Hematopoiesis of Indeterminate Potential and Atherothrombotic Risk

Andrew J. Murphy[1]   Dragana Dragoljevic[1]   Pradeep Natarajan[2,3]   Nan Wang[4]

[1] Haematopoiesis and Leukocyte Biology, Baker Heart and Diabetes Institute, Melbourne, Australia
[2] Cardiology Division, Department of Medicine, Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, United States
[3] Department of Medical and Population Genetics and the Cardiovascular Disease Initiative, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, United States
[4] Division of Molecular Medicine, Department of Medicine, Columbia University Medical Center, New York, United States

Address for correspondence Andrew J. Murphy, PhD, Haematopoiesis and Leukocyte Biology, Baker Heart and Diabetes Institute, Melbourne 3004, Australia (e-mail: andrew.murphy@bakeridi.edu.au).

Abstract

Hematopoiesis is the process of blood production, essential for the continued supply of immune cells and red blood cells. However, the proliferative nature of hematopoietic stem cells (HSCs) renders them susceptible to developing somatic mutations. HSCs carrying a mutation can gain a selective advantage over normal HSCs and result in hematological disorders. One such disorder is termed clonal hematopoiesis of indeterminate potential (CHIP), a premalignant state associated with aging, where the mutant HSCs are responsible for producing a small portion of mature immune cells in the circulation and subsequently in tissues. People with CHIP have been shown to have an increased risk of mortality due to cardiovascular disease (CVD). Why this occurs is under rigorous investigation, but the majority of the studies to date have suggested that increased atherosclerosis is due to heightened inflammatory cytokine release from mutant lesional macrophages. However, given CHIP is driven by several mutations, other hematopoietic lineages can be altered to promote CVD. In this review we explore the relationship between mutations in genes causing CHIP and atherothrombotic disorders, along with potential mechanisms of enhanced clonal outgrowth and potential therapies and strategies to slow CHIP progression.

Keywords
► clonal hematopoiesis
► atherothrombosis
► somatic mutations

Introduction

Hematopoiesis is the tightly regulated and hierarchical process of blood cell production. This process originates from hematopoietic stem cells (HSCs) through to lineage-committed progenitors and mature leukocytes, red blood cells, and platelets. The development of specific lineages is regulated through intrinsic factors, including precise combinations of transcription factors and epigenetic modifications, along with extrinsic cues such as cytokines and growth factors, resulting in the expression or repression of gene signatures to shape the morphological and functional capabilities of the mature cell. However, these processes can be altered with aging causing fundamental alterations to the hematopoietic

system.[1] One of the irreversible changes that occur in all somatic cells is the acquisition and persistence of mutations due to inefficiencies in DNA repair mechanisms. In HSCs, somatic mutations can accumulate with each division,[2–4] with most having no overt functional effect and many resulting in diminished HSC function initiating programmed cell death and clearance of the mutant cell. However, some mutations in HSCs evade clearance and can result in a competitive advantage, characterized by increased self-renewal, proliferation, survival, and biased lineage output.[5–7] To put this into numbers, HSCs are thought to acquire one to two mutations per division, which when extrapolated equates to approximately 10 mutations/year, and once we reach adulthood, modeling suggests that most of our HSCs will have two coding mutations and as many as 200,000 noncoding mutations. This suggests that by the time we are adults, each HSC is unique and keeps acquiring mutations.[2–4] These mutations can result in a process termed clonal hematopoiesis of indeterminate potential (CHIP), which is a form of clonal hematopoiesis, but is not driven by other mechanisms such as clonal mosaicism. Individuals with CHIP have an increased risk of mortality, which is now linked to cardiovascular disease (CVD). In this review, we will focus on CHIP mutations associated with thrombotic disease, the mechanisms contributing to this pathology, and potential therapies.

Clonal Hematopoiesis of Indeterminate Potential

Somatic mutations can result in several hematological disorders. CHIP occurs when HSCs acquire a somatic mutation providing them with a competitive growth advantage over normal HSCs. This results in a relative increased number of mutated hematopoietic cells in the bone marrow and blood. While the overall abundance of white blood cells (WBCs) is only modestly affected, over time the proportion of mutated cells in the blood grows at the expense of normal WBCs.[6] CHIP is an aging phenomenon, because a key feature of it is insufficient repair of damaged DNA which may then be differentially propagated depending on mutational fitness.[8] CHIP is defined as a variant allele frequency (VAF) of >2% in circulating WBCs (i.e., >4% of WBCs carry the mutation in one allele).[9,10] Largely based on whole exome sequence studies of blood DNA in various datasets, it is estimated that approximately 5% of individuals aged under 60 years display CHIP, which increases to approximately 10% aged over 60 years and is continued to increase with age.[11–13] Deep targeted sequencing indicates that hematological somatic leukemogenic mutations at very low VAF (i.e., median 0.2%) are almost ubiquitous in middle age healthy adults.[14] This finding infers that we are all at some point in our lives at risk of developing CHIP and other hematological disorders.

