant CH, a 5.4-fold increase in chronic liver disease with *TET2*-mutant CH, and a low risk for *DNMT3A* mutations. Causality was inferred by Mendelian randomization in human population cohorts and in *Tet2*-deficient mice, which had increased liver fat accumulation and enrichment of transcriptional programs associated with steatohepatitis and liver fibrosis.[104]

Proinflammatory cytokine signaling contributes to several other observed phenotypes in CHIP. A higher incidence of gout in CHIP/CCUS is driven by the exaggerated IL-1β signaling in response to urate crystal exposure in *Tet2*-deficient compared with in *Tet2*-proficient animals.[108] In CKD, CHIP is also a potential biomarker for worse outcomes in humans[105,107] because individuals with known CKD have a doubled risk of end-stage CKD at 5 years.[105] The osteoclastogenesis and accelerated bone loss observed in *Dnmt3a*-deficient mouse models is also linked to proinflammatory cytokines, offering a potential mechanism for the association between osteoporosis and CHIP in humans.[110] Other models have demonstrated exacerbated age- and obesity-associated insulin resistance with parallel enhancement of proinflammatory signaling in *Tet2*-deficient mice, suggesting that CHIP increases the risk of type 2 diabetes mellitus (T2DM).[112] However, despite a modest association between CHIP and T2DM in the initial report of adverse outcomes for CHIP,[4] causality has been difficult to establish for this association because T2DM diagnosis often precedes sample collection in sequenced CH cohorts.

Autoimmune diseases and chronic infections

There is growing evidence pointing to a shared pathophysiology for hematologic malignancy and diseases of immune dysregulation. In addition to aberrant inflammatory signaling in CH, CHIP is detected in ~30% of individuals with antineutrophil cytoplasmic antibody–associated vasculitis.[70] Additionally, overt myeloid malignancy risk is higher in people with autoimmune diseases[113] and autoinflammatory phenotypes are common in certain genotypes of myeloid malignancy.[114,115] However, although rheumatoid arthritis is prevalent in myeloid malignancy,[111] no observed differences in the gene distribution or rate of CHIP has been observed.[116] More recently, VEXAS (vacuoles, E1 enzyme, X-linked, autoimmune, somatic) syndrome was described as a clonal hematopoietic disorder driven by mutations in *UBA1* and characterized by multisystem, adult-onset autoinflammatory disease and MDS in 24% to 50% of cases.[117,118] Mutations in *UBA1* can emerge independently or from existing CH with typical CHIP/CCUS mutations.[119] AML transformation is rare in VEXAS syndrome, and treatment goals are centered around prevention of morbidity and mortality related to systemic inflammation.

In some cases, lymphoid CH may drive autoimmune disease through the hyperinflammatory activity of terminally differentiated T or B cells with CH mutations. Sequencing of $CD8^+$ T-cell isolates from patients with multiple sclerosis demonstrated mutations recurrent in L-CHIP[12] and in *STAT3*, a known driver of large granular lymphocytosis.[120] L-mCAs carry an increased risk of incident infection,[121] reflecting immune dysfunction, which is also observed in MBL[122] and CLL.[123] Chronic or severe

infections may be associated with other forms of CH including CHIP, although these associations are less clear.[68,124]

Alzheimer dementia

Epidemiologic data demonstrate that unlike cardiovascular and metabolic diseases, neurodegenerative diseases are less prevalent among individuals with myeloid malignancy than among controls.[111] Consistent with this finding, recent data demonstrate a reduced risk of Alzheimer dementia in CHIP.[125] This association could be due to altered phagocytic activity of bone marrow–derived microglial-like cells, which are enriched in the brains of individuals with CHIP compared with those in controls.

CH clinics: current approaches and priorities for clinical research

Several cancer centers have established "CHIP clinics" to counsel and monitor patients with CH.[126,127] Patients in these clinics are primarily being identified incidentally when CH is detected on next-generation sequencing ordered to work up cytopenia, evaluate solid tumors, diagnose germ line cancer predisposition syndromes, or investigate suspected donor cell leukemia after HSCT (Figure 5). Clinical management in CH involves work up and monitoring of any blood count abnormalities and counseling patients at risk of CH-associated adverse outcomes. For CHIP/CCUS in particular, the CHRS estimates myeloid malignancy risk[83] and may be used to guide risk-specific management. Patients with high-risk CHIP/CCUS may be monitored more frequently and considered for inclusion in clinical trials focused on preventing myeloid malignancy, whereas frequent monitoring and investigational therapeutic intervention is inappropriate for patients at low risk. Risk-stratification tools are needed for other CH subtypes.

Collaboration between hematologists and other subspecialists (eg, cardiologists or rheumatologists) may be helpful, given the

CHRS Prognostic Variable Scores

Prognostic Variable	0.5	1	1.5	2	2.5
Single *DNMT3A*	present	absent	–	–	–
High Risk Mutation	–	absent	–	–	present
Mutation Number	–	1	–	≥ 2	–
Variant Allele Fraction	–	< 0.2	–	> 0.2	–
Red Cell Distribution Width	–	< 15	–	–	≥ 15
Mean Corpuscular Volume	–	< 100	–	–	> 100
Cytopenia	–	CHIP	CCUS	–	–
Age	–	< 65y	≥ 65y	–	–

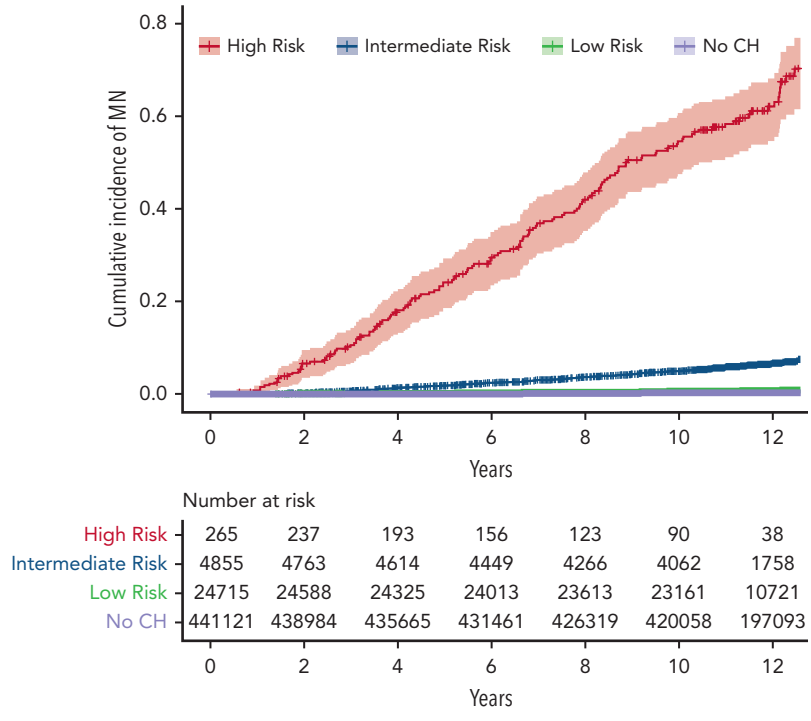

Figure 5. CHRS: a tool to predict risk of myeloid malignancy in CHIP/CCUS. (Top) Table of prognostic features and corresponding scores assigned in CHRS, and (bottom) cumulative incidence of hematologic malignancy among 470 960 participants in the UK Biobank stratified based on CHRS status. Top panel: From Weeks et al. Prediction of Risk for Myeloid Malignancy in Clonal Hematopoiesis. *New England Journal of Medicine Evidence*. 2023;2(5). Copyright ©(2023) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.[83]

risk of nonmalignant comorbidity accompanying various CH subtypes. In CHIP/CCUS, comorbidity risk correlates with CHRS risk groups suggesting that CHRS may also help guide risk-specific recommendations for nonmalignant comorbidity risk mitigation.[83] Age-appropriate screening, prevention, and management strategies for nonmalignant comorbidities are likely to provide universal benefit and should be used in patients with CH. Ongoing research efforts will determine whether additional benefit is derived from CH-specific lifestyle modifications[128] or therapies.[71] For patients with CH with solid tumors, there may soon be sufficient data to guide selection or timing of potentially genotoxic therapies and mitigate the risk of subsequent therapy-related MDS/AML (t-MDS/AML). Prospective randomized investigations of the differential risk of t-MDS/AML in CH patients with solid tumors treated with standard vs alternative therapies are needed.

Conclusions

Groundbreaking discoveries in CH have transformed our understanding of hematopoietic aging, leukemogenesis, and the role of bone marrow–derived immune cells in the etiology of age-related inflammatory disease. We now recognize CH as a collection of distinct subtypes, each with a risk of adverse outcomes that depends on genotype and clinical context. The accelerated pace of CH research is made possible by the availability of large genomic data sets, openly shared with long-term follow-up and detailed clinical annotation. In parallel, experimental model systems have allowed for basic understanding of cellular dependencies that etiologically link CH to adverse malignant and nonmalignant outcomes. Increasing clinical indications for next-generation sequencing has led to an exploding population of patients with incidentally diagnosed CH. The field is primed for rigorous prospective clinical investigations to validate and refine risk stratification in diverse clinical contexts and identify therapeutic interventions that counter the risk of malignant and nonmalignant outcomes in CH.

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Authorship

Contribution: L.D.W. and B.L.E. conceived the study design and wrote and approved the manuscript; and L.D.W. designed the figures.

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Footnote

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Clonal hematopoiesis of indeterminate potential (CHIP): Linking somatic mutations, hematopoiesis, chronic inflammation and cardiovascular disease

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ABSTRACT

Clonal hematopoiesis of indeterminate potential (CHIP) is the presence of a clonally expanded hematopoietic stem cell caused by a leukemogenic mutation in individuals without evidence of hematologic malignancy, dysplasia, or cytopenia. CHIP is associated with a 0.5–1.0% risk per year of leukemia. Remarkably, it confers a two-fold increase in cardiovascular risk independent of traditional risk factors. Roughly 80% of patients with CHIP have mutations in epigenetic regulators *DNMT3A, TET2, ASXL1*, DNA damage repair genes *PPM1D, TP53,* the regulatory tyrosine kinase *JAK2,* or mRNA spliceosome components *SF3B1,* and *SRSF2*. CHIP is associated with a pro-inflammatory state that has been linked to coronary artery disease, myocardial infarction, and venous thromboembolic disease, as well as prognosis among those with aortic stenosis and heart failure. Heritable and acquired risk factors are associated with increased CHIP prevalence, including germline variation, age, unhealthy lifestyle behaviors (i.e. smoking, obesity), inflammatory conditions, premature menopause, HIV and exposure to cancer therapies. This review aims to summarize emerging research on CHIP, the mechanisms underlying its important role in propagating inflammation and accelerating cardiovascular disease, and new studies detailing the role of associated risk factors and co-morbidities that increase CHIP prevalence.

1. Introduction

Clonal hematopoiesis refers to the presence of clonal populations of hematopoietic stem cells (HSCs) (Fig. 1). Hematopoiesis is generally a polyclonal process with HSCs of equipotential, giving rise to erythroid, lymphoid, myeloid, or megakaryocytic cells. Mutations may occur in genes that confer selective fitness advantage with aging HSCs less adept to correct for these errors, giving rise to clonally expanded populations of stem cells [1]. Clonal hematopoiesis may occur in the context of selective pressures such as cytotoxic therapies and tobacco smoking, inability to rectify DNA replication errors among aging HSCs or in the context of neutral drift, the random genetic drift of evolutionarily neutral alleles at the molecular level.

While clonal hematopoiesis can result in hematologic malignancy, cooperative mutations in additional genes are required to induce malignant transformation. Consequently, most people with clonal hematopoiesis never develop blood cancer. Therefore, these clonal populations are referred to as having "indeterminate potential." In 2015, a formal definition of clonal hematopoiesis of indeterminate potential (CHIP) was proposed with the following qualifiers: CHIP must occur in the absence of morphological variation in blood cells; a candidate driver gene mutation should be present at variant allele frequency of at least 2% in peripheral blood; and in the absence of diagnostic criteria for hematologic malignancy. Defining CHIP at a VAF of at least 2% reflects not only technical limits of sequencing technologies, but also a practical cutoff. With the emergence of extremely high-resolution sequencing, nearly all healthy 50–60 year-old patients tested had evidence of clonal hematopoietic populations with VAF of 0.03%, yet studies show that very small clones have minimal clinical consequence [2,3]. Nevertheless, the trajectory from smaller to larger clones currently remains ill-defined.

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2. Early epidemiologic evidence linking CHIP and cardiovascular disease

While CHIP carries an increased relative risk of incident hematologic malignancy (HR ~13) [4,5], the overall increase in absolute risk is comparatively small – roughly 0.5–1% per year [6]. Early epidemiologic studies noted that CHIP increased the risk of death by 40%, a magnitude far greater than could be explained by the risk of hematologic malignancy alone. Indeed, in these initial cohorts only one individual out of 246 carrying mutations associated with clonal hematopoiesis died of hematologic cancer [5]. Subsequent secondary analyses posited increased risk of coronary artery disease (HR 1.8–2.0), ischemic stroke (HR 2.6), and premature myocardial infarction (HR 4.0) independent of traditional risk factors for CVD which were later confirmed in independent datasets [5,7]. In fact, the magnitude of risk elevation from CHIP rivals that of traditional Framingham risk factors (Fig. 2) [5]. Subsequent studies have gone on to link CHIP carrier status with poorer prognosis in related cardiovascular conditions such as heart failure [8], and aortic stenosis [9]. (See Fig. 1.)

3. Mechanisms of CHIP candidate driver mutations in driving inflammation and CVD

The most commonly mutated candidate driver genes in CHIP are *DNMT3A*, *TET2,* and *ASXL1,* which increase the risk of atherosclerotic cardiovascular disease with HR of 1.7, 1.9 and 2.0 respectively (Table 1). These three mutations, referred to as "DTA mutations" in the myeloid leukemia literature, account for ~80% of all CHIP cases [7]. Additional mutations are seen in *JAK2*, which is particularly associated with increased rates of thrombosis, as well as the DNA damage response pathway genes *PPM1D* and *TP53*, and mRNA splicing factors *SRSF2* and *SF3B1*. Mutations in these sets of genes are commonly observed in myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPNs) and acute myeloid leukemia [10–13].

3.1. DNMT3A

DNA methyltransferase 3a (*DNMT3A)* is the most commonly mutated gene in CHIP. *DNMT3A* encodes a methyltransferase enzyme that catalyzes DNA methylation at CpG sites and is a critical epigenetic regulator of gene expression. The majority of pathogenic mutations are loss-of-function including disruptive missense mutations in regulatory and catalytic domains, nonsense mutations, insertions-deletions, and splice site mutations. *DNMT3A* pathogenic mutations enhance HSC self-renewal [14] and promote the expression of multipotency genes while suppressing differentiation factor expression [15]. This enables *DNMT3A* mutations to affect all hematopoietic lineages, inducing pro-inflammatory T-cell polarization and activating the inflammasome complex.

Mouse model experiments utilizing CRISPR gene editing establish that *DNMT3A* CHIP causes aberrant inflammation but may also be fostered by inflammation itself. Murine macrophage cell lines bearing *DNMT3A* mutation show increased inflammatory gene induction in response to lipopolysaccharide challenge, with increased expression of

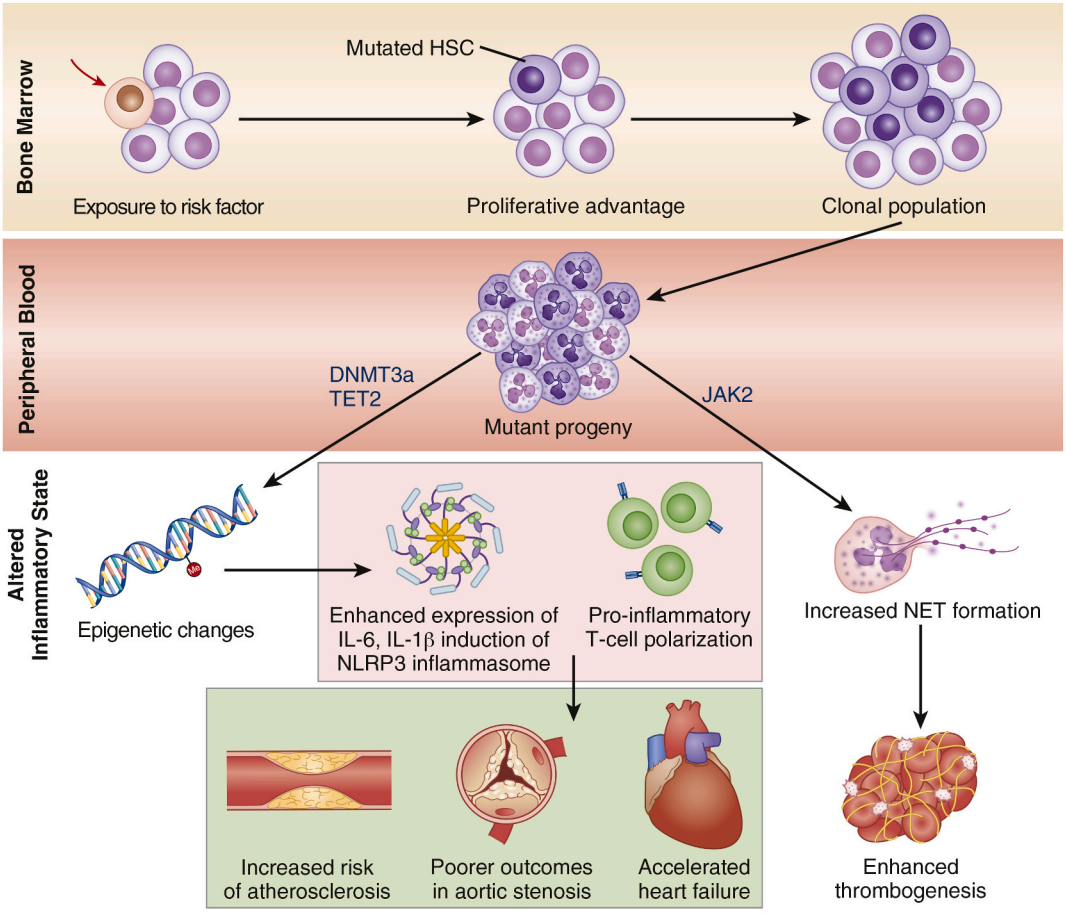

Fig. 1. CHIP associates with an altered inflammatory state, elevating cardiovascular risk.
The acquisition of driver mutations (in *DNMT3A*, *TET2*, and *JAK2* shown as a selection here) leads to clonal hematopoiesis of indeterminate potential (CHIP), which induces an altered inflammatory state that is associated with an increased risk of atherosclerosis, poorer outcomes in aortic stenosis and heart failure, and enhanced thrombogenesis. HSC = hematopoietic stem cell, NET = neutrophil extracellular traps Biorender.com

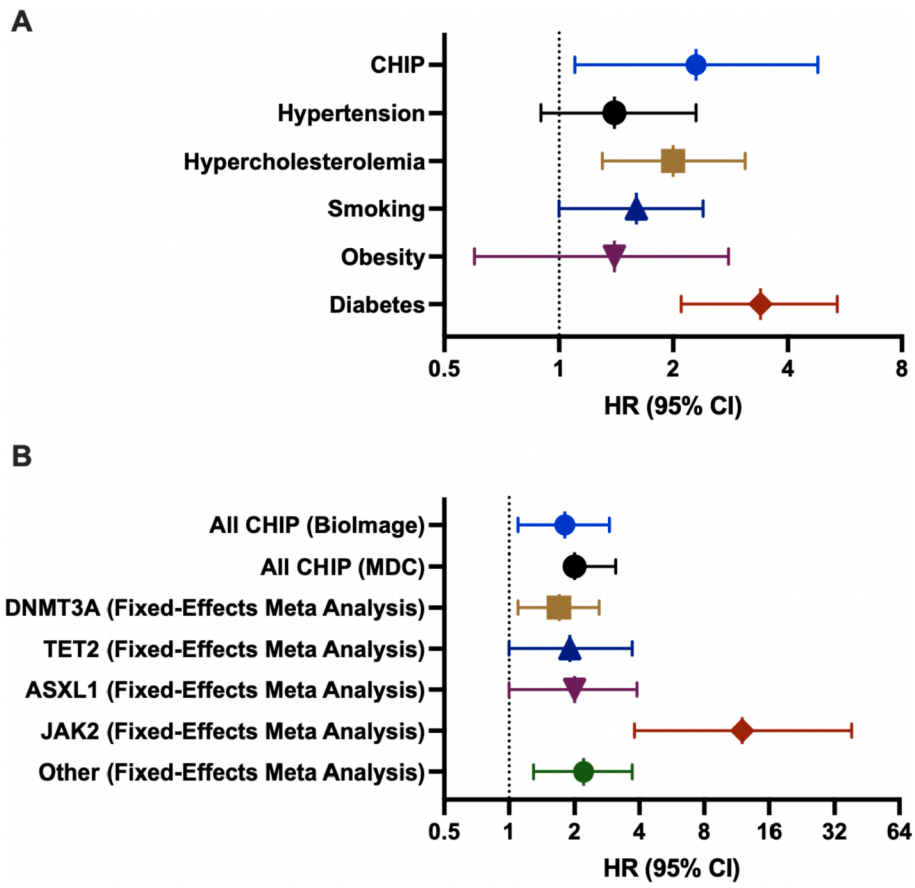

Fig. 2. Selected hazard ratios (HR) for CHIP and incident coronary heart disease are of similar magnitude to traditional risk factors.
(A) HR with 95% CI for incident coronary heart disease adjusted for age, sex, HTN, HLD, smoking, obesity, diabetes among Jackson Heart Study & FUSION participants, Summarized data from Jaiswal et al. 2014 [4].
(B) HR with 95% CI for incident coronary heart disease adjusted for age, sex, HTN, total and HDL cholesterol, triglycerides, smoking, and type 2 diabetes among BioImage, MDC (CHIP) and a fixed-effects meta-analysis for each gene (incorporating BioImage, MDC, JHS/Fusion/FHS). Summarized data from Jaiswal et al. 2017 [7].

Cxcl1, Cxcl2, IL-6 and Ccl5. Studies also show that a pro-inflammatory environment can reciprocally drive *DNMT3A* CH expansion. In chronic infection, *DNMT3A*-mutant HSCs outcompete wild-type HSCs and lead to *DNMT3A*-CH in peripheral blood, via increased resistance to stress-induced apoptosis and differentiation defects. Inflammatory interferon-gamma was sufficient to drive this clonal expansion of *DNMT3A*-mutant HSCs [16]. This pro-inflammatory environment has cardiac consequences: in a mouse model, *DNMT3A* deletion in HSCs leads to increased angiotensin II-mediated cardiac hypertrophy, reduced cardiac function, and greater cardiac and renal fibrosis [17].

Studies of *DNMT3A* CHIP in humans demonstrate similar downstream effects. Transcriptomic profiling of peripheral blood mononuclear cells derived from heart failure patients who were *DNMT3A*-mutant CHIP carriers demonstrated significantly increased expression of inflammatory interleukins IL-1β, IL-5, IL-8, activation of the NLRP3 inflammasome, macrophage inflammatory proteins CCL3 and CCL35, and resistin. Studies of this population also reveal a pro-inflammatory circulating monocyte signature and marked induction of T-cell stimulating genes like CD58, increased expression of T-cell alpha receptor and changes in T-cell subtype signature [18]. This pro-inflammatory T-cell polarization signature is observed in *DNMT3A* CHIP carriers in a cohort of aortic stenosis patients undergoing transcatheter aortic valve replacement, with a significantly increased Th17/Treg ratio in such patients [9].

3.2. *TET2*

The second most commonly mutated CHIP gene is DNA demethylase *TET2* (ten-eleven translocation-2). Notably while DNMT3A adds methyl groups at CpG sites, TET2 oxidizes the methyl group which is the first step in removing the mark. Remarkably these two biochemically opposing functions have a convergent stem cell phenotype. In mouse models, *TET2* loss of function enhances HSC self-renewal and preferentially leads to differentiation toward myeloid lineages [19]. *TET2* has an important role in restraining the expression of inflammatory genes in macrophages. *TET2*-deficient macrophages show increased inflammation, both spontaneous and in response to lipopolysaccharide, further potentiating an activated pro-inflammatory state [20]. *TET2* deficiency is associated with higher circulating levels of IL-1β through induction of the NLRP3 inflammasome [21], IL-6 [22], and IL-8 [7].

