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REVIEW ARTICLE

Clonal hematopoiesis, aging, and cardiovascular diseases

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Cardiovascular diseases (CVDs) remain the leading cause of death worldwide. Many studies have provided evidence that both genetic and environmental factors induce atherosclerosis, leading thus to cardiovascular complications. Atherosclerosis is an inflammatory disease, and aging is strongly associated with the development of atherosclerosis. Recent experimental evidence suggests that clonal hematopoiesis (CH) is an emerging cardiovascular risk factor that contributes to the development of atherosclerosis and cardiac dysfunction and exacerbates cardiovascular diseases. CH is caused by somatic mutations in recurrent genes in hematopoietic stem cells, leading to the clonal expansion of mutated blood cell clones. Many of the mutated genes are known in the context of myeloid neoplasms. However, only some individuals carrying CH mutations develop hematologic abnormalities. CH is clearly age dependent and is not rare: at least 10%–20% of people >70 years old carry CH. The newly discovered association between myeloid leukemia-driver mutations and the progression of CVDs has raised medical interest. In this review, we summarize the current view on the contribution of CH in different cardiovascular diseases, CVD risk assessment, patient stratification, and the development of novel therapeutic strategies.

Despite advances in the medical and interventional clinical management of patients, cardiovascular diseases (CVDs) remain the leading cause of death worldwide. It is well appreciated that atherosclerosis represents the underlying cause of most CVDs [1]. Atherosclerosis is a chronic inflammatory disease that leads to the formation of atheromatous lesions in the vessel associated with increased recruitment, adhesion, and proliferation of different leukocyte subsets to the endothelium [1]. Several cardiovascular risk factors (CRFs) have been found to enhance the risk of CVD (Figure 1), including hypercholesterolemia (HC), diabetes mellitus (DM), hypertension, metabolic syndrome, obesity, and smoking [2]. Inflammation plays a crucial role in the development of CVDs and several studies have reported that CRFs enhance production of myeloid cells and multipotent hematopoietic progenitors in the bone marrow and in this way may promote atherosclerosis and disease development [3].

Increasing evidence suggests that conventional CRFs are not fully predictive of the development of CVDs and, more importantly, that the incidence of CVDs increases with age [4,5]. Although the effect of aging on the development of atherosclerosis has been considered to be caused by cumulative exposure to classic CRFs, the exact molecular mechanisms of age predisposition to CVDs are not completely understood. Several studies have linked cardiovascular aging to genomic instability, telomere attrition, and accumulating irreversible epigenetic alterations, including DNA methylation, histone posttranslational modifications, and dynamic nucleosome occupancy [6–8].

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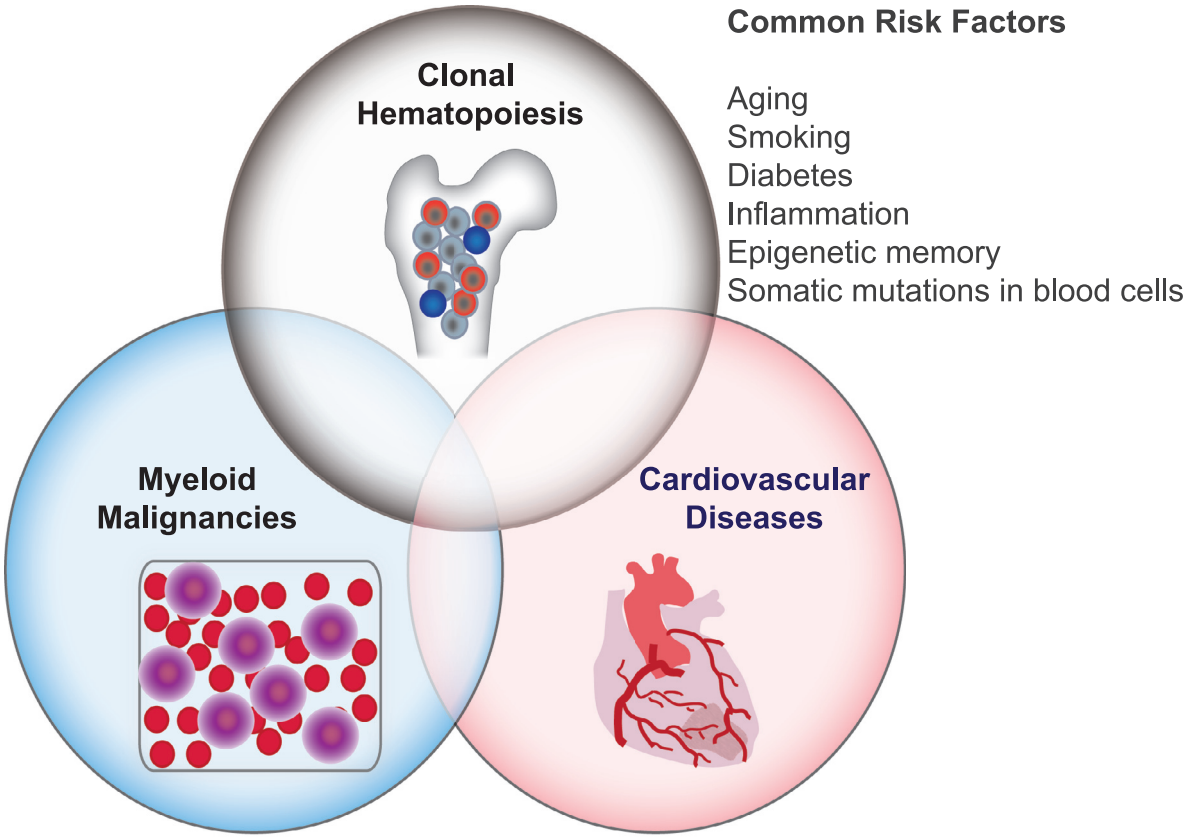