Mutations indicative of CHIP are most commonly observed in the genes DNMT3A, TET2, ASXL1, JAK2, and TP53.[15,16] Why mutations in the epigenetic modifiers DNMT3A and TET2 are such prevalent drivers of CHIP is not known. However, the relatively open chromatin structure of HSCs suggests that gene expression is largely governed by the methylation status and may reveal why mutations in DNMT3A (methylation) and TET2 (hydroxymethylation) cause such dominant changes in HSCs to promote their outgrowth.[17] Certainly, studies exploring the deletion or loss of function of these genes demonstrate the competitive advantage these mutant HSCs acquire.[18,19] The loss in DNMT3A and thus reduced DNA methylation result in the increased expression of genes involved in HSC proliferation and self-renewal, while the prevention of hydroxymethylation when TET2 is nonfunctional destabilizes key HSC maintenance genes which promotes both hyperproliferation and myeloid skewing.

CHIP and Cardiovascular Disease

CHIP-driving mutations are known to increase the risk of hematologic malignancy and carriers have 10 times the risk of hematologic cancer as those without such mutations do.[13] Initial analysis has found an association of CHIP with increased all-cause mortality, but the increased risk of hematological malignancies of 0.5 to 1% per year is not nearly enough to account for the 40% increase in mortality.[12,13] Further analyses identified a strong association of CHIP with a higher risk of CVD independent of age and other traditional risk factors.[11,13,20–22] The direct evidence for causality was first provided by animal studies. Hematopoietic $Tet2^{-/-}$ or $Tet2^{+/-}$ markedly increased atherosclerosis in hypercholesterolemic $Ldlr^{-/-}$ mice.[21,23] Mechanistically, $Tet2$-deficient macrophages showed increased NLRP3 inflammasome activation and elevated IL-1β production (►**Fig. 1A**).[21,23] Concentrations of related biomarkers are also increased among individuals with CHIP.[24,25] An NLRP3 inhibitor selectively reversed the increased atherosclerosis in the $Tet2^{-/-}$ CHIP model.[23]

$JAK2V617F$ ($JAK2^{VF}$) is less common than the mutations of epigenetic modifiers such as TET2, DNMT3A, or ASXL1.[13,21] Nevertheless, a recent study, with innovative deep targeted sequencing that had a screening sensitivity as low as VAF 0.01%, found that the $JAK2^{VF}$ mutation is detectable in almost 4% of a general European population.[26] Among the $JAK2^{VF}$ individuals in this population, approximately 60% had a VAF of >0.1% but most of whom did not have features of myeloproliferative neoplasm (MPN). CHIP-associated $JAK2^{VF}$ occurs at a younger age than the other CHIP variants[27] and dramatically increases risk of myocardial infarction by as much as 12-fold in younger people.[21] We found that $JAK2^{VF}$ increases atherosclerotic disease despite lowering LDL (low-density lipoprotein) cholesterol in both mice and humans.[28,29] Interestingly, it has been shown that mouse models of $JAK2^{VF}$ resembling a MPN (i.e., 100% $JAK2^{VF}$ bone marrow transplant [BMT]) or CHIP (20% $Jak2^{VF}$:80% WT BMT) both increase atherosclerosis.[29,30] $JAK2^{VF}$ causes altered functionality of multilineage blood cells.[29] Selective expression of $JAK2^{VF}$ in monocyte/macrophage increases atherosclerosis in association with increased generation of IL-1β and IL-18, the product of inflammasome activation.[30] Unlike $Tet2$ deficiency, knockout of NLRP3 has little effect, while
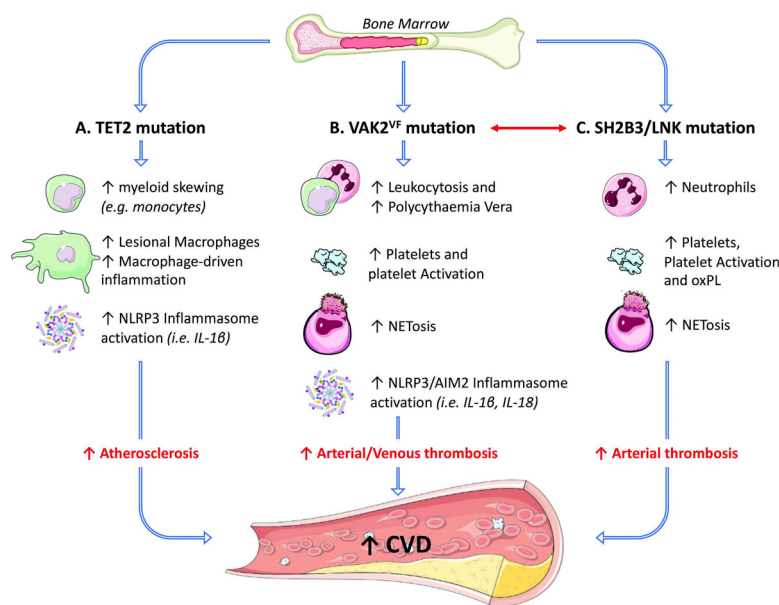
Fig. 1 Mechanistic features of hematopoietic mutations resulting in increased risk of atherosclerosis and atherothrombotic disease. (A) Somatic mutations in *TET2* have been shown to accelerate atherosclerosis through inflammatory macrophage signaling driven by the NLRP3/IL-1β axis, which when inhibited reduces atherosclerosis. (B) Somatic mutations in JAK2 can result in myeloproliferative neoplasms which influence atherothrombotic disorders, namely venous thrombosis. However, when JAK2 mutations result in CHIP, enhanced activation of the AIM2 inflammasome occurs to release inflammatory cytokines through gasdermin D pores. Whether this causes atherothrombosis is not yet known. (C) Germ line mutations in SH2B3 increase the risk of atherothrombotic disease. Inflammatory interactions between neutrophils and platelets occur, particularly driven by platelet release oxidized phospholipids (oxPL), causing NETosis. Interestingly, JAK2 mutations are linked with SH2B3 mutations, where the LNK(R262W, T allele) predisposes individuals to $JAK2^{VF}$ MPN and CHIP, along with coronary artery disease. CHIP, clonal hematopoiesis of indeterminate potential.

