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# Clonal haematopoiesis and dysregulation of the immune system

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## Abstract

Age-related diseases are frequently linked to pathological immune dysfunction, including excessive inflammation, autoreactivity and immunodeficiency. Recent analyses of human genetic data have revealed that somatic mutations and mosaic chromosomal alterations in blood cells — a condition known as clonal haematopoiesis (CH) — are associated with ageing and pathological immune dysfunction. Indeed, large-scale epidemiological studies and experimental mouse models have demonstrated that CH can promote cardiovascular disease, chronic obstructive pulmonary disease, chronic liver disease, osteoporosis and gout. The genes most frequently mutated in CH, epigenetic regulators *TET2* and *DNMT3A*, implicate increased chemokine expression and inflammasome hyperactivation in myeloid cells as a possible mechanistic connection between CH and age-related diseases. In addition, *TET2* and *DNMT3A* mutations in lymphoid cells have been shown to drive methylation-dependent alterations in differentiation and function. Here we review the observational and mechanistic studies describing the connection between CH and pathological immune dysfunction, the effects of CH-associated genetic alterations on the function of myeloid and lymphoid cells, and the clinical and therapeutic implications of CH as a target for immunomodulation.

## Introduction

Physiological haematopoiesis leads to the production of blood cells involved in tissue oxygenation, haemostasis and immunity[1]. Disruption of normal haematopoiesis can cause impaired production or function of red blood cells, platelets or immune cells and occurs in a wide variety of pathological states, including nutrient and growth factor deficiencies, exposure to toxic substances, infection, autoimmunity, neoplasia and inherited bone marrow failure syndromes[2–8]. In this context, aberrant haematopoiesis is often associated with clinical outcomes related directly to decreased abundance of specific haematopoietic cells (for example, increased bleeding in patients with thrombocytopenia)[9]. Similarly, clinical indicators of immunodeficiency in patients with aberrant haematopoiesis can

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also be attributed to cytopenias (for example, increased risk of infection in the setting of lymphopenia or neutropenia)[10,11]. Interestingly, aberrant haematopoiesis that occurs in patients with certain haematological neoplasms is often accompanied by clinical manifestations of immune dysregulation. For example, autoimmune or autoinflammatory conditions have been reported in 10–25% of patients with myelodysplastic syndromes (MDS) or chronic myelomonocytic leukaemia (CMML)[12], while autoantibodies leading to the destruction of red blood cells or platelets develop in approximately 25% of patients with chronic lymphocytic leukaemia (CLL)[13].

The mechanistic underpinnings of immune dysfunction in patients with haematological neoplasms have yet to be fully elucidated. However, it has been noted that autoimmune or autoinflammatory conditions can occur several years preceding the diagnosis of a haematological neoplasm, suggesting that pathological changes in immune function occur in a premalignant state. More recently, technological advances in nucleic acid sequencing combined with curation of large clinical datasets have enabled population-wide characterization of somatic genetic alterations in blood cells and the associated clinical phenotypes in hundreds of thousands of individuals. These analyses have revealed that somatic genetic alterations frequently found in patients with haematological malignancies are also common in aged individuals despite lack of evidence of cancer. This phenomenon, referred to as clonal haematopoiesis (CH), has now been linked to a wide range of conditions, including haematological neoplasms and diseases of inflammageing (Box 1). Clonal haematopoiesis of indeterminate potential (CHIP) comprises a subset of CH, defined specifically by the presence of somatic mutations in individual haematological malignancy-associated genes at an allele fraction greater than 2%. Mosaic chromosomal alterations (mCAs), which consist of somatic gains or losses affecting the entirety or parts of a chromosome, represent another type of CH detectable in blood cells. Here, we review the observational studies describing the association between autoimmunity, immunodeficiency and haematological neoplasms as well as the mechanistic connections between the somatic genetic alterations observed in CH and functional dysregulation of myeloid and lymphoid immune cells.

## Immune dysregulation in haematological neoplasms

Haematological neoplasms are often associated with concurrent or antecedent indicators of immune dysregulation, including autoimmunity and immunodeficiency. Evidence of immune dysregulation has been observed in patients with myeloid and lymphoid neoplasms, although there are particular diagnoses of autoimmunity and immunodeficiency that are associated with specific cell lineages. In the following sections, we provide a summary of the clinical observational studies describing the manifestations of immune dysregulation in the setting of myeloid and lymphoid neoplasms.

### Myeloid neoplasms.

The presence of laboratory and clinical indicators of immune dysregulation in myeloid neoplasms has been recognized since the 1960s[14–18]. Immune aplastic anaemia shares significant clinicopathological overlap with hypoplastic MDS[19], further emphasizing the

link between immune state and haematological disease. Several small case series have also suggested that frank rheumatic disease, including vasculitis, relapsing polychondritis, rheumatoid arthritis and polymyalgia rheumatica, are more common in patients with myeloid neoplasms[20–29].

Analyses of large, population-based cohorts have revealed associations between myeloid neoplasms and prior autoimmunity or age-related diseases with inflammatory components (i.e., diseases of inflammageing)[30–32] (Box 1). Two case-control studies of acute myeloid leukemia (AML) and MDS in the United States and Sweden found that patients were significantly more likely than controls to have antecedent diagnoses of autoimmunity[30,31]. A recent analysis of approximately 20,000 MDS and CMML cases demonstrated that such patients were more likely to have a history of diseases associated with inflammageing compared with either healthy controls or patients with solid malignancies[32]. In all three studies, these associations remained statistically significant even when the analyses were restricted to autoimmune or inflammageing-related diagnoses that occurred more than 3–5 years prior to the onset of a myeloid neoplasm. This temporal sequence suggests that autoimmunity may be driven by clonal premalignant cells. Indeed, myeloid clones have been detected within inflammatory skin lesions from patients with and without underlying myeloid neoplasms[33–37]. In addition, several lines of evidence have suggested that dysregulated inflammation and immune signalling contribute to the development of haematological neoplasms[38,39].

Increased secretion of proinflammatory cytokines by monocytes and macrophages also constitutes part of immune dysregulation in myeloid neoplasms. It has been long recognized that MDS, AML and CML show increased serum levels of tumour necrosis factor (TNF), IL-1β and IL-6[40–44]. Increased levels of TNF are associated with bone marrow failure and inferior prognosis in MDS[45,46]. Lower IL-1β levels predict favourable response to erythropoietin and better survival in MDS[47]. In CML, higher IL-1β is associated with poor prognosis and blast crisis[41]. Increased expression of proinflammatory cytokines directly influences the cell fate and function of haematopoietic progenitor cells in myeloid neoplasms. For example, chronic stimulation by IL-1β promotes haematopoietic stem cell (HSC) cell cycle exit and differentiation into myeloid cells in a process dependent on the myeloid transcription factor CEBPA[48,49]. HSCs lacking *Cebpa* are resistant to the pro-differentiation effect of IL-1β, thereby gaining a competitive advantage under IL-1 stimulation. IL-1 also selects for *Tet2* deficient clonal expansion by promoting HSC cell cycle progression and multilineage differentiation in mice[39]. Mutations in *Tet2*, *Dnmt3a* and *Asxl1*, which encode the mouse homologues of epigenetic modifiers that are frequently mutated in CH, MDS and myeloid malignancies in humans, further render HSCs more resistant to acute inflammatory stresses from lipopolysaccharide and IL-1β, thereby supporting clonal expansion and malignant evolution via different mechanisms[50–52]. Finally, increased IL-6 signalling in the setting of myeloid neoplasms has been associated with aggressive disease and poor outcomes in humans and mice[43,53–55].