Figure 1. Commonalities of clonal hematopoiesis, myeloid neoplasms, and cardiovascular diseases. Many shared risk factors suggest a causal relation between the origin and progression of blood-related alterations and cardiovascular diseases.

Recent elegant studies have provided evidence that clonal hematopoiesis (CH) is a novel causal CRF for CVDs in elderly individuals, as previously reviewed [9,10]. CH was initially described in elderly people who develop hematologic malignancies. CH originates from somatic mutations in hematopoietic stem cells (HSCs) in genes implicated in myeloid neoplasms and results in expansion of mutated blood cell clones. Clonal hematopoiesis of indeterminate potential (CHIP) is technically defined by the presence of somatic variants with a variant allele frequency (VAF) (i.e., variant prevalence among all blood cells) of at least 2%, but without the presence of hematologic malignancies or other hematologic abnormalities [11]. Genetic and epidemiology studies in humans revealed that CHIP correlated with increased mortality in individuals with CVDs. Preclinical animal models provided mechanistic evidence for the role of CHIP in the progression and development of CVD. In this review, we summarize the role of inflammation and inflammatory blood cell types in the development of atherosclerosis and CVDs. We also discuss the potential mechanisms by which CH contributes to increased cardiovascular risk in aging individuals and how increased inflammation, induced by cardiovascular risk factors, further promotes the clonal dominance of mutated HSC clones, leading to a feedback loop between CH and CVDs. Finally, we raise potential implications of these findings in CVD risk assessment, patient stratification, and the development of novel therapeutic strategies.

Role of myeloid cells and inflammation in the development of CVD

Atherosclerosis is a chronic inflammatory disease of the vasculature. It is well appreciated that monocyte-derived macrophages play a crucial role in the progression of atherosclerosis, plaque development, and the incidence of CVD [1,2]. Pathological stimuli such as hyperlipidemia and hypertension increase monocyte adhesion on the endothelium and their infiltration into the vessel wall during atherogenesis [1,2]. After entering the vessel wall, monocytes start to proliferate and differentiate into macrophages, which endocytose lipids and develop into foam cells, which contribute to plaque development [1,2]. It has been reported that increased numbers of circulating monocytes (monocytosis) correlate with plaque formation and the development of carotid artery disease (CAD) [1,12,13]. Experimental evidence has established a causal relationship between monocytosis and the

development of atherosclerosis in preclinical models [1,2]. Monocyte depletion or inhibition of monocyte recruitment results in reduced atherogenesis and plaque formation in experimental models of atherosclerosis [14–18]. Several CRFs have been found to increase the risk of CVDs by inducing myelopoiesis and hematopoiesis (Figure 1). Hypercholesterolemia, diabetes, and obesity increase the number of circulating monocytes, resulting thus in acceleration of atherosclerosis [19–21]. Moreover, hypercholesterolemia promotes hematopoietic stem and progenitor cell (HSPC) proliferation and, in this way, contributes to increased myelopoiesis and exacerbated atherosclerosis [22–26]. Similarly, other studies have found that diabetes and hyperglycemia influence HSC function, differentiation, and mobilization and in this way further contribute to the development of CVDs [27–29]. Diabetes results in reduced numbers of HSCs and interferes with their repopulation capacity in a competitive engraftment experiment and their cytokine expression patterns [29]. Furthermore, diabetes leads to increased expression of plasma levels of the alarmins S100A8 and S100A9 secreted by neutrophils, resulting in increased proliferation of granulocyte–monocyte progenitors (GMPs) and enhanced myelopoiesis [19,20].

