Clonal Hematopoiesis and Its Impact on Cardiovascular Disease

Soichi Sano, MD, PhD; Ying Wang, MD; Kenneth Walsh, PhD

A number of recent epidemiological studies have associated the clonal expansion of hematopoietic cells, a process referred to as clonal hematopoiesis, with increased mortality. Clonal hematopoiesis increases the risk of hematological cancer, but this overall risk cannot account for the increase in mortality in the general population. Surprisingly, these mutations have also been associated with higher rates of cardiovascular disease, suggesting a previously unrecognized link between somatic mutations in hematopoietic cells and chronic disease. Here, we review recent epidemiological and experimental studies on clonal hematopoiesis that relate to cardiovascular disease.

Key Words: Atherosclerosis; Clonal hematopoiesis; Heart failure; Hematopoietic stem cells

Traditional risk factors of cardiovascular disease (CVD) include hypertension, diabetes mellitus, hyperlipidemia, and smoking. However, the completeness of their predictive value has been questioned.[1,2] The occurrence of CVD increases exponentially with age, which is traditionally viewed as a non-modifiable risk factor.[3] Although aging is a major risk factor for CVD, we have poor understanding of how it promotes disease progression. These considerations lead to the question, "Are there age-associated, as-yet-unidentified, risk factors for CVD?"

Somatic DNA mutations accumulate in all cells with age, such that over time tissues become a mosaic of cells with slightly different genotypes.[4–7] In proliferating tissues, mutations in "driver" genes, which provide a selective advantage or "fitness" to cells, can lead to the clonal expansion of these single cells. Previously, there was little information about the role that mutant cell clonal expansion has on age-associated chronic disorders. However, a number of recent epidemiological studies have associated the clonal expansion of hematopoietic cells, a process referred to as clonal hematopoiesis (CH), with increased mortality.[8–11] CH increases the risk of a hematological cancer, but this overall risk is small and cannot account for the increase in mortality in the general population. Surprisingly, these mutations have also been associated with higher rates of CVD,[8,12] suggesting a previously unrecognized link between somatic mutations in hematopoietic cells and chronic disease. Thus, a series of experimental studies has been performed to assess the potential causal connection between mutations in hematopoietic system and CVD, and define the mechanisms involved in these processes.[12–15] Here, we review recent epidemiological and experimental studies on CH that relate to CVD.

Clonal Hematopoiesis: a Common Condition in the Elderly Population

Human bone marrow produces billions of mature blood cells every day. The traditional view is that the progeny blood cells are derived from 10,000–20,000 hematopoietic stem and progenitor cells (HSPC).[16,17] Typically, HSPC are equally capable of producing all of the differentiated blood cells, thereby maintaining the healthy state of polyclonal hematopoiesis. In contrast, CH is a condition in which a substantial proportion of mature blood cells are derived from a single dominant HSPC.[18] Presumably, this condition arises because HSPC and their immediate progenitors are subjected to Darwinian selective pressures within the stem cell niche, favoring the expansion of clones with somatic mutations that provide a competitive advantage. CH was initially detected as the skewing of X chromosome inactivation in the white blood cells of women. In one study, 37.9% women over the age of 60 years displayed skewed inactivation of the X chromosome, whereas little or no skewing was observed in younger women.[19] A notable advance was made when the skewing of X chromosome inactivation was attributed to somatic mutations in the epigenetic regulator TET2.[20] These data provided the first evidence that CH can result from somatic mutations in preleukemic or myelodysplastic syndrome (MDS) "driver" genes. Consistent with these findings, single nucleotide polymorphism (SNP) array analyses have detected somatic clonal mosaicism in peripheral blood of healthy individuals, and the frequency of this mosaicism steeply increased in individuals past the age of 50 years.[21,22] More recent studies analyzed exome sequences of more than 32,000 cancer-free individuals and found high frequencies of clonal expansion events that were associated with mutations in numerous

Study	Method	Sample	Population	Sensitivity	Prevalence of CH
Table. Studies of Clonal Hematopoiesis					
Xie et al (2014)[9]	Whole-exome sequencing	Peripheral blood	2,728 individuals with first-time primary cancers (selected from TCGA)	0.01 VAF	5–6% of >70-year-olds
Genovese et al (2014)[10]	Whole-exome sequencing	Peripheral blood	12,380 Swedish persons unselected for cancer of hematological phenotypes (6,245 controls, 4,970 with schizophrenia, 1,165 with bipolar disorder)	0.01 VAF	1% <50-year-olds 10% of >65-year-olds
Jaiswal et al (2014)[8]	Whole-exome sequencing	Peripheral blood	17,182 persons unselected for hematological phenotypes (selected from 22 population-based cohorts)	0.035 VAF	Rare <40 years old 11% of >70-year-olds
McKerrell et al (2015)[24]	Targeted sequencing (15 hotspot analysis)	Peripheral blood Cord blood	4,219 individuals (3,067 blood donors, 1,152 unselected individuals, 32 patients with hematopoietic stem cell transplant, 18 cord blood)	0.008 VAF	0.8% of <60-year-olds 19.5% of >90-year-olds
Young et al (2016)[25]	Targeted sequencing (error-corrected)	Peripheral blood	20 healthy female participants (selected from Nurse's Health Study)	0.0003 VAF	Mutation in DNMT3A and TET2 in 95% of individuals (56.6–68.1 years old)
Jaiswal et al (2017)[12]	Whole-exome sequencing	Peripheral blood	4,726 participants with coronary artery disease, 3,529 controls (selected from 4 case-control studies)	0.1 VAF	7% (mean age of 65 years)
Zink et al (2017)[11]	Whole-genome sequencing	Peripheral blood	11,262 Icelanders (in various disease projects at deCODE genetics)	0.1 VAF	0.5% of <35-year-olds >50% of >85-year-olds
Abelson et al (2018)[29]	Targeted sequencing (error-corrected)	Peripheral blood	1. Discovery Cohort: 95 pre-AML cases, 414 controls 2. Validation Cohort: 29 pre-AML cases, 262 controls (selected from EPIC Study)	0.005 VAF	Around 30% of >50-year-olds
Desai et al (2018)[28]	Targeted sequencing	Peripheral blood	181 age-matched control population against later AML group (selected from WHI)	0.01 VAF	20.75% of <64-year-olds 38.46% of >65-year-olds
Gibson et al (2017)[30]	1. Whole-exome sequencing 2. Targeted sequencing	1. (pre-and post-ASCT) Peripheral blood and bone marrow 2. Cryopreserved aliquots of autologous stem-cell products	1. 12 patients who underwent ASCT for Hodgkin or non-Hodgkin lymphoma 2. 401 patients who underwent ASCT for non-Hodgkin lymphoma	0.02 VAF	1. 50% 2. 29.9%
Coombs et al (2017)[31]	Targeted next-generation sequencing	Paired tumor and blood	8,810 patients	0.01 VAF	25.1%
Jongen-Lavrencic et al (2018)[32]	Targeted next-generation sequencing	Bone marrow or peripheral blood	428 patients with a confirmed diagnosis of previously untreated AML, 54 patients with refractory anemia with excess of blasts	0.02 VAF	89.2%

AML, acute myeloid leukemia; ASCT, autologous stem cell transplantation; VAF, variant allele fraction.

driver gene candidates that are recurrently mutated in hematologic cancers. The frequency of these somatic mutations increased with age and those most commonly detected included the epigenetic regulators DNMT3A, TET2 and ASXL1.[8–10] Despite the clonal expansion of peripheral blood leukocytes with one of these mutations, and less frequently two or three somatic mutations, these individuals did not exhibit significant differences in white blood cell counts, hemoglobin levels or platelet counts. In the study, men exhibited a higher incidence of CH, which is consistent with the difference in the frequency of MDS between sexes. Hispanics had lower frequency of CH compared with those of European ancestry. Given these

considerations, it would be of interest to evaluate the prevalence of CH in the Japanese population specifically, which has the second longest life expectancy worldwide (81 years for men and 87 years for women).

