Hematopoiesis of Indeterminate Potential and Atherothrombotic Risk

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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. 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, 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. 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. 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 throbocytic 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. 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. 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). 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. 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. 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. 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. Certainly, studies exploring the deletion or loss of function of these genes demonstrate the competitive advantage these mutant HSCs acquire. 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. 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. Further analyses identified a strong association of CHIP with a higher risk of CVD independent of age and other traditional risk factors. The direct evidence for causality was first provided by animal studies. Hematopoietic Tet2−/− or Tet2+/− markedly increased atherosclerosis in hypercholesterolemic LDLr−/− mice. Mechanistically, Tet2-deficient macrophages showed increased NLRP3 inflammasome activation and elevated IL-1β production. Concentrations of related biomarkers are also increased among individuals with CHIP. An NLRP3 inhibitor selectively reversed the increased atherosclerosis in the Tet2−/− CHIP model.

**JAK2V617F (JAK2VF)** is less common than the mutations of epigenetic modifiers such as TET2, DNMT3A, or ASXL1. Nevertheless, a recent study, with innovative deep targeted sequencing that had a screening sensitivity as low as VAF 0.01%, found that the JAK2VF mutation is detectable in almost 4% of a general European population. Among the JAK2VF 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 JAK2VF occurs at a younger age than the other CHIP variants and dramatically increases risk of myocardial infarction by as much as 12-fold in younger people. We found that JAK2VF increases atherosclerotic disease despite lowering LDL (low-density lipoprotein) cholesterol in both mice and humans. Interestingly, it has been shown that mouse models of JAK2VF resembling a MPN (i.e., 100% JAK2VF bone marrow transplant [BMT]) or CHIP (20% JAK2VF, 80% WT BMT) both increase atherosclerosis. JAK2VF causes altered functionality of multilineage blood cells. Selective expression of JAK2VF in monocyte/macrophage increases atherosclerosis in association with increased generation of IL-1β and IL-18, the product of inflammasome activation. Unlike Tet2 deficiency, knockout of NLRP3 has little effect, while...
deletion of AIM2, the essential component of AIM2 inflammasome, reduces the increased atherosclerosis in JAK2VF 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 JAK2VF 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 JAK2VF atherosclerosis models suggests that individuals with TET2 mutant or JAK2VF 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.⁴¹

**CHIP and Thrombosis**

Several somatic mutations have been linked to quantitative or qualitative abnormalities in platelets (reviewed by Veninga et al⁴⁰). Among the common CHIP-driving mutations that affect platelets, the evidence for increased risk of atherothrombosis primarily comes from studies of JAK2VF-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
confers a risk of thrombosis and venous thrombosis, which were observed in a mouse model. Various mouse models have been used to study the impact of JAK2V617F on arterial thrombosis. Different mouse models have demonstrated 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 Ldr−/− mice with hematopoietic Lnk deficiency display increased atherogenesis and accelerated atherothrombosis.57 Lnk deficiency and hypercholesterolemia act synergistically promoting platelet activation and myelopoiesis.58 More recently, we showed 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 that 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 > G) 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 cultures of human-induced pluripotent stem cell (iPSC)-derived neutrophils and activated platelets carrying isogenic LNK(TT) relative to LNK(R262W, T allele).59 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 JAK2V617F-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 JAK2V617F on arterial thrombosis, including mouse JAK2V617F knock-in, human JAK2V617F transgenic, and knock-in of human JAK2V617F cDNA into the mouse Jak2 allele, with constitutive or tamoxifen-induced expression in hematopoietic stem cell maintenance and proliferation.56,57

- **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.
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.\(^{13,21,25,63-65}\) (\(\text{Fig. 2}\)). In the initial studies linking CHIP to mortality due to CVD, an over-representation 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 (\(\text{Fig. 2}\)). 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 progenitor, 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 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 JAK2V6 MPNs benefit more from aspirin relative to MPN patients carrying no JAK2V6. Potentially, aspirin could be more effective in JAK2V6 CHIP carriers to reduce thrombosis risk. OxPL has long been considered as a risk factor for CVD. 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 managing clonal expansion. It has been shown 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. 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.

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, Öbro NF, Shepherd MS, et al. Population dynamics of normal human blood inferred from somatic mutations. Nature 2018;561(7724):473–478
4 Osorio FG, Rosenhell 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 Hick 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, Girmann 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, Dragoevic 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 cooperate 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-Fidal GA, 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
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

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

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

Cordua S, Kjaer L, Skov P, 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

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

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

Wang W, Liu W, Fidler T, et al. Macrophage inflammation, erythrophagocytosis, and accelerated atherosclerosis in Jak V617F mice. Circ Res 2018;123(11):e5–e47

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

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

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

Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout–disease. N Engl J Med 2017;377(12):1119–1128

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

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

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

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

Kwak HC, 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

Hurtado-Nedelec M, Csilag-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

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

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

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

Lamrani L, Lacout C, Ollivier V, et al. Hemostatic disorders in a CANTOS randomized clinical trial. Arterioscler Thromb Vasc Biol 2013;33(08):2032–2040

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

Bersenev A, Wu C, Bal cercjak 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

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

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

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

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

Cremer S, Kirs chbaum K, Berkowitzch 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 hematopoiesis 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 IncRNA 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 resistance 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 indeterminate 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 atherosclerosis. 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 myelofibrosis. 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 thrombocythemia with a CALR mutation. Haematologica 2016;101(08):926–931
80 Marin Oyarzún CP, Heller PG. Platelets as mediators of thromboinflammation 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 Immunol 2016;16(08):485–497