deletion of AIM2, the essential component of AIM2 inflammasome, reduces the increased atherosclerosis in $JAK2^{VF}$ models (►**Fig. 1B**). Inflammasome activation can lead to programmed cell death that is mediated by the pyroptosis executioner gasdermin D (Gsdmd), leading to release of inflammasome activation products such as IL-1β. $Gsdmd^{-/-}$ reduces atherosclerosis in $JAK2^{VF}$ mice.[30]

Chronic inflammation associated with atherosclerosis has long been thought to mediate atherosclerosis progression.[31] The recent Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) largely validated this notion.[32] IL-1β inhibition by canakinumab reduces incident CVD events in individuals with prevalent CVD and elevated high-sensitivity C-reactive protein (hsCRP), a marker of inflammation.[32] However, canakinumab therapy was associated with only a moderate clinical benefit and an increase in infections. Consequently, canakinumab has not been marketed for cardiovascular indications. Similarly, colchicine, which inhibits the microtubule-dependent assembly of the NLRP3 inflammasome and IL-1β secretion,[33,34] appeared to benefit CVD but also increased pneumonia.[35,36]

A more precise approach to identify patients who may benefit most from anti-inflammatory therapy, as well as identification of upstream therapeutic targets, could lead to more effective, safer anti-inflammatory treatments for CVD. Enhanced inflammation in NLRP3 or the AIM2 inflammasome/IL-1β axis in TET2 mutant or $JAK2^{VF}$ atherosclerosis models suggests that individuals with TET2 mutant or $JAK2^{VF}$ CHIP and increased risk of CVD could benefit more from anti-inflammation therapy. In the preclinical JAK2VF

CHIP model, administration of an anti-IL-1β antibody improved readouts of plaque stability, but did not change the overall lesion size and had no apparent effect in control mice.[30] Consistently, in an exploratory preliminary analysis, patients with a TET2 mutant have an improved response to canakinumab (hazard ratio [HR] = 0.38) relative to the response to overall response to canakinumab (HR = 0.93) in the CANTOS trial.[37] In addition to the pharmacological evidence, human genetic studies provide more support for a causal role of inflammation in CVD. IL-6 can be a downstream product of IL-1β signaling and increased IL-6 production and activity are considered to be a common mediator in chronic inflammation associated with CVD. A disruptive IL-6R missense variant is associated with 5% reduced CVD risk in a general population.[38,39] A more recent study indicates that this IL-6R variant attenuates CVD risk in individuals with CHIP by 54%, supporting the notion that individuals with CHIP may benefit most from IL-6 inhibition to reduce the risk of CHIP-associated CVD.[11]

CHIP and Thrombosis

Several somatic mutations have been linked to quantitative or qualitative abnormalities in platelets (reviewed by Veninga et al[40]). Among the common CHIP-driving mutations that affect platelets, the evidence for increased risk of atherothrombosis primarily comes from studies of $JAK2^{VF}$-associated MPNs and CHIP. Patients with MPNs have increased risk of arterial and venous thrombosis and thrombotic complications.[41–43] The studies of thrombotic risk

conferred by JAK2[VF] CHIP without apparent MPNs have significantly advanced relatively recently. In a population study of over 10,000 individuals without a known myeloid disorder, JAK2[VF] CHIP was associated with an increased incidence of thrombosis[44] (►**Fig. 1**). Polycythemia vera (PV), in which more than 90% of the patients are JAK2[VF] positive,[45] has increased hematocrit in association with increased blood viscosity,[46] a major risk factor of thrombosis in PV patients. Phlebotomy has been successfully used to maintain the hematocrit and reduce the risk of thrombosis in PV patients.[43] Thrombotic risk also is increased in JAK2[VF] MPN patients without apparently increased hematocrit. JAK2[VF] increases myeloid cell production, causing leukocytosis. An increased WBC count has been associated with an increased risk of thrombosis in MPNs. However, many with JAK2[VF] CHIP do not show signs of abnormal blood cell counts yet they still have increased risk of thrombosis.[44] JAK2[VF] is generally thought to only affect platelets, but platelets can interact with many cells types. In the circulation, neutrophils often form aggregates with platelets which can activate these cells. A consequence of neutrophil activation is the formation of neutrophil extracellular traps (NETs), where neutrophils release their contents leading to the formation of web-like structures (termed NETosis) made of DNA, myeloperoxidase, citrullinated histones and proteases that entrap and kill bacteria.[47] While NETs may help to suppress infections, the formation of NETs in blood vessels can promote atherosclerosis and thrombosis[48,49] (►**Fig. 1B**). NETs may serve as biomarkers predicting the risk of thrombosis.[49] Neutrophils from patients with MPNs display some features of enhanced activation.[50,51] Subsequent studies showed that neutrophils from MPN patients are primed for NETosis.[44] However, this is not always observed.[52] JAK2[VF]-modeled mice showed increased NET formation and venous thrombosis, which were reduced by DNase treatment or hematopoietic deficiency of peptidyl-arginine deiminase 4 (PAD4), the enzyme essential for citrullination of histones in NET formation.[44]