Somatic mutations in hematopoietic stem cells drive clonal hematopoiesis

Clearly, aging poses the largest risk for the development of CVDs; this is due not only to the accumulative effect of the CRFs and the increased inflammatory responses but also to the accumulation of epigenetic alterations and genetic mutations. It was recently reported that CHIP contributes to the development of cardiovascular diseases in elderly individuals [30–33]. Before discussing the role of CHIP in the development of CVD, it is important to review the basis of CH.

Hematological malignancies ensue from the clonal expansion of transformed blood cells, and are generally diseases of the elderly, as the median age for most of these diseases is between 60 and 70 years. Although, in certain cases, they can be associated with inherited genetic mutations, hematologic malignancies usually originate from recurrent somatic mutations in driver genes [34,35]. Interestingly, although most of the somatic mutations have no effect, certain mutations result in increased proliferation or, alternatively, reduced cell death and enhanced self-renewal and, as a result, confer a specific clonal expansion advantage to the HSCs carrying these mutations [36]; this may lead to hematologic malignancies. Clonality was also found to be a major characteristic of the aging hematopoietic system. Studies in women over the age of 65, with no hematologic malignancies, suggested that there is a skewed pattern of X-chromosome inactivation in peripheral blood cells, particularly within the myeloid compartment, which is age related [37–39]. Subsequently, Busque and colleagues demonstrated by exome sequencing the presence of somatic, recurrent *TET2* mutations in normal elderly individuals with clonal hematopoiesis but without hematological malignancies [40]. *TET2* mutations were previously reported to be associated with myeloid cancers [41].

Subsequent studies using whole-exome sequencing as well as gene-targeted sequencing assisted the detection of several somatic mutations with low VAF [31–33]. These studies provided evidence that the majority of the age-associated recurrent mutations were in genes such as *DNMT3A*, *TET2*, *JAK2*, *TP53*, *ASXL1*, *SF3B1*, *PPM1D*, and *BCORL1*, known to be associated with acute myeloid leukemia (AML), myeloproliferative neoplasms (MPNs), or myelodysplastic syndrome (MDS) [42]. Interestingly, other mutations in genes found to play an important role in AML development and progression, such as *FLT3*, *IDH1*, *IDH2,* and *NPM1,* were not so common in CH [30–32,42–44]. All identified mutations increased with age. Subsequent complementary studies of large cohorts of patients utilizing sensitive error-corrected, targeted deep sequencing [45,46] further supported these results, indicating that recurrent mutations in genes associated with leukemia are increased in aged individuals and correlated with clonal hematopoiesis [30–33,46–48], but without leading to hematological malignancies. This type of CH was technically defined as CHIP and is characterized by the presence of recurrent mutations with a VAF of at least 2% in aged individuals in the absence of hematologic cancer or other clonal disease [11]. Although the frequency of CHIP is less than 1% in individuals younger than 40 years, its prevalence increases to 15%–20% in people older than 70 years [31,33,42,43]. However, the prevalence of CH is highly dependent on the sensitivity of the detection method used. Although the initial studies used whole-exome sequencing, which is relatively insensitive to smaller clones, recent studies using more sensitive, error-corrected, deep sequencing approaches have found a much higher prevalence of CH with low VAF, even at younger age. The biological and medical importance of the smaller clones still remains unclear [45,49].