This pro-inflammatory state potentiated by *TET2* CHIP leads to accelerated atherosclerosis. Initial studies of *Ldlr*−/− atherosclerogenic mouse models marrow reconstituted with *Tet2*−/− HSCs via irradiation and bone marrow transplantation demonstrated greater atherosclerotic plaque burden [7]. In a similar mouse model, Fuster et al. mimicked the effect of variant allele frequencies observed in humans by studying mice with ~10% *Tet2* −/− bone marrow with 90% *Tet2 WT*, followed by ~10% *Tet2*+/− bone marrow with 90% *Tet2* WT. They observed a similar acceleration of atherosclerotic plaque burden at this clinically relevant VAF although speed of *Tet2* haploinsufficient clonal dominance is likely faster in the model than in humans [21]. Subsequent murine studies showed *Tet2* loss of function accelerates myocardial fibrosis and heart failure in pressure-overload- and ischemia-induced murine models of heart failure, modulated through the induction of the IL-1β/NLRP3 inflammasome [23].

Many findings extend to human populations to-date, with in silico analysis of the TOPMed cohort revealing significantly increased serum IL-1β levels among *TET2* CHIP carriers [24]. Cardiovascular risk conferred in *DNMT3A*- and *TET2*-mutant CHIP carriers could be abrogated by an inhibitory IL-6 receptor gene variant (*IL6R* p.Asp358Ala) [25], using a stratified Mendelian randomization approach to demonstrate the central role of pro-inflammatory mediators NLRP3, IL-1β and IL6 in mediating the development of CHIP-associated atherosclerosis. A cohort of patients with severe degenerative aortic stenosis undergoing

Table 1
Common CHIP driver mutations.

Candidate Driver	% of CHIP[a]	Mechanism	References
DNMT3A	**~58.5%**	Methyltransferase enzyme that catalyzes DNA methylation at CpG sites and alters epigenetic signature; tumor suppressor gene	[9,14,15,17,18]
TET2	**~20%**	DNA demethylase *TET2* (ten-eleven translocation-2) augments DNA methylation and affects transcription by recruiting histone deacetylases toward promoters; tumor suppressor gene	[9,20,21,23,25]
ASXL1	**~8.0%**	Epigenetic modulator and chromatin-binding protein, function relatively unknown	[27,29]
JAK2	**~3.2%**	Transmits intracellular signals downstream of cytokine receptors. *JAK2* tyrosine phosphorylates and activates *TET2* in response to cytokines, linking extracellular signals with epigenetic changes in hematopoiesis	[24,31,33,34]
PPM1D, TP53	**~3.8%, 1.9%**	DNA damage response pathway in regulatory feedback loop with the tumor suppressor p53.	[35,36]
SF3B1, SRSF2	**2%, 2%**	mRNA spliceosome complex components	[24]
No candidate driver mutation		Limits of detection methods, epigenetic changes not detectable, neutral drift, or mosaic chromosomal alterations	[37,40–42]

[a] Approximate percentages among CHIP with candidate driver mutations as detected in "Inherited causes of clonal haematopoiesis in 97,691 whole genomes, Bick et al *Nature* 2020" [24]

transcatheter aortic valve implantation (TAVI) noted an association between *TET2* CHIP carrier status and higher circulating levels of non-classical monocytes (CD14dimCD16++), which secrete higher concentrations of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-8. Compared to those without CHIP, these patients had an increased medium-term all- cause mortality following TAVI [9]. Deep-targeted amplicon sequencing of bone-marrow derived mononuclear cells among a cohort of chronic heart failure patients also showed an association between *TET2 CHIP* carrier status and HF progression and poorer clinical outcomes. This included a dose-response relationship with increasing *TET2* variant allele frequency [8].

3.3. ASXL1

ASXL1 (additional sex combs-like 1) is the third most commonly mutated gene in CHIP, with its gene product regulating polycomb-mediated transcriptional repression. *ASXL1* mutations are typically frameshift or nonsense mutations occurring near the 5′ end of the gene and likely lead to gain of function and aberrant histone modifications [26]. Observational studies detail a link between *ASXL1* mutations in blood cells with smoking [27], and among patients with HIV [28]. *ASXL1* deletion facilitates aberrant gene expression and results in myeloid transformation [29]. However, *ASXL1* deletion in mice also impairs HSC functioning, and so the mechanisms by which *ASXL1* mutations lead to clonal hematopoiesis are not clear.

Similarly, the mechanism by which ASXL1 enhances inflammation and atherosclerosis is also poorly understood. No in vivo data has demonstrated the downstream inflammatory effects of *ASXL1* CHIP carriers, but one can postulate that the shift toward myeloid transformation in *ASXL1* CHIP carriers may carry similar downstream effects to *TET2* loss of function.

3.4. JAK2

JAK2 is a non-receptor tyrosine kinase that transmits intracellular signals downstream of cytokine receptors. *JAK2* tyrosine phosphorylates and activates *TET2* in response to cytokines, linking extracellular signals with epigenetic changes in hematopoiesis [30]. *JAK2* p.V617F gain-of-function mutations in hematopoietic cells are associated with myeloproliferative neoplasm (MPNs) like polycythemia vera, essential thrombocytopenia and myelofibrosis, which are in turn associated with myocardial infarction, deep vein thrombosis and stroke. *JAK2* p.V617F mutations enhance formation of neutrophil extracellular traps (NET) that promote thrombosis. Indeed, in population studies, *JAK2* p.V617F CHIP carriers demonstrate increased incidence of deep vein thrombosis and pulmonary embolus [31]. *JAK2* CHIP carrier status is associated with higher levels of IL-18, and downstream increases in IL-6 production and inflammation [24].

JAK2 p.V617F mutations in CHIP tend to occur at a younger age and carry an up to a 10-fold increased risk of coronary artery disease – the strongest risk of premature cardiac disease among CHIP variants [7,24]. Interestingly, the induction of atherosclerosis in *JAK2* CHIP carriers occurs in the presence of reduced serum cholesterol: a negative correlation is observed between *JAK2* CHIP-carrier status and both total and LDL cholesterol among studies in large WGS databases [24].This is in contrast to the lack of association between CHIP overall and lipid profile [7].

Studies in *Ldlr*-deficient mice bearing *JAK2* p.V617F mutations demonstrate accelerated atherosclerosis [32]. *JAK2* mutant CH leads to increased proliferation of macrophages, and necrotic core formation in atherosclerotic lesions in mouse models. These necrotic core lesions demonstrate increased AIM2 inflammasome expression (as opposed to NLRP3), oxidative DNA damage, and DNA replication stress, effects blunted by IL-1β inhibition [33]. Additionally, murine heart failure models transplanted with *JAK2* p.V617F myeloid clones show evidence of accelerated pathologic remodeling, revealing a role for *JAK2* CHIP in potentiating fibrosis in heart failure [34].

3.5. CHIP with less common driver mutations: TP53, PPM1D, SF3B1, SRSF2

The next most frequent CHIP mutations are in DNA damage repair genes *TP53* and *PPM1D*. PPM1D (protein phosphatase Mn2+/Mg2 + −dependent 1D) is part of the DNA damage response pathway and in regulatory feedback loop with the tumor suppressor p53. Activated p53 induces PPM1D expression, leading to downstream dephosphorylation of p53 and downregulation of apoptosis. PPM1D loss-of-function mutations are in particular associated with CH in the context of prior exposure to cytotoxic chemotherapies such as cisplatin, etoposide and doxorubicin [35]. p53 mutations promote HSC expansion in response to radiation-induced stress, and mutant p53 interacts with EZH2 through epigenetic mechanisms to enhance its association with chromatin, increasing H3K27 tri-methylation of genes regulating differentiation and self-renewal of HSCs [36].

CHIP driver mutations in *SF3B1* and *SRSF2* are key components of the mRNA spliceosome. Mutations in these genes lead to defects in splicing and export of mRNAs encoding genes involved in translation. While these CHIP mutations are not well studied with respect to cardiovascular disease, studies have shown that patients with *SF3B1* mutant-CHIP have higher circulating levels of IL-18 [24].

3.6. CH without candidate driver mutations, neutral evolution, and mosaic chromosomal alterations

Despite the identification of multiple driver mutations associated with CHIP, in a significant proportion of cases of clonal hematopoiesis no clear candidate driver mutation is identified. As CHIP is defined as somatic mutation with VAF >2%, CH without known candidate driver

mutations is technically excluded from this classification. Despite this, clonal hematopoiesis without driver mutations carries increased risk of hematologic cancers and all-cause mortality, although its links to cardiovascular disease are poorly understood [37].

Whole-exome sequencing of peripheral blood DNA from an unselected cohort of 12,380 Swedish patients was among the first to identify that a large fraction of clonal hematopoiesis carriers (defined as at least 3 somatic mutations at detectable allele frequency) do not carry obvious driver mutations (none of the previously identified candidate driver mutations identified) [4]. Similarly, in whole-genome sequencing of 11,262 Icelandic patients (deCODE Genetics), the majority of subjects with clonal hematopoiesis (defined as >20 single somatic mutations with VAF 0.10–0.20) did not carry a clear driver mutation [37].

CH without driver mutations may be the result of limitations in detection methods (i.e. if driver mutations exist in non-exonic areas) or may be driven primarily by epigenetic encoding to enhance HSCs self-renewal and proliferation. CH without driver mutations might also be the consequence of neutral drift of small populations of active HSCs [37]. This theory focusing on genetic drift and neutral evolution has important potential implications – if the primary downstream effects of CHIP are mediated more by enhanced leukocytosis and less by the effects of individual driver mutations, then a focus on driver mutations as detailed above may not inform a therapeutic approach [38].

Mosaic chromosomal alteration (mCA) represents an additional mechanism of CH without driver mutations. mCAs include larger structural somatic alterations such as deletions, duplications, or copy number neutral loss of heterozygosity (CN-LOH). Similar to candidate driver mutations in CHIP, mCA accumulate with age, rising to a prevalence as high as 35% in the older than 90 population [39]. mCA-driven CH predisposes primarily to lymphoid malignancies like CLL and carries a roughly two-fold increase in all-cause mortality [40,41]. However, mCA-driven CH (even when associated with *DNMT3A* or *TET2* loss) did not appear to be associated with a significant increase in cardiovascular risk, with the notable exception of *JAK2*-related CN-LOH events [41]. In addition to an association with incident blood cancer, mCA-driven CH may lead to impaired immunity and predispose to infection [42].

4. Risk factors associated with clonal hematopoiesis of indeterminate potential

Epidemiologic and genetic studies have focused on identifying the interplay between germline variation, co-morbid conditions and lifestyle factors and their association with CHIP. These associations are important foundations for hypothesis generation regarding mechanisms, and for informing the clinical care of CHIP patients (Table 2).

4.1. Genotypic associations with CHIP

While CHIP driver mutations are acquired, somatic mutations, germline variation has an important role in predisposing the development of CHIP. Hinds et al. performed initial genome-wide association studies of 726 individuals with myeloproliferative neoplasms, 497 individuals with *JAK2* p.V617F clonal hematopoiesis, and 252,140 controls. This identified associations with germline genetic variants of *TERT, SH2B3, TET2, ATM, CHEK2, PINT* and *GF11B* [43]. Using whole genome sequencing data from 11,262 participants, Zink et al. detected a strong association with CHIP development and an 8-bp deletion in intron 3 of *TERT*, which correlated with shorter telomere length [37]. Extending these observations to a larger cohort unselected for candidate driver mutation, whole genome sequencing studies of 97,691 individuals identified three gene risk loci associated with a predilection to *TET2* CHIP. One set of loci were associated with genes facilitating genomic integrity and telomere length (*TERT* and *CHEK2*) that also raised the risk of neoplasm in multiple organ systems; another, with HSC self-renewal (*TET2*) was only associated with hematologic malignancies; and a final locus at the *TCL1A* promoter specifically associated with increased risk of *DNMT3A* CHIP alone [24].

Table 2
Emerging evidence of genotypic and phenotypic associations with CHIP.

Risk factor	Relevant studies
Germline Mutation	[24,37,43]
Aging and leukocyte telomere length	[24,37,48,50,51]
Smoking	[4,27,37,58,59]
Obesity, Insulin resistance & Type 2 Diabetes	[5,58,85]
Hyperlipidemia & Atherosclerosis	[53,54,58]
Sleep Deprivation	[38,62]
Premature Menopause	[64]
Chronic Inflammatory Conditions	[37] Systemic Sclerosis [65] Rheumatoid Arthritis [66] Ulcerative colitis [72]
Chronic Infection, including HIV	Chronic Infection [16] HIV [28,75]
Cancer therapy	[35,59,78]

4.2. Phenotypic associations with CHIP

There are numerous phenotypic associations with CHIP, ranging from aging to lifestyle factors. A selection of emerging research is summarized below highlighting the links between these conditions and CHIP risk. The directionality of causality is an area of active research, in part due to the paucity of longitudinal samples limiting the study design and methodologies used to establish these associations.

4.3. Aging: cumulative genomic damage and leukocyte telomere length

Aging is a potent risk factor for atherosclerotic cardiovascular disease [44], and aging is marked by the acquisition of somatic mutations in hematopoietic stem cells due to cumulative genomic DNA damage [45]. Multiple studies have demonstrated that the proportion of CHIP carriers increases exponentially with age [4,5,46].

Leukocyte telomere length and CHIP have a complex relationship. Patients with inherited telomeropathies show increased CH prevalence [47]. Common variants at *TERT* predisposing to prolonged leukocyte telomere length (LTL) are associated with increased CHIP odds [24], and CHIP carriers possess significantly shorter leukocyte telomere length (LTL) compared to those without [37]. Mendelian randomization studies in particular support an inverse relationship between LTL and coronary artery disease [48–50], and Nakao et al. now systematically show with bidirectional Mendelian randomization that processes promoting LTL lengthening at first increase the propensity for CHIP development, with CHIP then promoting accelerated LTL shortening [51]. LTL length additionally mediated a modest association between CHIP and CAD but this may be limited by the bidirectional opposing associations between LTL and CHIP [51].

Based on these observations, some have proposed a “telomere brink” hypothesis of CHIP. This posits that as individuals age, age-dependent telomere shortening particularly affects the highly proliferative hematopoietic system, and that candidate driver mutations enrich in hematopoietic cells as a means of delaying reaching their “telomere brink” when the replicative potential of HSCs is reached [52].

4.4. Complex interactions with hyperlipidemia and the “Atherosclerosis Trait Complex”

Chronic elevation of blood lipid levels promotes the formation of atherosclerotic plaques, potentiated by immune cell recruitment and local inflammation. Increased cholesterol levels stimulate proliferation and mobilization of HSCs as well as myeloid cell expansion [53,54], and high levels of HDL suppress HSC proliferation [55]. In mouse models, *Apoe* −/− and *Ldlr* −/− mice fed high-lipid diets demonstrate elevated HSC proliferation [56].

Despite these associations, in large-scale association studies CHIP is not consistently associated with lipid levels (triglycerides, total cholesterol, LDL-C or HDL-C) apart from the association with *JAK2* CHIP, which is in fact correlated with a decrease in total cholesterol and LDL-C despite elevated CAD risk [24,57]. Seeking to explain this discrepancy, Heyde et al. demonstrated that atherosclerosis induces HSC proliferation in both mice and humans, and that this induction of HSC proliferation in atherosclerosis is sufficient to drive clonal expansion of mutant *Tet2* −/− HSCs in *Ldlr*−/− mice. Notably, the induction of mild hypercholesterolemia in non-atherosclerotic wild-type did not induce *TET2*−/− clonal expansion [38], suggesting that in the absence of the inflammatory milieu of atherosclerosis, elevated cholesterol alone is not sufficient to drive clonal hematopoiesis. The extent to which blood cholesterol concentrations differentially promote atherogenesis in the context of CHIP versus no CHIP requires further study.

4.5. Smoking and obstructive airway disease

Smoking has been consistently observed to associate with CH [4,37], and never smoked status compared to current smoking status is associated with a reduced risk of developing CHIP [58]. Research suggests that *ASXL1* mutations in particular are enriched in current and past smokers [27,59]. While two groups have reported associations with CHIP and COPD, the association of CHIP and smoking likely confound these associations [37,60].

4.6. Obesity and type 2 diabetes

In a study of 8709 post-menopausal women, having a normal body mass index compared to being obese was associated with lower frequency of CHIP [58], and obesity-related insulin resistance appears to be associated with increased *TET2* CHIP status. Jaiswal et al. reported a 1.3 fold increased odds of CHIP in diabetes patients [5]. Subsequently, studies in mouse models showed that *TET2*-driven clonal hematopoiesis led to increased expression of IL-1β in white adipose tissue, aggravating age and obesity-related insulin resistance and worsening hyperglycemia [61]. Whether CHIP is associated with the risk for incident diabetes mellitus requires further investigation.

4.7. Sleep fragmentation

Sleep disruption increases the risk of cardiovascular disease, diabetes, obesity, and cancer, and studies in mouse models demonstrate increased atherosclerotic lesions in sleep-deprived mice, with systemic monocytosis and neutrophilia [62]. The emergence of CH of *Tet2*−/− clones in *Ldlr*−/− mouse models is accelerated by a factor of 1.6 with sleep fragmentation [38], suggesting that sleep deprivation may accelerate CHIP development. The association of addiction and psychiatric diseases with CHIP might be driven by the fact that sleep fragmentation is a critical component these conditions [37].

4.8. Premature menopause

Premature menopause, both natural and surgical, is associated with an increased risk of cardiovascular events in women (including coronary artery disease, heart failure, ischemic stroke, PAD, VTE, MR and aortic stenosis) [63]. A follow-up cohort study of women from the UK Biobank and Women's Health Initiative databases established premature menopause was associated with increased CHIP prevalence (OR 1.40) and incident CAD (HR 1.36, rising to 1.48 for VAF > 0.1), with a stronger association observed with natural premature menopause (HR 1.73) versus surgical premature menopause [64]. *DNMT3A* was the only candidate driver mutation found to be associated with premature menopause.

4.9. Chronic inflammatory conditions

A broad range of pro-inflammatory and rheumatologic conditions associate with CHIP, including chronic pulmonary disease [37]. Systemic sclerosis (SSc) is characterized by immune dysregulation, aberrant fibrosis and microangiopathy. A study of 90 SSc patients and 44 healthy donors found the prevalence of CHIP was elevated in SSc patients (25%) compared to healthy donors (4%), with *DNTM3A* mutations most common. However, no clinical differences were apparent between CHIP and non-CHIP carriers in this small cohort [65]. Increased CH prevalence was also found in rheumatoid arthritis (RA) patients. Savola et al. studied a cohort of 59 RA patients and found a 17% prevalence of CHIP, increasing to up to 25% among 70–79 year old patients studied. *DNMT3A* and *TET2* mutations were most common [66]. Whether CHIP associates with other closely related rheumatologic conditions such as systemic lupus erythematosus (SLE) – which itself carries a significantly increased risk of early MI and CVD [67] - is unknown. Limited data suggest an increased risk of myeloid neoplasms in both RA and SLE populations [68].

Ulcerative colitis (UC), an inflammatory bowel disease (IBD) marked by T-cell infiltration in the colon and overproduction of TNF-alpha and interferon-gamma, has also been associated with CHIP. While cardiovascular manifestations in IBD tend to cluster around immune-related phenomena (pericarditis, myocarditis, thromboembolism), there is also an increased risk of ischemic heart disease in UC patients [69–71]. UC patients with CHIP exhibited a distinctive mutational spectrum, with *DNMT3A* and *PPM1D* mutations most commonly seen in this patient cohort, and evidence of a correlation found between elevated levels of serum interferon-gamma and *DNMT3A* mutation [72]. Elevated levels of Th17 cells and Th17 related cytokines are important in the pathogenesis of mucosal damage in IBD [73], similar to observations of pro-inflammatory T cell polarization (increased Th17 ratio) in *DNMT3A*-CHIP carriers with severe degenerative aortic stenosis [9].

4.10. Chronic infection and HIV

Chronic infections may also increase the risk of developing CHIP. In mouse models, chronic mycobacterial infection led to *DNMT3A*-CHIP clone expansion via an interferon-gamma dependent mechanism [16], and mCA-driven CH was associated with an increased risk of sepsis, pneumonia, and coronavirus disease 2019 hospitalization [42].

Among HIV patients, coronary artery disease is a major source of morbidity, the consequence of accelerated biological aging, chronic inflammation and immune dysregulation. HIV is associated with a greater risk of myelodysplastic syndromes [74]. In a multi-ethnic sample of 600 people living with HIV (PLWH) derived from the Swiss HIV Cohort Study, a two-fold increased prevalence of CHIP was observed when compared to 8111 participants from the ARIC (Atherosclerotic Risk in the Community) cohort. In particular, there was a greater prevalence of *ASLX1* mutations among PLWH [28]. Similar findings were observed in the ARCHIVE study, which included 220 HIV positive and 226 HIV-negative Australian participants, and demonstrated a two-fold increased risk of CHIP with *DNMT3A* (48.5%), *TET2* (20.5%) and *ASXL1* (11.4%) mutations [75].

4.11. Cancer treatment

Clonal hematopoiesis has been observed in the context of a wide variety of solid and liquid malignancies, often considered to be a consequence of cytotoxic chemotherapies. Among patients treated with stem-cell transplantation for non-Hodgkin's lymphoma, the incidence of CH was 30% [76]. Cancer therapies shape the fitness landscape of clonal hematopoiesis, with ionizing radiation, topoisomerase II inhibitors and cisplatin selecting for mutations in DNA damage response genes *TP53, PPM1D* and *CHEK2* [35,59]. Importantly, immune checkpoint blockade did not appear to be associated with expansion of clonal hematopoiesis

[77]. A cohort of 135 invasive glioma patients undergoing next-generation sequencing of cfDNA treated with temozolomide treatment showed increased CH-type mutations, most commonly *TP53*, followed by *ATM*, *GNAS* and *JAK2*, that correlated with risk of shorter survival [78]. Understanding the degree of cardiovascular risk conveyed by higher CHIP prevalence in these populations will be important in informing oncologic survivorship care.

5. Conclusions and future directions for CHIP prevention and therapy

As studies detail new genotypic and phenotypic associations with CHIP, researchers and clinicians are confronted with the question of what preventative and therapeutic interventions could be taken to mitigate disease risk. Understanding which cohorts of patients could benefit from targeted CHIP testing could enable a precision-medicine approach to risk reduction. Additionally, many CHIP patients may be identified incidentally. Cell-free DNA analysis, intended to detect circulating tumor DNA to aid in early-cancer detection, is invariably confounded by the presence of CHIP, as the vast majority of cell-free DNA arises from hematopoietic cells [79–81].

An understanding of CHIP biology and its reciprocal relationship to a pro-inflammatory state presents numerous potential targets for therapy. As a genetic proxy of IL-6 inhibition, the presence of the inhibitory IL-6 receptor gene variant (*IL6R* p.Asp358Ala) reduced the CVD risk in *DNMT3A* and *TET2* CHIP carriers by ~50%, highlighting that inhibiting IL-6 signaling can decrease the risk of cardiovascular disease to a much greater degree among individuals with CHIP versus without [25]. This is in keeping with studies which have found that IL-1β blockade with canakinumab after MI reduced risk of death form cardiovascular disease, rates of nonfatal AMI and nonfatal stroke (CANTOS [Canakinumab Anti-inflammatory Thrombosis Outcomes Study]). Non-prespecified post hoc exploratory analyses demonstrated that a greater reduction was seen in those with *TET2* CHIP [82]. In a *JAK2* CHIP model, both non-selective IL-1 receptor blockade with anakinra and targeted IL-1β blockade led to reduced atherosclerotic plaque instability, with normalized macrophage proliferation and density in early atherosclerotic plaques, and reduced core necrosis and increased cap thickness in advanced atherosclerotic plaques [33]. Targeted inhibition of the inflammasome itself may also have protective benefits against atherogenesis. In mouse models of *TET2* deficient mice, pharmacologic inhibition of the NLRP3 inflammasome with MCC950 led to a 50% decrease in aortic atherosclerotic plaque size, greater than the non-significant reduction observed in wild-type mice [21]. Targeted inhibition of the AIM2 inflammasome, which is characteristic of *JAK2* mutant CHIP, might also have similar benefit [33].

Targeting candidate driver mutations in CHIP could represent an alternative therapeutic strategy. Vitamin C metabolites activate *TET2* and can mimic restoration of *TET2* via enhancing 5-hydroxymethycytosine formation in *TET2*-deficient mice to reverse aberrant HSC self-renewal, presenting a potential preventive therapy for *TET2*-CHIP carriers [83]. In *JAK2* mutant CHIP, treatment with the approved *JAK2* inhibitor ruxolitinib reduced abnormal neutrophil extracellular trap formation and deep vein thrombosis [31] as well as circulating IL-18 levels [33], and *JAK2* inhibition with fedratinib in *Apoe* −/− mice suppressed myelopoiesis and the development of atherosclerosis [84].

