



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Veiga, CB;Lawrence, EM;Murphy, AJ;Herold, MJ;Dragoljevic, D

Title:

Myelodysplasia syndrome, clonal hematopoiesis and cardiovascular disease

Date:

2021-04-02

Citation:

Veiga, C. B., Lawrence, E. M., Murphy, A. J., Herold, M. J. & Dragoljevic, D. (2021). Myelodysplasia syndrome, clonal hematopoiesis and cardiovascular disease. *Cancers*, 13 (8), <https://doi.org/10.3390/cancers13081968>.

Persistent Link:

<https://hdl.handle.net/11343/278139>

License:

CC BY

Review

Myelodysplasia Syndrome, Clonal Hematopoiesis and Cardiovascular Disease

Camilla Bertuzzo Veiga ^{1,2} , Erin M. Lawrence ^{3,4} , Andrew J. Murphy ^{1,5,6}, Marco J. Herold ^{3,4} and Dragana Dragoljevic ^{1,5,6,*}

- ¹ Division of Immunometabolism, Baker Heart and Diabetes Institute, Melbourne, VIC 3004, Australia; camillabertuzzo.veiga@baker.edu.au (C.B.V.); andrew.murphy@baker.edu.au (A.J.M.)
² Department of Anatomy and Physiology, University of Melbourne, Parkville, Melbourne, VIC 3010, Australia
³ Walter and Eliza Hall Institute of Medical Research, 1 G Royal Parade, Parkville, Melbourne, VIC 3052, Australia; lawrence.e@wehi.edu.au (E.M.L.); herold@wehi.edu.au (M.J.H.)
⁴ Department of Medical Biology, University of Melbourne, Parkville, Melbourne, VIC 3052, Australia
⁵ Department of Diabetes, Department of Immunology, Monash University, Clayton, VIC 3004, Australia
⁶ Baker Department of Cardiometabolic Health, University of Melbourne, Melbourne, VIC 3052, Australia
* Correspondence: dragana.dragoljevic@baker.edu.au

Simple Summary: The development of blood cancers is a complex process that involves the acquisition of specific blood disorders that precede cancer. These blood disorders are often driven by the accumulation of genetic abnormalities, which are discussed in this review. Likewise, predicting the rate of progression of these diseases is difficult, but it appears to be linked to which specific gene mutations are present in blood cells. In this review, we discuss a variety of genetic abnormalities that drive blood cancer, conditions that precede clinical symptoms of blood cancer, and how alterations in these genes change blood cell function. Additionally, we discuss the novel links between blood cancer development and heart disease.



Citation: Veiga, C.B.; Lawrence, E.M.; Murphy, A.J.; Herold, M.J.; Dragoljevic, D. Myelodysplasia Syndrome, Clonal Hematopoiesis and Cardiovascular Disease. *Cancers* **2021**, *13*, 1968. <https://doi.org/10.3390/cancers13081968>

Academic Editor: Adriano Venditti

Received: 18 March 2021

Accepted: 14 April 2021

Published: 19 April 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: The development of myelodysplasia syndromes (MDS) is multiphasic and can be driven by a plethora of genetic mutations and/or abnormalities. MDS is characterized by a hematopoietic differentiation block, evidenced by increased immature hematopoietic cells, termed blast cells and decreased mature circulating leukocytes in at least one lineage (i.e., cytopenia). Clonal hematopoiesis of indeterminate potential (CHIP) is a recently described phenomenon preceding MDS development that is driven by somatic mutations in hemopoietic stem cells (HSCs). These mutant HSCs have a competitive advantage over healthy cells, resulting in an expansion of these clonal mutated leukocytes. In this review, we discuss the multiphasic development of MDS, the common mutations found in both MDS and CHIP, how a loss-of-function in these CHIP-related genes can alter HSC function and leukocyte development and the potential disease outcomes that can occur with dysfunctional HSCs. In particular, we discuss the novel connections between MDS development and cardiovascular disease.

Keywords: myelodysplasia syndrome; clonal hematopoiesis and indeterminate potential (CHIP); DNMT3A; TET2; P53; ASXL1; JAK2; hematopoietic stem cell (HSC); cardiovascular disease



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Myelodysplasia syndrome (MDS) is primarily a disease of the elderly, with an average age of diagnosis at 70 years of age. MDS is a myeloid neoplasm that originates in hematopoietic stem cells (HSCs), resulting in dysregulation of hematopoiesis, bone marrow (BM) dysplasia, peripheral blood cytopenia (reduced blood cells) and predisposition to the development of acute myeloid leukemia (AML) [1,2]. Furthermore, patients with MDS generally die of conditions related to MDS, such as increased bleeding or susceptibility to infections due to the cytopenia's, or the progression to AML. However, patients can also

have co-morbidities related to ageing, such as cardiovascular disease (CVD), which may have a compounding effect on MDS related complications [3,4].

In this review, we discuss the disorders that precede MDS development, the vast number of genetic abnormalities that can cause MDS and how the dysfunction of MDS-related genes alters HSC activity and the hematological consequences. Additionally, we discuss the novel association of increased CVD in MDS patients.