Association of clonal hematopoiesis with CVDs

Although CH was initially associated with hematologic malignancies, it became evident that CH led to decreased patient survival, which could not be explained by the increase in hematological cancer [30–33,43] Different studies using next-generation sequencing analysis in large cohorts revealed that CHIP-driver mutations in leukemia-related genes *DNMT3A*, *TET2*, *ASXL1*, and *JAK2* were associated with an increased risk of incidence of coronary heart disease (CHD) or stroke and increased mortality [30–32]. Importantly it was reported

that the prevalence of CHIP was higher in individuals with early onset of myocardial infarction [30,32]. In a recent study, CHIP was also associated with chronic heart failure (CHF) caused by ischemic heart disease. It was found that the occurrence of CHIP was increased in CHF patients compared with published control cohorts, with a higher prevalence in aged patients with CHF. CHIP-driver mutations in *DNMT3A* or *TET2* were the most prevalent causes of CHIP, representing more than 60% of CHIP carriers with CHF. CHF patients with or without CHIP did not have any difference in other clinical parameters or common classifications of CHF. Most importantly, *DNMT3A* or *TET2* CHIP-driver mutations were associated with a poor outcome in these CHF patients, who had reduced survival and increased progression of their disease [50]. The dosage dependence of *DNMT3A*- or *TET2*-mutated cells on the outcome in these patients suggests a causative role of mutated blood cells and the progression of CHF. The presence of *DNMT3A* or *TET2* CHIP-driver mutations remained a significant new risk factor for CHF after multivariate correction of confounding factors. Interestingly, individuals with CHIP had increased coronary artery calcium scores, indicative of vascular wall inflammation and development of atherosclerosis [30,32]. In line with these results, a recent study reported that CHIP is also associated with degenerative aortic valve stenosis and that mutations in *DNMT3A* or *TET2* result in worse prognosis and increased mortality in patients undergoing transcatheter aortic valve implantation [51]. Moreover CHIP mutations in one of the driver genes, *JAK2,* have been associated with an increased risk of thrombosis, which correlated with increased numbers of leukocytes and acute coronary events [52–54]. Hence, CHIP is now considered an additional CRF that contributes to age-related CVD development and progression (Figure 2).

Role of CHIP-driver gene mutations in the development of atherosclerosis and CVDs

After establishment of a link between CHIP and CVD, several studies focused on the characterization of the fundamental mechanisms that may explain the functional association of CHIP with the development of CVD. Studies in humans and mice provided functional evidence for a differential role of distinct mutations in various CHIP-driver genes in CAD (Figure 2).

In this respect, *TET2* (ten-eleven translocation-2) is the most well-characterized gene. *TET2* is a methylcytosine dioxygenase that modulates DNA hydroxymethylation by converting 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) to promote DNA demethylation [55]. *TET2* is involved in DNA methylation and, in this way, regulates transcriptional activation or repression of many genes [55,56]. *TET2* loss of function leads to dysregulated expansion of HSCs, enhanced repopulating capacity of HSCs in vivo, and an altered cell differentiation skewing toward monocytic/granulocytic lineages [41,56–58]. *TET2* mutations have been associated with increased cardiovascular risk, and it was reported that in individuals carrying *TET2* mutations, levels of circulating interleukin (IL)-8 were increased twofold compared with levels in those without mutations [32]. In addition, patients with severe aortic valve stenosis carrying *TET2* mutations had increased numbers of nonclassic inflammatory monocytes, which secrete pro-inflammatory cytokines and in this way may contribute to development of CVD [50,51]. *TET2* CH-driver mutations lead to increased bone marrow (BM) leukocytes and increased HSPC numbers in patients with CHF following ischemia, while patients carrying *DNMT3A* CHIP-driver mutations do not exhibit any alteration in bone marrow blood cell lineage distribution and HSPCs [59]. Studies in preclinical models revealed that atheroprone mice transplanted with *TET2*-deficient BM cells exhibited robust expansion in vivo and increased macrophage infiltration and atherosclerotic plaque size [32,60]. Further characterization revealed that *TET2* deficiency results in increased expression of different chemokines such as Cxcl1, Cxcl2, and Cxcl3, as well as IL-6 and IL-1β [32,60,61]. Increased expression of IL-1β from *TET2*-deficient macrophages was found to be mediated by NLRP3 inflammasome, and treatment of atherosclerosis-prone mice, transplanted with *TET2*-deficient BM cells, with an NLRP3 inhibitor reduced the proatherogenic effect of *TET2* deficiency [60]. *TET2* deficiency in HSCs increased cardiac dysfunction and fibrosis in hearts subjected to left anterior descending artery ligation or pressure overload [62]. This was shown to be caused by increased IL-1β expression, and treatment with an NLRP3 inhibitor reversed the effects of *TET2* deficiency on cardiac dysfunction [62]. In another study, CRISPR-mediated editing of *TET2* introducing inactivating mutations into mouse HSCs increased cardiac dysfunction and fibrosis in mice with angiotensin II-induced heart failure by inducing the expression of IL-1β, IL-6, and Ccl5 [63]. Although *TET2* mutations result in a myeloid-bias lineage differentiation, recent studies have found that specific deletion of *TET2* in regulatory T cells in mice unleashed their effector function and skewed their phenotype toward Tfh/Th17, resulting in the development of dominant inflammatory disease, suggesting that TET2 inactivation may also affect the function of other immune cells involved in the development of CVDs [64].