Multiple studies have shown that somatic mutation-mediated CH is frequent in the elderly populations (**Table**). In one study, it was found that somatic mutations in driver genes occurred in 10% of individuals over 65 years old and >20% of individuals over 90 years old using methods with a variant allele fraction (VAF) limit of detection of 3.5% and 7.0% for single nucleotide variants (SNV) and small insertions and deletions (indels), respectively.[8] Clonal Hematopoiesis of Indeterminate Potential (CHIP) is a

term used to describe individuals who harbor a hematologic malignancy-associated somatic mutation in a driver gene that is present at a VAF of ≥2% in white blood cells, yet the individual does not meet the diagnostic criteria for any detectable hematologic malignancy. This distinction, while convenient, is largely based on the limits of mutation detection by the sequencing methodology rather than being based on epidemiological data or biological principles. In this regard, the estimated frequency of CH in the population doubles when it is deduced from the analysis of "passenger" gene expansion in whole-exome sequencing analyses.[10] Even more striking, whole-genome sequencing analysis of mosaic somatic mutations in peripheral blood cells reveals even higher levels of CH in the general population.[11] In this study, CH was observed in more than 50% of individuals older than 85 years, but only a small proportion could be attributed to a known driver gene candidate or could be detected by non-biased whole-exome analysis. Collectively, these studies highlight that genomic instability is prevalent in the hematopoietic cells of elderly individuals, and that the estimated frequencies of CH in the population depends on the methodology to detect this condition.

Etiology of Clonal Hematopoiesis

In describing the etiology of CH, the competition among clones can be viewed as a game of chance among gamblers.[23] If the odds favor some players they will become winners while other players will go bankrupt. With regard to CH, the odds of "winning" vs. "losing" are influenced by many factors, including the presence of driver gene mutations, alterations in the bone marrow microenvironment, cis-acting heritable loci, etc. Based on current knowledge, CH can be divided into several categories based on their etiological characteristics. We will briefly discuss 3 broad categories: age-associated CH, therapy-associated CH and neutral drift.

Age-Associated CH

As noted previously, numerous studies have shown a strong correlation between age and CH. As also discussed, an analysis of clonal mutations in 160 candidate driver genes revealed that CH occurs in 10% of individuals aged 70 years when the sequencing methodology has a detection level of >3.5% for SNVs and >7% for insertions/deletions.[8] However, an analysis of mutational hotspots within a subset of these driver genes using more sensitive detection methods has projected that the mean mutation frequency is >12% of individuals in their 60s, >19% in their 70s, >40% in their 80s and >74% in their 90s.[24] A whole-genome sequence analysis revealed that the frequency of CH increases from 0.5% in individuals younger than 35 years to more than 50% in those older than 85 years.[11] A study using highly sensitive targeted error-corrected sequencing, which enables the detection of CH at a very low VAF (~0.03%), revealed that 95% of individuals in their 50s harbor low levels of mutations in select driver genes.[25] Collectively, these studies suggest that initiating clonal mutations are ubiquitous by middle age and the expansion of these clones occurs in a portion of individuals at later ages. These findings are consistent with estimates made from the observed rate of exonic mutation accumulation in human HSPC.[26] It can be predicted that 44% of healthy 50-year-old individuals will possess a HSPC with a randomly generated "oncogenic" TP53 mutation.[27]

Although these considerations indicate that nearly everyone will develop mutations in driver genes by middle age, it is unclear why some individuals progress to CH with high VAF values whereas others will not display this clonal expansion. Presumably, genetics or environmental factors modulate the kinetics of HSPC clonal expansion. However, longitudinal data on CH in individuals are limited. To the extent that it has been examined, it appears that mutant clones are relatively stable over a ~10-year period.[25,28,29] These data suggest that the expansion of clones within an individual is episodic rather than linear, and it is conceivable that clone expansion is triggered by variations in exposure to external stressors that alter the bone marrow niche or a change in the demand for hematopoiesis. These hypotheses can be addressed by additional epidemiological and experimental studies.

Therapy-Associated CH

Studies have shown that CH is associated with prior radiation therapy or chemotherapy (Table).[30–32] A study investigating the prevalence of CH in patients who underwent autologous stem cell transplantation for non-Hodgkin lymphoma reported that 30% of patients harbored driver gene-associated CH at a low VAF at the time of transplantation.[30] Correspondingly, this cohort displayed an increased risk of all-cause death, later developing therapy-related myeloid neoplasm and a predisposition to death from CVD. Another study investigating 8,810 individuals with solid tumors identified CH in 25% of the patients, among whom some individuals harbored the presumptive mutation prior to radiation therapy.[31] Notably, CH resulting from prior exposure to cytotoxic therapy is associated with high frequencies of mutations in TP53 and PPM1D compared with age-related CH.[33] This feature is likely related to the ability of mutated TP53 and PPM1D to confer resistance to genotoxic stress, thus providing HSPC with a survival advantage under these conditions. In support of this notion, it has been reported that patients with therapy-related acute myeloid leukemia (AML)/MDS harbor low levels of TP53-mutated clones before the onset of the disease, and prior to the chemotherapy to treat the primary hematological disease.[27] These data suggest that TP53 mutations are selected for, but are not induced by the chemotherapy. Evidence for this hypothesis was provided by experiments in which mice underwent transplantation with either wild-type or p53-haploinsufficient HSPC. It was found that p53-mutant cells preferentially expanded after N-ethyl-N-nitrosourea administration.[27] Interestingly, in another study, the fitness advantage of TP53-deficient cells appeared to be non-cell autonomous, such that the outcompeted cells played a role for establishing clonal dominance of mutant cells.[34]

PPM1D is a member of the PP2C family of Ser/Thr protein phosphatase that functions to suppress the activation of TP53.[35] Although PPM1D is rarely mutated in a primary hematologic malignancy,[36] PPM1D mutations are observed in the CH that is enriched in patients who have been treated for ovarian,[37,38] breast,[37] prostate,[39] lung[40] and other solid tumors,[41] and in patients who relapse after therapy for hematological malignancy.[30] As with TP53, these data suggest that genotoxic stress promotes the expansion of HSPC that harbor PPM1D mutations.[30,31] Consistent with this notion, a recent study showed that PPM1D mutant clones display a competitive advantage under genotoxic stress.[42]

Neutral Drift

Neutral drift refers to a phenomenon of clonal selection among HSPC that originally possess an equal proliferative potential. This mechanism is analogous to the scenario of gamblers with equal odds of winning at a game of chance.[23] In a stochastic manner, a fraction of clones is favored to predominate, and as these clones continue to "win" (i.e. expand), other clones are lost and finally replaced by the "winning" clone. The expansion of HSPC clones in this manner is referred to as "neutral drift-mediated CH". In this scenario, the size of the active HSPC pool will affect the probability of neutral drift and its outcome.[11,43] For example, it is much more probable that a clone will outcompete its neighbors if the HSPC pool is small and finite. Although this scenario will lead to the "clonal collapse" of the HSPC pool, the pathological consequences of neutral drift-mediated CH remain to be elucidated.