Various mouse models have been generated to assess the impact of JAK2[VF] on arterial thrombosis, including mouse JAK2[VF] knock-in, human JAK2[VF] transgenic, and knock-in of human JAK2[VF] cDNA into the mouse *Jak2* allele, with constitutive or tamoxifen-induced expression in hematopoietic and endothelial cells.[53–55] These models have different levels of mouse or human JAK2[VF] expression in different types of cells and generate various thrombotic and hemostasis phenotypes, varying from increased platelet activation, reduced tail vein bleeding time, and increased thrombus formation in a flow chamber assay to decreased platelet activation in response to convulxin or TRAP4, increased tail vein bleeding, and accelerated arterial thrombosis with unstable thrombi in vivo. A common feature of these studies is modeling of MPNs with high JAK2[VF] burden but not CHIP. The impact of JAK2[VF] CHIP with a lower burden on platelet activity and arterial thrombosis in mice is not known (►**Fig. 1B**). We have some evidence that modeling CHIP in mice with a small fraction (2–20%) of hematopoietic cells expressing JAK2[VF] shows increased platelet activation to thrombin and accelerated

arterial thrombosis, without increased bleeding (*unpublished observations*).

SH2B3/LNK encodes an adaptor protein that acts as a negative regulator of JAK2-mediated hematopoietic cell proliferation.[56] *Lnk* deficiency promotes multilineage expansion of HSCs in mice.[56] *Ldlr*^{−/−} mice with hematopoietic LNK deficiency display increased atherogenesis and accelerated atherothrombosis.[57] *Lnk* deficiency and hypercholesterolemia act synergistically promoting platelet activation and myelopoiesis.[57] More recently, we show that the increased arterial thrombosis in hematopoietic *Lnk*-deficient mice is due to NETosis in the thrombi and accelerated thrombosis is completely reversed by neutrophil depletion or PAD4 deficiency.[58] Mechanistic studies have identified oxidized phospholipids (OxPLs) released and presented by activated platelets mediate neutrophil activation and NETosis (►**Fig. 1C**). *Lnk*^{−/−} mice show increased plasma OxPL levels and transgenic expression of E06-scFv, which specifically binds and neutralizes OxPL activity, selectively and completely reverses NETosis in thrombi, and accelerates thrombosis in *Lnk* deficiency.[58] A common SH2B3 polymorphism (p.R262W, c.784T > C) is a loss-of-function LNK variant in association with increased platelet and neutrophil counts and the risk of CVD.[59] Consistent with this, we observed increased NETosis in co-cultures of human-induced pluripotent stem cell (iPSC)-derived neutrophils and activated platelets carrying isogenic LNK(TT) relative to LNK(CC).[58] Interestingly, the LNK(R262W, T allele) predisposes to both JAK2[VF] MPN and CHIP.[60] We also confirmed this with data from UK Biobank, showing that individuals with the JAK2[VF] mutation only displayed increased CAD when also carrying the LNK(TT) allele.

Accelerators of CHIP

Clone size, as estimated by VAF, is strongly associated with the prognosis of CHIP, whether it be cancer, subclinical atherosclerosis, atherosclerotic CVD, or heart failure.[13,20,21,61,62] Thus, identifying CHIP early will provide a window of opportunity to slow clonal growth and avoid cardiovascular complications. However, there is a scarcity of longitudinal sampling to confidently explore and define drivers of clonal outgrowth over time, with much of our knowledge coming from preclinical models. Thus, a major outstanding question is what drives clonal outgrowth and how can this be halted? Given that CHIP is driven by somatic mutations providing a competitive advantage, one strategy could be to define driver genes and target their expression or protein function to slow proliferative rates or kill the mutant HSCs. This is a complicated option as the major genes mutated in CHIP are epigenetic modifiers and kinases which have a significant impact on a network of genes involved in stem cell maintenance and proliferation.[18,19] We suggest that understanding the interactions between the mutations and environmental drivers of CHIP is key in delaying clonal outgrowth and may reduce the risk of CVD in the context of CHIP.
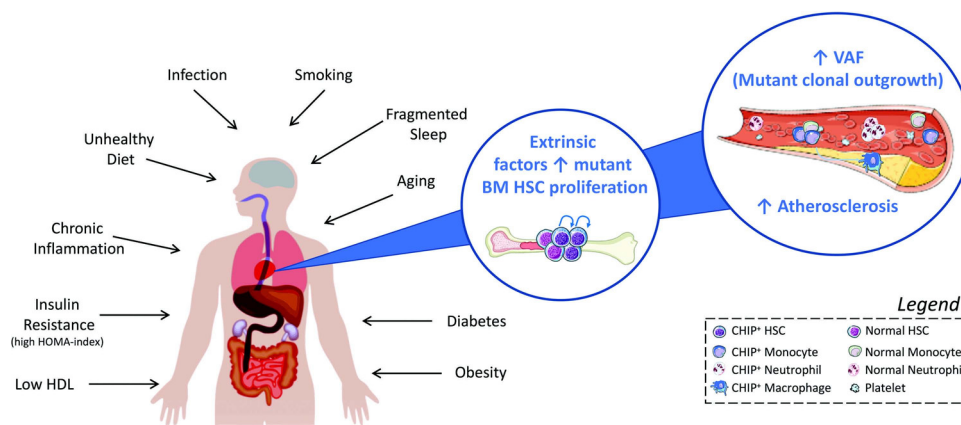
Fig. 2 Accelerators of clonal hematopoiesis. Somatic mutations are ubiquitous in people of middle age. However, what promotes the proliferation of these mutated cells and development of CHIP is relatively unknown. To date, some metabolic disorders/stressors, sleep fragmentation and chronic inflammation, and viral infections have been associated with CHIP. The extrinsic environment could aid in the increase of clonal outgrowth of mutated hematopoietic stem cells (HSCs), influencing VAF and contributing to atherosclerosis. CHIP, clonal hematopoiesis of indeterminate potential; VAF, variant allele frequency.