Additionally, understanding the associations of race, ethnicity and ancestry with CHIP prevalence is a crucial area for future research. The majority of early CHIP studies have been conducted in European Caucasian cohorts. However, a modestly lower prevalence of CHIP has been observed in individuals of Hispanic and East Asian ancestry compared to those of other ethnicities [7,25]. Additionally, the germline variant rs144418061, which conveys an increased risk of CHIP, is a variant present only in individuals of African ancestry [25], recognized in part due to the expanded diversity of the TOPMED cohort (40% European, 32% African, 16% Hispanic and 10% Asian). Further studies in racially and ethnically diverse populations will be important in understanding the implications of a CHIP diagnosis in broader populations and may help to identify further associations between certain ethnic groups and germline variants or specific candidate driver mutations.

Many questions remain in understanding the potent cardiovascular disease risk conveyed by CHIP. Ongoing research focused on elucidating the underlying genetic and biological mechanisms driving CHIP, and the environmental risk factors modulating CHIP risk will be critical in addressing this newly recognized risk factor. CHIP represents a unique shift in the study of cardiovascular genetics and atherosclerosis biology from inherited germline mutations to an understanding of the critical role of acquired somatic mutations. As identification of CHIP patients becomes more common, this represents a unique and emerging opportunity for multidisciplinary collaboration between hematologists, oncologists, and cardiologists.

Declaration of Competing Interest

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Assessing clonal haematopoiesis: clinical burdens and benefits of diagnosing myelodysplastic syndrome precursor states

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Diagnosing, surveilling, and understanding the biological consequences of clonal haematopoiesis poses a clinical challenge for both patients and clinicians. The relationship between peripheral blood cytopenias and myeloid neoplasms—such as myelodysplastic syndrome—is an area of active research, and understanding of clonal haematopoiesis has developed markedly on the basis of findings concerning somatic mutations in genes known to be associated with myelodysplastic syndrome. These findings have raised the conundrum of how to appropriately define and follow myelodysplastic syndrome precursor states, such as clonal haematopoiesis of indeterminate potential (CHIP) and clonal cytopenias of undetermined significance (CCUS). Identifying these conditions could allow earlier diagnosis of myelodysplastic syndrome, modify surveillance for myelodysplastic syndrome, and possibly guide therapies, but this information also comes at a cost to patients that might or might not be justified by our present understanding of clonal haematopoiesis. When faced with a diagnosis of clonal haematopoiesis, some patients and providers might be content to let the events unfold naturally, whereas others may insist on intense follow-up and early interventions. This Viewpoint assesses recent developments in clonal haematopoiesis and the related implications for affected patients and their providers.

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Introduction

A patient who presents to the clinic for investigation of peripheral blood cytopenia brings dilemmas of diagnosis, monitoring, and therapy. Refractory cytopenia in the context of a normocellular or hypercellular bone marrow can often raise concern for myelodysplastic syndromes and related disorders.[1,2] Myelodysplastic syndrome encompasses a heterogeneous collection of clonal haematopoietic malignancies affecting a predominantly older population. The disorder is characterised by poor overall survival due to ineffective haematopoiesis, progressive cytopenia, and transformation to acute myeloid leukemia.[3] Extremely rare in patients younger than 50 years, the prevalence of myelodysplastic syndrome increases with age. Between 30 000 and 40 000 cases are diagnosed per year,[4] with a median age at diagnosis of about 70 years.[5] The diagnosis of myelodysplastic syndrome has become more complex since myelodysplastic syndrome precursor states and other causes of unexplained cytopenias have been defined. The advent of next-generation sequencing technology has provided additional diagnostic information, which could allow important clinical insights, such as early identification of predisposition states to myelodysplastic syndrome and increased accuracy in diagnosis of myelodysplastic syndrome.[6,7] However, this added ability to assess a patient for evidence of clonal haematopoiesis poses inherent challenges of how to allow these results to affect the patient's health—both physical and emotional.

Patients with unexplained cytopenias are increasingly undergoing molecular testing by next-generation sequencing of peripheral blood or bone marrow to diagnose possible myelodysplastic syndrome precursor states. These tests can detect mutations in individuals without morphological or cytogenetic evidence of myeloid neoplasm or myelodysplastic syndrome, and new entities have therefore been defined to categorise these patients appropriately. Some of these precursor states could evolve to frank malignancy, making it important to define a path that explains these conditions and allows proper understanding of and context for a patient's health.[5,8,9]

Genesis and evolution of clonal haematopoiesis

Clinicians need to understand the biological explanation of positive next-generation sequencing results to guide their next steps, and patients also deserve and often require explanations to contextualise their new diagnosis. In truth, the acquisition of somatic mutations is an unavoidable consequence of cell division. Even though fewer than three somatic mutations occur per cell division, mutations can accumulate quickly in rapidly dividing haematopoietic progenitor pools.[10–13] The human haematopoietic system is one of the most proliferative tissues in the human body. Haematopoietic stem cells have an enormous task of providing nearly 10^{12} cells every day,[14] and thus the acquisition of somatic mutations with time is inevitable. The most rapidly dividing, mutation-prone haematopoietic progenitors lack the potential for self-renewal; therefore, any such mutation-carrying clone will usually disappear as a consequence of terminal differentiation and senescence. Occasionally, mutations can occur in self-renewing haematopoietic stem cells and be retained in the haematopoietic pool. As expected, these so-called single nucleotide variants accumulate with time and become relatively ubiquitous as people age beyond the fifth decade of life.[15]

Fortunately, because of their mostly random occurrence, most mutations affect non-coding regions and thus remain functionally silent (passenger mutations). However, somatic mutations occasionally fall within coding or regulatory regions of the genome and affect

genes crucial to cell fate determination, proliferation, or self-renewal, resulting in selective growth advantage and clonal expansion (driver mutations). Uncontrolled proliferation and incomplete maturation frequently result in substantial expansion of the clones and attrition of the healthy haematopoietic counterparts, leading to a clinically apparent haematological malignancy, such as myelodysplastic syndrome. In the past decade, several groups[8,9,16,17] have reported that mostly minor clones (marked by acquired mutations in genes frequently associated with haematological malignancies) are present in the blood of ageing individuals with no haematologic phenotype. The terms clonal haematopoiesis of indeterminate potential (CHIP) and age-related clonal haematopoiesis have been introduced to describe this intriguing and multifaceted condition.[5,18]

Assessment of clonality for CHIP

It is now widely accepted that cancers arise from a single progeny, as a result of uncontrolled growth of its daughter cells or clones. The clonal nature of cancer was first described in the 1960s using X-chromosome inactivation studies.[19,20] Most contemporary methods of clonality detection are based on genetic analysis and include gene rearrangements (T-cell and B-cell receptors), structural and numerical chromosomal changes, small copy number variants, and somatic point mutations. All these methods differ in terms of sensitivity and specificity, directly related to detection limits and number of markers used in each assay. In general, broad panels—such as whole genome or whole exome sequencing—provide a high sensitivity for the detection of clonal haematopoiesis given the extent of the genome tested.[21] Such broad approaches, although useful in research studies, are clinically impractical because of their cost, analytical challenges, and the difficulty of interpretation. By contrast, targeted panels, which are frequently limited to less than 100 cancer-relevant genes, are often more specific, affordable, and easier to interpret than their broader counterparts. Most clinically available panels for haematological malignancies also include genes frequently mutated in CHIP, such as *DNMT3A*, *TET*, *ASXL1*, *TP53*, *JAK2*, and around 25 other genes.[22]

The recent interest in clonal haematopoiesis stems from the broad application of next-generation sequencing in individuals without apparent haematological disease, and from the use of peripheral blood cells as a source of constitutional DNA. This approach resulted in incidental identification of somatic mutations in genes known to be frequently mutated in haematological malignancies. Thus, in most recent studies, clonal haematopoiesis in individuals with unremarkable haemograms was defined as a limited expansion of haematopoietic clones in peripheral blood, marked by the presence of somatic single nucleotide variants or small insertions or deletions (indels). This point is important to emphasise, because our references to clonal haematopoiesis will be based largely on the presence of somatic single nucleotide variants and indels, rather than on cytogenetic alterations determined by conventional cytogenetic studies and frequently used to diagnose haematological malignancies.

Definition of clonal haematopoiesis

Unfortunately, the term clonal haematopoiesis, outside its undisputable association with haematological malignancies, misses a degree of precision in its application. Clonal haematopoiesis is frequently defined as non-reactive, relative expansion of haematopoietic clones—regardless of magnitude—detected by any means and at any point in time. Although this definition could allow potential categorisation for some patients, the defining characteristics of clinically relevant clonal haematopoiesis remain unclear. Several questions need to be addressed as clinicians continue to apply incomplete knowledge of clonal haematopoiesis to the bedside. First, relative clonal dominance may be a consequence of genuine, uncontrolled expansion of cells due to clonal growth advantage or stem-cell attrition, which is frequently seen with ageing. A reliable method is needed to differentiate these two scenarios and their respective biological consequences.

Second, the minimum size of biologically relevant clones is yet to be established definitively. Initially, the proposed 2% variant allele frequency for the definition of CHIP was based on the lower limit of reliable detection of small somatic variants using whole exome sequencing.[5] This variant allele frequency is reasonable in these conditions, but it might not fully detail the biological and clinical relevance of this value. We discuss the nuances of variant allele frequency at diagnosis later in this Viewpoint. Additionally, the development of ultra-deep, error-corrected targeted sequencing approaches that are capable of detecting mutations in less than 0·5% of cells revealed the presence of miniscule clones in more than 95% of older individuals (aged >60–70 years). These clones were evenly distributed among haematopoietic lineages in peripheral blood and remained stable for decades, suggesting both an haematopoietic stem cell origin and insufficient expansion potential.[23] Third, it is unclear whether qualitative or quantitative (or both) characteristics of CHIP clones result in similar phenotypes across patients, or whether there are more diverse biological effects. To illustrate, cardiovascular complications have been associated with CHIP, and the likelihood of these events is increased when clonal monocytes are present in a patient's peripheral blood; this increase could in part be related to the qualitative characteristics of the clone effect, but malignant potential is probably related to quantitative burdens.[24–27] Furthermore, an exploration into phenotypes has shown that some mutations can result in spontaneous expansion and transformation, whereas others can require additional cell-extrinsic stressors such as chemotherapy, radiation, or environmental toxins, to produce the

disease.[28] Finally, there is the question of clonal persistence. Are all detectable clones permanent or transient? This last question has particular relevance to the establishment of a clinical monitoring plan once the diagnosis of clonal haematopoiesis has been made.

Classification of clonal haematopoiesis with and without cytopenias: axiom or linguistic mélange of four-letter acronyms?

It has been postulated that ageing haematopoietic stem cells acquire random somatic mutations, which could lead to clonal expansion, acquisition of secondary hits, and ultimate transformation to clinically apparent disease. In fact, nearly 90% of patients with myelodysplastic syndrome have identifiable somatic mutations in haematopoietic cells.[29] Moreover, mutations in putative cancer drivers such as *DNMT3A*, *TET2*, and *ASXL1* encompass more than 90% of clonal haematopoiesis and are among the most frequently affected genes in myelodysplastic syndrome. Even though clonal haematopoiesis is frequent, less than 1% of people with clonal haematopoiesis progress to clinically apparent disease. Thus, the presence of somatic mutations in haematopoietic cells might be of clinical importance or might represent an incidental finding in older patients with marginally abnormal haemograms that do not fulfill minimal diagnostic criteria for myelodysplastic syndrome.[30] What we do not yet know with precision is whether these incidental findings might evolve into clinically apparent disease. The associated uncertainly could lead to great anxiety in some patients and providers. The most recent attempts to classify these somewhat overlapping and potentially clinically important premalignant conditions encompass three major clinical and pathological findings: presence of clonal markers, dysplasia, and peripheral blood cytopenias (figure 1).

Despite subtle differences, which are perhaps more pertinent to research than clinical practice, the terms CHIP and age-related clonal haematopoiesis can be used interchangeably and denote the expansion of haematopoietic clones, harbouring specific—probably disruptive—and recurrent genetic variants in individuals with normal haemograms and without clear diagnosis of haematological malignancies.[5,18,31,32]

Patients with cytopenias can be categorised (panel 1) as either idiopathic cytopenia of unknown significance (ICUS), or clonal cytopenia of unknown significance (CCUS). Peripheral blood cytopenias are defined on the basis of standard laboratory values (haemoglobin <130 g/L [males], <120 g/L [females]; absolute neutrophil count $<1{\cdot}8\times10^9$/L; platelets $<150\times10^9$/L).[33] ICUS is defined as any degree of cytopenia in one or more lineages that persists for at least 6 months, does not fulfill the minimal diagnostic criteria for myelodysplastic syndrome, and cannot be explained by other haematological or non-haematological conditions.[34–36] A consensus panel of experts has proposed terminology for and classification

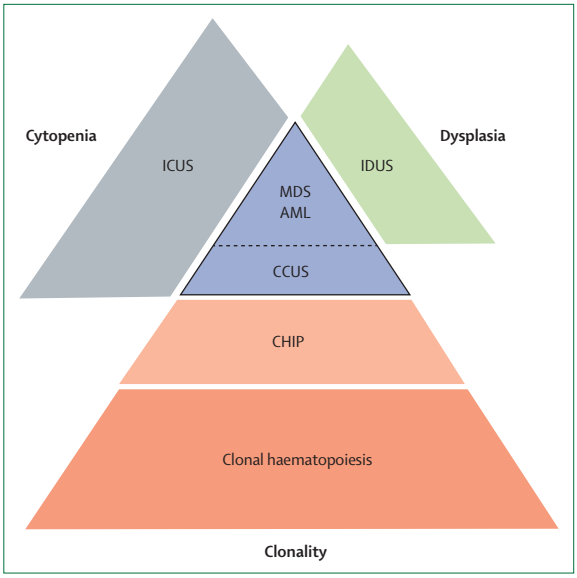

Figure 1: **Relationship between cytopenia, dysplasia, and clonality in precursor states and myelodysplastic syndrome**
Figure showing clinical and pathologic overlap of various diseases, and a hierarchy whereby patients can acquire increasing depth of cytopenia, dysplasia, or even additional clonality on the path to myelodysplastic syndrome. ICUS=idiopathic cytopenia of unknown significance. MDS=myelodysplastic syndrome. IDUS=idiopathic dysplasia of unknown significance. AML=acute myeloid leukaemia. CCUS=clonal cytopenia of unknown significance. CHIP=clonal haematopoiesis of indeterminate potential.

Panel 1: **Definitions of the precursor states to guide diagnosis**

Clonal haematopoiesis of indeterminate potential
- Presence of at least one somatic mutation that is relevant clinically and otherwise found in myelodysplastic syndrome (or other myeloid neoplasms)
- Absence of persistent cytopenia
- Exclusion of myelodysplastic syndrome and all other haematopoietic neoplasms (and other diseases) as the causal underlying condition

Idiopathic cytopenia of undetermined significance
- Presence of relevant cytopenia in one or more lineage for at least 6 months
- Not explained by any other disease
- Diagnostic criteria of myeloid neoplasm not fulfilled

Clonal cytopenia of undetermined significance
- Presence of one or more somatic mutations otherwise found in patients with myeloid neoplasms in bone marrow or peripheral blood cells with an allele burden of more than 2%
- Presence of persistent cytopenia (≥4 months) in one or more peripheral blood cell lineages
- Diagnostic criteria of myeloid neoplasm not fulfilled
- Exclusion of all other causes of cytopenia and molecular aberration

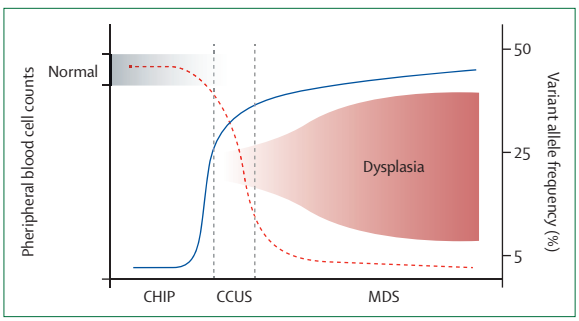

Figure 2: **Peripheral blood cell count, variant allele frequency, and dysplasia as a continuum from asymptomatic CHIP to clinically obvious myelodysplastic syndrome**
Clonal haematopoiesis of indeterminate potential (CHIP) is characterised by normal peripheral blood counts, low level clonal expansion (low variant allele frequency), and no evidence of dysplasia. With time, somatic mutations present in CHIP clones result in clonal outgrowth of cells (variant allele frequency >20–30%), with abnormal differentiation leading to peripheral blood cytopenias without morphological evidence of dysplasia (clonal cytopenia of unknown significance, CCUS) followed by clinically apparent myelodysplastic syndrome (MDS) with dysplastic features. Dashed red line indicates blood count; solid blue line indicates variant allele frequency.

of premalignant clonal conditions, which is useful for classification.[36] Patients with ICUS in whom clonal abnormalities have been identified can be classified as patients with CCUS.[31] This term is reserved only for patients with non-myelodysplastic syndrome or non-acute myeloid leukaemia, for defining cytogenetic abnormalities.

There is no standardised variant allele frequency cut-off for CCUS, and some authors propose the same as for CHIP (>2%). In our opinion, CCUS should imply that cytopenias are solely driven by the clonal process resulting in ineffective haematopoiesis. Since this causation cannot be fully explained by the presence of a minute clone, we propose to apply the variant allele frequency cut-off of the dominant clone (>20%) for CCUS diagnosis. This value is based on previously published data showing the variant allele frequency distribution in patients with CCUS with 95% cumulative progression to clinically apparent myeloid malignancy within 10 years.[7] In addition, using this higher variant allele frequency cut-off would possibly separate cytopenias that are due to the clonal process (such as CCUS) from other cytopenias that co-occur with incidental and inconsequential small CHIP clones (figure 2). Given a nearly 100% 10-year probability of CCUS progression to myeloid malignancies, perhaps the term unknown significance is not the most fitting and this condition ought to be placed in an early myeloid neoplasm category. These thresholds may allow for improvements in monitoring patients and in risk assessment, especially for those at presumed highest risk for earlier progression and evolution.

Idiopathic dysplasia of undetermined significance (IDUS) is defined as the presence of dysplasia in peripheral blood or bone marrow, the absence of cytopenias, no evidence of clonality, and no obvious cause.[31] Even though the offending condition may not be obvious at first, IDUS is usually a reactive process rather than primary marrow disorder.

Assessment of clonality in diagnosis

Since the first systematic reports in the late 1970s, numerical and structural chromosomal abnormalities and the use of various cytogenetic techniques have remained central to diagnostic testing, risk stratification, and therapeutic decision making. Beginning in 2001 (in recognition of chromosome 5q deletion syndrome), and then more broadly in 2007, WHO classification recognised cytogenetics as an essential diagnostic tool.[3] The diagnosis of some types of acute myeloid leukaemia with recurrent cytogenetic abnormalities—such as inv(16), t(8;21), or t(15;17)—can now be made without referring to the blast count. Similarly, myelodysplastic syndrome can be diagnosed in patients with cytopenia who have myelodysplastic syndrome-specific chromosomal aberrations without obvious dysplasia, or even by *SF3B1* without extensive ring sideroblasts.[30] Next-generation sequencing techniques have now made their way to the clinic and are not only widely applied for diagnostic and prognostic purposes, but also essential in the implementation of novel targeted therapies. This wide application has been fueled further by continually shrinking costs of disease-specific targeted panels, which are now less expensive than traditional metaphase karyotyping.

Unilineage or multilineage peripheral blood cytopenia often result from a wide range of haematological or non-haematological disorders. These include some haematological cancers and bone marrow failure syndromes, autoimmune conditions, viral infections, systemic diseases, medication toxicity, and vitamin deficiencies.[37] The term unexplained cytopenia is used to define a condition that is characterised by peripheral blood cytopenia whose origin is not attributable to causes that can be detected with conventional tests or to any concomitant diseases.[38]

The current diagnostic approach to a suspected myeloid neoplasm with myelodysplasia includes morphological studies of peripheral blood and bone marrow aspirate smears, bone marrow biopsy, and cytogenetic studies aimed at identifying selected chromosomal abnormalities or genetic lesions that WHO classification recognises to be of diagnostic value.[37,39] Tremendous progress in discovery of the genes associated with human disease combined with parallel sequencing of different genomic regions have resulted in wide use of next-generation sequencing in diagnostic schema for myeloid malignancies. The results of next-generation sequencing are now incorporated in the National Comprehensive Cancer Network guidelines, which provide the list of gene mutations likely to be somatic and disease-related, and therefore give presumptive evidence of myelodysplastic syndrome.[39] Although helpful under some circumstances, the incorporation of next-generation sequencing results into

a diagnostic process must be done with caution. Practising clinicians must be aware of the possible limitations of a test that is meant to complement proper diagnostic assessment, rather than act as a stand-alone diagnostic test (figure 3).

Diagnostic handling of CHIP

As with any new evolution in clinical classification systems, inherent limitations become apparent. It is in this way that clinicians must fully comprehend the consequences of labelling patients with precursor states. The prospective Myelodysplastic Syndrome Natural History Study[40] is currently enrolling patients with ICUS to address this very issue. Although underdiagnosis might not be a relevant problem because disease will declare itself with time, overdiagnosis can be anxiety provoking in patients and clinicians alike, and could result in serious consequences if acted on prematurely. Thus, we believe that healthy individuals with unremarkable haemograms should not be tested for clonal haematopoiesis. We recognise that this condition is frequently discovered incidentally, in research studies, cell-free DNA or solid tumour biopsies, and commercial DNA testing. Once discovered, we do not recommend monitoring for changes in clonal dynamics in asymptomatic individuals, especially given that there are no approved or investigational therapeutic interventions proven to change the natural history of CHIP in otherwise healthy individuals.

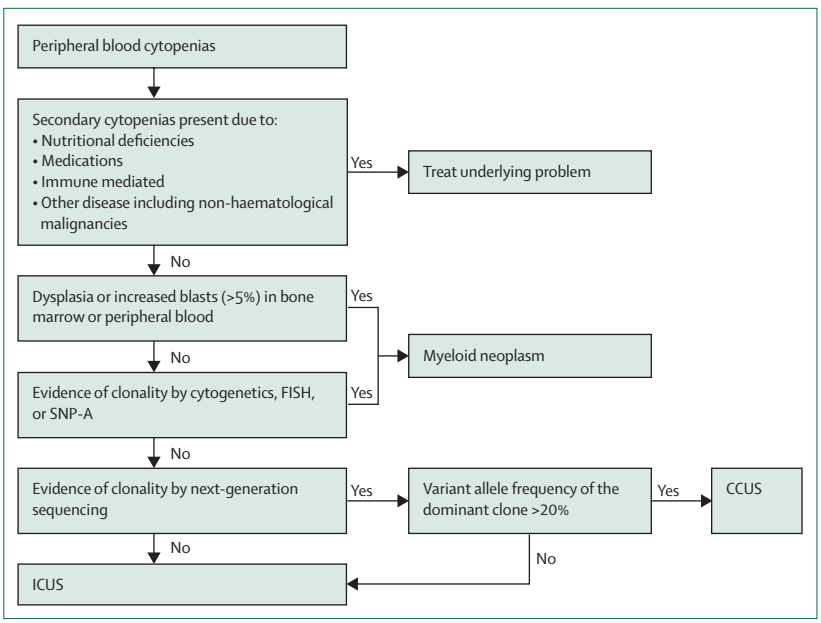

Figure 3: **Clinical diagnostic testing for peripheral blood cytopenias**
The flowchart represents our approach to the diagnosis of precursor conditions. We consider variant allele frequencies greater than 20% to be most akin to clonal cytopenia of unknown significance (CCUS). FISH=fluorescence in-situ hybridisation. SNP-A=single nucleotide polymorphism array. ICUS=idiopathic cytopenia of unknown significance.