CH, Mortality and CVD

A number of studies have associated CH with an increase in all-cause death. The initial reports were published in in 2014.[8,10] In those studies, exome sequence analysis was performed on peripheral blood mononuclear cells from individuals who were unselected for cancer or hematologic phenotypes. Although these somatic mutations often occurred in the epigenetic regulators DNMT3A, TET2 and ASXL1 and other recognized driver genes, the study by Genovese et al also reported that mutations in candidate driver genes could only account for half of the observed CH, based on a non-biased whole-exome sequence analyses.[10] The 2 studies found an increase in mortality during the follow-up period that was approximately 40% in individuals who exhibited CH.[8,10] Marked increases in the frequencies of hematologic cancer were observed in both studies, as would be expected because mutations in these driver genes can be viewed as an early step in the progression to hematologic malignancy. However, the conversion to a hematologic malignancy is low in the general population, and it could not account for the large increases in the CH-associated all-cause death in these studies. Expanding on this issue, recent studies have used deep DNA sequence analysis of candidate driver genes to identify features that distinguish individuals at particular risk of developing AML from those who exhibit age-related CH but do not progress to a hematologic malignancy.[28,29] Interestingly, AML was found to be associated with the total number of mutations exhibited by an individual, the magnitude of the VAF and the specific nature of the driver gene mutation. Clonal mutations in DNMT3A and TET2 showed a greater association with CH, whereas TP53 and U2AF1 mutations were more prognostic for AML.[28,29]

This leads to the question: what can account for most of the elevated mortality in individuals with CH? The increased risk of all-cause death is most likely associated with a relatively large increase in the risk for CVD. In their original study, Jaiswal et al (2014) reported that driver gene-associated CH corresponded to an increased risk of coronary artery disease (hazard ratio=2.0) and ischemic stroke (hazard ratio=2.6) after adjusting for age, sex, type 2 diabetes, systolic blood pressure, and body mass index.[8] In a subsequent study, Jaiswal et al (2017) reported that CH increased the risk of coronary artery disease (hazard ratio=1.9) in other patient cohorts after adjusting for age, sex, type 2 diabetes mellitus, total cholesterol, high-density lipoprotein, smoking, and hypertension.[12] That study also reported a significant association between CH and early onset (<50 years of age) myocardial infarction (odds ratio=4.0) after adjusting for age, sex, type 2 diabetes status, and smoking status. Consistent with these findings, individuals with CH exhibited higher coronary artery calcification scores.
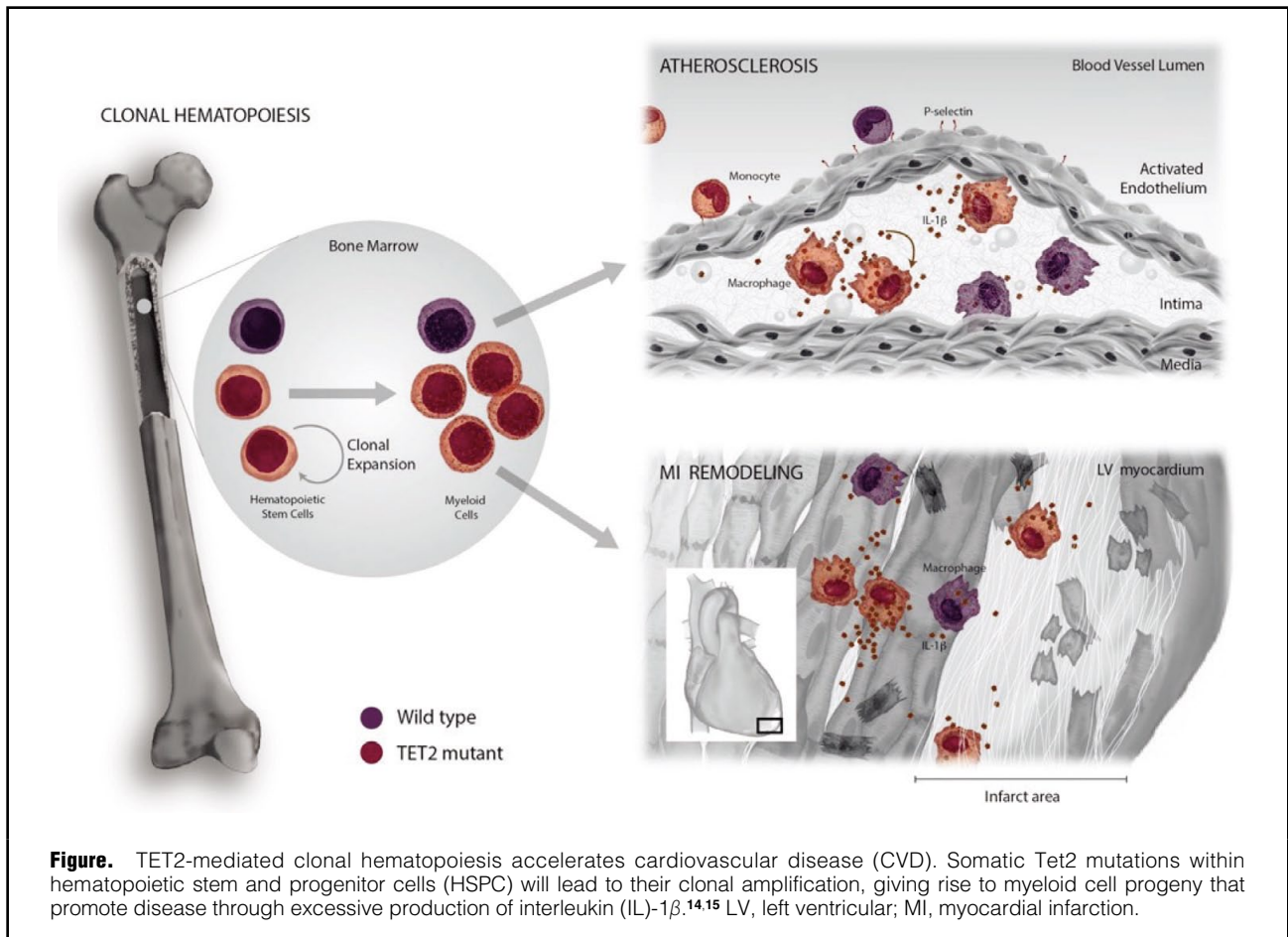
Whole-genome sequence analysis of 11,262 Icelanders also reported an association between CH and death.[11] In that study, CH was assessed by SNP-based computational analysis of genomic DNA of peripheral blood. A very high prevalence of age-associated CH was detected, but known candidate driver genes accounted for a relatively small fraction of the CH in this cohort. CH was associated with an increase in all-cause death regardless of whether or not it could be associated with a candidate driver gene mutation. Similarly, the non-biased whole-exome sequencing analysis by Genovese et al also associated CH with death regardless of whether individuals had a mutation within an identified candidate driver gene.[10] Although the Icelandic study did not report cardiovascular outcomes, significant associations were observed between CH and smoking, smoking-related disease and chronic pulmonary disease.[11] The study also detected a strong association between CH and the mosaic loss of the Y chromosome, suggesting that they are related phenomena.