An alternative approach to exploring the gene regulatory networks that are altered by somatic mutations is to identify the extrinsic drivers of clonal outgrowth. It is slowly emerging that clonal outgrowth is linked with extrinsic factors including comorbidities, diets, smoking along with inflammatory status and infections[13,21,25,63–65] (►Fig. 2). In the initial studies linking CHIP to mortality due to CVD, an overrepresentation of individuals with metabolic disorders, namely diabetes, was noted.[13] Indeed, diabetes has been linked with leukemia[66,67] and in murine models diabetes has been shown to cooperate with Tet2 heterozygosity to cause leukemia.[68] TET2 deficiency can also aggravate insulin resistance in mice.[69] Metabolic stressors such as unhealthy diets have now been linked to a higher prevalence of CHIP.[70,71] Additional evidence to support the hypothesis that altered lipid and glucose metabolism often seen in individuals consuming unhealthy diets or with diabetes is linked with clonal outgrowth was seen in the Swedish Obese Subjects (SOS) study.[65] In this preprint manuscript, the authors report that over a 20-year follow-up period, growing clones in the obese individuals were found to correlate with low high-density lipoprotein insulin levels and HOMA index as a readout of insulin resistance (►Fig. 2). However, these data were based on a small sample size of <40 individuals and a larger follow-up study is required. Nonetheless, the mechanism(s) responsible for this are unknown, but could relate to low-grade chronic inflammation, which is a known consequence of obesity, providing cytokines that would enhance the proliferation of the HSC-harboring mutations. This hypothesis was also illustrated by Naxerova's group, revealing that disorders underpinned by enhanced hematopoietic activity (i.e., atherosclerosis and sleep fragmentation) accelerated clonal outgrowth.[64] However, it is important to note in the SOS study that hsCRP was not associated with clonal outgrowth.[65] We hypothesize that clonal outgrowth in metabolic disorders is due to an alternative mechanism.[72] First, we have previously shown that obesity and diabetes promote increased hematopoiesis at the level of the common myeloid progenitor and granulocyte-macrophage progeni-

tor, while HSCs are largely unaffected in respect to abundance.[73,74] This suggests that enhanced hematopoiesis at the level of the HSCs due to extrinsic signaling is unlikely to be responsible. Instead, high-energy environments such as obesity and diabetes could reduce the activity of epigenetic modifiers particularly TET2, which is reliant on AMPK activity.[75] Through this mechanism, further loss of TET2 function in TET2 mutant cells (i.e., 1 mutant allele) or combination with mutations in other genes may synergize to cause myeloid skewing and increased clonal outgrowth. This is consistent with hematopoietic TET2 heterozygote mice that display transition to leukemia in a model of hyperglycemia.[68] If this is true, treating individuals with agents such as metformin or novel AMPK activators along with life-style interventions may be effective in slowing the expansion of mutant cells in these individuals. However, the impact on extrinsic stimuli in promoting clonal outgrowth is largely limited to mice and requires large longitudinal clinical studies to address our hypothesis more accurately.

Interventions for Individuals with CHIP

There is no approved treatment for CHIP-related CVD risk. CANTOS[32] and genetic evidence[11] suggest that anti-IL-1β and anti-IL-6 could be particularly effective and potentially broadly beneficial to CHIP carriers. Targeting NLRP3 or AIM2 inflammasome may need to be tailored to the specific genetic factors responsible for CHIP. While ruxolitinib, a JAK1/2 inhibitor, has been approved for JAK2[VF] MPNs, it shows no benefit in atherogenesis in the JAK2[VF]-modeled mice.[30] Fedratinib, a selective JAK2 inhibitor, is newly approved for MPN-associated myelofibrosis.[76] We showed that Fedratinib reduced atherogenesis in *Apoe*^{−/−} mice at least partly by reducing aberrant myelopoiesis[77] but its impact on CHIP-driven atherogenesis is not known. The ASPREE trial indicates that aspirin use in the healthy elderly does not provide benefit against CVD but increases the risk of major hemorrhage,[78] suggesting the need for more targeted therapy. Interestingly, evidence exists suggesting that individuals

with JAK2[VF] MPNs benefit more from aspirin relative to MPN patients carrying no JAK2[VF].[79,80] Potentially, aspirin could be more effective in JAK2[VF] CHIP carriers to reduce thrombosis risk. OxPL has long been considered as a risk factor for CVD.[81] Anti-OxPL therapy could potentially be particularly beneficial for LNK mutant carriers or individuals with LNK risk polymorphism who have increased atherothrombotic risk.