Other studies focused on the effect of *DNMT3A* deficiency on CVDs. DNMT3A is a DNA methyltransferase mediating DNA methylation and regulating gene expression. Interestingly, *DNMT3A* is the most prevalent driver gene associated with CH [31–33].

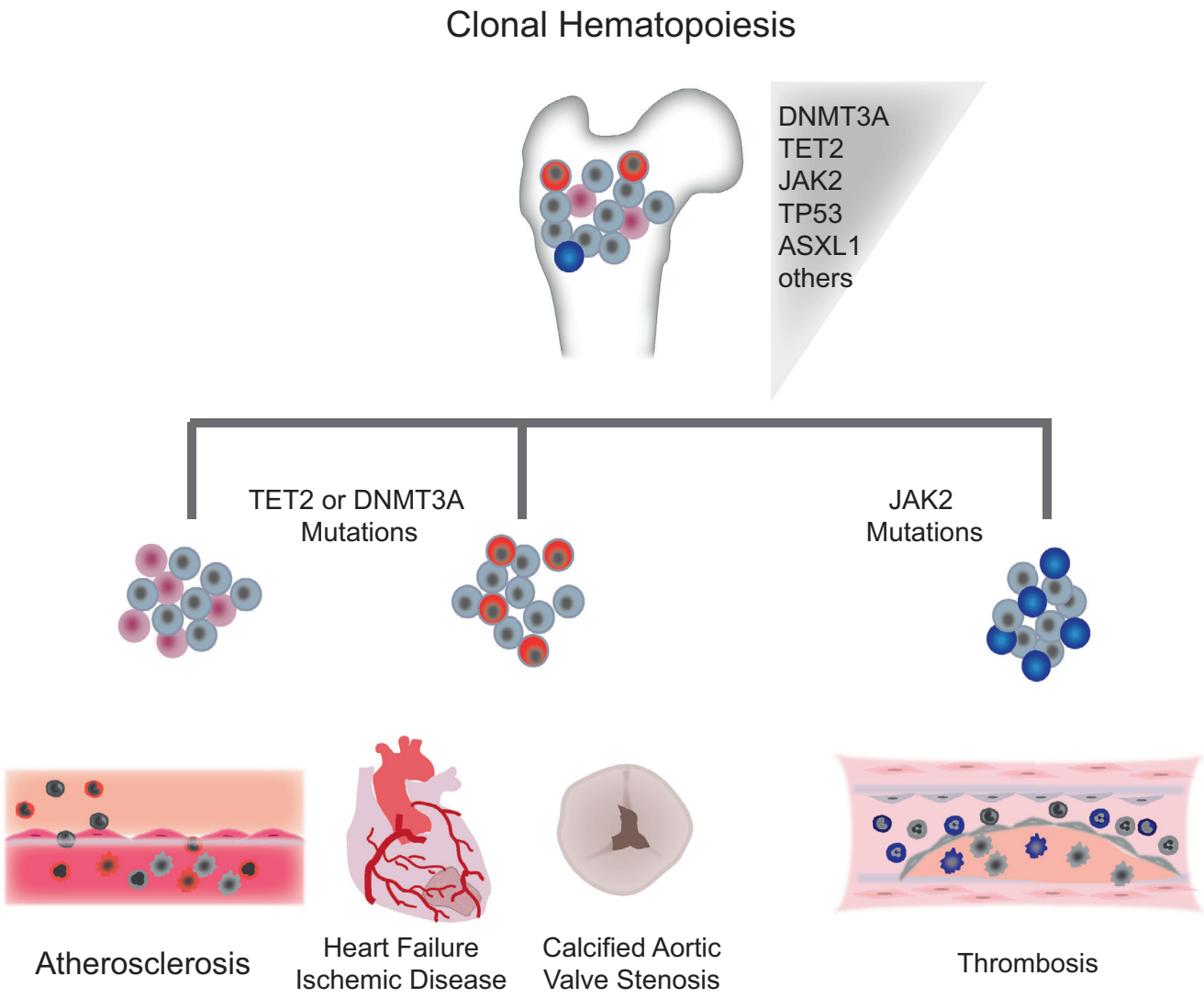

Figure 2. Clonal hematopoiesis is a potent cardiovascular risk factor for cardiovascular diseases. Mutations in myeloid leukemia-associated driver genes in HSCs confer an advantage in the mutated blood cell clones, leading to clonal hematopoiesis. Mutations in distinct genes are associated with increased risk and progression of atherosclerosis, heart failure, aortic stenosis, and thrombosis.