### Lymphoid neoplasms.

It is well-established that CLL is associated with increased risk of infectious complications[56] due to reduced levels of protective immunoglobulin[57,58] and impaired capacity to mount a robust antibody response[59]. Patients often have a significant clinical history of infection years before the diagnosis of CLL, suggesting that abnormal lymphopoiesis or lymphocyte function creates an immunocompromised state during the premalignant period[60]. Indeed, the diagnosis of CLL is frequently preceded by a monoclonal B cell lymphocytosis (Box 1), which is also associated with increased risk of infection and abnormalities in serum immunoglobulin levels[61,62].

Clinical manifestations of hyperinflammatory immune dysregulation are commonly observed in patients with T and natural killer (NK) cell neoplasms. Angioimmunoblastic T cell lymphoma (AITL) often presents with signs of rheumatic disease, including polyarthritis, leukocytoclastic vasculitis, serositis and autoimmune cytopenias[63]; NK cell large granular lymphocyte (LGL) leukemia and chronic lymphoproliferative disorder of NK cells (CLPD-NK) are typically associated with autoimmune cytopenias, especially neutropenia; and the macrophage activation syndrome haemophagocytic lymphohistiocytosis (HLH), which is characterized by fever, elevated ferritin, hepatosplenomegaly and pancytopenia, has been especially linked to extranodal NK/T cell lymphoma (ENKTL)[64]. Altogether, there is a broad spectrum of immune dysregulation and clinical features of inflammation observed in the setting of T and NK cell malignancies.

## Clonal haematopoiesis

There is now ample evidence demonstrating that haematological malignancies arise via serial acquisition of mutations by a founding clone[65–69], implying the existence of premalignant CH in which genetic lesions are detectable in the absence of disease (Box 2). Additional evidence of CH emerged from studies that reported skewing of X-inactivation and clonal mutations in *TET2*[70–72]. Subsequent analyses of several genome-wide association studies also observed mCAs in peripheral blood cells from individuals with no evidence of haematological malignancy[73,74]. In the sections below, we review the genetic, demographic and clinical features of both CH defined by mutations in single genes associated with haematological malignancies and CH defined by mCAs (Fig. 1).

### Clonal haematopoiesis associated with individual haematological malignancy genes.

Several independent analyses of more than 30,000 exomes derived from cohort studies of type 2 diabetes and schizophrenia, as well as The Cancer Genome Atlas, demonstrated that ageing individuals without haematological malignancies harbour clonal mutations in their peripheral blood[75–77]. Mutations were more common in older individuals, occurring in 10–20% of individuals over the age of 70 compared with <5% of individuals under the age of 50. Notably, the majority of CH mutations were in genes associated with MDS and myeloid malignancies. Consistent with the idea of clonal evolution from a premalignant state, the detection of a mutation at the time of DNA collection was associated with a dramatically increased risk of a subsequent myeloid malignancy (Box 2).

Several follow-up analyses with even larger numbers of subjects confirmed that the presence of blood cells harbouring clonal mutations in genes associated with myeloid neoplasia is common in aged individuals and confers an increased risk of mortality, malignancy and non-malignant conditions[78–85]. The number of mutations, size of the clone(s) and the identity of the gene(s) mutated seem to affect the likelihood of these outcomes, but it is still unclear which individuals with CH are at highest risk. Analysis of peripheral blood DNA collected years before the diagnosis of AML demonstrated that risk of malignant transformation was higher among individuals with a greater number of mutations and among individuals with larger, rapidly expanding clones[84,86–90]. The risk of AML also varied among different CH-associated genes, likely corresponding to the mechanisms by which mutations promote clonal advantage and/or impact differentiation. For example, mutations in splicing factor genes or *TP53* conferred a significantly greater risk of malignant transformation than mutations in *DNMT3A* or *TET2*, even though the latter mutations occur more frequently. Future analyses of larger patient cohorts and mechanistic studies will facilitate the discovery of other genetic, demographic, clinical, and laboratory variables that will aid in the risk stratification of individuals with CH.

Another recent study mined exome sequencing data from over 50,000 individuals using a set of target genes somatically mutated in either myeloid or lymphoid malignancies[79]. Similar to myeloid malignancy-associated mutations, the frequency of somatic mutations in lymphoid malignancy-associated genes increased with age. Lymphoid malignancy-associated mutations were less common than myeloid malignancy-associated mutations, occurring in 1% and 6% of individuals, respectively. Among individuals with lymphoid malignancy-associated mutations, *DUSP22*, *FAT1*, *KMT2C*, *KMT2D*, *SYNE1* and *ATM* were the genes most frequently affected; consistent with previous studies, *DNMT3A*, *TET2* and *ASXL1* were the most frequently mutated myeloid malignancy-associated genes. There was a striking correlation between incident haematological malignancies and the type of premalignant mutation detected. The presence of a lymphoid malignancy-associated mutation conferred an increased risk of developing a lymphoid malignancy (hazard ratio 4.2) but not a myeloid malignancy. Conversely, the presence of a myeloid malignancy-associated mutation conferred an increased risk of developing a myeloid malignancy (hazard ratio 7) but not a lymphoid malignancy, although somatic mutations in *DNMT3A* and *TET2* have also been widely described as oncogenic drivers in several lymphoid neoplasms (Box 3).

### Clonal haematopoiesis associated with mosaic chromosomal abnormalities.

In addition to clonal mutations in individual haematological malignancy genes, CH can be defined by clonal cytogenetic aberrations. In 2012, two independent groups analyzing SNP microarray data from more than 100,000 individuals in total observed that mCAs detected in peripheral blood cells were associated with ageing and conferred an increased risk of developing a haematological malignancy[73,74]. More recently, analyses of several additional cohorts comprising more than 700,000 individuals in total confirmed these findings and provided novel insights into the laboratory findings and incident clinical diagnoses associated with the presence of specific mCAs[91–93]. For example, detection of mCAs commonly observed in CLL (for example, events involving chromosomes 3, 12 and 13) were associated with elevated lymphocyte counts and incident diagnosis of CLL;

mCAs defined by 9p loss of heterozygosity including the *JAK2* locus were associated with elevated levels of red blood cells, platelets and neutrophils and incident diagnosis of a myeloproliferative neoplasm[91]. By contrast, the most common mCA, loss of chromosome Y, has been associated with increased platelet counts but not consistently with increased risk of neoplasia[91,92,94,95].

Multiple studies have described CH with both mCAs and individual gene mutations in the same individual[79,81,96]. mCAs were more frequent among those with individual CH gene mutations compared to those without individual CH gene mutations. In addition, mCAs defined by loss of heterozygosity tended to affect loci containing individual CH-associated gene mutations. For example, *DNMT3A*, *TET2*, *TP53* and *JAK2* mutations were associated with loss of heterozygosity at chromosomes 2p, 4q, 17p and 9p, respectively.