Another important point to consider is surveillance for and managing clonal expansion. It has been shown that individuals with larger clones are at great risk of mortality and that people with small stable clones generally live healthy lives. As discussed above there is growing knowledge surrounding what lifestyle factors and co-morbidities drive clonal outgrowth.[18,34,64,73] We suggest another approach, to avoid small clones becoming problematic, could be to effectively treat comorbidities, alter lifestyles (i.e., diets, cessation of smoking), or activate pathways that might slow the proliferation of the mutated cells, which will likely be dependent on the mutated gene.

Nonetheless, since discovering the link between CHIP and CVD, experimental interventions targeting inflammation may find an indication in individuals with CHIP. With the movement toward precision medicine in the cardiovascular field, it may be important to define the genetic drivers of CHIP to treat these individuals effectively and significantly reduce their risk of CVD.

Conflict of Interest

P.N. reports grants from Amgen, Apple, AstraZeneca, Boston Scientific, and Novartis; personal fees from Apple, Astra-Zeneca, Blackstone Life Sciences, Foresite Labs, Genentech/Roche, Novartis, and TenSixteen Bio; equity in geneXwell, TenSixteen Bio, and Zizi; and spousal employment at Vertex. P.N. is a co-founder of TenSixteen Bio, a company focusing on somatic mutations in blood cells to reduce risks for blood cancer and atherosclerotic cardiovascular disease; his interests were reviewed and are managed by Massachusetts General Hospital and Mass General Brigham in accordance with their conflict of interest policies. All other authors report no conflict of interest.

References

1 Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. Nat Rev Immunol 2013;13(05):376–389

2 Challen GA, Goodell MA. Clonal hematopoiesis: mechanisms driving dominance of stem cell clones. Blood 2020;136(14):1590–1598

3 Lee-Six H, Øbro NF, Shepherd MS, et al. Population dynamics of normal human blood inferred from somatic mutations. Nature 2018;561(7724):473–478

4 Osorio FG, Rosendahl Huber A, Oka R, et al. Somatic mutations reveal lineage relationships and age-related mutagenesis in human hematopoiesis. Cell Rep 2018;25(09):2308–2316.e4

5 Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat Genet 2012;44(11):1179–1181

6 Shlush LI, Zandi S, Mitchell A, et al; HALT Pan-Leukemia Gene Panel Consortium. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 2014;506 (7488):328–333

7 Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell 2012;150(02): 264–278

8 Watson CJ, Papula AL, Poon GYP, et al. The evolutionary dynamics and fitness landscape of clonal hematopoiesis. Science 2020;367 (6485):1449–1454

9 Natarajan P, Jaiswal S, Kathiresan S. Clonal hematopoiesis: somatic mutations in blood cells and atherosclerosis. Circ Genom Precis Med 2018;11(07):e001926

10 Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood 2015;126(01):9–16

11 Bick AG, Pirruccello JP, Griffin GK, et al. Genetic interleukin 6 signaling deficiency attenuates cardiovascular risk in clonal hematopoiesis. Circulation 2020;141(02):124–131

12 Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371(26):2477–2487

13 Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371(26):2488–2498

14 Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nat Commun 2016;7:12484

15 Jaiswal S, Libby P. Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease. Nat Rev Cardiol 2020;17 (03):137–144

16 Veiga CB, Lawrence EM, Murphy AJ, Herold MJ, Dragoljevic D. Myelodysplasia syndrome, clonal hematopoiesis and cardiovascular disease. Cancers (Basel) 2021;13(08):13

17 Chambers SM, Boles NC, Lin KY, et al. Hematopoietic fingerprints: an expression database of stem cells and their progeny. Cell Stem Cell 2007;1(05):578–591

18 Izzo F, Lee SC, Poran A, et al. DNA methylation disruption reshapes the hematopoietic differentiation landscape. Nat Genet 2020;52 (04):378–387

19 Zhang X, Su J, Jeong M, et al. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. Nat Genet 2016;48(09):1014–1023

20 Dorsheimer L, Assmus B, Rasper T, et al. Association of mutations contributing to clonal hematopoiesis with prognosis in chronic ischemic heart failure. JAMA Cardiol 2019;4(01):25–33

21 Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017; 377(02):111–121

22 Pascual-Figal DA, Bayes-Genis A, Díez-Díez M, et al. Clonal hematopoiesis and risk of progression of heart failure with reduced left ventricular ejection fraction. J Am Coll Cardiol 2021;77(14): 1747–1759

23 Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science 2017;355(6327):842–847

24 Abplanalp WT, Mas-Peiro S, Cremer S, John D, Dimmeler S, Zeiher AM. Association of clonal hematopoiesis of indeterminate potential with inflammatory gene expression in patients with severe degenerative aortic valve stenosis or chronic postischemic heart failure. JAMA Cardiol 2020;5(10):1170–1175

25 Bick AG, Weinstock JS, Nandakumar SK, et al; NHLBI Trans-Omics for Precision Medicine Consortium. Inherited causes of clonal haematopoiesis in 97,691 whole genomes. Nature 2020;586 (7831):763–768

26 Cordua S, Kjaer L, Skov V, Pallisgaard N, Hasselbalch HC, Ellervik C. Prevalence and phenotypes of JAK2 V617F and calreticulin mutations in a Danish general population. Blood 2019;134(05): 469–479

27 Khetarpal SA, Qamar A, Bick AG, et al. Clonal hematopoiesis of indeterminate potential reshapes age-related CVD: JACC review topic of the week. J Am Coll Cardiol 2019;74(04):578–586