Nevertheless, *DNMT3A*-deficient BM cells do not exhibit selective expansion in vivo [63,65] and mainly expand in vivo after sequential BM transplantations in aged mice [66–68]. CRISPR-mediated mutagenesis of *DNMT3A* in mouse HSCs resulted in increased cardiac dysfunction and fibrosis in mice following angiotensin II-induced heart failure by promoting the expression of Cxcl1, Cxcl2, IL-6, and Ccl5 but not Il-1β [63]. *DNMT3A* deficiency may also promote a pro-atherogenic phenotype in different immune cells such as activation of mast cells and increased interferon-γ production by T cells [69–73]. Moreover, it was recently reported that patients with aortic valve stenosis carrying *DNTM3A* recurrent mutations exhibited an increased Th17/Treg ratio, suggesting that *DNMT3A* mutations might promote T-cell polarization toward a pro-inflammatory phenotype that can contribute to atherosclerosis development and CVDs [51,74]. The exact role of *DNMT3A* deficiency in atherosclerosis development remains to be further investigated.

JAK2 (Janus kinase 2) is a signaling tyrosine kinase that associates with the signaling cascades of a variety of cytokine receptors; it has been associated with cell growth and division and is especially important for controlling blood cell production in the BM [75]. In addition, several studies have implicated JAK2 in the development of atherosclerosis [76,77]. *JAK2* is one of the genes frequently mutated in individuals with CHIP [31–33] and leads to proliferation of HSCs. Early studies provided evidence suggesting that patients carrying the *JAK2* V617F gain-of-function have a higher risk of thrombosis [78]. Interestingly, studies in mice suggested that *JAK2* V617F drives CH toward the myeloid and

granulocytic lineage and enhances the pro-inflammatory activities of macrophages and neutrophils, providing a potential mechanism by which *JAK2* V617F affects cardiovascular function [79–81]. Moreover, it was reported that hematopoietic *JAK2* V617F in atheroprone mice leads to acceleration of atherosclerosis and plaque instability [82].

Further studies are awaited that shed light on the functional role of somatic mutations in other CH-driver genes, such as *TP53*, *ASXL1*, *SF3B1*, *PPM1D*, and *BCORL1,* in the development of CVDs.

Association between cancer and CVDs and the possible role of CH therein

There is substantial biological evidence that cancer and CVD share biological mechanisms underlying their pathogenesis [83,84]. Numerous studies have proposed that there are similar risk factors for cancer (solid and hematological) and CVDs (Figure 1). The incidence of both cancer and CVDs increases with advancing age [83–85]. Chronic inflammation plays a major role in the development of CVDs [1], and now it is well appreciated that inflammation in the tumor microenvironment can promote malignant transformation and cancer progression [86–88]. Interestingly, a number of CRFs such as diabetes, obesity, and smoking have been also reported to increase the prevalence of cancer [89]. Although advances in medical care have improved longevity, they have increased the overlap between cancer and CVDs; cancer patients are at high risk of developing CVDs, and patients with CVDs or heart failure have a higher rate of cancer than healthy control subjects [83–85,90]. It was found that patients with myelodysplastic syndrome, leukemia, and Hodgkin's lymphoma have an increased risk of cardiovascular complications [91–93]. Moreover, arterial thrombosis is preceding cancer diagnosis in older patients [94,95], and vascular calcifications, CAD, and other cardiovascular complications are prevalent in patients with colorectal cancer [96]. There is a causal relationship between heart failure/myocardial infarction (MI) and development of cancer. Analysis of the distributions of different cancer sites among patients with MI revealed that the most frequent types of cancers were colorectal cancer (22%), prostate cancer (22%), and lung cancer (16%) [97]. Furthermore, the induction of MI in APCmin mice, which are prone to developing precancerous intestinal tumors, resulted in increased tumor formation and accelerated tumor growth [83,90].