Variant allele frequency

The analysis of molecular reports must take into account not only the binary information of the presence or absence of particular mutations, but also the percentage of affected cells (clonal burden). Variant allele frequency represents the percentage of mutated DNA molecules relative to the total DNA input. Most cancer-associated somatic mutations affect only one allele (heterozygous), and thus a variant allele frequency of 50% implies that 100% of tumour cells carry somatic mutations. Variant allele frequency depends on the size of the clone, tumour heterogeneity, the amount of non-clonal healthy cells (eg, non-clonal lymphocytes or stromal cells from bone marrow biopsy), and the co-occurrence of numerical chromosomal alterations. Particularly close attention should be paid to variant allele frequency in diagnostic investigation of unexplained cytopenias, because association between somatic mutations and cytopenias does not always prove causally significant. This could be one of the hardest principles for both patients and providers. For example, the presence of anaemia and *DNMT3A* mutation with variant allele frequency of 5% is probably very different from the same mutation at variant allele frequency of 40% in a patient receiving transfusions. For patients receiving transfusions, anaemia is probably due to clonal processes affecting 80% of haematopoietic cells, leading to ineffective erythropoiesis, but when transfusion is not involved, other causes of anaemia should be investigated thoroughly. Thus, we propose to incorporate a higher variant allele frequency cutoff (>20%) than is often used to distinguish CCUS from other conditions (panel 2). Another example could be the presence of unexplained anaemia and a small *JAK2* Val617Phe clone. Most clinicians would agree that the two are probably independent processes and that alternative explanations of anaemia should be considered.

Driver versus passenger mutations

Given the vast number of somatic alterations detectable by next-generation sequencing, discriminating between leukaemia-initiating driver mutations and incidental passenger mutations lacking functional consequences can be extremely challenging. This judgment relies heavily not only on the sequencing method, but also on the analytical pipeline, filtering strategies, variant annotations, and in silico prediction of pathogenicity. In reality, only a small proportion of somatic gene alterations passes these strict criteria and is reported by molecular laboratories. Even these highly refined lists frequently contain what are currently termed variants of unknown significance. With few exceptions of clearly deleterious recurring hot-spots (eg, *JAK2* Val617Phe, *SF3B1* Lys700Glu) or canonical truncating mutations (*ASXL1* or *CALR*), most loss-of-function mutations are scattered across the affected genes.[29] The limitations of currently available prediction algorithms and scarcity of experimental data make the distinction between pathological driver mutations and silent passenger mutations extremely challenging. To

***Panel 2:* Recommendations for diagnosis and monitoring of precursor states**

Diagnosis

- For diagnosis of clonal haematopoiesis of indeterminate potential (CHIP), the current cutoff for variant allele frequency of the dominant clone is more than 2%
- We propose to apply a cutoff of more than 20% variant allele frequency of the dominant clone for diagnosis of clonal cytopenias of undetermined significance

Monitoring

- Bone marrow and peripheral blood counts twice per year after CHIP diagnosis, with bone marrow evaluation dictated by change in peripheral counts
- If diagnostic criteria for myelodysplastic syndrome or acute myeloid leukaemia are not met and cytopenia persists, we recommend monitoring haemograms at least once every 3–6 months
- We propose next-generation sequencing testing once per year for symptomatic patients to observe changes in clonal burden
- We do not recommend further testing or excessive monitoring for changes in clonal dynamics in haematologically asymptomatic individuals

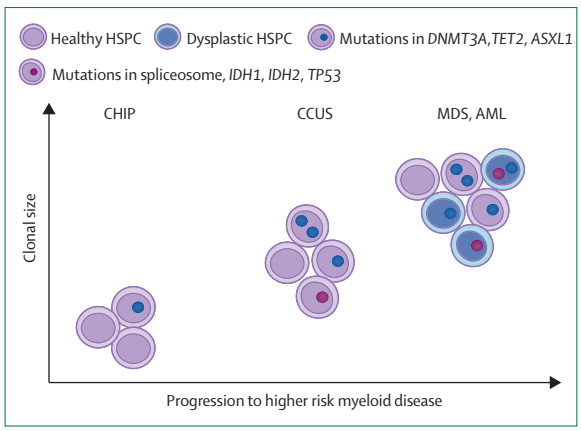

***Figure 4:* Features of clonal haematopoiesis of indeterminate potential (CHIP) considered at high risk for transformation to haematological malignancy**

The evolution of CHIP to clinically apparent haematological malignancies is represented by clonal expansion or variant allele frequency (y axis), by acquisition of additional somatic mutations, somatic mutations in specific genes (*TP53*, *IDH1*, *IDH2*, spliceosome machinery), or multiple mutations in epigenetic modifiers (*DNMT3A*, *TET2*), and by increased morphological features of dysplasia. HSPC=hematopoietic stem and progenitor cell. CHIP=clonal haematopoiesis of indeterminate potential. CCUS=clonal cytopenia of unknown significance. MDS=myelodysplastic syndrome. AML=acute myeloid leukaemia.

address this important and constantly evolving problem, attempts to standardise the interpretation and reporting of sequence variants have been undertaken.[41]

Somatic versus germline

When reviewing the results of next-generation sequencing panel testing, some germline variants could be present in somatic reports. How these are codified and interpreted can vary by report and can be a potential source of confusion. Thus, most molecular laboratories are slowly starting to incorporate concurrent DNA testing from non-haematopoietic tissues (eg, skin fibroblasts) as germline controls. In the absence of germline control, variant allele frequency could occasionally help to distinguish between germline and somatic mutations, especially if clones constitute less than 80% of tested tissue (variant allele frequency <40%). Unfortunately, in some conditions, the vast majority of nucleated haematopoietic cells are derived from a single pathological clone, which frequently makes this distinction infeasible. Moreover, mutations in certain genes (eg, *RUNX1, DDX41, ETV6*) can be present as inherited germline variants or as acquired somatic events. Interpretation of dedicated germline sequencing should be done according to guidelines for variant classification from the American College of Medical Genetics and Genomics.[42,43] A detailed review of inherited haematopoietic disorders extends beyond the scope of this Viewpoint and can be found elsewhere.[44]

Following the CHIPs

Malignant consequences of CHIP

With regards to the risk of malignant consequences, CHIP can apply the multiple hit theory in cancer evolution.[45] People with CHIP generally have a single somatic mutation and do not have an overt malignancy. The mutations found in people with CHIP are also common in myeloid malignancies, including acute myeloid leukaemia, myelodysplastic syndrome, myeloproliferative neoplasms, and some lymphomas.[46] In most cases, transformation to malignancy requires the sequential acquisition of multiple mutations.

Therapy-related myeloid neoplasms represent a specific clinical scenario in which chemotherapy or radiation can select for a mutant haematopoietic stem-cell clone, increasing the risk that this clone will acquire additional mutations and progress to malignancy.[47,48] Individuals with CHIP who are treated for solid tumours have an elevated risk of therapy-related myeloid neoplasms and increased overall mortality.[26,28,49]

Individuals with CHIP have approximately ten times the risk of developing a haematological malignancy compared with people without CHIP, with the risk increasing with the size of the clone.[8,9] Overall, the risk of transformation to malignancy is approximately 0·5–1% per year, which is roughly the same as the risk of transformation of monoclonal gammopathy of undetermined significance to multiple myeloma.[45]

The risk of transformation to acute myeloid leukaemia has been evaluated specifically in large, retrospective cohort studies. Specific features predicted the risk of developing acute myeloid leukaemia to be three to five times higher than in individuals without CHIP. In particular, mutations in *TP53* and genes encoding splicing factors were associated with an elevated risk of developing leukaemia (figure 4).[50,51]

Non-malignant consequences of CHIP

CHIP is associated with increased overall mortality.[8,9] An increased risk of haematological malignancies alone

	Myelodysplastic syndrome	Idiopathic cytopenia of unknown significance	Clonal haematopoiesis of indeterminate potential	Clonal cytopenia of unknown significance
Cytopenias	+	+	-	+
Dysplasia	+	-	-	-
Clonality	+	-	+	+
Risk of transformation to AML	++	↓↓↓	↓(↓)	↓↑
Monitoring	Per consensus guidelines by disease stage	No need to reassess next-generation sequencing; monitoring 2–4 times per year CBC	Observation; routine CBC for health maintenance	Observation; monitoring 2–4 times per year; supportive care

The proposed guidelines are based on currently available data for surveillance of these conditions; certainly all monitoring plans should be guided by a patient's clinical scenario including age, expectation, specific mutations, and comorbid conditions. CBC=cell blood count. + sign=present. - sign= absent. Arrows indicate increase or decrease.

Table: **Characteristic features of precursor states and proposed monitoring guideline**

does not explain this mortality risk, because overall blood cancers are relatively rare. In large genetic studies, CHIP has been associated with myocardial infarction, with a hazard ratio greater than many of the established risk factors for cardiovascular disease, such as blood pressure, cholesterol levels, and smoking.[9] CHIP approximately doubles the risk of myocardial infarction,[25] and studies indicate that CHIP plays a direct functional role in the pathogenesis of atherosclerosis.[24,52] Altered inflammatory response in the blood cells of patients with CHIP has also been shown to influence a wide range of disease biology, particularly in diseases of ageing that are linked to inflammation.[24,52–54] These associations could lead to providers outside of the discipline of haematology ordering more next-generation sequencing testing to determine whether CHIP could be contributing to the underlying pathophysiology. This could present further challenges, not only with interpretation of next-generation sequencing results, but also with subsequent requests for haematological evaluation of clinically insignificant conditions.

Monitoring

CHIP, ICUS, and CCUS are all currently considered to be premalignant conditions that can progress to myelodysplastic syndrome, acute myeloid leukaemia, or other haematological malignancies. The main implication of making these diagnoses is that the monitoring and clinical follow-up will change so that malignancy, if it occurs, will be diagnosed efficiently in a patient (table). Caution is required, however, as progression to malignancy is not a foregone conclusion and each precursor state does not carry the same level of risk.[55] Clinical scenarios at increased risk for early progression and malignant evolution must be highlighted to a patient (figure 4). Additionally, the form of monitoring should be age-dependent. A patient younger than age 60 years, with more life years ahead, should be surveyed once per year, whereas a patient aged 80 years with comorbid conditions could require discussions for their expectations, but might have less use of monitoring. Precursor states also need to be factored in if a patient with CHIP requires chemotherapy for another malignancy. Care should be taken to avoid prescription of medications that could further predispose patients to acquiring additional somatic mutations that could further escalate the patient's evolution to acute myeloid leukaemia.

The clinical course of ICUS is variable and unpredictable. In a subset of patients, progression to myelodysplastic syndrome or acute myeloid leukemia is observed after a variable time period.[56] In some patients with ICUS, a smaller clone carrying typical chromosome abnormalities (otherwise found in myelodysplastic syndrome or acute myeloid leukemia) is initially detected by a modality such as fluorescence in-situ hybridisation.[57] It is important to repeat cytogenetic and molecular studies during follow-up in patients with ICUS, IDUS, and CCUS, especially when clinical signs of clinical progression are found.[58]

CHIP at the bedside

Providers of clinical care value the capacity to contextualise disease manifestations (and the patients they affect) into categories to help to create a path forward. The aforementioned aid this goal and have the added use of predicting with greater accuracy which patients might develop myeloid neoplasms.[7] Additionally, these entities in many ways justify the use of next-generation sequencing at diagnosis. This form of molecular genetic testing promotes and confirms the diagnosis of a clonal disorder in a patient with unexplained cytopenia. Furthermore, a negative test result can also influence diagnostic assessment, because of the high negative predictive values of a normal result in ruling out a disorder such as myelodysplastic syndrome.[59]

In clinical practice, using molecular genetic testing and applying these acronyms at the individual patient level is complex.[59,60] During the past few years, the emerging concept of premyelodysplastic syndrome conditions has received attention and acceptance from the medical community, owing to fact that the clinical implications of such conditions are becoming clear. It is clinically appropriate, in our opinion, to use the panels in patients with cytopenia to rule in, rule out, or predict myeloid neoplasms. This practice has increased such that it is now considered standard care by nearly all haematologists and

Search strategy and selection criteria

We identified references for this Viewpoint through searches of PubMed databases and abstracts of the American Society for Hematology and the American Society of Clinical Oncology, using the search terms "CHIP", "CCUS", "ICUS", and "clonal haematopoiesis". We included articles that were published from Jan 1, 2010, to August 1, 2019. We considered results from all papers published in English only when drafting the manuscript, but included what we currently view as many of the most seminal papers to date for this Viewpoint in the reference list.

oncologists. Overall, the recommendation is to follow premyelodysplastic syndrome conditions proactively and based on the risk of evolution, as best estimated by the clinician for the patient at the time. However, the frequency of next-generation sequencing repeat monitoring (beyond marrows and blood counts) has not been established clinically. We favour once per year to avoid an overdiagnosis burden (panel 2). In the future, it is likely that our colleagues in cardiology, rheumatology, or other providers specialising in diseases with inflammation will send these panels looking for causality of the comorbid condition. We do not recommend further testing or close monitoring in haematologically asymptomatic individuals. Bone marrow biopsy with repeat next-generation sequencing analysis should be done in individuals with CHIP who develop unexplained cytopenias. If diagnostic criteria for myelodysplastic syndrome or acute myeloid leukaemia are not met and cytopenia persists, we recommend monitoring haemograms at least once every 6 months (as recommended by the myelodysplastic syndrome National Comprehensive Cancer Network guidelines).[39]

Conclusions

Clonal haematopoiesis is an exciting area of research, leading to better knowledge and further diagnosis of precursor status for patients. However, comprehension of clonal haematopoiesis is ongoing, and as yet, we sometimes struggle to interpret clonal haematopoiesis results with complete diagnostic and prognostic certainty. When faced with uncertainty, some patients and providers will be content to let events unfold beyond their control. However, other patients (or physicians) might feel compelled to be more proactive and pursue either more intense monitoring or, more worryingly, earlier intervention without evidence. This approach could lead to earlier detection and treatment of malignancy or to unnecessary and potentially harmful overtreatment. Ongoing and future studies will help to refine understanding of clonal haematopoiesis and of its implications.

Contributors

LPG and AED were responsible for the concept, literature search, analysis and synthesis of the data with interpretation, and writing.

Declaration of interests

We declare no competing interests.

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Review

Clonal Hematopoiesis with Oncogenic Potential (CHOP): Separation from CHIP and Roads to AML

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Abstract: The development of leukemia is a step-wise process that is associated with molecular diversification and clonal selection of neoplastic stem cells. Depending on the number and combinations of lesions, one or more sub-clones expand/s after a variable latency period. Initial stages may develop early in life or later in adulthood and include premalignant (indolent) stages and the malignant phase, defined by an acute leukemia. We recently proposed a cancer model in which the earliest somatic lesions are often age-related early mutations detectable in apparently healthy individuals and where additional oncogenic mutations will lead to the development of an overt neoplasm that is usually a preleukemic condition such as a myelodysplastic syndrome. These neoplasms may or may not transform to overt acute leukemia over time. Thus, depending on the type and number of somatic mutations, clonal hematopoiesis (CH) can be divided into CH with indeterminate potential (CHIP) and CH with oncogenic potential (CHOP). Whereas CHIP mutations *per se* usually create the molecular background of a neoplastic process, CHOP mutations are disease-related or even disease-specific lesions that trigger differentiation and/or proliferation of neoplastic cells. Over time, the acquisition of additional oncogenic events converts preleukemic neoplasms into secondary acute myeloid leukemia (sAML). In the present article, recent developments in the field are discussed with a focus on CHOP mutations that lead to distinct myeloid neoplasms, their role in disease evolution, and the impact of additional lesions that can drive a preleukemic neoplasm into sAML.

Keywords: premalignant states; neoplastic stem cells; clonal evolution; cancer

1. Introduction

It is generally appreciated that cancer evolution is a step-wise process that is associated with molecular diversification and clonal selection of neoplastic stem cells [1–6]. Blood cancer evolution may begin early in life or later in adulthood and includes premalignant and malignant stages. Thus, in many instances, the development of (blood) cancer is a long-lasting process that takes several years or even decades [1–8]. In chronic (indolent) myeloid neoplasms, such as the myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), or chronic myeloid leukemia (CML), the disease is separable into premalignant (non-aggressive) phases and a terminal phase that resembles secondary acute myeloid leukemia (sAML) [9–15]. However, even before an overt myeloid neoplasm, such as a MDS, develops, a clonal pre-phase of the disease may be detected by chance or by screening apparently healthy individuals [8,16–22]. Such pre-phase is defined by normal or near normal blood counts and detection of one or more somatic mutations, which *per se* (as isolated lesion) exhibit low oncogenic potential [8,16–22]. Whereas, in some of these individuals, a myeloid neoplasm develops in the follow-up, others will never develop an overt myeloid disorder during their lifetime. The risk of disease evolution (risk to develop a myeloid neoplasm) depends on the type of mutations and other factors. Clonal hematopoiesis (CH) and the related mutations can thus be divided into CH with indeterminate potential (CHIP) and CH with oncogenic potential (CHOP) [8].

We and others have recently proposed a model of cancer evolution where the earliest stages of carcinogenesis are defined by expression of somatic mutations in small-sized (yet stem cell-containing) cell fractions [8,19–25]. Later, over time, one or more sub-clone(s) expand(s) and replace normal cells, depending on additional somatic lesions (Figure 1) [8]. As long as the neoplastic cells retain their full differentiation and maturation potential and can be controlled by the niche and the immune system, the involved clones will remain indolent and may, over time, replace or even mimic (at least to a degree) the normal organ in functional and morphological terms. However, as soon as the dominant clone(s) escape(s) most control mechanisms, the disease will further expand and progress into an aggressive malignancy. The end-stage of such malignancy (sAML) is usually resistant not only against most endogenous control systems, but also against diverse therapeutic maneuvers [9–15].

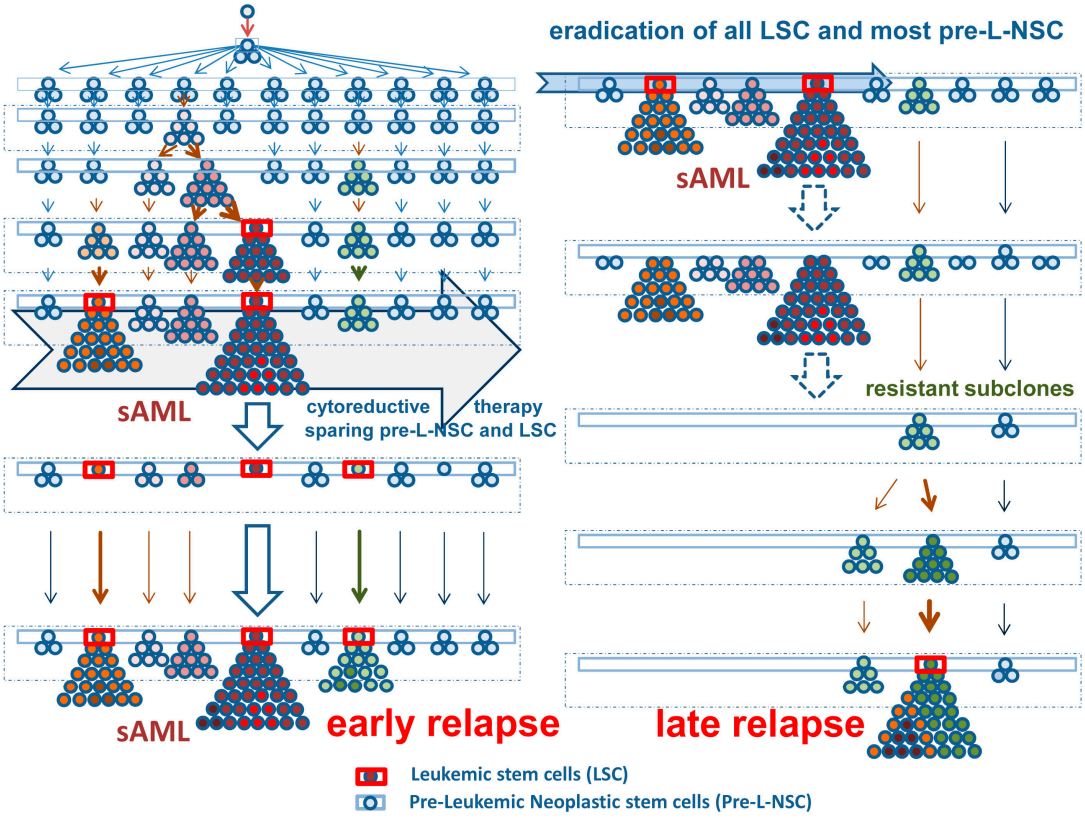

Figure 1. Development and diversification of leukemic stem cells in secondary acute myeloid leukemia (sAML). Left panel, upper part: An initial oncogenic event transforms a normal stem cell into a premalignant (preleukemic) neoplastic stem cell (Pre-L-NSC) (blue boxes). These cells or their daughter cells acquire early somatic mutations. Usually, these have low oncogenic potential (blue-colored cells) and are thus slowly cycling or dormant cells so that the mutation is not detectable. After some time, more daughter clones develop and the somatic lesions may be detected and classified as clonal hematopoiesis with indeterminate potential (CHIP). After several years or decades, one or more daughter clones and their stem cells expand and may replace normal hematopoiesis. At that time, some of the stem cell clones may have acquired disease-specific oncogenic driver lesions (red-colored cells). Still, these cells may be indistinguishable from normal cells by morphology and in functional terms. In a next step, one or more of the sub-clones acquire additional driver mutations or lose tumor suppressor genes. As a result, the stem cells are now cycling and the neoplastic process forms a visible overt myeloid neoplasm (red-colored prominent clones—upper left panel). In most instances, these neoplasms still behave as indolent driver-positive neoplasm for some time. However, unless treated, many of these conditions will finally transform into a secondary acute myeloid leukemia (sAML). At that time, long-term disease propagating cells are called leukemic stem cells (LSC—red boxes). Note that all of the Pre-L-NSC-derived clones are also still present and can be detected (as small-sized sub-clones) in an overt sAML. Left lower panel: Nonspecific cytoreductive (palliative) therapy (example: hydroxyurea) can suppress the growth of cycling stem and progenitor cells for some time but cannot eradicate any of the Pre-L-NSC or LSC. After a variable (usually short) time period, a relapse develops. Right panel: Most interventional therapies (intensive chemotherapy, targeted drugs, or stem cell transplantation) are able to eradicate most or all of the LSC and their progeny, but not all Pre-L-NSC. When all LSC are killed, the patient enters complete remission and operational cure. In these patients, the Pre-L-NSC may or may not be detected as minimal residual disease. These Pre-L-NSC may (or may not) produce a late relapse after several months or years. Although some of the early mutations (rarely even drivers) of the original sAML clone may be detected in such relapsing disease, the molecular aberration profiles usually differ substantially from the initial molecular make-up of the sAML clone.

Depending on the disease variant and the types and numbers (combinations) of somatic mutations acquired, the premalignant phases of a myeloid neoplasm bear a low or high risk to transform to sAML [8,19–25]. Interestingly, certain myeloid neoplasms, such as advanced MDS, most MDS/MPN overlap disorders, and chronic phase CML bear a (relatively) high risk, whereas other clonal conditions are at a clearly lower risk, but may eventually also transform to sAML. These include, among others, chronic eosinophilic leukemia (CEL), MDS with isolated del(5q), or indolent systemic mastocytosis (ISM). So far, it remains unknown why some conditions are associated with a relatively high risk of sAML transformation. Possible explanations are the differential oncogenic potential of various driver mutations and the presence of additional germline or somatic mutations.

In the current article, we review the mutational landscape of myeloid neoplasms and try to link individual mutations and mutation-combinations to clinical phenotypes and the risk of progression. We also discuss why CHOP mutations can trigger disease evolution and why some CHOP mutants are less oncogenic than others.

2. Clonal Hematopoiesis (CH) of Indeterminate Potential (CHIP)

The term CHIP was coined to describe the presence of clonal somatic mutations (otherwise detected in myeloid neoplasms: MDS, AML, and others) in leukocytes obtained from apparently healthy individuals or subjects with minimal blood count abnormalities [8,21,22,26–28]. In patients with CHIP, diagnostic criteria for MDS, MPN, or other myeloid neoplasms are not fulfilled even if the size of the 'CHIP clone' is substantial. Most patients with CHIP are older healthy individuals. Therefore, the term age-related clonal hematopoiesis (ARCH) was also proposed [19]. In patients with CHIP, the risk to develop a myeloid (hematopoietic) neoplasm is slightly elevated compared to controls without CH [18–21,26–28]. In addition, these patients may be at relatively high risk to develop progressive atherosclerosis and related cardiovascular disorders [19,29]. In a subset of patients with CHIP/ARCH, however, no malignancy and no severe cardiovascular disease develop.