28 Liu DJ, Peloso GM, Yu H, et al; Charge Diabetes Working Group EPIC-InterAct Consortium EPIC-CVD Consortium GOLD Consortium VA Million Veteran Program. Exome-wide association study of plasma lipids in >300,000 individuals. Nat Genet 2017;49(12): 1758–1766

29 Wang W, Liu W, Fidler T, et al. Macrophage inflammation, erythrophagocytosis, and accelerated atherosclerosis in Jak V617F mice. Circ Res 2018;123(11):e35–e47

30 Fidler TP, Xue C, Yalcinkaya M, et al. The AIM2 inflammasome exacerbates atherosclerosis in clonal haematopoiesis. Nature 2021;592(7853):296–301

31 Murphy AJ, Febbraio MA. Immune-based therapies in cardiovascular and metabolic diseases: past, present and future. Nat Rev Immunol 2021;21(10):669–679

32 Ridker PM, Everett BM, Thuren T, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017;377(12):1119–1131

33 Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 2006;440(7081):237–241

34 Misawa T, Takahama M, Kozaki T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol 2013;14(05):454–460

35 Tardif JC, Kouz S, Waters DD, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. N Engl J Med 2019;381 (26):2497–2505

36 Newby LK. Inflammation as a treatment target after acute myocardial infarction. N Engl J Med 2019;381(26):2562–2563

37 Svensson EC, Madar A, Campbell CD, et al. TET2-driven clonal hematopoiesis and response to canakinumab: an exploratory analysis of the CANTOS randomized clinical trial. JAMA Cardiol 2022;7(05):521–528

38 Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium Swerdlow DI, Holmes MV, et al. The interleukin-6 receptor as a target for prevention of coronary heart disease: a Mendelian randomisation analysis. Lancet 2012;379:1214–1224

39 Cai T, Zhang Y, Ho YL, et al; VA Million Veteran Program. Association of interleukin 6 receptor variant with cardiovascular disease effects of interleukin 6 receptor blocking therapy: a phenome-wide association study. JAMA Cardiol 2018;3(09): 849–857

40 Veninga A, De Simone I, Heemskerk JWM, Cate HT, van der Meijden PEJ. Clonal hematopoietic mutations linked to platelet traits and the risk of thrombosis or bleeding. Haematologica 2020;105(08):2020–2031

41 Panova-Noeva M, Marchetti M, Russo L, et al. ADP-induced platelet aggregation and thrombin generation are increased in Essential Thrombocythemia and Polycythemia Vera. Thromb Res 2013;132(01):88–93

42 Panova-Noeva M, Marchetti M, Spronk HM, et al. Platelet-induced thrombin generation by the calibrated automated thrombogram assay is increased in patients with essential thrombocythemia and polycythemia vera. Am J Hematol 2011; 86(04):337–342

43 Spivak JL. Myeloproliferative neoplasms. N Engl J Med 2017;376 (22):2168–2181

44 Wolach O, Sellar RS, Martinod K, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci Transl Med 2018;10(436):10

45 Verstovsek S, Silver RT, Cross NC, Tefferi A. JAK2V617F mutational frequency in polycythemia vera: 100%, >90%, less? Leukemia 2006;20(11):2067

46 Kwaan HC, Wang J. Hyperviscosity in polycythemia vera and other red cell abnormalities. Semin Thromb Hemost 2003;29(05): 451–458

47 Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303(5663):1532–1535

48 Martinod K, Demers M, Fuchs TA, et al. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. Proc Natl Acad Sci U S A 2013; 110(21):8674–8679

49 Borissoff JI, Joosen IA, Versteylen MO, et al. Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. Arterioscler Thromb Vasc Biol 2013;33(08):2032–2040

50 Hurtado-Nedelec M, Csillag-Grange MJ, Boussetta T, et al. Increased reactive oxygen species production and p47phox phosphorylation in neutrophils from myeloproliferative disorders patients with JAK2 (V617F) mutation. Haematologica 2013;98 (10):1517–1524

51 Kushnir M, Cohen HW, Billett HH. Persistent neutrophilia is a marker for an increased risk of venous thrombosis. J Thromb Thrombolysis 2016;42(04):545–551

52 Marin Oyarzún CP, Carestia A, Lev PR, et al. Neutrophil extracellular trap formation and circulating nucleosomes in patients with chronic myeloproliferative neoplasms. Sci Rep 2016;6:38738

53 Hobbs CM, Manning H, Bennett C, et al. JAK2V617F leads to intrinsic changes in platelet formation and reactivity in a knock-in mouse model of essential thrombocythemia. Blood 2013;122(23):3787–3797

54 Lamrani L, Lacout C, Ollivier V, et al. Hemostatic disorders in a JAK2V617F-driven mouse model of myeloproliferative neoplasm. Blood 2014;124(07):1136–1145

55 Etheridge SL, Roh ME, Cosgrove ME, et al. JAK2V617F-positive endothelial cells contribute to clotting abnormalities in myeloproliferative neoplasms. Proc Natl Acad Sci U S A 2014;111(06): 2295–2300

56 Bersenev A, Wu C, Balcerek J, Tong W. Lnk controls mouse hematopoietic stem cell self-renewal and quiescence through direct interactions with JAK2. J Clin Invest 2008;118(08): 2832–2844