CH has been also associated with nonhematological cancers [42,43,98], although not so much is known regarding the driver genes or molecular mechanisms involved therein. Nevertheless, it has been reported that cancer patients with CH have worse prognosis than the ones without CH [43,99]. Interestingly, CH-driver gene mutations have been identified in tumor-supporting lymphocytes and macrophages in the tumor stroma but not in the tumor epithelium. The same mutations were found in the blood cells of the same patients although at a lower VAF [100].

Although the exact relationship between CH, CVD and solid cancers is not yet clear, there is growing apprehension that CH may be a risk factor linking CVDs and cancer progression. Future studies using advanced sequencing technologies are awaited to shed light on the interrelationship between CH, cancer, and cardiovascular complications.

Future perspectives on the role of CH in the development of atherosclerosis and CVDs

CH has emerged as an age-dependent CRF although the underlying mechanisms are far from been completely understood. CH-driver gene mutations give an advantageous proliferation to specific HSC clones and promote monocytic skewing and increased production of inflammatory cytokines such as IL-1β and IL-6 and in this way accelerate inflammation in CVDs (Figure 3). The expansion of mutated HSC clones may be boosted by classic CRFs, considering evidence that hypercholesterolemia and diabetic conditions promote the expansion of HSCs and myelopoiesis. There may be a feedback loop in which CH induces inflammation and accelerates atherosclerosis, which in turn further stimulates the expansion of mutated HSPC clones and their progeny and further induces inflammatory responses and CVD progression (Figure 3).

Mutations in different CH-driver genes result in different pro-inflammatory profiles, suggesting that the underlying mechanisms may be different. Thus, it is important to characterize the molecular mechanisms by which the various CH-driver gene mutations contribute to CVDs or other inflammatory diseases and cancer. Preclinical studies have reported that NLRP3 inhibitors that block IL-1β production can inhibit atherosclerosis in mice with TET2-deficient BM cells [62]. Recently, the CANTOS clinical trial found that anti-inflammatory treatments using a neutralizing antibody against IL-1β improve CVD patient outcome and, in addition, lower total cancer mortality [101,102]. In line with this, Bick and colleagues reported that individuals with CHIP and a genetic deficiency in IL-6 signaling (by carrying the IL6R p.Asp358Ala allele variant) had a decreased CVD risk compared with CHIP patients without defective IL-6 signaling [103]. As targeted therapies against CHIP-driver gene products are technically more challenging to implement, it may be more effective to design therapies targeting their causal effects. Future approaches are likely to include a combination of anti-inflammatory treatments and clonal selective immunotherapies.

The prevalence of CH is increasing in an aging population, as are the numbers of coding and non-coding