More recently, a study examined large clonal mosaic chromosomal alterations, ranging from 50 kb to 249 Mb, in blood-derived DNA using SNP array data from 151,202 UK Biobank participants.[44] These chromosome alterations increased with age and were associated with a doubling in the risk for all-cause death. Notably, this increase in mortality could only partly be explained by an increase in cancer deaths. Although a number of these loci are predicted to ablate tumor suppressor genes, including DNMT3A and TET2, this study provided evidence that multiple DNA mutations beyond those of target candidate known driver genes can give rise to CH with consequences on mortality.

In patients undergoing autologous stem cell transplantation for lymphoma, driver gene-associated CH was associated with an increased risk of overall mortality, an increased risk of therapy-related myeloid neoplasm, and a predisposition to death from CVD.[30] In this cohort, hematopoietic stem cells are presumably under extreme hematopoietic stress, leading to a distinct mutational spectrum of candidate drivers, including high frequencies of mutations in TP53 and PPM1D. Driver gene-associated CH is also found to be enriched in solid tumor patients, and this condition is associated with inferior survival.[31] In this cohort, CH is associated with an increased risk of hematologic cancer, but the most common cause of death was progression of the primary non-hematologic cancers.

Experimental Studies on CH

Epidemiological analyses are inherently descriptive, and it is not possible to determine whether CH and CVD are causally linked or whether they are epiphenomena of the aging process. Furthermore, it is often difficult to address issues of directionality. For example, it has been suggested that chronic inflammation and other stresses associated with CVD can promote somatic mutagenesis and CH.[45] At the current stage of research, animal models have particular utility in addressing questions of causality and directionality.

Figure. TET2-mediated clonal hematopoiesis accelerates cardiovascular disease (CVD). Somatic Tet2 mutations within hematopoietic stem and progenitor cells (HSPC) will lead to their clonal amplification, giving rise to myeloid cell progeny that promote disease through excessive production of interleukin (IL)-1β.[14,15] LV, left ventricular; MI, myocardial infarction.

TET2

TET2 is a regulator of HSPC self-renewal and proliferation.[46–48] Over 130 TET2 mutations have been reported in cancer-free individuals, most of which are predicted to lead to a loss-of-function.[49] TET2 is an epigenetic regulator that can activate or repress transcription depending on the gene target and molecular context. TET2 is widely recognized to catalyze the conversion of 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), leading to DNA demethylation and transcriptional activation.[50–52] TET2 can also promote the repression by proinflammatory genes by recruiting histone deacetylases to their promoters.[53]

Murine studies by our group initially revealed a potential causative link between somatic TET2 mutations in blood cells and atherosclerotic CVD[15] and heart failure[14] (**Figure**). In these studies, a competitive bone marrow transplantation approach was undertaken to simulate the clonal expansion of mutant cells. Tet2-deficient HSPC displayed progressive expansion into all immune cell progeny in the bone marrow, spleen, and blood. A slight myeloid bias was detected, with a preferential expansion into the Ly6C^{high} classical monocyte population, a feature that is more pronounced in humans.[54] Notably, the expansion of the Tet2-deficient cells did not affect white blood cell numbers, consistent with what is observed in individuals with TET2-mediated CH.[8,20] In studies of experimental atherosclerosis, Tet2-mediated clonal expansion led to a marked increase in plaque size in hyperlipidemic low-density lipoprotein receptor-deficient

mice.[15] Similarly, an increase in plaque size was observed when cells heterozygous for Tet2-deficiency were transplanted into the atherogenic mouse model. Under these conditions, the degree of clonal expansion was less than what was observed with the homozygous-deficient mice and there was a smaller increase in plaque size, indicative of a dose-response relationship. It was also shown that myeloid-specific ablation of Tet2 was sufficient to promote atherosclerosis development.[15] Consistent with these observations, it was also reported that full hematopoietic ablation of Tet2 increased plaque size in a murine atherosclerosis model.[12]

To corroborate and extend these studies, the effects of partial hematopoietic cell Tet2-deficiency were assessed in murine models of heart failure.[14] Partial hematopoietic Tet2-deficiency was achieved by bone marrow reconstitution techniques or by myeloid-specific ablation. The inactivation of hematopoietic Tet2 led to increased pathological cardiac remodeling, as indicated by diminished cardiac function and greater myocardial hypertrophy, fibrosis and inflammation in both the left anterior descending (LAD) ligation model of myocardial infarction and in the transverse aortic constriction (TAC) model of pressure overload hypertrophy. In these heart failure models, as in experimental atherosclerosis, accelerated pathology could be observed under conditions of Tet2 haploinsufficiency. Additionally, hematopoietic Tet2-deficiency, achieved by the transplantation of lineage-negative bone marrow

cells that underwent lentivirus-mediated, CRISPR/Cas9-mediated gene editing, was shown to promote greater detrimental remodeling in mice that were treated with angiotensin II (AngII) infusion.[13]

Experimental studies have shown that the overactivation of interleukin (IL)-1β signaling contributes to the enhanced cardiovascular pathogenesis that is associated with the expansion of Tet2-deficienct hematopoietic cells[14,15] (**Figure**). In the atherosclerosis model, it was found that exacerbated IL-1β production by Tet2-deficient plaque cells promoted P-selectin expression and endothelial cell activation in the vascular lesion.[15] Similarly, hematopoietic deficiency of Tet2 led to an increase in myocardial expression IL-1β in the models of LAD ligation and TAC.[14] In cell culture studies, Tet2-deficient macrophages express higher levels of the IL-1β transcript and the inactive precursor protein pro-IL-1β.[15] Tet2 deficiency also upregulates components of the NLRP3 inflammasome, leading to the increased processing and secretion of active IL-1β protein. Mechanistic studies have revealed that Tet2-mediated of IL-1β regulation is mediated by its ability to modulate the recruitment of histone deacetylase to the IL-1β promoter.[15,53] The functional significance of IL-1β in the Tet2-mediated pathology was indicated by experiments in which treatment with an NLRP3 inflammasome inhibitor eliminated the accelerated pathologies associated with partial hematopoietic Tet2 ablation in atherosclerosis and heart failure models.[14,15]

The experimental studies on Tet2 regulation of the IL-1β/NLRP3 inflammasome may shed light on the findings of the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) trial. This study found that treatment with an IL-1β neutralizing antibody (canakinumab) reduced major adverse cardiovascular events in high-risk patients with a previous history of myocardial infarction and elevated levels of C-reactive protein.[55] Although canakinumab showed efficacy, its general use in the clinic poses challenges because it increases the risk of infection. Furthermore, it has been reported that a fraction of study participants do not respond to canakinumab with C-reactive protein reduction, and this subgroup did not show cardiovascular benefits.[56] Based on experimental studies, one can speculate that individuals with CH mediated by mutations in TET2, and perhaps related driver genes, may respond more favorably than the general population to IL-1β/NLRP3-targeted therapies. Thus, an evaluation of CH in CANTOS trial participants has merit because it could indicate a path for personalized therapy for the prevention of CVD in individuals carrying somatic mutations in their hematopoietic system.