## [H2] Clonal haematopoiesis, mortality and non-malignant disease.

In addition to an increased risk of developing a myeloid malignancy, individuals with mutations in haematological malignancy-associated genes consistently show higher all-cause mortality compared with individuals without mutations[75,76,78–84]. Surprisingly, the increased risk of death among individuals with mutations is not attributable to haematological malignancy but rather to atherosclerotic cardiovascular disease[76,81,82,84,97]. Interestingly, all-cause mortality and cardiovascular disease were significantly increased only among individuals with myeloid malignancy-associated mutations; the risk of these adverse outcomes was the same among individuals with lymphoid malignancy-associated mutations and those without any malignancy-associated mutations[79].

An increase in mortality unrelated to haematological malignancy was also observed among individuals with mCAs. The cause of this mortality increase has not been determined although it could be linked to an increased risk of infection in individuals with mCAs[93], suggesting that mCAs are associated with an immunodeficient phenotype. In this context, higher lymphocyte counts and increased incidence of CLL were observed among individuals with mCAs, indicating that these genetic alterations are markers of the CLL premalignant condition monoclonal B cell lymphocytosis (Box 2). Similar to lymphoid malignancy-associated mutations, mCAs did not confer an increased risk of death due to cardiovascular disease[79,81,98], implying that the mechanisms by which mCAs affect the development of non-malignant disease is also distinct from CH with mutations in individual myeloid malignancy-associated genes. It remains unclear if the difference in non-malignant phenotypes between different types of CH is due to differences in the genes mutated or the cell types affected. Interestingly, in the setting of CH with combined mCAs and individual mutations in myeloid malignancy-associated genes, mortality due to incident haematological malignancies or cardiovascular disease was increased compared with either type of CH alone[81]. This observation was independent of the total number of CH genetic alterations and the presence of individual gene mutations and mCAs affecting the same locus. Further investigation is required to determine the mechanisms by which the co-occurrence of different types of CH alter clonal evolution and non-malignant phenotypes.

Although CH encompasses a wide range of cytogenetic and genetic aberrations, including a substantial fraction of cases with as yet unknown driver mutations[75,80], the study of disease

associations in CH have focused on CHIP due to the relative consistency and reproducibility of identifying recurrent, high-allele-fraction mutations in genes implicated in haematological malignancies. Since the initial identification of CHIP as a risk factor for cardiovascular disease, CHIP has been found to be significantly associated with other common disorders typified by robust inflammation, ageing or both (Table 1). Using a combination of large scale population-based genetic analysis and mouse models of human disease, CHIP has been associated with heart failure[99,100], atrial fibrillation[83], chronic kidney disease[84,101,102], chronic liver disease[103], chronic obstructive pulmonary disease[104], gout[105], inflammatory bowel disease[106], autoimmunity[107], osteoporosis[108], premature menopause[109], venous thromboembolism[110], pulmonary hypertension[111], post-transplant liver disease[112], type II diabetes[113], solid cancers[83,84,114,115], stroke[116] and Alzheimer disease[117]. Epidemiological tools such as Mendelian randomization have allowed causal inferences to be made regarding the role of CHIP in various diseases[83,101,103,118,119].

Analyses of epigenetic ageing, which has been associated with a wide range of adverse outcomes in humans[120], have refined the relationship between CHIP and non-malignant phenotypes. Individuals with CHIP showed evidence of accelerated epigenetic ageing[121,122]. Moreover, the increase in all-cause mortality and atherosclerotic cardiovascular disease among individuals with CHIP was observed in those with epigenetic age acceleration but not in those without[121]. In this context, the likelihood of detecting an association between CHIP and certain phenotypes may depend on factors other than the presence of somatic mutations alone. Indeed, a recent Mendelian randomization analysis to identify diseases and biomarkers linked to CHIP did not find an association with atherosclerotic cardiovascular disease or stroke[83]. It remains to be determined if additional analyses of CHIP with and without accelerated epigenetic ageing specifically highlight those individuals with significantly increased risk of atherosclerosis or other clinical phenotypes.

Apart from disease associations, population-based studies have shown that individuals with CHIP exhibit overall higher levels of IL-6 and C-reactive protein[78,123,124]. Specifically, *TET2* mutated CHIP is significantly associated with increased serum IL-1β, whereas *JAK2* and *SF3B1* mutated CHIP correlated with increased serum IL-6 levels[123]. In a separate study, *DNMT3A* mutated CHIP was specifically associated with elevated serum interferon-γ (IFNγ) levels in patients with ulcerative colitis[106]. Although patients with myeloid malignancies have been known to exhibit high levels of pro-inflammatory cytokines[43,125,126], these new studies suggest a pattern of gene-specific effects on the inflammatory cytokine milieu in CHIP and related haematological diseases. Another strand of evidence highlighting the influence of CHIP on the inflammatory state lies in the finding that CHIP-mutated haematopoietic cells can be identified at sites of inflammation. *TET2*, *JAK2* and *TP53* mutant myeloid cells have been identified in the synovial fluid from individuals with CHIP who have rheumatoid arthritis[127]. In mice transplanted with *Tet2* deficient bone marrow cells, $Tet2^{-/-}$ myeloid cells were enriched in the heart and correlated with increased cardiac hypertrophy and fibrosis[128]. To summarize, the study of CHIP provides a mechanistic framework for understanding the long-recognized association between myeloid neoplasia and autoimmune and inflammatory conditions as well as molecular insights into diseases of ageing.

## Mechanisms of myeloid dysregulation in clonal haematopoiesis

CH has been shown to play a causal role in disease pathogenesis using mouse models transplanted with *Tet2*$^{-/-}$ bone marrow haematopoietic cells and exposed to specific metabolic and environmental conditions. *Tet2*-deficient haematopoietic cells exacerbate the development of atherosclerosis in *Ldlr*$^{-/-}$ mice[97,129]; interestingly, this effect was observed in the context of myeloid-specific *Tet2* deficiency, implying that the presence of a mutation in myeloid cells was sufficient to promote atherosclerosis[97,129]. Mice transplanted with *Tet2*-deficient bone marrow cells show increased immune infiltration in models of gout, chronic liver disease and chronic obstructive pulmonary disease[103–105]. Furthermore, *Tet2*-deficient macrophages expressed increased levels of several inflammatory chemokines and cytokines compared with wild-type macrophages[97,129–131], corroborating human studies in which patients with *TET2* mutations had higher circulating levels of the inflammatory chemokine IL-8[97]. Together, these data indicated that increased myeloid-derived inflammation may underlie the association between clonal haematopoiesis and cardiovascular disease.

Mechanistically, the best characterized *Tet2*-dependent proinflammatory mediator is the NLRP3 inflammasome. The NLRP3 inflammasome is a macromolecular complex that integrates responses to a wide range of damage-associated molecular patterns (DAMPs) that are released into the extracellular space upon cellular damage, including extracellular adenosine triphosphate (ATP), uric acid, reactive oxygen species (ROS), heat shock proteins (HSP), small calcium binding proteins S100A8 and S100A9, nuclear histones and extracellular nucleic acids[132]. The activation of NLRP3 occurs in two sequential steps. First, priming of the inflammasome occurs via nuclear factor-kB-dependent transcription of NLRP3, caspase 1 and the inactive cytokine forms pro-IL-1β and pro-IL-18. A DAMP or pathogen-associated molecular pattern (PAMP) comprises the second signal, which activates NLRP3 via intracellular potassium and calcium fluxes. Upon activation, NLRP3 rapidly oligomerizes into a multimeric scaffold, recruiting ASC and procaspase 1 into a large perinuclear speck complex. Active caspase 1 cleaves pro-IL-1β and pro-IL-18, causing secretion of biologically active IL-1β and IL-18. NLRP3-mediated inflammatory cytokine release has received intensive study after the discovery that CH is associated with increased IL-1β and IL-6[78].