In some individuals, the CHIP clones are small-sized and may thus escape detection by conventional screening/sequencing approaches. However, most next generation sequencing (NGS) assays have sufficient sensitivity to detect relatively small clones (mutant allele burden 1–5%) and thus represent the preferred method for the diagnostic assessment of CHIP. NGS assays can also be modified to reliably detect even smaller clones (mutant allele burden clearly <1%). These very small hematopoietic cell clones are currently not considered as CHIP per definition since their prevalence is even higher and their clinical impact remains unclear. The generally accepted definition of CHIP includes a minimal allele burden of 2%, the absence of persistent ($\geq$4 months) cytopenia and exclusion of an underlying overt pathology associated with the somatic lesion [21,22]. The term CHIP should thus only be applied to individuals who have normal blood counts. In the case that slight cytopenia is also detected and the criteria for MDS or other myeloid neoplasms are not fulfilled, the diagnosis changes to clonal cytopenia of undetermined potential (CCUS), which is a rare condition [21,22]. When detected as an isolated defect (in the absence of other lesions or loss of tumor suppressors), CHIP mutations are indicative of a rather good prognosis regarding clonal stability, and only a small subset of these individuals will eventually develop a hematopoietic neoplasm over time [18–21]. A list of frequently reported CHIP mutations is provided in Table 1.

Table 1. Examples of mutations that have been described in the context of clonal hematopoiesis of indeterminate potential (CHIP) or age-related clonal hematopoiesis (ARCH).

	Reported Frequency (% of Cases) in Patients with					
Mutated Gene	**CHIP**	**MDS**	**CMML**	**MPN**	**AML**	**AdvSM**
DNMT3A	50–60	5–15	1–10	1–12 *	15–35	5–15
TET2	10–15	20–30	50–60	18–45 *	<1–10 **	30–40
ASXL1	8–10	15–20	35–40	5–35 *	1–10 **	15–20
SF3B1	2–5	20–30 ***	5–10	5–10	<1–10	<1
GNB1	3–4	<1	<1	<1	<1	<1
SRSF2	1–2	15–17	45–50	<1–18 *	5–10	35–40
GNAS	1–2	<1	<1	<1	<1	<1

* The broad range in patients with myeloproliferative neoplasms (MPN) is due to a variable distribution of these mutations among the three major entities: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF—where these mutations occur more frequently). ** The broad range is due to a different prevalence of these mutations in various AML categories. In general, these mutations are more frequently detected in secondary AML, following MDS or CMML. *** The mutant *SF3B1* status is associated with deletions in the long arm of chromosome 11 and with the presence of ring sideroblasts in MDS. Abbreviations: MDS, myelodysplastic syndromes; CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasms; AML, acute myeloid leukemia; AdvSM, advanced systemic mastocytosis.

CHIP-like mutations can also be detected in patients with overt myeloid neoplasms, including MDS, AML, and mast cell neoplasms (Tables 1 and 2) [30–39]. However, although some of these mutations are more frequently detected in certain myeloid neoplasms than others, most are not disease-specific. Another interesting aspect is that CHIP-like mutations can sometimes also be detected after successful therapy when the predominant clones have been eradicated [40–43]. This is best explained by the stem model of cancer evolution where early sub-clones are formed and acquire these mutations, but only a few of these sub-clones expand after acquiring driver lesions and additional mutations over time (Figure 1) [8,23,44–48]. After debulking, the early sub-clones may still be present because of their slow proliferation rate and drug resistance (Figure 1). Such early clones exhibiting CHIP-like lesions may either remain silent for the lifetime of the host or they become relevant clinically: First, they may produce a late and usually driver-negative relapse (Figure 1). Second, they may contribute to the risk to develop a severe cardiovascular (vascular occlusive) disease [19,29,40–43].

In myeloid neoplasm, isolated CHIP-like mutations may still be indicative of a rather good prognosis regarding clonal stability [30–39]. However, in the context of an overt myeloid neoplasm, CHIP-like mutations are often indicative of a poor prognosis, especially when multiple CHIP-like mutations are expressed, or the CHIP-like mutation is accompanied by clonal hematopoiesis of oncogenic potential or loss of a tumor suppressor gene (Table 2) [30–39].

It was also described that the prognostic impact of CHIP-like mutations regarding survival and AML evolution depends on (i) the type of the mutation, (ii) the dynamics of clonal evolution (rapid expansion of sub-clones carrying CHIP-like mutations is a poor prognostic sign), and (iii) the underlying primary neoplasm. For example, a stable *TET2* mutation in low-risk MDS may be indicative of a good prognosis, whereas a rapidly expanding *ASXL1*-mutated clone in CMML-2 has to be regarded as a poor prognostic sign concerning AML evolution.

Table 2. Some clinically relevant somatic mutations detectable in myeloid neoplasms based on oncogenic potential and risk to transform to secondary acute myeloid leukemia (sAML).

Myeloid Neoplasm	Clinically Relevant Somatic Mutations		
	Early (CHIP-Like)	**Specific/Driver**	**Late/Transforming**
MDS [49–52]	*TET2*, *DNMT3A*, *IDH1/2* *	*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2* **	*ASXL1* ***, *RUNX1*, *TP53*, *EZH2*, *SETBP1*, *STAG2*, *NPM1*, *FLT3*, *PTPN11*, *N/KRAS*, *CBL*, *WT1*, *PHF6*
PV	*TET2* ****, *DNMT3A*, *ASXL1* §	*JAK2* V617F, *JAK2 exon 12*	*TP53*, *RUNX1*, *SRSF2*, *U2AF1*, *IDH1/2*, *CBL*,
ET	*TET2*, *DNMT3A*, *ASXL1*	*JAK2* V617F, *CALR*, *MPL*	*EZH2*, *FLT3*, *N/KRAS*, *NPM1*, *ETV6*, *SETBP1*
PMF	*TET2*, *DNMT3A*, *ASXL1*	*JAK2* V617F, *CALR*, *MPL*	[13,51,53,54]
CMML [55–58]	*TET2*, *SRSF2*, *ASXL1*, *DNMT3A* §§	*TET2*, SRSF2, *ASXL1*, *N/KRAS*, *CBL*, *SETBP1*, *EZH2*	*RUNX1*, *N/KRAS*, *CBL*, *EZH2*, *U2AF1*, *SETBP1* §§§
CML	*TET2*, *DNMT3A*, *ASXL1*	*BCR-ABL1*	*BCR-ABL1* mutations, *ASXL1*, *IKZF1*, *TP53*, *RUNX1*, *SETD1B*
AML [59,60]	*DNMT3A*, *TET2*, *ASXL1*, *IDH1/2* *	*PML-RARA*, *MYH11-CBFB*, *RUNX1-RUNX1T1*, *MLLT3-KMT2A*, *DEK-NUP214*, *RUNX1*, *NPM1*, *CEBPA*, *GATA2*	*FLT3*, *N/KRAS*, *KIT*, *PTPN11*, *TP53*, *PHF6*, *SRSF2*, *STAG2*, EZH2, *RAD21*
AdvSM/MCL [38]	*TET2*, *ASXL1*, *SRSF2*	*KIT* D816V/H/Y	*RUNX1*, *CBL*, *N/KRAS*, *SRSF2*, *IDH1/2*

* Although *IDH1/2* mutations are associated with an unfavorable clinical outcome in myelodysplastic syndrome (MDS), they appear to be early events in the clonal evolution in MDS and acute myeloid leukemia (AML). In MPN, *IDH1/2* mutations usually appear as late events leading to leukemic transformation. Of note is that these mutations have not been identified in the context of CHIP/ARCH [19,61]. ** Although the *SF3B1* mutation shows a clear association with the presence of ring sideroblasts in MDS, other mutations are not specific for MDS and appear at various frequencies across other myeloid malignancies. In MDS, they represent most frequently mutated genes and are usually detectable in the founding clone. These mutations are listed here as driver mutations, but not under CHIP-like mutations, because in the context of unexplained cytopenia, their presence is highly predictive of development of a myeloid neoplasm within 5 years (95%) [62]. *** *ASXL1* gene mutations are commonly found in MDS patients. As in other myeloid malignancies these mutations are associated with an unfavorable outcome. Although they are found at similar frequencies in MDS and post-MDS sAML, they are more often sub-clonal mutations and were therefore marked here as late events [50]. **** *TET2* mutations can both precede and follow the acquisition of *JAK2* V617F in MPN. Ortmann et al. postulated that the order of acquisition of *JAK2* and *TET2* mutations has an effect on the phenotype, and that patients who acquire *JAK2* V617F mutation first and *TET2* mutation at a later time point are more likely to present with PV and have an increased risk of thrombosis [63]. § *ASXL1* mutations can occur as early events, following the acquisition of *JAK2* V617F/*CALR* mutations in MPN or as separate clones in MPN as demonstrated by Lundberg et al. [13]. They were found at higher frequency in post-PV and post-ET myelofibrosis, indicating their role in disease progression in MPN. §§ These four mutations were described by Patel et al. as ancestral events in the clonal evolution of CMML [56]. All of them, except *DNMT3A*, can also appear in sub-clones, indicating that they can also be late events in the clonal evolution of CMML. Some authors consider *TET2*- and *ASXL1* mutations to be driver mutations in CMML due to their high frequency among the reported cases, and in particular the combination of *TET2* and *SRSF2* mutations which is highly prevalent in CMML. §§§ Despite many articles describing the genetic basis of CMML, no mutation was clearly associated with disease progression. *RUNX1* is more frequently detected in post-CMML sAML than in CMML, however due to its high frequency in CMML the difference was not statistically significant [55]. Abbreviations: MDS, myelodysplastic syndromes; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; CMML, chronic myelomonocytic leukemia; CML, chronic myeloid leukemia; MPN, myeloproliferative neoplasms; AdvSM, advanced systemic mastocytosis; MCL, mast cell leukemia; CHIP, clonal hematopoiesis of indeterminate potential; ARCH, age-related clonal hematopoiesis.

3. Clonal Hematopoiesis of Oncogenic Potential (CHOP) and Separation from CHIP

Whereas isolated CHIP-type mutations can be detected in healthy individuals who stay healthy for their lifetime, CHOP mutations are usually associated with manifestation of an overt neoplasm. In fact, most of these individuals will develop a hematopoietic malignancy, although CHOP-positive cases presenting with a long-lasting disease-free survival have been described. For example, although *BCR-ABL1* can be detected in small-sized clones in a few healthy individuals [16], most patients with a persistent *BCR-ABL1*+ clone have or will develop a *BCR-ABL1*-positive leukemia. Even in those with low mutant burden, *BCR-ABL1* must be regarded as a high-risk condition (CHOP) that is likely to transform to an overt leukemia after a variable latency period. As in other myeloid neoplasms, the CML clone acquires additional lesions and hits when the disease progresses into accelerated phase and blast phase [64–66]. In addition, early CHIP-like lesions may be detected in the CML clone [64–66]. These lesions may also persist during successful treatment with BCR-ABL1 tyrosine kinase inhibitors (TKI) and may confer a potential risk for the development of cardiovascular events during treatment with certain TKI, such as nilotinib [67].

In the *JAK2*-mutated MPN, the situation is similar compared to CML. However, in contrast to *BCR-ABL1*, the *JAK2* V617F mutation status *per se* confers a high risk for the development of cardio-vascular events [17,68–71]. Therefore, *JAK2* V617F+ hematopoiesis is often detected quite early, sometimes long before an overt MPN is diagnosed. However, again, the risk for the development of an MPN is high and most patients will eventually develop such a disease over time [17,68–71]. Therefore, although *JAK2* V617F exhibits some features of a CHIP-like mutation, it is generally considered to belong to the group of CHOP mutations.

In patients with MPN, other driver lesions may also be detected, including mutations in the calreticulin (*CALR*) gene or in the *MPL* gene [72–74]. In addition, apart from these drivers and *JAK2* V617F, a number of additional mutations may be detected in patients with MPN. These include, among others, mutations in *TET2*, *ASXL1*, *SRSF2*, *DNMT3A*, *U2AF1*, *CBL*, *KIT*, *RUNX1*, and *EZH2* [13,75,76] (Tables 1 and 2). Moreover, neoplastic cells in MPN patients may acquire mutations in *TP53*, which is usually associated with disease progression to sAML [13,75,76].

In MDS, no classical recurrent driver lesions, such as *BCR-ABL1* or *JAK2* V617F, have been identified. Rather, in these patients, a number of different molecular lesions and combinations of somatic mutations are found [30–35]. Based on clinical correlates regarding progression and survival, these lesions may be divided into CHIP-like mutations (Tables 1 and 2) and more oncogenic (CHOP-like) driver mutations (Tables 2 and 3). The latter are associated with a substantial risk to transform to AML, and include, among other, mutations in *FLT3*, *RUNX1*, *WT1*, *NPM1*, *NRAS*, and *TP53* [30–35,77]. However, these CHOP mutations are usually not detected in a pre-diagnostic phase (in healthy subjects) but only (mostly) in the context of a full-blown MDS or AML.

In chronic myelomonocytic leukemia (CMML), a classical MPN/MDS overlap disorder, mutations can also be divided into CHIP-like and more oncogenic (CHOP-like) mutations (Table 2). Genetic lesions that are typically detectable in CMML include mutations in *SRSF2* (about 50% of patients), *TET2* (50–60%), and *ASXL1* (35–49%) (Table 1). Mutations associated with disease progression to sAML include mutations in *CBL*, *NRAS*, *KRAS*, *RUNX1*, and *SETBP1* [55,78–82]. These mutations are detectable in at least 10% of all patients with CMML and are associated with poor prognoses. Especially RAS-pathway mutations and complex mutation patterns are highly predictive for progression to sAML. Mutations in *ASXL1* and *TET2* are also detected frequently in CMML. As an isolated lesion in CMML, a *TET2* mutation may be regarded as a CHIP-like mutation. However, in the CMML-context, *ASXL1* mutations are indicative of a poor prognosis, especially when other mutations are also expressed in CMML cells [83–85]. The same holds true for other rare driver mutations, such as *BRAF* mutations or *FLT3* mutations [86,87]. An overview of CHOP-type mutations is provided in Table 2.

Table 3. Somatic mutations producing clonal hematopoiesis of oncogenic potential (CHOP).

	Effects of the Mutant on Clonal Cells			Affected
Mutation	**Differentiation**	**Proliferation**	**Oncogenesis**	**Myeloid Neoplasm**
BCR-ABL1$_{p210}$	+	+	+ *	Ph+ CML
JAK2 V617F	+	+/-	-	MPN
CALR mutations	+	+/-	-	MPN
MPL mutations	++	+/-	-	
KIT D816V	++	+/-	-	ISM and AdvSM
FIP1L1-PDGFRA	+	+/-	-	CEL, MPN-eo
RUNX1- RUNX1T1	+/-	++	+	AML
CBFβ-MYH11	+/-	++	+	AML
FLT3 ITD mutations	+/-	+	+/-	AML
NPM1 mutations	-	++	+/-	AML
KRAS, *HRAS* mutations	-	++	+	AML
TP53 mutations	-	+	+	MPN, CMML, AML

* The oncogenic potential of *BCR-ABL1* is well documented and correlates with the invariable transition of (untreated) chronic phase CML into accelerated and blast phase CML. Abbreviations: Ph+ CML, Philadelphia chromosome-positive chronic myeloid leukemia; MPN, myeloproliferative neoplasms; ISM, indolent systemic mastocytosis; AdvSM, advanced systemic mastocytosis; CEL, chronic eosinophilic leukemia; MPN-eo, MPN with eosinophilia; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia.

In *de novo* AML, a number of disease-related or even AML-specific driver mutations, such as *RUNX1-RUNX1T1* (*CBFB-MYH11*), *PML-RARa*, *FLT3* mutations, *KIT* D816V, or *NPM1* mutations, have been identified [37,88–92]. Some of these mutations are even diagnostic and serve as diagnostic AML criteria in the World Health Organization (WHO) classification [37,89,90]. Other mutations contribute to clonal expansion of leukemic cells and are indicative of a poor prognosis (examples: *TP53* mutations, RAS pathway mutations, multiple somatic mutations) similar to the situation in CMML [37,89,90]. In addition, CHIP-like mutations can be detected in both *de novo* AML patients and (even more frequently) in patients with sAML [37,89–91].

Based on preclinical data and clinical studies, the low oncogenic potential of individual CHIP-like mutations may change to a higher oncogenic potential when the lesion is expressed together with other CHIP-like mutations or with a CHOP-type driver lesion. Indeed, in most disease models analyzed, the presence of multimutated sub-clones is usually associated with disease progression and an unfavorable outcome [36–39,82–85,89,90]. An exception may be the presence of two CHIP-like mutations in two coexisting and clearly separable (independent) sub-clones.

Another important aspect is that oncogenic (CHOP-like) mutations may also be expressed in the germline, thereby leading to a familial predisposition to develop a myeloid neoplasm, including AML. Such germline mutations were described in *RUNX1*, *CBL, KIT*, and other target genes [92–95]. In addition, it was described that certain gene constellations (genetic patterns or gene variations) predispose for the acquisition of CHOP mutations, such as *JAK2* V617F (MPN) or *KIT* D816V (mastocytosis) [96–99]. In these conditions, familial clustering of MPN or mastocytosis is found, but the oncogenic driver lesion is a somatic defect [96–99].

Finally, inherited gene defects may be associated with loss of tumor suppressor genes or tumor suppressor function and may thereby contribute to the development of myeloid neoplasms, including MDS, CMML, and AML.

4. Classification of CHOP: Drivers of Differentiation, Proliferation, Maturation, and/or Oncogenesis

The term 'oncogenic' is often used to describe a somatic process that converts a non-neoplastic or a premalignant neoplastic condition into an overt malignancy. This definition implies that these mutations (or other events) provide a growth and survival advantage over normal cells. However, in many instances, the driver mutation *per se* is promoting differentiation and maturation rather than proliferation, unless additional mutations are also expressed by neoplastic cells.

In general, CHOP mutations can be divided into (a) disease specific drivers that trigger differentiation and maturation without promoting substantial proliferation (weak oncogenes) like *KIT* D816V, (b) disease-specific drivers of differentiation and proliferation of hematopoietic (stem) cells, like *BCR-ABL1*, and (c) drivers that preferentially trigger the proliferation of hematopoietic stem and progenitor cells but do not or only marginally promote differentiation, such as mutant forms of *RAS*. In most instances, tumor suppressor loss (functionally by mutations or loss of genetic material) is also associated with enhanced proliferation of neoplastic (stem) cells.

The Ba/F3 model with doxycycline-inducible expression of oncogenes is a useful tool to define the differentiation and proliferation capacity of disease-related drivers. For example, expression of *KIT* D816V induces histamine production and (mast cell) differentiation, but not proliferation in Ba/F3 cells, whereas expression of *BCR-ABL1* induces both histamine production and proliferation in Ba/F3 cells [100]. Correspondingly, patients with indolent systemic mastocytosis, where *KIT* D816V is usually the only driver lesion, accumulate their mast cell aggregates over years or even decades, and have a stable disease course with normal life expectancy. In these patients, most neoplastic mast cells are mature cells that do not proliferate and even the immature mast cell progenitor cells show no enhanced proliferative capacity over normal cells (the total burden of mast cells remains stable) unless additional mutations are acquired. By contrast, in *BCR-ABL1*+ chronic phase CML, uncontrolled proliferation of myelopoietic cells is a key feature, and when untreated, the disease rapidly transforms to accelerated phase and blast phase with short survival times.

Another important aspect is that some of the oncogenic driver mutants, such as BCR-ABL1 or JAK2 V617F, are *per se* capable of promoting clonal instability. For example, it was described that BCR-ABL1 promotes the acquisition of secondary mutations by a pathway involving STAT5 and increased production of reactive oxygen species (ROS), with consecutive DNA damage and clonal instability [101]. Similarly, JAK2 V617F was described to induce clonal instability and loss of heterozygosity via mutant-induced generation of ROS [102].

These additional lesions and hits are required for leukemogenesis and are indeed found in high-risk patients and those who actually progress to sAML. Sometimes, the primary driver may even suppress evolution to sAML because of its differentiation-inducing (and thus proliferation-hindering) effects. Therefore, it is not unexpected that in patients with ISM or MPN who progress to sAML, the AML clone often becomes or is negative for the molecular driver lesion. Especially when these patients are treated with drugs directed against these drivers, progression to sAML is often accompanied by a 'loss' of the driver, which can be explained by the selection of more malignant, 'initial driver-negative' sub-clones. A summary of CHOP mutations and secondary driver lesions and their functional impact in oncogenesis in myeloid neoplasms is provided in Table 2.

5. CHIP and CHOP in the Context of Leukemic Stem Cells

The concept of leukemic stem cells (LSC) has been coined to explain cellular hierarchies, sub-clone formation, and the directed diversification of clonal cell populations in diverse malignancies [44–48,103,104]. In addition, the LSC concept was propagated with the idea to focus research work and translational approaches on disease-initiating and -propagating cells. In fact, it is clear that antineoplastic therapies can only be curative in nature when eliminating most or all neoplastic stem cells fractions in a given malignancy [103–105].

In the past few years, neoplastic stem cells have been classified into premalignant (preleukemic) neoplastic stem cells (pre-L-NSC) and leukemic (malignant) neoplastic stem cells (LSC), also termed cancer stem cells in the context of a solid cancer [45–48]. Pre-L-NSC give rise to small-sized clones and usually behave very similar compared to normal hematopoietic stem cells. By contrast, LSC give rise to an overt leukemia or another advanced blood cell cancer. It is generally accepted that at least one or more CHIP mutations are required to convert a normal (stem) cell into a Pre-L-NSC. CHOP mutations may also be expressed in Pre-L-NSC, especially in chronic myeloid neoplasms. However, in these patients, Pre-L-NSC may convert into LSC within short time. Based on the model of step-wise

evolution of cancer/leukemic stem cells (Figure 1), LSC and their progeny (= leukemic cells) must be expected to contain a mixture of CHIP and CHOP mutations. However, in rare cases, CHOP-negative sub-clones expand, especially under targeted therapy (Figures 1 and 2). In these cases, other oncogenic mutant forms may be expressed or the loss of certain tumor suppressors may introduce an additional oncogenic player.

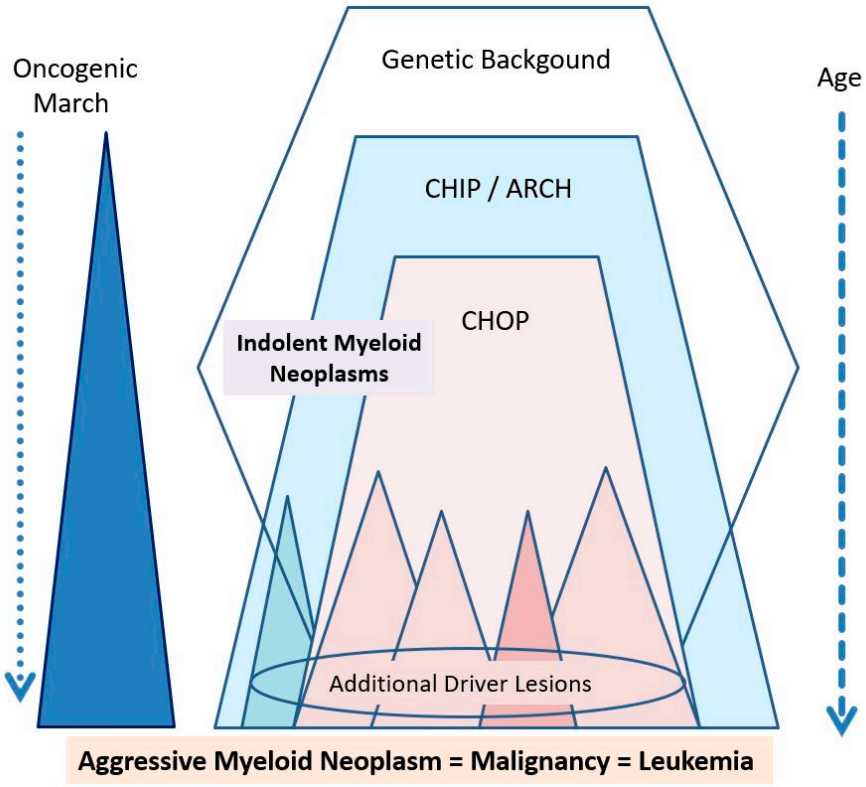

Figure 2. Major players contributing to the 'oncogenic march' in myeloid neoplasms. The genetic background may form the basis of a familiar predisposition to the development of hematopoietic (and thus also myeloid) neoplasms. In some of these families, more or less specific of even disease-related mutations (with low or even high oncogenic potential) are found. CHIP develops later during lifetime—the related somatic mutations *per se* (as isolated lesions) have a low oncogenic potential and are more frequently detectable at higher age. Therefore, these lesions are also called age-related clonal hematopoiesis (ARCH). Later, somatic mutations with CHOP may be acquired and usually lead to an overt myeloid neoplasm (at least after some time). This neoplasm may manifest as an indolent (chronic) myeloid neoplasm unless additional drivers (driver lesions) and other oncogenic hits (loss of tumor suppressors) are acquired. In a few cases, such additional driver lesions may be acquired in a CHIP status (blue triangle) or even a pre-CHIP status and may then lead to the immediate formation of primary (*de novo*) AML.