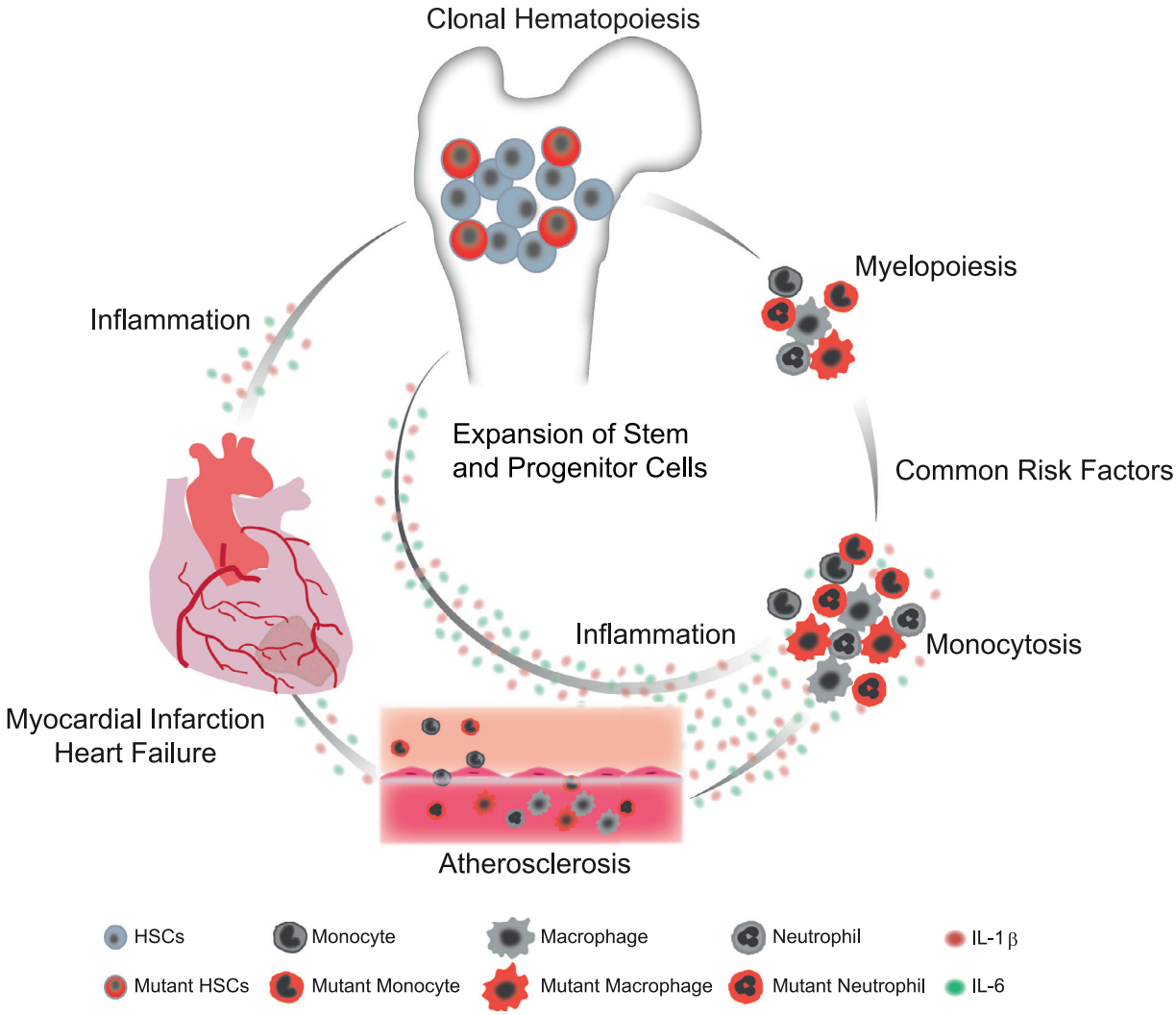

Figure 3. Schematic of a vicious circuit of inflammation driven by somatic mutations in myeloid blood cells. CH-driver gene mutations endorse an expansion of mutated HSC clones and empower myeloid skewing. Mutated myeloid cells have a pro-inflammatory profile that results in increased production of inflammatory cytokines, such as IL-1β, IL-6, and IL-8, which contribute to increased inflammation and acceleration of atherosclerosis and thrombosis, as well as a poor prognosis following degenerative aortic stenosis and heart failure. In turn, cardiovascular risk factors such as diabetes and hypercholesterolemia, by inducing the expression of inflammatory cytokines, can induce activation of HSCs and, by this vicious circuit, further promote the clonal dominance of mutated HSC clones, leading to a feedback loop between CHIP and CVDs.

driver events of CH. Not all mutations leading to CH will also have a consequence on the development and progression of CVDs. Future studies must delineate specific mutated genes and even distinct mutations in these genes that are truly associated with and clinically relevant for various diseases in the cardiovascular system having a high predictive value, as recently evidenced for the prediction of AML progression [45,104]. Careful evaluation of driver mutations, also within one particular gene, will help us to distinguish cooperating events from passenger events. Moreover, mechanistic studies, by generating functional mutation-specific models of these driver mutations, must be applied to identify the functional consequences of these mutations in the context of CVD to enlighten the causative effects that can be targeted in the future. Shared mechanisms of different mutated genes may be identified as common targets in the treatment of different CVDs.

These recent findings have raised several questions for clinicians as well as for patients. Although screening of patients with a high prevalence of CHIP or hematological malignancies is well established, screening of CVD patients for the presence of predictive CHIP-driver mutations is not presently customary. Based on the association between CH and the development of CVDs, as well as the poor prognosis of patients with CVDs, it may be reasonable to screen individuals with CVDs for CHIP, especially individuals with CAD in the absence of traditional CRFs. Based

on developments in the field of next-generation sequencing, genotyping for CH may become routine in the near future. In addition, a logical consequence would be to initiate a more intense screening for CVD blood parameters and echocardiography, angiography, or [^{18}F]fluorodeoxyglucose positron emission tomography (FDG-PET) imaging for people with CH mutations, and recommend lifestyle changes to reduce confounding factors.

Despite the challenges, future studies are awaited to shed more light on the functional causalities of distinct CH-driver mutations and their associated diseases, including CVDs, and on the clinical management of complications associated with CH in these patients.

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Conflict of interest disclosure

The authors do not have any conflicts of interest to declare.

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