Studies implicating NLRP3 inflammasome activity in CH have relied on pharmacological inhibitors of NLRP3, such as the sulfonylurea derivative MCC950, a specific inhibitor of NLRP3 inflammasome that blocks ASC oligomerization[133]. The pro-atherosclerotic effects of haematopoietic *Tet2* deletion in mice can be rescued by MCC950 treatment[129]. MCC950 treatment also abrogated *Tet2*-dependent cardiomyocyte hypertrophy and resultant heart failure[99]. The same group also found that haematopoietic Tet2 deficiency increased the expression of IL-1β in white adipose tissue and led to systemic insulin resistance, and that these effects were suppressed upon NLRP3 inhibition with MCC950[113]. However, despite its encouraging activity in preclinical models, clinical development of MCC950 was limited by hepatotoxicity in early phase trials. In a study of osteoarthritis, researchers found that genetic knockdown of *DNMT3A* in chondrocyte and osteoblast cell lines led to promoter hypomethylation of C terminal binding protein genes *CtBP1* and *CtBP2*, resulting in

overexpression of CtBP and transcriptional upregulation of NLRP3[134]. Furthermore, single cell sequencing of peripheral blood cells from patients with heart failure with DNMT3A-mutated CH showed upregulation of NLRP3 and IL-1β expression in monocytes[135]. These findings suggest that *DNMT3A* loss-of-function mutations may directly stimulate priming of the NLRP3 inflammasome via transcriptional changes.

In addition to studies of the NLRP3 inflammasome, several reports have implicated a wide range of molecular mechanisms and myeloid cell types involved in CH-related inflammation[131,136–146] (Fig. 2). For example, haematopoietic *Jak2* V617F mutation also promotes atherosclerosis in hyperlipidemic mice[147,148]. However, unlike *Tet2* and *Dnmt3a* knockout models of CH, macrophage-specific gain of JAK2 function led to enrichment of mutant macrophages within atherosclerotic plaques. *Jak2* gain-of-function macrophages secrete higher levels of IL-1β via activation of the AIM2 inflammasome in response to DNA damage caused by oxidative stress[148]. *Trp53*-mutated CH aggravates doxorubicin-related cardiotoxicity via the recruitment of p53-deficient neutrophils, which showed upregulation of genes encoding inflammasome proteins and chemokines[149]. In a study of osteoporosis, one of the most frequent complications of chronic inflammation, *Dnmt3a* loss promoted osteoclast differentiation and subsequent bone loss via IRF3–NF-κB-mediated upregulation of IL-20[108]; interestingly, increased osteoclastogenesis and decreased bone mass were also observed in the setting of *Asxl1* deficiency[137]. *Dnmt3a*-deficient macrophages showed impaired production of type I IFNs after viral infection or stimulation with various Toll-like receptor agonists; in this context, mice with myeloid cell-specific loss of *Dnmt3a* were also more susceptible to vesicular stomatitis virus infection[145]. Altogether, these findings indicate that different genetic drivers in CH promote cardiovascular and other inflammatory phenotypes through various mechanistic pathways to dysregulate the immune response.

## Mechanisms of lymphoid dysregulation in clonal haematopoiesis

CH mutations in myeloid malignancy-associated genes can be detected in both myeloid and lymphoid cell subsets[150,151], consistent with the occurrence of these mutations in HSCs[151–155]. Among those with CH mutations in lymphoid cells, *DNMT3A* and *TET2* mutations have been observed most frequently, consistent with their prevalence, and the VAF in lymphoid cells is lower compared with myeloid cells from the same individual. Interestingly, whereas *DNMT3A* mutations are detectable in myeloid, NK, B and T cells, *TET2* mutations appear to be absent from T cells[150,151], indicating that different CH mutations may undergo differential selection depending on the haematopoietic lineage. It has long been appreciated that epigenetic modifications play a key role in lymphocyte gene expression[156–161]. Moreover, studies in both humans and mice have clearly demonstrated that DNMT3A and TET2 regulate lymphocyte differentiation and function, suggesting that lymphoid cells with *DNMT3A* or *TET2* mutations may contribute to CH-associated phenotypes in humans.

### [H2] TET2 and DNMT3A in lymphocyte differentiation and function.

Insight into the roles of TET2 and DNMT3A in lymphocytes has come from analysis of mouse models. In mice with T cell-specific *Tet2* or *Dnmt3a* deficiency, T cell subsets

develop normally and control of acute viral infection with lymphocytic choriomeningitis virus (LCMV) is unaffected[162–164]. However, *Tet2* or *Dnmt3a* deficiency is associated with skewing of $CD8^+$ T cells towards a central memory phenotype with relative depletion of short-lived effector cells. This change in differentiation is T cell-intrinsic and leads to increased cytolytic function and control of viral infection upon re-challenge[163–166] (Fig. 3). Aberrant cytokine production may also contribute to altered T cell function in the setting of *Tet2* or *Dnmt3a* deficiency. Although *Tet2* or *Dnmt3a* deficiency does not affect the potential of naive $CD4^+$ T cells to differentiate into T helper 1 ($T_H1$), T helper 2 ($T_H2$), T helper 17 ($T_H17$) or regulatory T ($T_{reg}$) cell subsets *in vitro*[162,167], *Tet2*-deficient $T_H1$ and $T_H17$ cells produce less IFNγ and IL-17A, respectively; *Dnmt3a*-deficient $CD4^+$ T cells produce IFNγ aberrantly across all subsets generated *in vitro*[162,167,168] and increased IL-13 upon $T_H2$ cell differentiation *in vitro* and *in vivo*[169] (Fig. 3).

Peripheral B cells and innate lymphoid cell (ILC) subsets also develop normally in unmanipulated mice with cell type-specific deficiency in *Tet2* or *Dnmt3a*[162,170–172]. Upon model antigen exposure, *Tet2*-deficient B cells develop a cell-intrinsic increase in germinal centre B cells with impaired capacity to exit the germinal centre reaction, resulting in reduced plasma cell generation and lower circulating antigen-specific antibodies[173] (Fig. 3). By contrast, *Dnmt3a*-deficient B cells respond normally to model antigen exposure but develop an aggressive CLL-like disease with high penetrance[170,171]. In NK cells, *Tet2* is dispensable for activation and maturation during murine cytomegalovirus infection, although cytolytic protein expression and activity against target tumour cells is modestly reduced[172]. Though the abundance of ILC subsets is unchanged in *Tet2*-deficient mice, IL-17A production by inflammatory group 2 ILC and group 3 ILC subsets is significantly reduced in the absence of *Tet2*, similar to $T_H17$ cells. *Tet2*-deficient group 3 ILCs also produce less IL-22 and are less effective in controlling infection by the enteric bacterial pathogen *Citrobacter rodentium*[172].