All in all, the CHIP vs. CHOP concept is in line with and nicely explains the classification of NSC into Pre-L-NSC and fully malignant/leukemic NSC = LSC. Whereas Pre-L-NSC are more likely to contain one CHIP mutation, LSC are more likely to express two or more CHIP mutations or (more frequently) at least one CHOP mutation.

An important aspect, when considering potentially curative concepts, is LSC resistance. In fact, LSC are known to exhibit multiple forms of drug resistance, including intrinsic stem cell resistance (natural defense against toxins), acquired drug resistance (often mediated by secondary mutations in driver target genes or mutations in additional genes), niche-mediated resistance, and immune-mediated resistance (often triggered by checkpoint molecules). In addition, pharmacologic

resistance may occur. Finally, LSC sub-populations may confer resistance because of the lack of major drug targets [45,46].

6. CHOP-Mutant Forms as Major Targets of Therapy

Because of their specificity and obvious function as major drivers of oncogenesis in various myeloid neoplasms, CHOP mutant forms were recognized as major targets of therapy. It started with the notion that BCR-ABL1-targeting TKI, such as imatinib, could effectively suppress growth and survival of leukemic cells in patients with Ph+ CML [106]. Later, imatinib was also found to block the kinase activity of FIP1L1-PDGFRA, the major driver of oncogenesis in chronic eosinophilic leukemia (CEL) and in a subset of related myeloid malignancies [107,108]. Subsequently, a number of different driver mutants and drugs directed against these drivers have been examined. In the classical MPN, inhibitors of JAK2 V617F were applied with considerable success [109]. In advanced systemic mastocytosis, drugs targeting the oncogenic KIT D816V mutant have been developed [110,111]. Finally, in AML, drugs directed against FLT3 ITD, IDH2 mutants and other oncogenic mutants were developed and are applied together with poly-chemotherapy in these patients.

All in all, targeting of CHOP-like mutant forms seems to be an effective approach in many patients. However, not all patients respond or show long lasting remissions. Rather, neoplastic cells are often resistant or develop resistance against these drugs. A number of different mechanisms of resistance against driver mutants have been deciphered in recent years. A full description and review of these mechanisms is beyond the scope of this article. The reader is referred to the available literature. In general, fusion genes encoding for CHOP-like drivers can acquire secondary mutations through which drug resistance develops. Second, driver-negative sub-clones can emerge. Third, niche-related and immunological forms of resistance may contribute to overall drug resistance. Finally, intrinsic stem cell resistance and pharmacological resistance may occur [45,46]. All these forms of resistance can be found in myeloid neoplasms and are often critically involved in MDS, CMML, and AML.

A logical way to overcome the multiple forms of resistance against CHOP driver-directed drugs is the application of drug combinations. Such combinations are currently being tested in preclinical and clinical studies.

7. Concluding Remarks and Future Perspectives

The term CHOP was proposed for somatic mutations that drive oncogenesis in various hematopoietic neoplasms as a single hit or cooperative hit that acts pro-oncogenic in somatic aberration networks. Whereas some of these drivers may directly induce the proliferation of neoplastic stem and progenitor cells, others induce differentiation in distinct hematopoietic lineages and are therefore disease-specific and lineage-related and often detected in premalignant chronic neoplasms. Some of these drivers may *per se* promote oncogenesis through the induction of clonal instability. Finally, in the context of multimutated sub-clones, oncogenic drivers contribute to the transformation to sAML. CHOP-related mutants have also been recognized as promising targets of therapy in myeloid neoplasms. However, complete suppression of oncogenesis and eradication to cure require the elimination of all premalignant and malignant neoplastic stem cells.

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Review

Clonal hematopoiesis: Mutation-specific adaptation to environmental change

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SUMMARY

Clonal hematopoiesis of indeterminate potential (CHIP) describes a widespread expansion of genetically variant hematopoietic cells that increases exponentially with age and is associated with increased risks of cancers, cardiovascular disease, and other maladies. Here, we discuss how environmental contexts associated with CHIP, such as old age, infections, chemotherapy, or cigarette smoking, alter tissue microenvironments to facilitate the selection and expansion of specific CHIP mutant clones. Further, we consider major remaining gaps in knowledge, including intrinsic effects, clone size thresholds, and factors affecting clonal competition, that will determine future application of this field in transplant and preventive medicine.

INTRODUCTION

Hematopoietic stem cells (HSCs) reside in the bone marrow where they divide, differentiate, and self-renew to maintain all blood cells throughout an organism's life (Pinho and Frenette, 2019). Over time, HSCs naturally acquire somatic mutations, some of which enable selective advantages over other HSCs in a context-dependent fashion (Figure 1). As a result of this competition, oligoclonality in the bone marrow has been observed, initially based on patterns of X chromosome inactivation and later confirmed in normal individuals harboring *TET2* mutations (Busque et al., 2012). The term clonal hematopoiesis of indeterminate potential (CHIP) was first coined in 2015 by Steensma et al. to define a phenotype in which hematopoietic cells harboring somatic mutations clonally expand in the absence of hematological disease (Steensma et al., 2015). A number of studies have defined the genetic landscape of CHIP, with most mutations occurring in pre-leukemic driver genes that are recurrently implicated in the pathogenesis of hematological malignancies and disorders (Bolton et al., 2020; Genovese et al., 2014; Miller and Steensma, 2020; Jan et al., 2017). Although CHIP was originally defined by detection of mutations in genes related to hematological neoplasms at a variant allele frequency (VAF) of $\geq$2%, newer methods now enable detection of CHIP at a much lower VAF (Steensma et al., 2015).

CHIP is found, by conservative measures, in more than 10% of individuals by the eighth decade of life and poses increased risks of adverse outcomes including severe infection, kidney disease, hematological and non-hematological cancers, cardiovascular disease (CVD), and all-cause mortality (Jaiswal et al., 2014, 2017; Genovese et al., 2014; Zink et al., 2017; Kessler et al., 2022; Kar et al., 2022; Bolton et al., 2021; Zekavat et al., 2021a; Dawoud et al., 2020; van Deuren et al., 2021). Despite the increased prevalence of CHIP with age, CHIP mutations appear to be relatively stable over time in the absence of additional stressors (Midic et al., 2020). This stability implies that extrinsic factors provide opportunities for somatic mutations to undergo "somatic evolution," facilitating the selection and expansion of clones in specific microenvironments (Laconi et al., 2020). There is an increasing body of evidence showing that particular stressors can drive expansion of specific CHIP mutant clones (Figure 1). Studies exploring the mechanisms by which specific mutant clones outcompete other HSCs and expand in certain contexts have provided insight into the selective environments that drive their expansion. Understanding the differential fitness landscapes that promote the selective expansion of CHIP variants will enhance our ability to not only manage clonal expansion but also reduce risks associated with CHIP (King et al., 2020).

CHIP and disease risk

CHIP is associated with a widening array of diseases, many of which are age-associated (Table 1). Patients with CHIP have an increased risk of developing hematological malignancies, with the effect size varying according to VAF, clonal complexity,

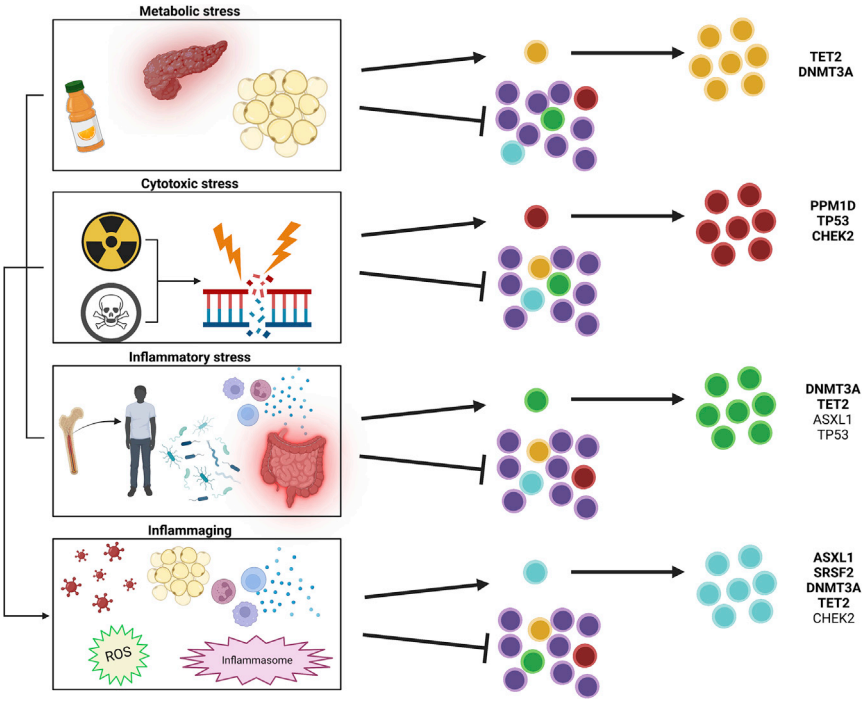

Figure 1. Environmental stressors promote mutation-specific clonal expansion

Over an organism's lifetime, HSCs encounter cellular stresses that may induce somatic mutations in common driver genes. Depending on the context, somatic mutations may confer a selection advantage for certain clones to persist and expand. Environmental effects on clonal competition are mutation specific. Genotoxic stressors such as chemotherapy and radiation provide a selection advantage for clones bearing mutations in TP53, PPM1D, and CHEK2, whereas infection and inflammation promote the expansion of clones with mutations in TET2 and DNMT3A. Studies on the metabolic effects of clonal expansion suggest that metabolites, like vitamin C, may impact the function of mutations, such as TET2.

mutation type, and gene(s) mutated (Desai et al., 2018; Abelson et al., 2018; Zink et al., 2017; Genovese et al., 2014; Jaiswal et al., 2014). Deep sequencing analysis has revealed that the risk of individuals with CHIP developing acute myeloid leukemia (AML) may occur at VAFs as low as 0.005–0.01 (Young et al., 2019; Desai et al., 2018; Abelson et al., 2018), with further increase in AML risk proportionate to VAF (Abelson et al., 2018; Genovese et al., 2014). Mutations commonly identified in CHIP include those in epigenetic modifiers (*TET2*, *DNMT3A*, *IDH1*, *IDH2*, and *ASXL1*), splicing factors (*U2AF1* and *SRSF2*), DNA damage repair genes (*PPM1D* and *TP53*), and inflammatory mediators (*JAK2*, *STAT3*, and *MYD88*), most of which are known drivers of hematological cancers. The risk of future blood cancers and overall decreased survival varies by mutation, with mutations in *DNMT3A* and *TET2* conferring a lesser risk of progression compared to *PPM1D* and *TP53* (Genovese et al., 2014; Abelson et al., 2018; Chou et al., 2011; Gelsi-Boyer et al., 2012; Park et al., 2020; Yuan et al., 2016a; Ley et al., 2010; Jaiswal et al., 2014; Desai et al., 2018).

DNMT3A and *TET2* are the most commonly mutated genes in CHIP (Buscarlet et al., 2017; Feusier et al., 2021; Genovese et al., 2014; Jaiswal et al., 2014; Xie et al., 2014). *DNMT3A* mutations are present in at least 20% of AML patients (Gaidzik et al., 2013; Lauber et al., 2020; Park et al., 2020; Yan et al., 2011; Ley et al., 2010), with roughly 60% of these occurring at the R882 codon (Gaidzik et al., 2013; Lauber et al., 2020; Ley et al., 2010; Park et al., 2020; Ribeiro et al., 2012). Mutations at this hotspot locus, located within the methyltransferase catalytic domain, have been linked with a higher risk of AML development and poorer outcomes compared to other mutations within the same gene (Gaidzik et al., 2013; Kumar et al., 2018; Lauber et al., 2020; Ley et al., 2010; Renneville et al., 2012; Ribeiro et al., 2012; Yuan et al., 2016a; Young et al., 2019). Similarly, mutations in *TET2*, a methylcytosine dioxygenase, occur at high frequencies in myeloid neoplasms (Abdel-Wahab et al., 2009; Delhommeau et al., 2009; Xie et al., 2014), and patients harboring mutations in *TET2* tend to have decreased survival and response to therapy (Wang et al., 2019). *DNMT3A* and *TET2* regulate methylation and demethylation of the genome, respectively, though through different mechanisms (Izzo et al., 2020). Despite these mechanistic differences, functional studies suggest that mutations in both *DNMT3A* (Lauber et al., 2020; Park et al., 2020; Russler-Germain et al., 2014; Sandoval et al., 2019; Yan et al., 2011) and *TET2* (Lopez-Moyado et al., 2019; Rasmussen et al., 2015; Cimmino et al., 2017) alter the expression of genes involved in proliferation, differentiation, and oncogenesis. These epigenetic changes allow the mutant cells to propagate and persist, enabling the acquisition of other mutations that further drive leukemogenesis (Bezerra et al., 2020; Celik et al., 2015; Guryanova et al., 2016; Kronke et al., 2013; Loberg et al., 2019; McKerrell et al., 2015; Meyer et al., 2016; Yang et al., 2016; Jan et al., 2017). While the mechanistic roles of *DNMT3A* and *TET2* in malignant hematopoiesis have been thoroughly summarized elsewhere (Chaudry and Chevassut, 2017; Im et al., 2014; Venugopal et al., 2021; Yang et al., 2015; Bowman and Levine, 2017; Nakajima and Kunimoto, 2014; Feng et al., 2019), it is worth noting that blood cells carrying CHIP mutations may not only increase the risk of AML but also impact non-hematological tumors by modulating the immune microenvironment (Kleppe et al., 2015). Indeed, CHIP has been associated with a variety of solid tumors in multiple large cohort studies (Bolton et al., 2020; Kar et al., 2022; Kessler et al., 2022), strongly suggesting that CHIP may also affect the progression of solid tumors.

In addition to its impact on cancer development and prognosis, CHIP has been noted to affect autologous stem-cell transplant (ASCT) outcomes for lymphoma. Patients with CHIP mutations had higher incidence of therapy-related myeloid neoplasms (TMNs) following ASCT. Furthermore, within this cohort, patients harboring *PPM1D* mutations had overall lower survival (Gibson et al., 2017b). An increased prevalence of unexplained cytopenias in patients receiving ASCT of hematopoietic cells harboring CHIP mutations has been reported, suggesting graft

Table 1. Suspected CHIP-mediated diseases by driver mutation

Gene	Function(s) in HSCs	Suspected CHIP-associated outcomes
DNA damage response		
PPM1D	protein phosphatase Mn2+/Mg2+-dependent 1D; negative regulator of DDR pathway	t-MN (Hsu et al., 2018) t-MDS (Lindsley et al., 2017) heart failure (Yura et al., 2021) CKD (Dawoud et al., 2021)
	regulator of cellular proliferation and differentiation	MDS (Papaemmanuil et al., 2013; Haferlach et al., 2014) t-MN (Bolton et al., 2020; Wong et al., 2015)
TP53	transcriptional factor regulating cellular stress	heart failure (Sano et al., 2021) t-MDS (Lindsley et al., 2017; Wong et al., 2015)
	epigenetic modifier	atherosclerosis (Zekavat et al., 2021b)
Chek2	checkpoint kinase 2; regulator of DDR	MDS (Janiszewska et al., 2018)
Epigenetic modifiers		
DNMT3a	*de novo* DNA methyltransferase	MDS (Haferlach et al., 2014; Papaemmanuil et al., 2013)
		MPN (Lundberg et al., 2014)
		atherosclerosis (Rauch et al., 2018)
		renal fibrosis (Sano et al., 2018a)
		heart failure/CVD/MI (Bick et al., 2020a; Abplanalp et al., 2021; Jaiswal et al., 2017; Sano et al., 2018a; Dorsheimer et al., 2019)
		stroke (Bhattacharya et al., 2021; Jaiswal et al., 2014)
		AML (Young et al., 2019)
		osteoporosis (Kim et al., 2021)
		pre-mature menopause (Honigberg et al., 2021)
		liver-GVHD (Newell et al., 2021)
		aortic valve stenosis (Mas-Peiro et al., 2020)
		acute-GVHD SCT (Oran et al., 2021)
TET2	DNA methylcytosine dioxygenase	COPD (Buscarlet et al., 2017; Zink et al., 2017; Miller et al., 2021a)
		stroke (Bhattacharya et al., 2021; Jaiswal et al., 2014)
		gout (Agrawal et al., 2021)
		lung cancer progression (Nguyen et al., 2021)
		MDS (Haferlach et al., 2014; Papaemmanuil et al., 2013)
		heart failure/CVD/MI (Sano et al., 2018b; Wang et al., 2020; Bick et al., 2020a; Jaiswal et al., 2017; Dorsheimer et al., 2019; Yu et al., 2021)
		renal fibrosis (Sano et al., 2018a)
		atherosclerosis (Fuster et al., 2017; Rauch et al., 2018)
		MPN (Lundberg et al., 2014)
		CKD (Dawoud et al., 2021)
ASXL1	Polycomb-associated protein involved in epigenetic regulation and transcription	MDS (Haferlach et al., 2014)
		MPN (Lundberg et al., 2014; Nangalia et al., 2013; Vannucchi et al., 2013)
		heart failure/CVD/MI (Jaiswal et al., 2014, 2017; Yu et al., 2021)
		stroke (Jaiswal et al., 2014)
IDH1/IDH2	isocitrate dehydrogenase 1/2; metabolizes isocitrate to α-ketoglutarate	MPN (Lundberg et al., 2014; Vannucchi et al., 2013)
Cellular signaling		
JAK2	non-receptor tyrosine kinase; signals through JAK/STAT pathway	MPN (Wolach et al., 2018; Lundberg et al., 2014)
		thrombosis (Wolach et al., 2018)
		atherosclerosis (Wang et al., 2018)
		CVD (Jaiswal et al., 2017)
		heart failure (Sano et al., 2019; Yu et al., 2021)
		aortic aneurysms (Yokokawa et al., 2021)
		pulmonary HTN (Kimishima et al., 2021)
		CKD (Dawoud et al., 2021)

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Table 1. *Continued*

Gene	Function(s) in HSCs	Suspected CHIP-associated outcomes
Spliceosome		
SF3B1	RNA splicing	MDS (Haferlach et al., 2014; Papaemmanuil et al., 2013)
SRSF2	RNA splicing	MDS (Haferlach et al., 2014; Papaemmanuil et al., 2013)
		MPN (Vannucchi et al., 2013)
U2AF1	RNA splicing	MDS (Haferlach et al., 2014; Papaemmanuil et al., 2013)

dysfunction (Gibson et al., 2017a). However, others report no adverse outcomes with CHIP post-ASCT, and even improved survival, suggesting that adverse effects may be mutation specific (Grimm et al., 2019; Heini et al., 2021).

CHIP also has recently been implicated in impacting both risk and response to infections. Patients harboring CHIP were found to have increased risk of severe coronavirus disease 2019 (COVID-19), sepsis, and other infections (Bolton et al., 2021; Dawoud et al., 2020; Jaiswal and Ebert, 2019; Kessler et al., 2022; Zekavat et al., 2021a). These risks may be attributable to impaired function of downstream immune cells, which has been reported for cells bearing CHIP mutations (Jaiswal and Ebert, 2019; Zekavat et al., 2021a).

One phenomenon of great interest related to CHIP is its link to CVD and all-cause mortality (Jaiswal et al., 2014; Sano et al., 2018c; Libby et al., 2019; Dorsheimer et al., 2019; Cremer et al., 2020; Abegunde et al., 2018; Kar et al., 2022; Kessler et al., 2022). One of the earliest studies found that CHIP carriers had a 2-fold risk of coronary heart disease and ischemic stroke (Jaiswal et al., 2014). Subsequent studies in patient cohorts found a 4× greater risk of having early myocardial infarction (MI) (Jaiswal et al., 2017) and congestive heart failure in CHIP patients (Dorsheimer et al., 2019), independent of traditional cardiovascular risk factors. Recently, however, two large epidemiological studies using the UK Biobank indicate that the increased risks of CVD may be less dramatic (hazard ratio: 1.1, 1.03–1.17) (Kar et al., 2022; Kessler et al., 2022). Specific mutations, including *TET2*, *DNMT3*A, and *ASXL1*, showed an increased incidence of coronary artery disease, coronary artery calcifications, and early MI, with risks of CVD that correlate with VAF (Jaiswal et al., 2014, 2017; Bick et al., 2020a; Kar et al., 2022). Studies using both human samples and mouse models revealed that the pro-inflammatory environment generated by the mutant clones drives adverse cardiovascular outcomes (Jaiswal et al., 2017; Fuster et al., 2017; Sano et al., 2018a; Bick et al., 2020a). However, differing pro-inflammatory phenotypes drive cardiovascular dysfunction and remodeling in CHIP with mutations in *PPM1D* (Yura et al., 2021), *TP53* (Sano et al., 2021), *DNMT3A* (Abplanalp et al., 2021; Heyde et al., 2021; Sano et al., 2018c), and *TET2* (Sano et al., 2018b; Wang et al., 2020), respectively. CHIP also has been linked to chronic obstructive pulmonary disease (COPD) (Buscarlet et al., 2017; Miller et al., 2021a; Zink et al., 2017), diabetes (Fuster et al., 2020; Jaiswal et al., 2014), psychiatric illnesses (Zink et al., 2017), early menopause (Honigberg et al., 2021), osteoporosis (Kim et al., 2021), liver disease (Wong et al., 2022), renal dysfunction (Dawoud et al., 2021), and epigenetic aging (Robertson et al., 2019). The correlation between these diseases and CHIP could be manifestations of tissue dysfunction due to common causes, like aging, smoking, and inflammation; however, it is possible that CHIP-driven inflammation contributes to the development of these diseases. As we discuss below, inflammation itself is a driver of CHIP, setting up a feedforward loop of CHIP and disease. Thus, CHIP has important implications for the future management of patients, most immediately in relation to cardiovascular outcomes and precision oncology (Libby et al., 2019; Miller and Steensma, 2020; Steensma, 2018; Sidlow et al., 2020).

It is worth noting that the factors that drive CHIP may have little to do with the mechanisms by which mutant clones promote disease. Epidemiologic studies will continue to be important to uncover key disease associations. Importantly, understanding what drives CHIP and its health consequences will provide two different avenues for preventive medicine and therapeutic management, respectively. With an eye toward prevention, here, we will highlight known associations between environmental stressors and CHIP, including mechanisms of clonal expansion where known.

Chemotherapy and radiation as drivers of CHIP

Chemotherapies and radiation destroy malignant cells by inducing DNA damage, impairing DNA replication, damaging DNA repair mechanisms, inhibiting pro-survival signaling pathways, impairing transcription and translation, and evoking metabolic and cellular stress (Krisl and Doan, 2017). In addition to cancer treatments, chemotherapy and radiation are useful in managing bone marrow diseases (Lee et al., 2018), transplants (Krisl and Doan, 2017), immunodeficiencies (Lum et al., 2019), and autoimmune diseases (Moreland, 2004) in which cytotoxic conditioning regimens make space for engraftment, prevent rejection, and dampen an overactive immune system. These reagents universally impair the hematopoietic system either as the primary goal or as an off-target effect of tumor targeting (Shao et al., 2013). Quiescent HSCs are more protected against the acute stress of chemotherapy and radiation in part via induction of a strong TP53-dependent DNA damage response (DDR), which promotes senescence instead of cell death (Mohrin et al., 2010). However, HSCs eventually lose clonogenic activity, undergo cell death, and exhibit decreased fitness, defined as overall ability to persist and expand, in a TP53-dependent manner (Marusyk et al., 2010; Bondar and Medzhitov, 2010). Furthermore, the quiescent nature of HSCs limits DNA repair primarily due to the use of error-prone non-homologous end joining (Mohrin et al., 2010). Thus, HSC survival comes at the expense of increased risk of mutagenesis and dampened HSC expansion (Mohrin et al., 2010; Schoedel et al., 2016). Specific pre-existing CHIP mutants are evolutionarily advantaged to expand and persist under the stress of chemotherapy and radiation. Multiple reports have shown that patients with a prior history of cancer

and cancer treatment are more likely to have certain CHIP mutations (Bolton et al., 2020; Boucai et al., 2018; Coombs et al., 2017; Olszewski et al., 2019; Hsu et al., 2018; Kahn et al., 2018; Wong et al., 2018), with selective clonal expansion dependent on the mechanism of the cytotoxic stressor as described below (see also Table 2).