57 Wang W, Tang Y, Wang Y, et al. LNK/SH2B3 loss of function promotes atherosclerosis and thrombosis. Circ Res 2016;119(06): e91–e103

58 Dou H, Kotini A, Liu W, et al. Oxidized phospholipids promote NETosis and arterial thrombosis in LNK(SH2B3) deficiency. Circulation 2021;144(24):1940–1954

59 Deloukas P, Kanoni S, Willenborg C, et al; CARDIoGRAMplusC4D Consortium DIAGRAM Consortium CARDIOGENICS Consortium MuTHER Consortium Wellcome Trust Case Control Consortium. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 2013;45(01):25–33

60 Hinds DA, Barnholt KE, Mesa RA, et al. Germ line variants predispose to both JAK2 V617F clonal hematopoiesis and myeloproliferative neoplasms. Blood 2016;128(08):1121–1128

61 Cremer S, Kirschbaum K, Berkowitsch A, et al. Multiple somatic mutations for clonal hematopoiesis are associated with increased

mortality in patients with chronic heart failure. Circ Genom Precis Med 2020;13(04):e003003

62 Yu B, Roberts MB, Raffield LM, et al; National Heart, Lung, and Blood Institute TOPMed Consortium. Supplemental association of clonal hematopoiesis with incident heart failure. J Am Coll Cardiol 2021;78(01):42–52

63 Dharan NJ, Yeh P, Bloch M, et al; ARCHIVE Study Group. HIV is associated with an increased risk of age-related clonal hemato-poiesis among older adults. Nat Med 2021;27(06):1006–1011

64 Heyde A, Rohde D, McAlpine CS, et al. Increased stem cell proliferation in atherosclerosis accelerates clonal hematopoiesis. Cell 2021;184(05):1348–1361.e22

65 van Deuren RC, Andersson-Assarsson JC, Kristensson FM, et al. Clone expansion of mutation-driven clonal hematopoiesis is associated with aging and metabolic dysfunction in individuals with obesity. bioRxiv 2021:2021.2005.2012.443095

66 Castillo JJ, Mull N, Reagan JL, Nemr S, Mitri J. Increased incidence of non-Hodgkin lymphoma, leukemia, and myeloma in patients with diabetes mellitus type 2: a meta-analysis of observational studies. Blood 2012;119(21):4845–4850

67 Shu X, Ji J, Li X, Sundquist J, Sundquist K, Hemminki K. Cancer risk among patients hospitalized for Type 1 diabetes mellitus: a population-based cohort study in Sweden. Diabet Med 2010;27(07):791–797

68 Cai Z, Lu X, Zhang C, et al. Hyperglycemia cooperates with Tet2 heterozygosity to induce leukemia driven by proinflammatory cytokine-induced lncRNA Morrbid. J Clin Invest 2021;131(01):131

69 Fuster JJ, Zuriaga MA, Zorita V, et al. TET2-loss-of-function-driven clonal hematopoiesis exacerbates experimental insulin resis-tance in aging and obesity. Cell Rep 2020;33(04):108326

70 Bhattacharya R, Zekavat SM, Uddin MM, et al. Association of diet quality with prevalence of clonal hematopoiesis and adverse cardiovascular events. JAMA Cardiol 2021;6(09):1069–1077

71 Haring B, Reiner AP, Liu J, et al. Healthy lifestyle and clonal hematopoiesis of indeterminate potential: results from the women's health initiative. J Am Heart Assoc 2021;10(05):e018789

72 Lee MKS, Dragoljevic D, Bertuzzo Veiga C, Wang N, Yvan-Charvet L, Murphy AJ. Interplay between clonal hematopoiesis of indeter-minate potential and metabolism. Trends Endocrinol Metab 2020;31(07):525–535

73 Nagareddy PR, Kraakman M, Masters SL, et al. Adipose tissue macrophages promote myelopoiesis and monocytosis in obesity. Cell Metab 2014;19(05):821–835

74 Nagareddy PR, Murphy AJ, Stirzaker RA, et al. Hyperglycemia promotes myelopoiesis and impairs the resolution of atheroscle-rosis. Cell Metab 2013;17(05):695–708

75 Wu D, Hu D, Chen H, et al. Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer. Nature 2018;559(7715):637–641

76 Talpaz M, Kiladjian JJ. Fedratinib, a newly approved treatment for patients with myeloproliferative neoplasm-associated myelofi-brosis. Leukemia 2021;35(01):1–17

77 Tang Y, Liu W, Wang W, et al. Inhibition of JAK2 suppresses myelopoiesis and atherosclerosis in Apoe$^{-/-}$ mice. Cardiovasc Drugs Ther 2020;34(02):145–152

78 McNeil JJ, Wolfe R, Woods RL, et al; ASPREE Investigator Group. Effect of aspirin on cardiovascular events and bleeding in the healthy elderly. N Engl J Med 2018;379(16):1509–1518

79 Alvarez-Larrán A, Pereira A, Guglielmelli P, et al. Antiplatelet therapy versus observation in low-risk essential thrombocythe-mia with a CALR mutation. Haematologica 2016;101(08):926–931

80 Marin Oyarzún CP, Heller PG. Platelets as mediators of throm-boinflammation in chronic myeloproliferative neoplasms. Front Immunol 2019;10:1373

81 Binder CJ, Papac-Milicevic N, Witztum JL. Innate sensing of oxidation-specific epitopes in health and disease. Nat Rev Immu-nol 2016;16(08):485–497