Mechanistically, it has become clear that *Tet2* and *Dnmt3a* affect the expression and genomic localization of master transcription factors in lymphocytes after antigen stimulation, leading to alterations in cell fate decisions[162,163,171–173]. For example, mice with T cell-specific deletion of *Dnmt3a* show increased $CD8^+$ T cell expression of *Tcf7*, a transcription factor known to drive memory differentiation[174]; increased *Tcf7* expression is associated with reduced methylation at the *Tcf7* promoter, and knockdown of *Tcf7* partially rescues the differentiation of effector $CD8^+$ T cells from *Dnmt3a* knockout[164–166,175]. In comparison, *Tet2*-deficient T cells display increased expression of a different transcription factor, *Eomes*, also involved in the differentiation of memory $CD8^+$ T cells[176]. Finally, in mice with B cell-specific deletion of *Tet2*, reduced expression of the plasma cell-defining transcription factor *Prdm1* correlated with hypermethylation of the *Prdm1* gene locus and impaired plasma cell differentiation[173] (Fig. 3).

Epigenetic changes to select cytokine loci in lymphocytes also correlate with altered production of T cell and ILC signature cytokines. In $CD4^+$ T cells differentiated *in vitro*, TET2 co-localizes on chromatin with the $T_H1$ cell-defining transcription factor T-BET or the $T_H17$ cell-defining transcription factor RORγt to promote targeted DNA hydroxymethylation and expression of IFNγ or IL-17A, respectively[162]. Conversely,

DNMT3A binding and methylation within the *Ifng* gene locus in $T_H2$-, $T_H17$- and $T_{reg}$-polarized $CD4^+$ T cells is associated with restrained IFNγ production[167] (Fig. 3).

Collectively, these data indicate that *Tet2*- and *Dnmt3a*-mediated changes in the epigenetic landscape can promote aberrant lymphocyte differentiation and function (Fig. 3). The extent and role of altered lymphocyte differentiation and function in CH-associated human disease remains largely unclear.

## Clonal haematopoiesis and antitumour immunity.

Recent observations indicate that CH can affect outcomes in patients receiving cellular therapies for haematological malignancies. Two independent studies of patients undergoing allogeneic HSC transplant for haematological malignancies reported that the presence of *DNMT3A* mutant CH in the donor cell product was associated with reduced risk of disease relapse and increased risk of chronic graft-versus-host disease (GVHD)[152,155]. Interestingly, CH-associated clinical outcomes were dependent on GVHD prophylaxis, as a T cell-depleting post-transplant cyclophosphamide but not calcineurin inhibitor-based regimen, completely abrogated the effect of donor *DNMT3A* mutant CH on disease relapse and chronic GVHD[152]. Donor-derived myeloid cells involved in T and NK cell activation might also play a role in the observed effects of donor CH on clinical outcomes in allogeneic transplant. Indeed, recipients of products with *DNMT3A* mutant donor CH had elevated serum levels of IL-12p70[152], which is known to promote the differentiation of IFNγ-producing $CD4^+$ T cells and the cytolytic activity of $CD8^+$ T cells and NK cells[177–180].

CH has also been linked to clinical outcomes in patients receiving chimeric antigen receptor (CAR) T cell therapy for haematological malignancies. The presence of CH was associated with increased rates of cytokine release syndrome and neurotoxicity severity in patients receiving CAR T cell therapy for non-Hodgkin lymphoma[181,182]. Lentiviral integration-mediated disruption of loci encoding genes mutated in CH has also been associated with cell-intrinsic alterations in CAR T cell expansion and differentiation[183–185]. In a patient receiving anti-CD19 CAR T cell therapy for CLL, lentiviral integration into one *TET2* allele, on the background of a heterozygous germline *TET2* variant associated with reduced catalytic activity on the other allele, led to dramatic clonal outgrowth with increased central memory $CD8^+$ T cell differentiation, suggesting that the effects *TET2* disruption in CAR T cells were the result of biallelic impairment in TET2 function[183]. Phenotypically, *ex vivo* $CD8^+$ CAR T cells expressed higher levels of the proliferation marker Ki-67, the cytolytic protein granzyme B and the transcription factor EOMES (Fig. 3); in addition, levels of the senescence marker KLRG1 were reduced[186]. Notably, knockdown of *TET2*[183] or knockout of *DNMT3A*[187] in experimental models of CAR T cells also led to increased differentiation of central memory $CD8^+$ T cells and antitumour responses dependent on CAR stimulation (Fig. 3), suggesting that inhibition of TET2 or DNMT3A activity could be exploited to enhance the efficacy of CAR T cell therapy.

## Future directions

The link between CH and various clinical phenotypes has revealed that immune cells harbouring clonal mutations drive immune dysfunction and age-related diseases in humans. Although the cell lineages and molecular mechanisms involved in aberrant immune function have been partially elucidated in mouse models, there are many outstanding questions related to the cell biology, therapeutic implications and range of immune phenotypes associated with CH.

CH-associated genetic alterations are detectable in both myeloid and lymphoid cells, but it is unclear how the cell-intrinsic effects of a mutation alter immunological interactions with cells that do not have a mutation. In addition, the manner in which cells without clonal mutations participate in driving inflammation and disease in the setting of CH is unknown. Among most individuals with detectable CH, only a small fraction of peripheral blood cells has clonal mutations, and wild-type cells may also contribute to immunopathology. Alternatively, it is possible that enrichment of immune cells with clonal mutations in tissues allows for a relatively small population of cells to drive inflammation and tissue injury independently of wild-type cells.

Several studies have implicated activation of the NLRP3 inflammasome and increased IL-1β production as causal drivers of non-malignant disease observed in human CH with *TET2* mutations and mouse models of CH with *Tet2* mutations[78,97,99,103,105,113,129,188,189]. In this context, the existence of pharmacological inhibitors and monoclonal antibodies against multiple components of the inflammasome pathway highlights the possibility of treating CH-associated non-malignant conditions using targeted anti-inflammatory therapies. Indeed, among patients with cardiovascular disease who received the IL-1β neutralizing antibody canakinumab, those with *TET2* mutant CH showed a greater reduction in incident major adverse cardiovascular events compared with those with non-*TET2* mutant CH and those without any CH[190]. This finding not only suggests that specific CH-associated mutations may serve as a prognostic biomarker for the efficacy of targeted anti-inflammatory therapies, but also that CH-associated mutations in different genes may require different anti-inflammatory therapies.

Given the distinct cytokine and immune cell profiles observed among individuals with different CH-associated mutations[78], it is likely that the inflammatory mechanisms driving non-malignant disease are correlated with the mutated gene. Whether other therapies targeting NLRP3, IL-1β, AIM2 or IL-6 would be effective in treating active CH-associated inflammation has yet to be tested. With the advent of CH-focused clinical programmes[191], the role of prophylactic therapy for patients with CH but without evidence of frank disease also remains to be addressed. Importantly, the use of any therapies in the setting of CH alone will require accurate predictive algorithms to identify individuals at highest risk of developing clinically significant malignant and/or inflammatory disease.

Recently, other diseases driven by pathological immune dysfunction have been associated with somatic mutations in haematopoietic cells. Singh et al. reported that autoreactive B cell clones from patients with Sjogren’s syndrome-associated cryoglobulinemic vasculitis

harboured mutations in genes recurrently mutated in B cell lymphomas, despite the absence of malignancy at the time of analysis[192]. Likewise, Beck et al. recently described a severe autoinflammatory condition called VEXAS syndrome, which occurs in late adulthood and is genetically defined by recurrent somatic mutations in *UBA1*[193]. Whether there are other autoimmune conditions defined by pathogenic somatic mutations in haematopoietic cells remains to be discovered.