DDR genes

Mutations in DDR genes (*TP53*, *PPM1D*, and *CHEK2*) have the strongest association with prior cancer treatment, and clones with mutations in these genes expand more under the stress of specific cytotoxic therapies (Bolton et al., 2020; Coombs et al., 2017; Swisher et al., 2016; Wong et al., 2015). Mutations in DDR genes are infrequent in the absence of prior cytotoxic exposure (Xie et al., 2014; Genovese et al., 2014; Jaiswal et al., 2014; Eskelund et al., 2020) but prevalent in patients with TMN, indicating the selective advantage under cytotoxic stress (Bolton et al., 2020; Gibson et al., 2017b; Hsu et al., 2018; Lindsley et al., 2017).

Studies have shown that *PPM1D* mutations often occur in exon 6 and confer resistance to chemotherapeutics, especially platinum-based therapies (Bolton et al., 2020; Hsu et al., 2018; Kahn et al., 2018; Kim et al., 2019). *PPM1D* mutations are typically gain-of-function truncation mutations that stabilize the protein and suppress the DDR, enabling mutant clones to resist apoptosis (Hsu et al., 2018; Kahn et al., 2018). Similar to the selective advantages observed in large patient cohorts (Bolton et al., 2020), *in vitro* studies show that *PPM1D* mutants are resistant to specific cytotoxic therapies (cisplatin, etoposide, doxorubicin, cytarabine) but not others (5-fluorouracil [5-FU], vincristine) (Hsu et al., 2018; Kahn et al., 2018). These differences are likely explained by these treatments' differing mechanisms of action. For instance, vincristine impairs mitosis by microtubule interference and inhibits trafficking of DNA repair proteins, including TP53, which interacts with PPM1D (Kahn et al., 2018; Poruchynsky et al., 2015). It is plausible that microtubule interference blocks PPM1D from interacting with downstream DDR mediators, thereby preventing its ability to inhibit apoptosis (Kahn et al., 2018; Poruchynsky et al., 2015).

Mutations in *TP53* are a strong risk factor in the development of TMN (Abelson et al., 2018; Desai et al., 2018; Gillis et al., 2017; Wong et al., 2015). TP53 has multiple functions and may regulate HSC response to cytotoxic stress by regulating apoptosis (Wu et al., 2005), proliferation (Bondar and Medzhitov, 2010; Wlodarski et al., 1998; Wu et al., 2005), quiescence, and self-renewal (Chen et al., 2008; Liu et al., 2009). Cytotoxic drivers of *TP53* mutant expansion include platinum-, radiation-, and taxane-based therapies (Bolton et al., 2020). Furthermore, *in vitro* studies suggest that 5-FU (Wlodarski et al., 1998) and doxorubicin (Sano et al., 2021) confer a selective advantage for TP53 mutant clones. The expansion of *TP53* mutant clones may be dependent on mutation type and may occur via multiple mechanisms, as *TP53* mutations have been suggested to cause both gain and loss of function. Interestingly, *TP53* mutation has been reported to promote DNA H3K27 trimethylation via enhanced interaction with EZH2, thereby reinforcing HSC self-renewal during radiation stress (Chen et al., 2019). Whether this mechanism underlies selective CHIP expansion of *TP53* mutants during other cytotoxic stresses remains unknown, though it is likely that both DDR impairment and epigenetic changes underlie the selective advantages of this mutant.

Epigenetic modifiers

The role of cytotoxic stress on clones bearing mutations in epigenetic modifiers is less clear. A few small studies have shown clonal expansion in non-leukemic HSC clones harboring mutations in the epigenetic modifiers DNMT3A and TET2 (Wong et al., 2016). Coombs et al. showed that cytotoxic therapy was associated with TET2 (Coombs et al., 2017), but this was not recapitulated in later large cohort studies (Bolton et al., 2020). Conversely, mutations in DNMT3A and TET2 are known to confer treatment resistance to chemotherapies in AML, and numerous studies have linked to specific DNMT3A^{mut} in AML with poor clinical outcomes (Lauber et al., 2020; Ley et al., 2010; Ribeiro et al., 2012; Yuan et al., 2016a). For example, DNMT3A^{R882mut} protects against anthracycline-mediated cell death in AML via an impaired chromatin remodeling response that prevents an appropriate DDR (Guryanova et al., 2016). Restoration of this pathway sensitized DNMT3A^{R882mut} cells to anthracycline (Guryanova et al., 2016). Conversely, administration of azacytidine, a DNA methyltransferase inhibitor, suppressed Dnmt3A^{R882mut} clones and prolonged survival in AML patients harboring DNMT3A^{R882mut} (Scheller et al., 2021). In mouse models, DNMT3A^{null} cells expand following exposure to busulfan, but not 5-FU, highlighting the specificity of certain stressors to drive clonal expansion (Chen et al., 2020). The effects of chemotherapies on DNMT3A^{mut} clonal selection may also be specific to the type of mutation (Yuan et al., 2016b).

For TET2, one proposed mechanism driving treatment resistance is that hypermethylation caused by *TET2* deletion promotes a quiescent stem-cell-like state, desensitizing cells to chemotherapies such as cytosine arabinoside and doxorubicin (Morinishi et al., 2020). The use of hypomethylating agents to target specific TET2^{mut} may prove beneficial in sensitizing TET2^{mut} cells to chemotherapy (Stölzel et al., 2021).

The impact of mutations in *IDH1/2* and *ASXL1* on chemoresistance in AML remains unclear (Brunner et al., 2019; Molenaar et al., 2018; Ni et al., 2020; Paschka et al., 2010, 2015). Determining the mechanisms by which chemotherapy interacts with *DNMT3A* and other epigenetic modifier mutant clones will provide greater insights on how these mutant clones respond to cytotoxic pressures (Chaudry and Chevassut, 2017; Metzeler et al., 2012; Venugopal et al., 2021; Yang et al., 2015, 2021).

Spliceosome complex proteins

Clones with mutations in spliceosome proteins (*SRSF2 SF3B1*, and *U2AF1*) are more likely to persist in AML patients following chemotherapy and are observed in a high proportion of secondary AMLs (Lachowiez et al., 2021). Still, whether spliceosome mutants have selective advantages with certain chemotherapeutics or whether selection is a consequence of other genetic lesions within the leukemic clones remains unknown.

Finally, the clinical correlation between the selective advantage of non-leukemic epigenetic and spliceosome driver mutations in CHIP under cytotoxic therapy may be limited by the type of cytotoxic therapies used in large cancer patient cohort studies (Bolton et al., 2020; Coombs et al., 2017). Although prior studies in leukemic patients suggest that epigenetic modifiers and spliceosome regulators play important roles in response to therapy, how various cytotoxic therapies regulate non-leukemic CHIP clones remains unclear. Further clinical and mechanistic studies are needed to determine whether cytotoxic drivers

Table 2. Extrinsic mechanisms of clonal expansion

Class	Gene	Mechanism	Description	Model	Reference(s)
Aging					
	CHIP	Next-generation sequencing	cross sectional study association	human	Jaiswal et al., 2014, Xie et al., 2014, Buscarlet et al., 2017, van Zeventer et al., 2021
	DNMT3A, TET2	exome sequencing	association study between CHIP risk and longer leukocyte telomere length	human, n = 200,453	Kar et al., 2022
	ASXL1	truncated ASXL1	truncated ASXL1 expands in murine model of aging via interactions with BAP1 to activate mTORC for survival	mouse	Fujino et al., 2021
Chemotherapy/radiation					
	CHIP	radioactive iodine (RAI)	cross-sectional study showed RAI was linked with increased prevalence of CHIP	human, n = 279	Boucai et al., 2018
	CHIP	chemotherapy followed by ASCT	longitudinal association study found MRD− samples treated with chemotherapy-ASCT was linked to CHIP expansion	human, n = 149	Eskelund et al., 2020
	PPM1D	chemotherapy (including topoisomerase I and II inhibitors, taxanes, platinum-based therapies, etoposide cytarabine, doxorubicin), XRT, radionuclide	multiple cross-sectional studies, mouse model, and *in vitro* experiments linked PPM1D to specific cytotoxic stressors	human, n = 10,138 human, n = 5,649 human, n = 119 human, n = 686 human, n = 1,185 mouse	Bolton et al., 2020; Coombs et al., 2017; Wong et al., 2018; Swisher et al., 2016; Kim et al., 2019; Hsu et al., 2018; Kahn et al., 2018
	CHEK2	chemotherapy (topoisomerase II inhibitors, platinum-based therapies)	cross-sectional study	human, n = 10,138	Bolton et al., 2020
	TP53	chemotherapy (including platinum-based therapies, taxanes, 5-FU), XRT	multiple cross-sectional studies and mouse models that show TP53 selective advantage under these cytotoxic stressors	human, n = 10,138 human, n = 5,649 human, n = 119 mouse models	Bolton et al., 2020; Coombs et al., 2017; Wong et al., 2018; Bondar and Medzhitov, 2010; Marusyk et al., 2010; Wlodarski et al., 1998
	TP53	n-ethyl-*n*-nitrosourea	treatment of *Tp53*$^{+/-}$ HSPCs showed competitive advantage	mouse	Wong et al., 2015
	TET2	chemotherapy (including doxorubicin and cytosine arabinoside)	TET2^{KO}AML cells are less sensitive to treatment; cross-sectional study linked to TET2	AML cell lines; human, n = 5,649	Morinishi et al., 2020; Coombs et al., 2017
	TET2	space flight (XRT)	astronauts that experienced space flight had earlier expansion of TET2	human, n = 2	Mencia-Trinchant et al., 2021
	DNMT3A	busulfan	Dnmt3a$^{-/-}$ HSCs expand with treatment	mouse	Chen et al., 2020

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Table 2. *Continued*

Class	Gene	Mechanism	Description	Model	Reference(s)
Tobacco/smoking					
	ASXL1	current/former smoking history	multiple cross-sectional studies	human, n = 5,649 n = 502,524 n = 200,453 n = 628,388 n = 10,138	Coombs et al., 2017; Dawoud et al., 2020; Kar et al., 2022; Kessler et al., 2022; Bolton et al., 2020
	DNMT3A, TET2	current/former tobacco use and smoking	cross-sectional study	human, n = 5,649 n = 502,524	Coombs et al., 2017; Dawoud et al., 2020
	PPM1D	current/former tobacco use	cross-sectional study	human, n = 5,649	Coombs et al., 2017
	DNMT3A, SRSF2, SR3B1	smoking status (current)	cross-sectional study	human, n = 200,453	Kar et al., 2022
	CHIP	smoking history	cross-sectional study	human, n = 12,380	Genovese et al., 2014
Inflammation					
	DNMT3A	elevated serum MIP1a	Cross sectional study showing increased risk of DNMT3A-CHIP	human, n = 200,453	Kar et al., 2022
	DNMT3A	chronic IFNγ signaling via *M. avium* infection	infection promotes DNMT3A KO expansion due to resistance to inflammation-mediated myeloid differentiation.	mouse	Hormaechea-Agulla et al., 2021
	TET2	LPS injection	TET2 clones selectively expanded via lncRNA *Morrbid* and a*TLR4/NK-kB/IL-6/STAT3* pathway	mouse	Cai et al., 2018
	TET2	TNFα signaling	TET2 selectively expanded with direct TNFα signaling	mouse	Abegunde et al., 2018
	TET2	disruption of intestinal barrier integrity	increased microbial translocation and signaling, particularly from *Lactobacillus*, promotes inflammation and TET2 expansion	mouse	Meisel et al., 2018
	TET2	broad-spectrum antibiotics	antibiotics slowed the expansion of TET2-deficient clones	mouse	Zeng et al., 2019
	CEBPa	direct injection of IL-1β	IL-1β promotes KO CEBPa MPP3 clonal expansion, while CEBPa activates WT competitors for myeloid expansion and exhaustion	mouse	Higa et al., 2021
	ASXL1	"Tissue editing With Inducible Stem cell Tagging via Recombination" (TWISTR) knockin of CHIP genes and single-cell analysis	single-cell sequencing of knocked-in CHIP homologs zebrafish HSCs demonstrated resistance to inflammation in CHIP stem cells and promotion of cytokine production in myeloid lineage cells	zebrafish	Avagyan et al., 2021

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Table 2. ***Continued***

Class	Gene	Mechanism	Description	Model	Reference(s)
	TP53	poly:IC serial injection	TP53 clones were significantly expanded via IFNγ production caused by poly:IC treatment in competitive transplant models	mouse	Rodriguez-Meira et al., 2022
Autoimmunity					
	DNMT3A and PPM1D	inflammatory environment caused by inflammatory bowel disease	cross-sectional study found higher prevalence of CHIP in patients with ulcerative colitis	human, n = 187	Zhang et al., 2019
HSCT					
	DNMT3A	autologous HSCT	cross-sectional study found that DNMT3A associated with HSCT	human, n = 35	Wong et al., 2018
	DNMT3A	murine competitive transplant	$DNMT3A^{null}$ HSCs outcompete WT HSCs in murine transplant settings	mouse	Challen et al., 2011; Jeong et al., 2018
	PPM1D	murine competitive transplant	truncated PPM1D R451X HSCs fails to outcompete WT HSCs in competitive murine transplant settings	mouse	Hsu et al., 2018
	TET2	serial HSC transplant	$Tet2^{null}$ HSCs exhaust similarly to WT HSCs in serial transplant settings	mouse	Ostrander et al., 2020
	ASXL1	murine competitive transplant	truncated ASXL1 are at a disadvantage in engraftment and cell function in a murine transplant setting	mouse	Fujino et al., 2021
	SRSF2	murine competitive transplant	heterozygous Srsf2 P95H HSCs cannot outcompete WT HSCs in engraftment or lineage output in competitive murine transplants	mouse	Kon et al., 2018
	SF3B1	murine competitive transplant	Sf3b1-K700E mutant HSCs are at a disadvantage compared to WT HSCs in both young or old recipient mice	mouse	Mupo et al., 2017
	U2AF1	murine competitive transplant	U2AF1 chimerism in transplanted mice is significantly reduced in mutant U2AF1 variants: S34F, Q157P, and Q157R	mouse	Yoshida et al., 2011
Metabolic					
	TET2	hyperglycemia	hyperglycemia drives TET2 myelopoiesis and MPN	mouse	Cai et al., 2021
	TET2	vitamin C deficiency	Cross-sectional study where lower plasma vitamin C concentrations was associated with a higher frequency of TET2 mutations	human, n = 215	Chen et al., 2021

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Table 2. ***Continued***

Class	Gene	Mechanism	Description	Model	Reference(s)
	TET2	apolipoprotein B	high ApoB was associated with TET2 clonal expansion, whereas alcohol consumption was protective in this cross-sectional study	human, n = 200,453	Kar et al., 2022
	TET2, DNMT3A, ASXL1	BMI	high BMI was protective against DNMT3A clonal expansion but associated with TET2 and ASXL1 CHIP in this cross-sectional study	human, n = 628,388	Kessler et al., 2022
	DNMT3A	fatty bone marrow model	Self-renewal and engraftment advantage was observed in DNMT3A mutant cells and was mediated by IL-6	mouse	Zioni et al., 2021
	CHIP	unhealthy diet, low LDL, low HDL, insulin resistance, and hypertension are associated with increased risk of CHIP	several cross-sectional or -longitudinal association studies link CHIP with metabolic syndrome	human n = 44,111	Bhattacharya et al., 2021; Kar et al., 2022; van Deuren et al., 2021
Other					
	CHIP	Down syndrome	observational study found early signs of CHIP in Down syndrome patients	human, n = 51	Liggett et al., 2021
	DNTM3A, PPM1D	growth factor usage, RBC transfusion	cross-sectional study-linked association	human, n = 5,649	Coombs et al., 2017
	TET2	atherosclerosis	atherosclerosis accelerates HSC proliferation and expansion of TET2$^{-/-}$ cells	mathematical modeling, mouse	Heyde et al., 2021

King and colleagues analyze how environmental contexts associated with clonal hematopoiesis of indeterminate potential (CHIP) alter tissue microenvironments to facilitate the selection and expansion of specific CHIP mutant clones. They also consider major remaining gaps in knowledge that will determine future application of this field in transplant and preventive medicine.

negatively or positively impact these classes of CHIP mutants. Expanding our understanding of these selective processes could revolutionize personalized medicine, such that treatment regimens are carefully chosen to avoid the selection of certain mutant clones.

Immunotherapies

Current cancer research has focused on the development of immunotherapies to prime, augment, or sustain immune responses to cancer. These therapies pose a greater risk of immune-mediated stress compared to the cytotoxic therapies described above (Esfahani et al., 2020). Immune checkpoint inhibitors (ICIs) may induce autoimmunity, including immune-mediated destruction of the hematological compartment (Davis et al., 2019), while chimeric antigen receptor T cell (CAR T) therapy is known to induce cytokine release syndrome mediated largely by interleukin-1 (IL-1) and IL-6 (Norelli et al., 2018). To date, the impact of immunotherapies on CHIP remains controversial, with some studies indicating that immunotherapies may promote expansion of CHIP clones but others reporting no impact on clonal expansion (Bolton et al., 2020; Miller et al., 2020).

Although there is no consensus about the impact of immunotherapy on CHIP emergence, CHIP mutations are known to affect CAR T cell function. A case study found that CAR T cells harboring a *TET2* CHIP mutation had enhanced expansion, prolonged activation, increased cytokine production, and memory-like properties (Fraietta et al., 2018), consistent with a known role for DNA methylation in promoting memory-like enhanced and sustained T cell function (Akbari et al., 2021; Ladle et al., 2016; Wang et al., 2021); similar results were observed in a pre-clinical mouse model (Jain et al., 2021). Deletion of *DNMT3A* in CAR T cells also promotes cell survival, limits exhaustion, and enhances therapeutic efficacy (Prinzing et al., 2021). One study suggested that CHIP mutations may influence CAR T therapy, as patients with CHIP had a higher rate of complete response to CAR T therapy, but with increased rates of cytokine release syndrome (Miller et al., 2021b). Aside from CHIP mutations in CAR T cells themselves, tumor-infiltrating leukocytes harboring CHIP mutations have also been observed in solid tumor samples (Kleppe et al., 2015). Thus, CHIP could impact the efficacy of immunotherapy via non-cell-autonomous mechanisms that have not yet been explored.

Smoking

Cigarette smoking induces cytotoxic DNA damage and causes substantial tissue damage and inflammation. Smoking damages the HSC niche and subsequently impairs HSC homing, causes proliferative exhaustion of HSCs, and induces extramedullary hematopoiesis (Morales-Mantilla et al., 2020; Siggins et al., 2014; Tura-Ceide et al., 2017). Increased risk of CHIP has been linked with a history of smoking (Bolton et al., 2020; Genovese et al., 2014; Coombs et al., 2017; Kar et al., 2022), as well as smoking-associated pulmonary diseases such as COPD (Buscarlet et al., 2017; Zink et al., 2017) and lung cancer (Zajkowicz et al., 2015; Coombs et al., 2017; Kessler et al., 2022). Among common CHIP mutations, mutations in *ASXL1* have the most significant association with smoking status (Bolton et al., 2020; Coombs et al., 2017; Kar et al., 2022; Kessler et al., 2022; Dawoud et al., 2020). Mutant ASXL1 regulates DNA transcription by enhancing the ubiquitinase BAP1, resulting in decreased histone ubiquitination and increased AKT/mTOR signaling. This results in increased cell-cycle progression even in the setting of DNA damage (Fujino and Kitamura, 2020). Likely, smoking not only contributes to increased mutational burden of *ASXL1* mutant clones but also provides a selective advantage for this clone via further induction of DNA and tissue damage and inflammation; further mechanistic studies of the relationship between smoking and *ASXL1* mutations are still pending.

Inflammation

Inflammation is a common source of stress that induces the regulation and repair of physiological insults. Inflammation can be triggered by many sources, including old age, infections, cancer, radiation, and underlying pathogenic conditions like atherosclerosis (Bick et al., 2020b; Gartung et al., 2019; Hansson et al., 2006; Matatall et al., 2016; Pietras, 2017). Inflammation is mediated by various signals, such as pathogen- and damage-associated molecular patterns (PAMPs and DAMPs), as well as pro-inflammatory cytokines including tumor necrosis factor alpha (TNFα), interferon alpha (IFNα), interferon gamma (IFNγ), IL-1β, and IL-6. As reviewed elsewhere, these signals influence HSCs via both direct and indirect mechanisms that impact quiescence, promote differentiation at the expense of self-renewal, and affect cell survival (King and Goodell, 2011; Baldridge et al., 2010; Essers et al., 2009; Caiado et al., 2021; Pietras, 2017; Hormaechea-Agulla et al., 2021). Long-term consequences of chronic inflammation include impaired HSC self-renewal and subsequent HSC terminal exhaustion (Matatall et al., 2016).

Several studies in both humans and animal models demonstrate that selected CHIP clones can resist inflammation-induced proliferative stress and gain a selective advantage in inflammatory conditions. Studies in patients with HIV (Bick et al., 2020b; Dharan et al., 2021) and aplastic anemia (Ogawa, 2016; Zhang et al., 2019; Yoshizato et al., 2015) indicate that inflammation can lead to the selection of specific CHIP clones and can predispose to progression to AML and myelodysplastic syndrome (MDS) (Kristinsson et al., 2011).

Resistance to inflammation-mediated depletion

Loss-of-function *TET2* clones have been extensively studied in murine models, and these *TET2* mutants selectively expand, particularly in the myeloid compartment, in response to pro-inflammatory stimuli TNFα and lipopolysaccharide (Abegunde et al., 2018; Cai et al., 2018). In a key study by Meisel et al., the presence of *Tet2* null hematopoietic cells disrupted intestinal barrier integrity and increased systemic microbial signaling, particularly from *Lactobacillus*, which was critical in promoting further clonal expansion (Meisel et al., 2018). Conversely, broad-spectrum antibiotic therapy slowed the expansion of *Tet2*-deficient clones (Zeng et al., 2019). Mechanistically, the long noncoding RNA (lncRNA) *Morrbid* and the TLR4/NF-κB/IL-6/Stat3 pathway may be crucial for *Tet2* clonal expansion and persistence (Cai et al., 2018). Pharmacological inhibition of Shp2 with inhibitor SHP099 or inhibition of Stat3 with E3330 led to a loss of *Morrbid* hyperactivity in *Tet2*-deficient hematopoietic stem and progenitor cells (HSPCs), suppressing selective clonal expansion (Cai et al., 2018). This study highlights the role

of lncRNAs in modifying cell death to inflammation by making them more resistant to inflammation-induced apoptosis.

Although *DNMT3A* mutations are not as extensively studied as *TET2*, our recent findings shed light on mechanisms by which inflammation can positively select for *Dnmt3a* knockout (KO) clones. We found that *Dnmt3a* KO murine clones outcompete wild-type (WT) murine cells in response to chronic IFNγ stimulation caused by *Mycobacterium avium* infection (Hormaechea-Agulla et al., 2021). Much like *TET2* clones, chronically IFNγ-stimulated *Dnmt3a* null HSCs are resistant to apoptosis, as caspase 3/7 activity is significantly decreased compared to IFNγ-stimulated WT HSCs. Most strikingly, *Dnmt3A* clones showed dampened differentiation in response to IFNγ compared to WT cells. Because we previously showed that excessive IFNγ-stimulated myeloid differentiation is a major mechanism of HSC depletion during chronic infection (Matatall et al., 2016), reduced IFNγ-dependent differentiation provides a clear selective advantage to *Dnmt3*a KO clones. Indeed, *Dnmt3*a KO clones do not upregulate pro-differentiation genes, nor do they display significant myeloid differentiation upon IFNγ stimulation *in vitro*. Consistent with this phenotype, the promoter regions of pro-differentiation genes were hypermethylated in *Dnmt3a* KO HSCs from infected mice compared to WT (Hormaechea-Agulla et al., 2021). Further, a recent study showed that IFNγ-dependent *DNMT3A*^{mut} clonal expansion was attributable to DNA hypomethylation and altered expression of *Txnip* and *p21*, allowing preservation of HSC quiescence (Zhang et al., 2022). These findings demonstrate that epigenetic changes underlie transcriptional activation of inflammation-driven HSC differentiation and provide a clear mechanism by which *Dnmt3a* KO clones are selected to expand in inflammatory conditions (Hormaechea-Agulla et al., 2021; Zhang et al., 2022).

While these studies provide a potential mechanism by which CHIP mutants, such as DNMT3A, expand in the setting of inflammatory stress, some parallel studies of humans and human cells have not mirrored exactly these findings. For instance, the lineage output of naive Dnmt3a KO HSCs in our murine model differed from what has been observed in human DNMT3A R882-mutated HSPCs (Nam et al., 2022). Single-cell multi-omics of DNMT3A R882 CD34 $^{+}$ HSC populations in multiple myeloma patients revealed a myeloid-biased differentiation and megakaryocytic expansion within humans, suggesting that broad transcriptional consequences of DNMT3A mutations affect differentiation even in the absence of a strong exogenous source of inflammation (Nam et al., 2022). Altered methylation of human DNMT3A R882 mutant clones showed aberrant methylation status, but the differentially methylated regions included *MYC* as opposed to the ATF transcription factors that we highlighted. Thus, although murine models can shed light on the mechanisms by which DNMT3A mutant clones are positively selected during inflammation, mechanistic studies in humans must be done to confirm relevance.