DNMT3A

DNA methyltransferase 3A (DNMT3A) is a de novo DNA methyltransferase that modulates gene transcription in various cell types including HSPC.[57–61] Accumulating human studies show that DNMT3A is the most prevalent mutated driver gene associated with CH in the elderly population,[8–11] and DNMT3A-mediated CH has been associated with an increased risk of CVD.[12] However, whether somatic mutation in Dnmt3a causally contributes to CVD was unknown until recently.

Our group has demonstrated that partial Dnmt3a loss-of-function in hematopoietic stem cells worsens the cardiovascular phenotype in a model of heart failure induced by

AngII infusion.[13] To conduct this study, a facile system of mutagenesis was developed in which lentiviral vectors were used transduce lineage-negative bone marrow cells prior to bone marrow transplantation with Cas9 and a guide RNA to create insertion and deletion mutations in the *Dnmt3a* gene.[62] In contrast to mutations in Tet2, Dnmt3a-disrupted HSPC did not display selective expansion during the 4-month time course of these experiments. This behavior is consistent with previous reports showing that Dnmt3a-null HSPC only expand in aged mice or after sequential bone marrow transplantations,[61,63,64] and these findings raise concerns about using murine models as the sole system to study CH. Regardless, mice transplanted with Dnmt3a-edited HSPC showed significantly greater cardiac hypertrophy, diminished ejection fraction, and more fibrosis after AngII administration despite a relatively low level of chimerism ($\approx$5–10%).

From the perspective of mechanism, Dnmt3a has been reported to regulate inflammation in multiple cell types and in multiple ways. In the experimental heart failure studies, partial hematopoietic deficiency of Dnmt3a was associated with greater macrophage accumulation and increased expression of immune cell markers in myocardium, suggesting that this condition impairs the resolution of inflammation.[13] Consistent with this hypothesis, studies with a macrophage cell line showed that Dnmt3a-deficiency promotes inflammation by upregulating the expression of specific cytokines and chemokines. In other systems, Dnmt3a-deficiency has been shown to accelerate proinflammatory activation of mast cells, dysregulation of T cell polarization, and modulate peritoneal macrophage function.[65–68] Taken together, these findings support the concept that hematopoietic cell Dnmt3a-deficiency promotes a proinflammatory state. However, given the complex immunomodulatory properties of Dnmt3a, additional experimental studies are warranted to develop a more comprehensive understanding of how hematopoietic cell Dnmt3a-deficiency affects the pathogenesis of CVD.

Other CH-Related Genes

Similar to TET2 and DNMT3A, ASXL1 is an epigenetic regulator. ASXL1 controls epigenetic marks through interaction with polycomb complex proteins and various other transcriptional regulators.[69] *ASXL1* gene mutations are detected in variety of myeloid neoplasms, and they are associated with poor prognosis.[70–72] These mutations are also frequently observed in CH.[8–11] Most alterations in ASXL1 are nonsense mutations or frameshift mutations located in or near the last exon that result in a premature stop codon. A truncation in the C-terminus of murine Asxl1 has been reported to lead to an unstable protein that gives rise to a MDS-like phenotype.[73–75] However, more recent data suggest that the truncation of Asxl1 may lead to a gain-of-function in which the truncated Asxl1 enhances the activity of BAP1, leading to reduced ubiquitination of H2AK119.[76] Consistent with this notion, truncated ASXL1 protein can be detected in leukemia cell lines.[77] In experimental mouse studies, it was found that the retroviral overexpression of a truncated form of Asxl1 in bone marrow cells leads to an MDS-like disease and a reduction in H3K27me3 levels.[78] More recently, analysis of a "knock-in" mouse model of truncated Asxl1 revealed that mice were void of overt hematological malignancies, a finding that is more consistent with the concept that ASXL1 functions as a CH driver gene.[79,80] Collectively,

current data suggest that the truncated Asxl1 protein can display dominant-negative and gain-of-function effects depending on context. To date, however, it is unknown whether mutations in hematopoietic cell Asxl1 contribute to CVD.

JAK2 associates with a variety of receptors and acts as a signaling kinase.[81] The constitutively active allele JAK2[V617F] is present in leukocytes of a majority of patients with myeloproliferative neoplasms (MPN), including polycythemia vera (PV) and essential thrombocytosis (ET), which result from the dysregulated expansion of red blood cells and platelets, respectively.[82] Currently, the role of JAK2[V617F] in the development of CH is controversial, but there is evidence that some JAK2[V617F] mutant carriers do not show signs of blood count abnormalities or progress to MPN.[8,10,12,29,83–86] The phenotypic heterogeneity of JAK2[V617F] carriers could be from the highly heterogeneous nature of HSPC.[87] For example, a JAK2[V617F] mutation in platelet-biased HSPC might result in an ET phenotype, whereas a mutation in myeloid-biased HSPC, which gives rise to erythroid progenitors, might result in a PV phenotype. Along these lines, it is possible that JAK2[V617F] CH occurs when the mutation arises in a more restricted HSPC population. Although further mechanistic studies are required, JAK2[V617F]-mediated CH could affect CVD in multiple ways depending on the cell types that express this allele. Although the effect of JAK2[V617F] mutations on erythroid cells and megakaryocytes has been well studied, its effect on myeloid populations has only recently been investigated. It has been reported that JAK2[V617F] will activate β1 and β2 integrin expression in neutrophils and promote thrombus formation in mice.[88,89] The JAK2[V617F]-mutant neutrophil can also be more prone to neutrophil extracellular trap (NET) formation, a process referred to as NETosis, contributing to thrombosis.[90] Given that neutrophils promote arterial plaque erosion and thrombosis via NETosis,[91–93] JAK2[V617F]-mediated CH may accelerate the onset of ischemic events at culprit lesions. This concept is consistent with the observation of deaths of young individuals with JAK2[V617F]-mediated CH caused by coronary artery disease.[12] Also of note, a recent exome-wide study associated the JAK2[V617F] allele with lower triglyceride and LDL-cholesterol levels despite increased risk of coronary artery disease, suggesting that the JAK2[V617F] allele can promote CVD via mechanisms that are independent of lipid metabolism.[94] Although further studies are required to understand the effect of JAK2[V617F]-mediated CH on CVD, current mouse models have limited value because the JAK2[V617F] mutation in hematopoietic cells leads to strong PV and ET phenotypes. Thus, novel expression systems that target JAK2[V617F] to specific cell types may be required to create an appropriate model to study CH.