## Competing interests

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**Box 1.**

## Inflammageing

Ageing is accompanied by gradual changes in the immune landscape. Adaptive immune function declines: B cells display reduced antibody diversity, and T cells develop a more restricted T cell receptor repertoire, impaired effector cell function and skewed memory to effector cell ratio[195–197]. The result is an overall impaired response to antigenic challenge known as immunosenescence. On the other hand, innate immune responses progress to a chronic baseline activity level, characterized by increased levels of circulating proinflammatory cytokines and low-level infiltration by innate immune cells, such as neutrophils and macrophages. This condition of persistent low-grade sterile inflammation is termed inflammageing[198].

Inflammageing is largely mediated by the innate immune system, which mounts immediate, nonspecific immune defenses in response to a wide range of pathogens via the recognition of external pathogen-associated molecular patterns (PAMPs) and intrinsic damage-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs). Stimulation of PRRs leads to the formation of macromolecular platforms, known as inflammasomes, which activate caspase 1-mediated processing of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. During ageing, increasing PAMP and DAMP signals resulting respectively from gut epithelial barrier changes and accumulating genomic damage and oxidative stress induce Toll-like receptor- and inflammasome-mediated inflammatory responses. Senescent cells acquire a hypersecretory phenotype consisting of increased expression of proinflammatory cytokines, chemokines, angiogenic factors, proteases and matrix metalloproteinases, known as the senescence associated secretory phenotype (SASP)[199]. The expression of proinflammatory cytokines IL-6 and IL-8 is a consistent feature of the SASP and constitutes a significant driver of inflammageing by priming the transcription of the NLRP3 inflammasome in an age-dependent manner.

Several studies have shown that ageing haematopoietic stem cells (HSCs) upregulate NLRP3 inflammasome activity. Aged HSCs demonstrate heightened NLRP3-dependent inflammatory responses to lipopolysaccharide and ATP stimulation compared with young HSCs due to increased mitochondrial oxidative stress[200]. Similarly, NLRP3 priming and activation are exaggerated in aged human monocytes[201]. In a timecourse of ageing, mice overexpressing the proinflammatory molecule S100A9 develop increasing NLRP3 activation in bone marrow cells between 2 to 11 months of age[202]. These studies reveal that inflammageing occurs within both differentiated and undifferentiated haematopoietic compartments.

Broader health implications of inflammageing include an increased susceptibility to latent common viral infections, such as by cytomegalovirus, herpes simplex virus 1 and varicella zoster virus (VZV). Inflammageing also attenuates vaccination responses, for example by promoting skin recruitment of peripheral blood monocytes in response to a VZV challenge, leading to higher levels of T cell-suppressing cyclooxygenases and prostaglandins and reduced VZV-specific T cell responses[203]. Furthermore, many chronic

diseases of ageing, such as metabolic syndrome and atherosclerosis, rheumatic and neurodegenerative diseases, osteoporosis and cancer, are involved in a maladaptive cycle of immune cell infiltration, tissue damage and release of proinflammatory signals[204]. Many of these diseases are also associated with haematological neoplasia, including clonal haematopoiesis, likely through dysregulated immune signalling by infiltrating haematopoietic cells.

**Box 2.**

### Clonal evolution of haematological neoplasms

In normal haematopoiesis, haematopoietic stem cells differentiate into multipotent progenitors, which eventually give rise to all differentiated cell lineages comprising blood. This cellular hierarchy becomes disrupted in malignant transformation, during which somatic mutations cause an impairment in cell differentiation together with enhanced survival and proliferation. The serial acquisition of driver genetic mutations and chromosomal aberrations in these cancer-initiating clones have been reported in significant detail in several recent studies[205–208]. Mutation selection in pre-leukemic cells results in an iterative process of clonal expansion and genetic complexity, favouring the accumulation of further mutations and the development of disease. Many of these mutation events are reproducible both in the genes affected and the temporal sequence in which they occur. For example, myeloid diseases are typified by recurrent, early mutations in chromatin modifiers (*DNMT3A*, *TET2*, *ASXL1*, *IDH1, IDH2* and *EZH2*) and RNA splicing machinery (*SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2*). Mechanistically, DNMT3A catalyzes de novo DNA methylation whereas TET2 oxidizes methylated CpG to 5-hydroxymethyl cytosine (5-hmC) facilitating further modification to unmodified cysteine; mutations thus cause dysregulated DNA methylation and demethylation, respectively, altering the expression of genes involved in proliferation, differentiation and inflammation via chromatin-level changes[209,210]. Mutations involving the DNA damage response (*TP53* and *PPM1D*) are strongly associated with therapy-related myeloid neoplasms arising in individuals who had received chemotherapy in the past, indicating a distinct pathogenetic mechanism in this group. With large scale sequencing analyses now being commonplace, multiple studies have demonstrated that these driver mutations, especially those occurring early in disease, are shared with pre-leukemic myeloid conditions such as myelodysplastic syndrome and myelodysplastic or myeloproliferative neoplasms, emphasizing the progressive nature of haematological cancers[66,68,194,211]. In later stages of acute myeloid leukemia (AML) leukemogenesis, mutations in signal transduction genes (*KRAS*, *NRAS*, *CBL*, *JAK2, GNB1, KIT*, *FLT3* and *NPM1*) and myeloid transcription factors (*EVI1*, *CEBPA* and *RUNX1*) predominate[77,212].

The recognition that clonal haematopoiesis bearing somatic mutations is common among older individuals has established an early signpost in myeloid neoplasia[88,213]. A formal definition has been established for recognizing clonal haematopoiesis of indeterminate potential (CHIP) as a clinical entity characterized by the presence of somatic mutations in genes associated with haematological malignancies at greater than 2% allele fraction in asymptomatic individuals[214–217]. With the improvement in sequencing capability, it is now possible to detect clonal haematopoiesis at much greater sensitivity[218]. However, it is unlikely that these diminutive clones will result in either disease progression or significant morbidity. Early outcome studies based on CHIP ≥2% variant allele frequency (VAF) showed a progression rate of 0.5–1% per year[76]. In this context, CHIP is analogous to such asymptomatic precursor lesions as high-count monoclonal B cell lymphocytosis and monoclonal gammopathy of undetermined significance, which

respectively precede chronic lymphocytic leukemia and small lymphocytic lymphoma and multiple myeloma.

Figure legend: *The rate of progression from CHIP to a myeloid neoplasm varies across different genes. For example, multiple studies have reported that mutations in splicing factor genes or *TP53* confer a higher risk of progression[83,86–88].

**Among individuals who develop myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), or either disorder after CHIP, the rate of progression to acute myeloid leukemia (AML) varies significantly depending on the identity and characteristics of the pre-AML diagnosis. For example, over a one-year follow-up period, the rate of transformation to AML is 1–2% among patients with the MPN essential thrombocythemia[219] but more than 30% among patients with high-risk MDS[220].

***The rate of progression from smouldering multiple myeloma (SMM) to multiple myeloma (MM) varies significantly depending on specific SMM disease characteristics, including M protein quantity, percentage of monoclonal plasma cells in the bone marrow and disease cytogenetics[221]. Over a two-year follow-up period, patients with SMM with low-risk features have a progression rate of less than 10%, whereas those with high-risk features have a progression rate greater than 60%.