Depletion of WT competitors

Other CHIP clones encode transcriptional alteration(s) that promote positive selection and suppression of competing WT clones (Higa et al., 2021). Our recent study showed that *Cebpa* KO MPP3 clones expand in response to chronic IL-1β signaling. Transcriptionally, *Cebpa* deficiency prevents IL-β-driven repression of self-renewal genes like *Foxo3* and *Mycn*, which are directly regulated by *Cebpa*, thereby giving mutant clones a self-renewal advantage over WT cells. Interestingly, *Cebpa* KO clones can also suppress WT competitors by promoting excessive myeloid differentiation in a competitive transplant model. Indeed, RNA sequencing analysis demonstrated that WT MPP3s exposed to chronic IL-1β and in contact with *Cebpa* KO clones showed a significant increase in myeloid differentiation genes compared to WT MPP3s stimulated by chronic IL-1β alone (Higa et al., 2021).

Avagyan et al. (2021) expanded upon the ability of mutant clones to promote differentiation of their competitors. Utilizing a novel technique called ''Tissue editing With Inducible Stem cell Tagging via Recombination'' (TWISTR) in a zebrafish model, Avagyan et al. created a mosaic zebrafish that expressed 12 mutated CHIP genes during development. Interestingly, clones bearing frameshift mutations in *asxl1*, the zebrafish homolog of human *ASXL1*, demonstrated clonal dominance. Single-cell RNA sequencing of marrow cells showed that *asxl1* mutant macrophage and neutrophil clones were highly inflammatory, with upregulation of inflammatory cytokines *il1β* and *tnfβ.* More strikingly, the *asxl1* mutant progenitors showed an inflammation-resistant molecular profile, upregulating genes that modulate the effects of inflammatory stress such as *socs3a* and *nr4a1*. These findings suggest that CHIP clones contribute to the generation of inflammatory cytokines known to deplete WT HSCs. At the same time, the clonal mutant progenitor cell types resist the deleterious effects of these inflammatory signals and expand (Avagyan et al., 2021). It is interesting to note that some CHIP mutations (e.g., in MYD88, STAT3) play a key role in transmitting inflammatory signaling, suggesting a direct route for inflammation resistance. These findings highlight how mutant HSCs can promote their own selection by preserving self-renewal while WT competitors are outcompeted or exhausted through excessive myeloid differentiation.

CHIP as a cause and consequence of inflammation

While CHIP mutants have survival and selective advantages in response to inflammation, CHIP itself also drives inflammation, setting up a feedforward loop, as noted in the above example of asxl1 mutant zebrafish and reported in association with other mutations. For instance, *PPM1D* and *TET2* mutant clones have been shown to produce immune cell progeny that generate significantly higher-than-normal levels of the pro-inflammatory cytokine IL-6 upon exposure to chemotherapy or lipopolysaccharide, respectively (Cai et al., 2018; Jaiswal et al., 2017; Yura et al., 2021). *TET2* mutant clones contribute to atherosclerosis and heart disease via increased production of IL-6 by downstream macrophages, and *DNMT3A* has recently been suggested to contribute to inflammation in the heart by the same mechanism (Abplanalp et al., 2021; Bick et al., 2020a). As previously noted, TET2 mutations may also contribute to systemic inflammation by causing intestinal barrier compromise. As genetic mosaicism is now recognized to occur widely across tissues (Hsieh et al., 2020), additional pro-inflammatory consequences of CHIP mutations are likely to arise.

Human studies support the notion that CHIP can contribute to inflammation-mediated diseases such as severe COVID-19,

sepsis, and CVD (Bolton et al., 2021; Dawoud et al., 2020; Jaiswal and Ebert, 2019; Kessler et al., 2022; Zekavat et al., 2021b). Although increased IL-6 has been linked to heart disease in animal models and blockade of IL-6 signaling can reduce cardiovascular risk (Kessler et al., 2022), no empirical evidence has been published suggesting that IL-6 itself is capable of promoting CHIP. Using the presence of the IL-6R p.Asp358Ala coding mutation as a proxy to assess the potential for IL-6R blockade in individuals with CHIP, Bick et al. (2020a) found that impaired IL-6R signaling reduced CVD risk but did not impact CHIP status, particularly DNMT3A and TET2 CHIP. Furthermore, transcriptomic studies have not shown upregulation of canonical inflammatory pathways in CHIP, and the role of IL-6 signaling in CHIP-associated CVD risk remains an open question (Kessler et al., 2022; Weinstock et al., 2021). Thus, although mechanistic studies in murine models highlight ways that inflammation drives CHIP, the role of inflammation as a driver of CHIP in humans requires further experimental validation. Such a role may be even more complex to identify in humans because of the impact of cell-intrinsic genetic modifiers such as TCL1A on the selective expansion of CHIP clones (Weinstock et al., 2021).

The extent to which inflammation suppresses competitor cells or changes the niche to allow for favorable expansion of CHIP clones has yet to be fully explored (Rodriguez-Meira et al., 2022). While we have shown that CHIP clones resist the differentiation and exhaustion effects of inflammation, future studies could highlight more active processes by which CHIP clones suppress their competitors (Rodriguez-Meira et al., 2022).

Autoimmunity

Autoimmune disease mediated in part by dysregulated cytokine production, including IL-1, IFNγ, and TNFα (Italiani et al., 2018; Muzes et al., 2012; Lubberts and van den Berg, 2003), generates a chronic inflammatory environment that could facilitate the expansion of selected CHIP clones. Autoimmune diseases like inflammatory bowel disease, systemic lupus erythematosus, and rheumatoid arthritis are well known to carry increased risk of myeloid malignancies (Bekele and Patnaik, 2020; Boddu and Zeidan, 2019; Ramadan et al., 2012). The risk of hematological malignancy in these diseases has often been attributed to the use of therapies such as cyclophosphamide, methotrexate, and TNFα blockade (Ramadan et al., 2012). However, increased risk of leukemia has been observed in patients with arthritis with no prior history of treatment, suggesting that inflammation itself may have a role in hematological malignancy risk (Boddu and Zeidan, 2019). One small study found an increased prevalence of CHIP mutations in arthritis patients, with higher VAFs correlated with more severe disease, although this was not replicated in a separate study (Tariq et al., 2020; Savola et al., 2018). Another small cohort study of individuals with inflammatory bowel disease found increased VAF for *DNMT3A* and *PPM1D* compared to prior cohort studies, which was independent of treatment history. Additionally, serum levels of IFNγ were elevated in patients with $DNMT3A^{mut}$ CHIP (Zhang et al., 2019), further implicating this inflammatory cytokine in the selection of $DNMT3A^{mut}$ clones. Indeed, altered epigenetic profiles are known to facilitate immune dysfunction in autoimmune diseases (Mazzone et al., 2019). Altogether, mutations in epigenetic modifiers could provide a selective advantage for CHIP expansion and subsequent autoimmune disease progression. It is important to note that most of these studies on autoimmune diseases are in small cohorts that are underpowered. Further mechanistic studies are warranted to elucidate the role of autoimmunity and its treatment in CHIP expansion.

HSC transplants

HSC transplant (HSCT) is a last-resort treatment for hematological diseases like AML, MDS, aplastic anemia, and sickle cell disease (SCD). However, the stress of HSCT promotes loss of HSC proliferative and regenerative capabilities (Flach et al., 2014; Harrison and Astle, 1982) through telomere shortening (Colla et al., 2015; Notaro et al., 1997) and epigenetic changes (Soraas et al., 2019), decreasing overall HSC fitness. Furthermore, preconditioning for bone marrow transplant via irradiation and cytotoxic regimens can damage the niche (Pinho and Frenette, 2019) and generate an inflammatory environment, further impairing HSPC fitness. Several studies have demonstrated that some CHIP clones have a selective repopulation advantage during transplantation stress.

Both murine and human studies have shown that *TET2*-deficient HSCs have increased self-renewal and myeloid-biased repopulation expansion after transplantation (Kunimoto and Nakajima, 2020; Moran-Crusio et al., 2011; Ortmann et al., 2019; Ostrander et al., 2020; Shide et al., 2012). A study by Abegunde et al. showed that *TET2* mutant clones avoid HSC suppression by inhibiting TNFα signaling (Abegunde et al., 2018). However, contradictory studies suggest that $TET2^{null}$ HSCs unexpectedly exhaust at the same rate as control HSCs in serial transplantation (Ostrander et al., 2020). Furthermore, Yamashita et al. found that TNFα plays a key role in facilitating HSC survival and myeloid differentiation (Yamashita and Passegue, 2019); therefore, if TET2 loss of function inhibits TNFα signaling, one might expect impaired HSC survival. Overall, the precise mechanism of *TET2*-mutant clonal expansion after transplantation remains poorly defined and may involve pathways other than TNFα.

Murine studies have shown that *Dnmt3a* mutant clones have virtually limitless self-renewal and competitive repopulating capacities (Challen et al., 2011; Kunimoto and Nakajima, 2020), allowing them to outcompete their normal counterparts over time, at least in the context of stem cell transplantation after irradiation preconditioning. Indeed, *DNMT3A* loss-of-function HSCs can regenerate over at least 12 transplant generations despite the stress of serial transplantations (Jeong et al., 2018). Allsopp et al. suggested that $DNMT3A^{null}$ embryonic stem cells have elongated telomeres that can extend the replicative lifespan of serially transplanted HSCs (Allsopp et al., 2003; Gonzalo et al., 2006). Resistance to inflammation-mediated decay may also contribute to persistence of *Dnmt3a* mutant clones after transplantation, a topic of ongoing exploration.

In contrast to *TET2* and *DNMT3A*, *ASXL1*, *SRSF2*, *SF3B1*, *U2AF1*, and *PPM1D* are unable to outcompete WT HSPCs in murine transplant settings (Fujino et al., 2021; Yoshida et al., 2011; Hsu et al., 2018; Kon et al., 2018; Mupo et al., 2017). *PPM1D* mutant clones lack proliferative advantage and have overall decreased fitness after HSC transplantation, as shown in a series of patients after autologous transplant (Hsu et al., 2018; Wong et al., 2018). These studies suggest that stabilization of the DDR and resistance to apoptosis conferred by *PPM1D*

mutations confer little to no benefit in the context of the proliferative stress of HSCT (Hsu et al., 2018; Kahn et al., 2018; Wong et al., 2018).

Although gain-of-function mutations in *PPM1D* were not selected following HSCT, *TP53* mutant HSC clones have a competitive expansion advantage under transplant stress (Chen and Liu, 2019; Chen et al., 2019). Chen et al. demonstrated that a gain-of-function mutation allows TP53 to have enhanced interaction with EZH2, reinforcing H3K27 trimethylation and thereby enhancing the mutant clone's self-renewal and differentiation capacity (Chen and Liu, 2019; Chen et al., 2019). Interestingly, *TP53* HSC mutant expansion also occurs in a non-irradiated transplant model in absence and, to a greater extent, in the presence of doxorubicin chemotherapy (Sano et al., 2021). Thus, both mechanisms of altered epigenetics and DDR pathway dysfunction may facilitate selective advantage of *TP53* mutant clones due to both transplantation and chemotherapy stress.

CHIP has been observed in patients following autologous (Chitre et al., 2018; Gibson et al., 2017b) and allogeneic stem cell transplants (Frick et al., 2019) and may affect stem cell repopulation and engraftment. Accumulating evidence shows that HSCs harboring CHIP mutations can be found in the donor and can be transplanted to the recipient (Gibson et al., 2017a; Nawas et al., 2021). Recipients who received donor grafts with CHIP mutations experience a more biased myeloid lineage expansion, increasing the risk of the development of adverse hematological outcomes, including therapy-related myeloid neoplasms and cytopenias (Chitre et al., 2018; Gibson et al., 2017a; McNerney and Le Beau, 2018). Furthermore, Alfonso Pierola et al. showed, in an *in vivo* study, that CHIP mutant HSC clones lacking DDR systems can survive chemotherapy-induced stress and DNA damage while acquiring secondary mutations, ultimately leading to secondary malignancies (Alfonso Pierola et al., 2016). Altogether, these discoveries are leading to increased scrutiny of the mutational landscape in HSCT donors.

Aging

Aging is the stressor that is most strongly associated with CHIP (Bolton et al., 2020; Genovese et al., 2014; Jaiswal et al., 2014; Xie et al., 2014). Sequencing studies in the elderly reveal that clonal hematopoiesis is most prevalent in those aged >70. Although many CHIP clones can emerge, the most prevalent age-associated clones are *DNMT3A*, *TET2*, *ASXL1*, *SF3B1*, and *SRSF2* (Buscarlet et al., 2017; Genovese et al., 2014; McKerrell et al., 2015; Jaiswal et al., 2014; Xie et al., 2014).

Although aging can lead to the expansion of various CHIP clones, it is noteworthy that the expansion of these clones is not directly correlated with CHIP-associated clinical outcomes. A study by van Zeventer et al. (2021) investigated CHIP in individuals over 80 years old and found no association between cardiovascular morbidity and the presence of CHIP. There were, however, associations between CHIP and deaths due to hematological malignancies (hazard ratio: 1.48, confidence interval: 95%) (van Zeventer et al., 2021). Of note, the most prevalent clones in this study were *DNMT3A* and *TET2* mutants, both of which are associated with CVDs (Jaiswal et al., 2017). These opposing findings indicate that aging can lead to the expansion of clones but that the major impacts on health occur before a threshold age, suggesting that the age CHIP onset may be the most relevant factor.

Mechanistically, aging can be considered a multi-factorial stressor that leads to various effects on the hematopoietic compartment, as explored in several reviews (Jaiswal and Ebert, 2019; Lee et al., 2019; Libby et al., 2019). The mechanisms underlying age-associated clonal expansion likely overlap with inflammatory stimuli discussed above. Indeed, there is a baseline increase in production of pro-inflammatory cytokines such IFNγ, TNFα, and IL-6 (Chung et al., 2006; Li et al., 2011; Sanada et al., 2018) over time, frequently called "inflammaging," which could promote the expansion of *DNMT3A* (Hormaechea-Agulla et al., 2021) and *TET2* (Abegunde et al., 2018; Meisel et al., 2018) CHIP clones. In addition, aged HSCs have increased histone and DNA methylation, resulting in activation of self-renewal signatures and silencing of differentiation programs (Sun et al., 2014; Nachun et al., 2021; Beerman, 2017). As a result, aged HSCs display defective homing to the bone marrow niche, impaired lymphocytic differentiation, and increased myeloid and platelet lineage priming (Franceschi and Campisi, 2014; Kovtonyuk et al., 2016; Lee et al., 2019). This overproduction of myeloid cells may contribute to another feedforward loop of increased inflammation and CHIP during aging (Franceschi et al., 2000).

Interestingly, Kar et al. (2022) recently reported that longer leukocyte telomere length was associated with increased DNMT3A and TET2 mutant CHIP (Kar et al., 2022). Long telomeres may be yet another mechanism by which these clones persist despite inflammation and replicative stress, but this proposed mechanism has not yet been formally demonstrated.

A study by Fujino et al. (2021) showed that C-terminally truncated ASXL1 clones expanded in aged mice (Fujino et al., 2021). Mechanistically, truncated Asxl1 interacts with BAP1 to deubiquitinate Akt and activate mTORC, leading to the survival and expansion of these aged *Asxl1* HSCs. However, *Asxl1* clones exhibited impaired engraftment, myeloid-biased hematopoiesis, and significantly decreased homing ability to the bone marrow niche compared to WT transplanted HSCs, consistent with the observation that *ASXL1* clones do not survive the stresses of HSCT (Fujino et al., 2021).

Other investigators have used modeling to argue that a constant fitness advantage conferred by the CHIP mutation is sufficient to model the CHIP seen in humans (Fabre et al., 2021; Watson et al., 2020). In fact, Fabre et al. conclude that "many clones grew more rapidly early in life compared with the rate in old age" (Fabre et al., 2021). In contrast, Monte Carlo modeling has been used to show that cancer-causing mutations have greater positive selection late in life, thus explaining the age dependence of leukemias and other cancers (Rozhok and DeGregori, 2019). Additional studies, such as using mouse and non-human primate models, will be required to determine whether CHIP occurs simply as a matter of time or whether extrinsic pressures are required.

Metabolic stress

Metabolism plays a crucial role in maintaining and regulating HSC homeostasis (Ito et al., 2019), and there has been increasing interest in uncovering how metabolic factors impact CHIP. In particular, *TET2* mutant clones have been known to interact with endocrine and metabolic axes, although the reports of these associations have been mixed. In a type 2 diabetes

mouse model, a *TET2* mutant clone was shown to facilitate insulin resistance in mice (Fuster et al., 2020). In addition, elevated glucose levels have been found to destabilize TET2 in human peripheral blood samples (Wu et al., 2018), and increased glucose levels drove leukemia formation in mice harboring *TET2* mutations (Cai et al., 2021). Two recent large cohort studies found a higher prevalence of CHIP in people with an unhealthy diet (Bhattacharya et al., 2021) and in postmenopausal women with a high body mass index (BMI) and poor diet quality (Haring et al., 2021). A longitudinal study of 20 patients showed that clonal expansion was associated with lower high-density-lipoprotein (HDL) cholesterol and insulin resistance but not with BMI, hypertension, hyperglycemia, or total cholesterol (van Deuren et al., 2021). However, UK BioBank data revealed hypertension, low total cholesterol, and low low-density-lipoprotein (LDL) cholesterol to be associated with CHIP but not obesity or diabetes (Kar et al., 2022). A separate analysis of UK BioBank data showed that elevated BMI was negatively associated with *DNMT3A* mutation but positively associated with *TET2* and *ASXL1* mutant clones (Kessler et al., 2022). One confounder that may explain these mixed results is that vitamin C has been shown to regulate HSC homeostasis by activating TET2 (Lee et al., 2020). Lower plasma vitamin C concentrations were associated with a higher frequency of TET2 mutations in an elderly cohort (Chen et al., 2021). For DNMT3A, murine models showed that a fatty bone marrow environment can provide a selective advantage for *DNMT3A*^{mut} cells, which may be mediated by IL-6 (Zioni et al., 2021). Altogether, early studies suggest an important role for metabolic and dietary stresses in the pathogenesis of CHIP.

Other stresses that expand CHIP clones

Although, in this review, we focused on specific DNA-damaging and -inflammatory environmental drivers of clonal expansion, CHIP has also been linked with various other potential drivers including psychiatric diseases (Zink et al., 2017), gout (Kessler et al., 2022), and Down syndrome (Liggett et al., 2021; Tong et al., 2018). In individuals with Down syndrome, clonal hematopoiesis is observed much earlier in life with the most common mutations being found in *TET2,* although, notably, this CHIP did not reach levels observed in the elderly (Liggett et al., 2021). Trisomy 21 has been shown to induce an enhanced IFN signature in the hematopoietic compartment, which may facilitate early clonal expansion (Sullivan et al., 2016), and CHIP in individuals with Down syndrome is associated with dysregulated immune patterns in peripheral leukocytes (Liggett et al., 2021). Selective clonal expansion in other diseases, like SCD, is less clear (Pincez et al., 2021; Liggett et al., 2022). A recent report suggests that space flight may accelerate clonal expansion (Mencia-Trinchant et al., 2021), and many more associations and drivers of CHIP are constantly being discovered.

CONCLUDING REMARKS

The natural acquisition of mutations throughout life generates a range of genetically distinct HSPCs. Despite the ubiquity of CHIP-associated mutations, most individuals never develop CHIP, suggesting that environmental pressures are critical determinants of clonal expansion (King et al., 2020). Here, we reviewed the contexts in which some genetically variant clones expand over time. We highlight specific examples of imposed external stress such as platinum-based chemotherapies that drive the expansion of clones containing *PPM1D* mutations, which in turn increase the risk of secondary malignancy. As the field increasingly identifies environmental factors that may provide a selective advantage for certain clones, the clinical implications of these associations and whether they can be modulated to improve human health continue to be explored.

Among genes known to be associated with CHIP, DDR factors such as *TP53* and *PPM1D* have been associated with chemotherapies and radiation. Mutations affecting epigenetic modifiers—*DNMT3A*, *TET2*, and *ASXL1*—and *JAK2* have been associated with bacterial translocation, infections, and inflammatory conditions, and one would expect that *MYD88* and *STAT3* mutant clones are similarly selected in the setting of inflammation. Relatively little is known about the environmental conditions that drive expansion of clones containing mutations in splicing factors such as *SF3B1, SRSF2*, and *U2AF1*, which are among the top 10 genes mutated in CHIP. These mutations have been reported to drive inflammatory responses, but further work will be required to determine whether they follow the pattern of inflammation-driven selection that has been reported for *TET2*, *DNMT3A*, *ASXL1*, and *JAK2*.

Whereas some variant clones may contribute to heart disease or stroke, others may be neutral in their impact on human health or may be beneficial by enabling long-term hematopoiesis in the elderly. Indeed, despite our tendency toward binary thinking, mutant clones may live in the liminal space between pathogenic and beneficial. For instance, whereas *TET2* clones drive increased IL-6-mediated inflammation, they may also enable persistent hematopoiesis in the challenging conditions of advanced age or serve to suppress competing clones with more serious adverse health effects, essentially constituting non-malignant "fitness peaks." Although mutant VAF >10% is strongly associated with increased heme malignancy risk, what other factor(s) govern progression to pre-malignant states like clonal cytopenia of undetermined significance (CCUS) and/or leukemia in this setting remain to be clearly identified. For some mutations, downstream consequences of mutations on differentiated cell function may have an outsized effect on health. Further, most mechanistic studies to elucidate environmental drivers of CHIP have been done in mice. While informative, these models have limitations, the most obvious of which is the short lifespan of the mouse relative to humans and the clean (if not sterile) and non-challenging housing conditions. Paired epidemiologic association studies have been a tremendous strength in this respect, but utilization of clinical trials and non-human primate studies will also be important.

Although a range of studies highlights how the environment (e.g., obesity, infection, prior exposure to chemotherapy) significantly alters which clones are selected to survive and expand, the extent to which changes in environment can shape clonal selection or modify disease risk remains to be seen. Lessons from the CANTOS trial examining IL-1β inhibition to reduce cancer risk are a case in point: while greatly reducing lung cancer risk and potentially reducing major adverse cardiovascular events in patients with TET2 mutant CHIP, canakimumab leaves patients vulnerable to potentially deadly infections (Ridker et al., 2017; Svensson

et al., 2022). Outcomes from such trials indicate that endpoints for intervention must be carefully defined. Furthermore, basal inflammatory signaling, such as is mediated by the microbiome (Josefsdottir et al., 2017), is essential for normal hematopoiesis and must be taken into account when considering anti-inflammatory approaches. Perhaps it is more productive to address the aberrant immunometabolic features of immune cells derived from mutant clones than to reduce the size of the clone itself. Similarly, one can consider more nuanced methods (beyond brute inhibition of inflammation) to modulate the bone marrow microenvironment to reduce the selective advantage of certain CHIP clones. Finally, although an intervention may prevent the expansion of a specific CHIP clone, it also may enhance the emergence of an alternate clone with unknown impact on health.

Evidence that *PPM1D* mutant clones expand only in the context of certain chemotherapies raises the potential of using personalized medicine approaches in the selection of therapy. One can imagine an array of decision-making algorithms to guide therapeutic approaches to cancer or other conditions while taking a patient's somatic mutational background into account. Innumerable studies, including an assignment of the relative risk of various mutations and mutant genes, will be needed to make such a personalized approach truly evidence based.

The relative accessibility of blood enabled the discovery of CHIP and its health implications. Recent studies show that clonal expansion also occurs in other tissues and may be a precursor to solid tumors (Kakiuchi and Ogawa, 2021; Wijewardhane et al., 2021). Non-oncologic health consequences of clonal expansion in other tissues have yet to be defined, and knowledge in this area will undoubtedly expand in the coming years. These future studies will shape our understanding of how systemic therapies or lifestyle modifications impact clonal evolution in non-hematologic tissues.

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AUTHOR CONTRIBUTIONS

M.A.F., B.T.T., and T.K.W wrote the initial draft and made the figures and tables. K.Y.K. conceived the project, provided funding, and edited the final draft. E.M.P. and J.D. edited the initial and final drafts.

DECLARATION OF INTERESTS

The authors have no conflicts of interest to declare.

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