Hematopoietic mutations in TP53 are also associated with CH. TP53 encodes a tumor suppressor that is widely recognized to protect against genomic instability through its abilities to regulate DNA repair, cell cycle arrest, and apoptosis.[95] Many studies have examined the link between TP53 and CVD, but they have mainly focused on the effects of p53 on cardiac myocytes,[96,97] endothelial cells,[98,99] and vascular smooth muscle cells,[100] and relatively little is known about the contribution of hematopoietic TP53 mutations to CH-associated CVD. However, prior to its appreciation as a CH driver gene, it was reported that the transplantation of p53-deficient bone marrow into hyper-

lipidemic LDLR-KO mice leads to larger atherosclerotic plaques with increased macrophage proliferation in the plaque.[100] Several studies have investigated the effects of p53-deficiency in various inflammatory processes in immune cells. Murine neutrophils and macrophages deficient for p53 express more TNFa, IL-6, and CXCL-2 after LPS stimulation, and p53-deficient neutrophil also upregulate elastase expression.[101,102] Given the current interest in CH, additional experiments that examine the functional role of TP53 are warranted.

Perspectives

Hematopoietic cells with somatic mutations are prevalent in the adult population. These mutations generally enhance the fitness of HSPC such that they allow for the clonal amplification of the mutant cell in the absence of changes in blood cell counts. These clonal events increase with age and they are associated with all-cause death and CVD. Some of these mutations occur in driver genes that are recurrently mutated in hematologic malignancies. Recent experimental work has delineated how mutations in the Tet2 and Dnmt3a driver genes contribute to pathology in models of heart failure and/or atherosclerosis. However, many other candidate driver genes have yet to be investigated. Furthermore, epidemiological studies suggest that a large fraction of the observed CH cannot be attributed to mutations in candidate hematologic driver genes, suggesting that multiple mechanisms of genome instability can contribute to this condition. CH represents a new mechanism of CVD, and this avenue of research is in its infancy. Continued studies in this burgeoning area may offer new therapeutic opportunities that can be personalized based on specific gene mutations.

References

1. Fernandez-Friera L, Penalvo JL, Fernandez-Ortiz A, Ibanez B, Lopez-Melgar B, Laclaustra M, et al. Prevalence, vascular distribution, and multiterritorial extent of subclinical atherosclerosis in a middle-aged cohort: The PESA (Progression of Early Subclinical Atherosclerosis) Study. *Circulation* 2015; **131:** 2104–2113.
2. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, et al. Prevalence of conventional risk factors in patients with coronary heart disease. *JAMA* 2003; **290:** 898–904.
3. Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, et al. Quantification of biological aging in young adults. *Proc Natl Acad Sci USA* 2015; **112:** E4104–E4110.
4. Lodato MA, Rodin RE, Bohrson CL, Coulter ME, Barton AR, Kwon M, et al. Aging and neurodegeneration are associated with increased mutations in single human neurons. *Science* 2018; **359:** 555–559.
5. Goodell MA, Rando TA. Stem cells and healthy aging. *Science* 2015; **350:** 1199–1204.
6. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, et al. Tumor evolution: High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 2015; **348:** 880–886.
7. Vijg J. Somatic mutations, genome mosaicism, cancer and aging. *Curr Opin Genet Dev* 2014; **26:** 141–149.

8. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014; **371:** 2488–2498.

9. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 2014; **20:** 1472–1478.

10. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014; **371:** 2477–2487.

11. Zink F, Stacey SN, Norddahl GL, Frigge ML, Magnusson OT, Jonsdottir I, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* 2017; **130:** 742–752.

12. Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med* 2017; **377:** 111–121.

13. Sano S, Oshima K, Wang Y, Katanasaka Y, Sano M, Walsh K. CRISPR-mediated gene editing to assess the roles of Tet2 and Dnmt3a in clonal hematopoiesis and cardiovascular disease. *Circ Res* 2018; **123:** 335–341.

14. Sano S, Oshima K, Wang Y, MacLauchlan S, Katanasaka Y, Sano M, et al. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1beta/NLRP3 inflammasome. *J Am Coll Cardiol* 2018; **71:** 875–886.

15. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* 2017; **355:** 842–847.

16. Orkin SH, Zon LI. Hematopoiesis: An evolving paradigm for stem cell biology. *Cell* 2008; **132:** 631–644.

17. Laurenti E, Gottgens B. From haematopoietic stem cells to complex differentiation landscapes. *Nature* 2018; **553:** 418–426.

18. Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: From clonal haematopoiesis to secondary leukaemia. *Nat Rev Cancer* 2017; **17:** 5–19.

19. Busque L, Mio R, Mattioli J, Brais E, Blais N, Lalonde Y, et al. Nonrandom X-inactivation patterns in normal females: Lyonization ratios vary with age. *Blood* 1996; **88:** 59–65.

20. Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet* 2012; **44:** 1179–1181.

21. Laurie CC, Laurie CA, Rice K, Doheny KF, Zelnick LR, McHugh CP, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. *Nat Genet* 2012; **44:** 642–650.

22. Jacobs KB, Yeager M, Zhou W, Wacholder S, Wang Z, Rodriguez-Santiago B, et al. Detectable clonal mosaicism and its relationship to aging and cancer. *Nat Genet* 2012; **44:** 651–658.

23. Klein AM, Simons BD. Universal patterns of stem cell fate in cycling adult tissues. *Development* 2011; **138:** 3103–3111.

24. McKerrell T, Park N, Moreno T, Grove CS, Ponstingl H, Stephens J, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep* 2015; **10:** 1239–1245.

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

26. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell* 2012; **150:** 264–278.

27. Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature* 2015; **518:** 552–555.

28. Desai P, Mencia-Trinchant N, Savenkov O, Simon MS, Cheang G, Lee S, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. *Nat Med* 2018; **24:** 1015–1023.

29. Abelson S, Collord G, Ng SWK, Weissbrod O, Mendelson Cohen N, Niemeyer E, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. *Nature* 2018; **559:** 400–404.

30. Gibson CJ, Lindsley RC, Tchekmedyian V, Mar BG, Shi J, Jaiswal S, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. *J Clin Oncol* 2017; **35:** 1598–1605.

31. Coombs CC, Zehir A, Devlin SM, Kishtagari A, Syed A, Jonsson P, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. *Cell Stem Cell* 2017; **21:** 374–382.e4.

32. Jongen-Lavrencic M, Grob T, Hanekamp D, Kavelaars FG, Al Hinai A, Zeilemaker A, et al. Molecular minimal residual disease in acute myeloid leukemia. *N Engl J Med* 2018; **378:** 1189–1199.