**Box 3.**

### *DNMT3A* and *TET2* mutations in lymphoid malignancies

Although typically associated with the development of myeloid neoplasia, recurrent somatic mutations in the epigenetic modifiers *DNMT3A* and *TET2* occur commonly in lymphoid malignancies characterized by autoimmune features, including angioimmunoblastic T cell lymphoma (AITL)[222–225], clonal lymphoproliferative disorders of NK cells (CLPD-NK)[226], large granular lymphocyte (LGL) leukemias[227], T cell acute lymphoblastic leukemia (T-ALL)[228–230], and extranodal NK/T cell lymphoma (ENKTL)[231] and diffuse large B cell lymphoma (DLBCL)[232,233].

In some cases, lymphoid neoplasia appears to develop from an haematopoietic stem cell (HSC) carrying a *DNMT3A* or *TET2* mutation. In AITL, *TET2* mutations have been detected in $CD34^+$ progenitor cells with both myeloid and lymphoid differentiation potential[225]. Similarly, *TET2* mutations in patients with CLPD-NK have been detected in both NK and myeloid cells[226]. Interestingly, families with germline heterozygous *TET2* or *DNMT3A* mutations develop predominantly myeloid neoplasms[234–238], whereas rare individuals with germline homozygous *TET2* mutations develop either B or T cell lymphomas along with a lymphoproliferative syndrome characterized by autoimmune cytopenias and immunodeficiency[239]. Finally, several studies have described co-occurrence of lymphoid and myeloid malignancies harbouring *DNMT3A* or *TET2* mutations within the same patient[226,240,241]. Altogether, these observations strongly suggest that *DNMT3A* or *TET2* mutant HSCs can give rise to both lymphoid and myeloid malignancies; the molecular basis of initiating one or both malignant outcomes has yet to be fully elucidated.

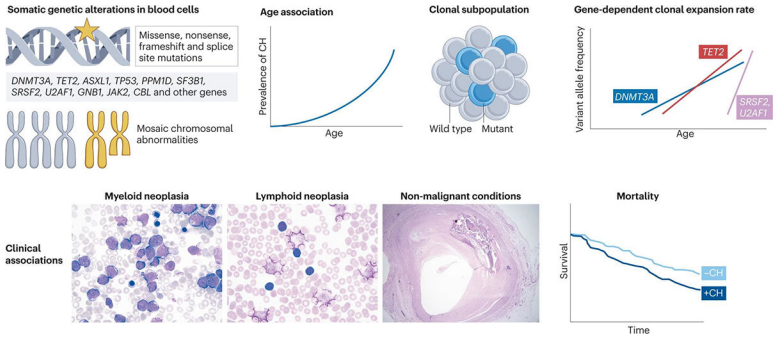


**Figure 1 |. Features of clonal haematopoiesis.**

Analysis of human genetic data has led to the discovery of somatic mutations in peripheral blood cells. These mutations comprise non-synonymous variants in genes frequently altered in myeloid malignancies (for example, *DNMT3A*, *TET2* and *ASXL1*) as well as mosaic chromosomal abnormalities. These genetic alterations occur in a clonal subpopulation of blood cells and are detected more frequently in older individuals. Mutations in different genes tend to follow unique trajectories of clonal expansion over time[88–90,194], implying distinct mechanisms of positive selection for different mutations. Clonal haematopoiesis has also been linked to several clinical phenotypes, including incident myeloid and lymphoid neoplasms, non-malignant diagnoses (such as atherosclerosis) and overall mortality. Images courtesy of the Department of Pathology at the Brigham and Women's Hospital, Boston, USA.

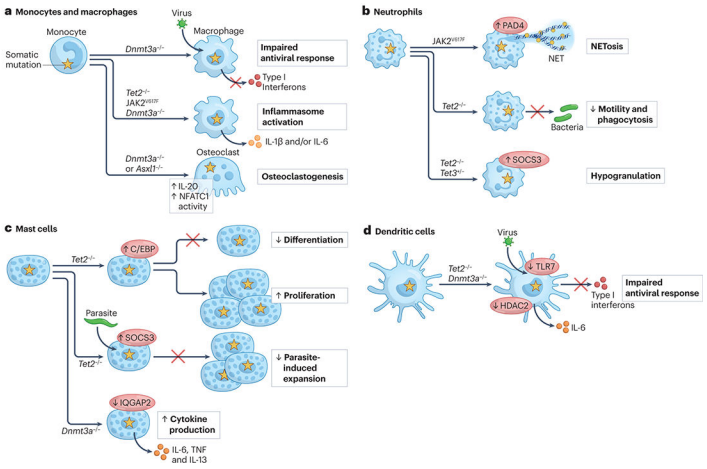


**Figure 2 |. Functional effects of clonal haematopoiesis-associated mutations in myeloid cells.**

Evidence from mouse models and human observational studies indicate that mutations in genes associated with clonal haematopoiesis can affect the differentiation and function of mature myeloid cells. a | In monocytes and macrophages, the effects of genetic alterations in *Tet2*, *Dnmt3a* and *Jak2* have been studied most extensively, revealing increased chemokine expression, hyperactivation of the inflammasome and increased IL-1β as common features[97,129–131,136,148,188]. In response to viral infection and Toll-like receptor (TLR) stimuli, *Dnmt3a* mutant macrophages produce less type I interferons (IFNs), correlating with reduced survival of virally-infected mice with *Dnmt3a*-deficient myeloid cells[145]. Elevated myeloid production of IL-20 in the setting of *Dnmt3a* deficiency is associated with increased in osteoclastogenesis and osteoporosis[108], whereas *Asxl1* loss in myeloid cells correlates with increased NFATc1 activity and osteoclast differentiation[137]. b | In neutrophils, JAK2 V617F is associated with neutrophil extracellular trap (NET) formation and thrombosis in both mouse and human studies[138,139]. Mouse and zebrafish models have also demonstrated a role for *Tet2* and *Tet3* in neutrophil mobility, phagocytosis and granule formation[140]. c | In mast cells, *Tet2* loss has contrasting effects – either suppressing mast cell differentiation and stimulating mast cell proliferation via C/EBP transcription factors[141], or suppressing mast cell expansion in response to Schistosoma infection[142]. Mast cells lacking *Dnmt3a* demonstrated higher cytokine production upon acute stimulation[143], whereas *Asxl1* truncating mutations promote mast cell differentiation in a multipotent haematopoietic precursor cell line[146]. d | In dendritic cells, *Tet2* loss resulted in higher IL-6 levels and reduced type I IFN production in response to challenge with herpes simplex virus or vesicular stomatitis virus (VSV)[131,144]. Similarly, *Dnmt3a* loss reduces type I IFN production in response to VSV[145].