33. Wong TN, Miller CA, Jotte MRM, Bagegni N, Baty JD, Schmidt AP, et al. Cellular stressors contribute to the expansion of hematopoietic clones of varying leukemic potential. *Nat Commun* 2018; **9:** 455.

34. Bondar T, Medzhitov R. p53-mediated hematopoietic stem and progenitor cell competition. *Cell Stem Cell* 2010; **6:** 309–322.

35. Fiscella M, Zhang H, Fan S, Sakaguchi K, Shen S, Mercer WE, et al. Wip1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. *Proc Natl Acad Sci USA* 1997; **94:** 6048–6053.

36. Bowman RL, Busque L, Levine RL. Clonal hematopoiesis and evolution to hematopoietic malignancies. *Cell Stem Cell* 2018; **22:** 157–170.

37. Ruark E, Snape K, Humburg P, Loveday C, Bajrami I, Brough R, et al. Mosaic PPM1D mutations are associated with predisposition to breast and ovarian cancer. *Nature* 2013; **493:** 406–410.

38. Swisher EM, Harrell MI, Norquist BM, Walsh T, Brady M, Lee M, et al. Somatic mosaic mutations in PPM1D and TP53 in the blood of women with ovarian carcinoma. *JAMA Oncol* 2016; **2:** 370–372.

39. Cardoso M, Paulo P, Maia S, Teixeira MR. Truncating and missense PPM1D mutations in early-onset and/or familial/hereditary prostate cancer patients. *Genes Chromosomes Cancer* 2016; **55:** 954–961.

40. Zajkowicz A, Butkiewicz D, Drosik A, Giglok M, Suwinski R, Rusin M. Truncating mutations of PPM1D are found in blood DNA samples of lung cancer patients. *Br J Cancer* 2015; **112:** 1114–1120.

41. Artomov M, Rivas MA, Genovese G, Daly MJ. Mosaic mutations in blood DNA sequence are associated with solid tumor cancers. *NPJ Genom Med* 2017; **2:** 22.

42. Kahn JD, Miller PG, Silver AJ, Sellar RS, Bhatt S, Gibson C, et al. *PPM1D*-truncating mutations confer resistance to chemotherapy and sensitivity to PPM1D inhibition in hematopoietic cells. *Blood* 2018; **132:** 1095–1105.

43. Mon Pere N, Lenaerts T, Pacheco JM, Dingli D. Evolutionary dynamics of paroxysmal nocturnal hemoglobinuria. *PLoS Comput Biol* 2018; **14:** e1006133.

44. Loh PR, Genovese G, Handsaker RE, Finucane HK, Reshef YA, Palamara PF, et al. Insights into clonal haematopoiesis from 8,342 mosaic chromosomal alterations. *Nature* 2018; **559:** 350–355.

45. Hasselbalch HC. Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: Is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer? *Blood* 2012; **119:** 3219–3225.

46. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* 2011; **20:** 11–24.

47. Quivoron C, Couronne L, Della Valle V, Lopez CK, Plo I, Wagner-Ballon O, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer Cell* 2011; **20:** 25–38.

48. Li Z, Cai X, Cai CL, Wang J, Zhang W, Petersen BE, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood* 2011; **118:** 4509–4518.

49. Fuster JJ, Walsh K. Somatic mutations and clonal hematopoiesis: Unexpected potential new drivers of age-related cardiovascular disease. *Circ Res* 2018; **122:** 523–532.

50. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 2009; **324:** 930–935.

51. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* 2011; **333:** 1300–1303.

52. He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* 2011; **333:** 1303–1307.

53. Zhang Q, Zhao K, Shen Q, Han Y, Gu Y, Li X, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. *Nature* 2015; **525:** 389–393.
54. Buscarlet M, Provost S, Zada YF, Bourgoin V, Mollica L, Dube MP, et al. Lineage restriction analyses in CHIP indicate myeloid bias for TET2 and multipotent stem cell origin for DNMT3A. *Blood* 2018; **132:** 277–280.
55. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017; **377:** 1119–1131.
56. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: A secondary analysis from the CANTOS randomised controlled trial. *Lancet* 2018; **391:** 319–328.
57. Hu N, Strobl-Mazzulla P, Sauka-Spengler T, Bronner ME. DNA methyltransferase3A as a molecular switch mediating the neural tube-to-neural crest fate transition. *Genes Dev* 2012; **26:** 2380–2385.
58. Wu Z, Huang K, Yu J, Le T, Namihira M, Liu Y, et al. Dnmt3a regulates both proliferation and differentiation of mouse neural stem cells. *J Neurosci Res* 2012; **90:** 1883–1891.
59. Xie W, Schultz MD, Lister R, Hou Z, Rajagopal N, Ray P, et al. Epigenomic analysis of multilineage differentiation of human embryonic stem cells. *Cell* 2013; **153:** 1134–1148.
60. Dhawan S, Tschen SI, Zeng C, Guo T, Hebrok M, Matveyenko A, et al. DNA methylation directs functional maturation of pancreatic beta cells. *J Clin Invest* 2015; **125:** 2851–2860.
61. Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* 2011; **44:** 23–31.
62. Heckl D, Kowalczyk MS, Yudovich D, Belizaire R, Puram RV, McConkey ME, et al. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. *Nat Biotechnol* 2014; **32:** 941–946.
63. Zhang X, Su J, Jeong M, Ko M, Huang Y, Park HJ, et al. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. *Nat Genet* 2016; **48:** 1014–1023.
64. Cole CB, Russler-Germain DA, Ketkar S, Verdoni AM, Smith AM, Bangert CV, et al. Haploinsufficiency for DNA methyltransferase 3A predisposes hematopoietic cells to myeloid malignancies. *J Clin Invest* 2017; **127:** 3657–3674.
65. Gamper CJ, Agoston AT, Nelson WG, Powell JD. Identification of DNA methyltransferase 3a as a T cell receptor-induced regulator of Th1 and Th2 differentiation. *J Immunol* 2009; **183:** 2267–2276.
66. Yu Q, Zhou B, Zhang Y, Nguyen ET, Du J, Glosson NL, et al. DNA methyltransferase 3a limits the expression of interleukin-13 in T helper 2 cells and allergic airway inflammation. *Proc Natl Acad Sci USA* 2012; **109:** 541–546.
67. Li X, Zhang Q, Ding Y, Liu Y, Zhao D, Zhao K, et al. Methyltransferase Dnmt3a upregulates HDAC9 to deacetylate the kinase TBK1 for activation of antiviral innate immunity. *Nat Immunol* 2016; **17:** 806–815.
68. Leoni C, Montagner S, Rinaldi A, Bertoni F, Polletti S, Balestrieri C, et al. Dnmt3a restrains mast cell inflammatory responses. *Proc Natl Acad Sci USA* 2017; **114:** E1490–E1499.
69. Micol JB, Abdel-Wahab O. The role of additional sex combs-like proteins in cancer (Review). *Cold Spring Harb Perspect Med* 2016; doi:10.1101/cshperspect.a026526.
70. Thol F, Friesen I, Damm F, Yun H, Weissinger EM, Krauter J, et al. Prognostic significance of ASXL1 mutations in patients with myelodysplastic syndromes. *J Clin Oncol* 2011; **29:** 2499–2506.
71. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* 2014; **28:** 241–247.
72. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med* 2011; **364:** 2496–2506.
73. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell* 2012; **22:** 180–193.
74. Wang J, Li Z, He Y, Pan F, Chen S, Rhodes S, et al. Loss of Asxl1 leads to myelodysplastic syndrome-like disease in mice. *Blood* 2014; **123:** 541–553.