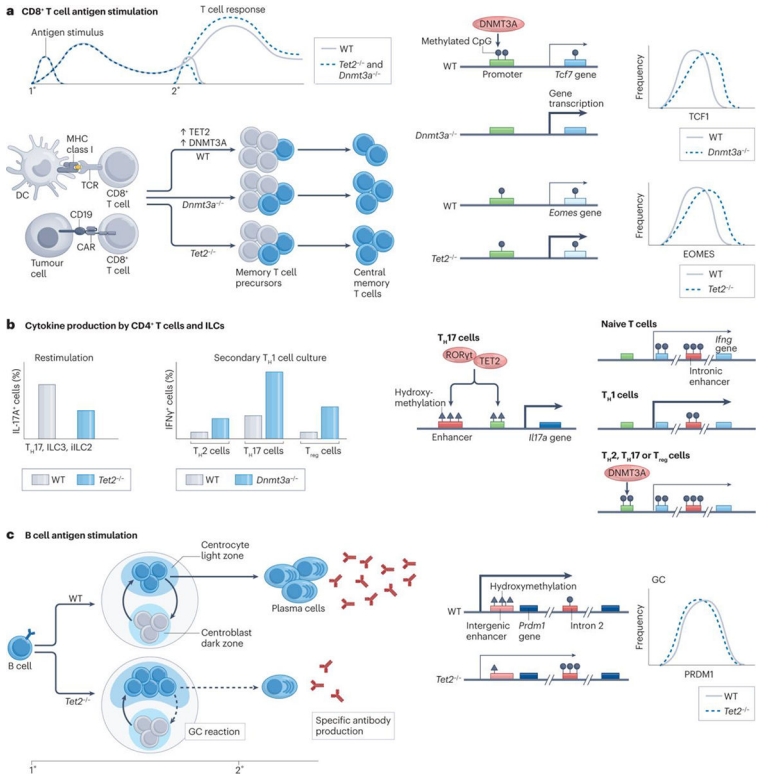


**Figure 3 |. Impact of TET2 and DNMT3A loss in lymphocytes.**

a | In antigen-stimulated mouse CD8$^+$ T cells and human chimeric antigen receptor (CAR) T cells, deficiency in DNMT3A or TET2 is associated with normal primary immune responses but enhanced secondary responses with increased memory precursor and central memory T cell differentiation[163–166,183,187]. These phenotypic changes correlate with increased expression of the master transcription factors TCF1 (encoded by *Tcf7*) or EOMES in absence of DNMT3A or TET2, respectively[164–166,183,187]. While TET2 deficiency does not result in significant methylation changes at the *Eomes* locus[163], DNMT3A deficiency leads to a reduction in *Tcf7* promoter methylation, consistent with DNMT3A localization to the *Tcf7* promoter in DNMT3A wild-type T cells[164]. b | In mouse CD4$^+$ T cells and innate lymphoid cell (ILC) subsets, TET2 is recruited to the *Il17a* enhancer CNS2 via RORγt, leading to increased enhancer and promoter hydroxymethylation and increased *Il17a* expression[162]. In this context, IL-17A production by T helper 17 ($T_H17$) cells, group 3 innate lymphoid cells (ILC3s) and inflammatory group 2 innate lymphoid cells (iILC2s) is significantly reduced in the setting of TET2 deficiency[162,172]. Similarly, DNMT3A localization to the *Ifng* promoter in T helper 2 ($T_H2$) cells, T helper 17 ($T_H17$) cells and regulatory T ($T_{reg}$) cells generated *in vitro* is associated with increased methylation and reduced *Ifng* expression; reduced *Ifng* methylation in DNMT3A-deficient $T_H2$, $T_H17$ and $T_{reg}$ cells leads to aberrant IFNγ production by all three subsets upon restimulation with the $T_H1$ cytokine IL-12[167]. c | In B cells, TET2 deficiency during primary immunization is associated with accumulation of germinal centre B cells, reduced expression of the plasma cell-defining transcription factor PRDM1, impaired plasma cell differentiation and decreased production of antigen-specific antibodies upon secondary immunization[173]. Reduced PRDM1 expression in the absence of TET2 correlates with hypermethylation

within *Prdm1* intron 2, which contains a locus that undergoes progressive demethylation during plasma cell differentiation, as well as decreased hydroxymethylation of an intergenic enhancer.

**Table 1 |**

Non-malignant conditions associated with clonal haematopoiesis of indeterminate potential

| Diagnosis | Patient cohorts | Odds ratio | Mouse models | *In vivo* mouse phenotypes | Refs. |
|---|---|---|---|---|---|
| Alzheimer disease | TOPMed, ADSP | ~0.5–0.8 | NA | NA | 117 |
| Atherosclerotic cardiovascular disease | TOPMed, UKB | ~1.1–1.5 | *Tet2*$^{-/-}$ bone marrow → *Ldlr*$^{-/-}$mice fed a high fat diet (myeloid-restricted *Tet2*$^{-/-}$sufficient mice) | ↑plaque size; ↑IL-1β, CXCL1, CXCL2, CXCL3 | 76,81,82,84,97,129 |
| Atrial fibrillation | UKB | ~1.1–1.2 | NA | NA | 83 |
| Heart failure | TOPMed, UKB | ~1.1–1.5 | *Tet2*$^{-/-}$ bone marrow → wild-type mice with subsequent vascular ligation or constriction *Tet2* or *Dnmt3a* CRISPR-Cas9 edited bone marrow → wild-type mice treated with angiotensin II (myeloid-restricted *Tet2*$^{-/-}$sufficient mice) | ↑systolic dysfunction; ↓ejection fraction; ↑fibrosis; ↑IL-1β, CCL2, CCL5 | 99,100 |
| Cerebrovascular accidents | TOPMed, UKB, MGBB | ~1.1–1.2 | NA | NA | 116 |
| Chronic kidney disease | UKB | ~1.1–1.5 | *Tet2* or *Dnmt3a* CRISPR-Cas9 edited bone marrow → wild-type mice treated with angiotensin II | ↑fibrosis | 101,102 |
| Chronic liver disease | FHS, ARIC, UKB, MGBB | ~2–4 | *Tet2*$^{-/-}$ or *Dnmt3a*$^{-/-}$ bone marrow → *Ldlr*$^{-/-}$ mice fed a high fat diet (myeloid-restricted *Tet2*$^{-/-}$ sufficient mice) | ↑steatohepatitis; ↑fibrosis; ↑IL-6, CXCL1, CCL22 | 103 |
| Chronic obstructive pulmonary disease | TOPMed, ICGN, EOCOPD, UKB | ~1.5–2 | *Tet2*$^{-/-}$ bone marrow → wild-type mice exposed to cigarette smoke and poly(I:C) | ↑emphysema; ↑IFNγ and type I IFNs | 104 |
| Gout | UKB, MGBB | ~1.2–1.7 | *Tet2*$^{-/-}$ bone marrow → wild-type mice injected with monosodium urate crystals in the footpad | ↑paw oedema; ↑IL-1β, CCL5, CCL22 | 105 |
| Osteoporosis | UKB | ~1.2–1.5 | *Tet2*$^{-/-}$, *Dnmt3a*$^{-/-}$ or *Dnmt3a* R878H bone marrow → *Ldlr*$^{-/-}$mice fed a high fat diet or wild-type aged mice fed a regular diet | ↓bone mass; ↑osteoclasts | 108 |
| Premature menopause | UKB, WHI | ~1.1–1.7 | NA | NA | 109 |

ARIC, Atherosclerosis Risk in Communities; ADSP, Alzheimer's Disease Sequencing Project; CCL, CC-chemokine ligand; CXCL, CXC-chemokine ligand; EOCOPD, Boston Early-Onset COPD Study; FHS, Framingham Heart Study; ICGN, International COPD Genetics Network; IFN, interferon; MGBB, Mass General Brigham Biobank; NA, not available; TOPMed, Trans-Omics for Precision Medicine program; UKB, UK Biobank; WHI, Women's Health Initiative.