75. Abdel-Wahab O, Gao J, Adli M, Dey A, Trimarchi T, Chung YR, et al. Deletion of Asxl1 results in myelodysplasia and severe developmental defects in vivo. *J Exp Med* 2013; **210:** 2641–2659.
76. Balasubramani A, Larjo A, Bassein JA, Chang X, Hastie RB, Togher SM, et al. Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1-BAP1 complex. *Nat Commun* 2015; **6:** 7307.
77. Inoue D, Matsumoto M, Nagase R, Saika M, Fujino T, Nakayama KI, et al. Truncation mutants of ASXL1 observed in myeloid malignancies are expressed at detectable protein levels. *Exp Hematol* 2016; **44:** 172–176.e1.
78. Inoue D, Kitaura J, Togami K, Nishimura K, Enomoto Y, Uchida T, et al. Myelodysplastic syndromes are induced by histone methylation-altering ASXL1 mutations. *J Clin Invest* 2013; **123:** 4627–4640.
79. Nagase R, Inoue D, Pastore A, Fujino T, Hou HA, Yamasaki N, et al. Expression of mutant Asxl1 perturbs hematopoiesis and promotes susceptibility to leukemic transformation. *J Exp Med* 2018; **215:** 1729–1747.
80. Hsu YC, Chiu YC, Lin CC, Kuo YY, Hou HA, Tzeng YS, et al. The distinct biological implications of Asxl1 mutation and its roles in leukemogenesis revealed by a knock-in mouse model. *J Hematol Oncol* 2017; **10:** 139.
81. Quintas-Cardama A, Kantarjian H, Cortes J, Verstovsek S. Janus kinase inhibitors for the treatment of myeloproliferative neoplasias and beyond. *Nat Rev Drug Discov* 2011; **10:** 127–140.
82. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005; **434:** 1144–1148.
83. Nielsen C, Birgens HS, Nordestgaard BG, Bojesen SE. Diagnostic value of JAK2 V617F somatic mutation for myelo-proliferative cancer in 49 488 individuals from the general population. *Br J Haematol* 2013; **160:** 70–79.
84. Nielsen C, Birgens HS, Nordestgaard BG, Kjaer L, Bojesen SE. The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. *Haematologica* 2011; **96:** 450–453.
85. Wang J, Hayashi Y, Yokota A, Xu Z, Zhang Y, Huang R, et al. Expansion of EPOR-negative macrophages besides erythroblasts by elevated EPOR signaling in erythrocytosis mouse models. *Haematologica* 2018; **103:** 40–50.
86. Hinds DA, Barnholt KE, Mesa RA, Kiefer AK, Do CB, Eriksson N, et al. Germ line variants predispose to both JAK2 V617F clonal hematopoiesis and myeloproliferative neoplasms. *Blood* 2016; **128:** 1121–1128.
87. Mead AJ, Mullally A. Myeloproliferative neoplasm stem cells. *Blood* 2017; **129:** 1607–1616.
88. Edelmann B, Gupta N, Schnoder TM, Oelschlegel AM, Shahzad K, Goldschmidt J, et al. JAK2-V617F promotes venous thrombosis through beta1/beta2 integrin activation. *J Clin Invest* 2018; doi:10.1172/JCI90312.
89. Gupta N, Edelmann B, Schnoeder TM, Saalfeld FC, Wolleschak D, Kliche S, et al. JAK2-V617F activates beta1-integrin-mediated adhesion of granulocytes to vascular cell adhesion molecule 1. *Leukemia* 2017; **31:** 1223–1226.
90. Wolach O, Sellar RS, Martinod K, Cherpokova D, McConkey M, Chappell RJ, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. *Sci Transl Med* 2018; **10:** eaan8292.
91. Quillard T, Araujo HA, Franck G, Shvartz E, Sukhova G, Libby P. TLR2 and neutrophils potentiate endothelial stress, apoptosis and detachment: Implications for superficial erosion. *Eur Heart J* 2015; **36:** 1394–1404.
92. Franck G, Mawson TL, Folco EJ, Molinaro R, Ruvkun V, Engelbertsen D, et al. Roles of PAD4 and NETosis in experimental atherosclerosis and arterial injury: Implications for superficial erosion. *Circ Res* 2018; **123:** 33–42.
93. Franck G, Mawson T, Sausen G, Salinas M, Masson GS, Cole A, et al. Flow perturbation mediates neutrophil recruitment and potentiates endothelial injury via TLR2 in Mice: Implications for superficial erosion. *Circ Res* 2017; **121:** 31–42.
94. Liu DJ, Peloso GM, Yu H, Butterworth AS, Wang X, Mahajan A, et al. Exome-wide association study of plasma lipids in >300,000 individuals. *Nat Genet* 2017; **49:** 1758–1766.
95. Kastenhuber ER, Lowe SW. Putting p53 in context. *Cell* 2017; **170:** 1062–1078.
96. Matsusaka H, Ide T, Matsushima S, Ikeuchi M, Kubota T, Sunagawa K, et al. Targeted deletion of p53 prevents cardiac

rupture after myocardial infarction in mice. *Cardiovasc Res* 2006; **70:** 457–465.
97. Naito AT, Okada S, Minamino T, Iwanaga K, Liu ML, Sumida T, et al. Promotion of CHIP-mediated p53 degradation protects the heart from ischemic injury. *Circ Res* 2010; **106:** 1692–1702.
98. Lee CL, Moding EJ, Cuneo KC, Li Y, Sullivan JM, Mao L, et al. p53 functions in endothelial cells to prevent radiation-induced myocardial injury in mice. *Sci Signal* 2012; **5:** Ra52.
99. Gogiraju R, Xu X, Bochenek ML, Steinbrecher JH, Lehnart SE, Wenzel P, et al. Endothelial p53 deletion improves angiogenesis and prevents cardiac fibrosis and heart failure induced by pressure overload in mice. *J Am Heart Assoc* 2015; doi:10.1161/JAHA.115.001770.
100. Merched AJ, Williams E, Chan L. Macrophage-specific p53 expression plays a crucial role in atherosclerosis development and plaque remodeling. *Arterioscler Thromb Vasc Biol* 2003; **23:** 1608–1614.
101. Liu G, Park YJ, Tsuruta Y, Lorne E, Abraham E. p53 Attenuates lipopolysaccharide-induced NF-kappaB activation and acute lung injury. *J Immunol* 2009; **182:** 5063–5071.
102. Madenspacher JH, Azzam KM, Gowdy KM, Malcolm KC, Nick JA, Dixon D, et al. p53 Integrates host defense and cell fate during bacterial pneumonia. *J Exp Med* 2013; **210:** 891–904.