2. MDS Pathophysiology

MDS results in disordered hematopoiesis as a consequence of genetic abnormalities in HSCs. Hematopoiesis is a tightly regulated and hierarchically structured process of blood production [5] (Figure 1A). HSCs give rise to all blood cells, and hence are classified as having multilineage potential. Generally speaking, HSCs can differentiate into either common myeloid progenitors (CMPs), which give rise to myeloid cells such as monocytes, neutrophils and platelets, or can differentiate into common lymphoid progenitors (CLPs) which generate B and T-cells.

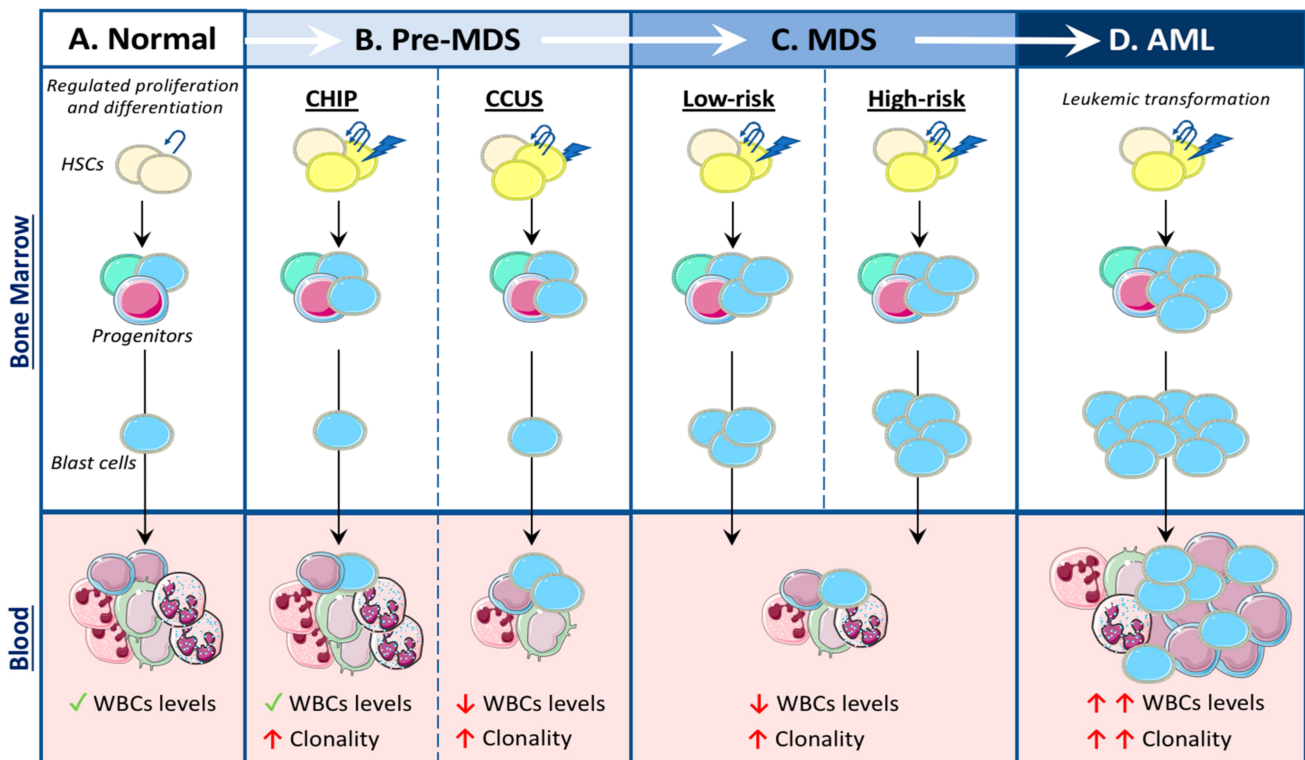


Figure 1. Transformations from normal hematopoiesis to acute myeloid leukemia (AML) development. (A) Normal: Normal hematopoiesis is highly regulated, whereby hematopoietic stem cells (HSCs) can self-renew and differentiate into progenitor cells. Progenitor cells differentiate into immature blood cells (blast cells) and then into mature white blood cells (WBCs). (B) Pre-MDS (myelodysplasia syndrome): Clonal hematopoiesis of indeterminate potential (CHIP) occurs when a HSC acquires a somatic mutation resulting in a proliferative advantage; giving rise to a mutated clone. While the levels of WBCs do not change, there are mutated clonal WBCs detected in the blood. Clonal cytopenia of undetermined significance (CCUS) presents with CHIP, as well as with cytopenia. (C) MDS: The development of significant bone marrow (BM) blast cells, BM dysplasia and cytopenia is usually associated with at least 1 genetic abnormality (either a gene mutation or chromosomal defect). MDS is considered low risk (transformation potential) when blast cell numbers are relatively low, and high risk when BM dysplasia is advanced. (D) AML: A leukemic transformation occurs that results in excessive BM expansion, BM dysplasia and pathological WBC production.

The development of MDS is a multiphasic process. Firstly, an “initiating mutation”, or other genetic abnormality, is acquired in an HSC, which primes the cell but is not sufficient to induce clinical hematological disease. Subsequently, an additional mutation

or multiple mutations are acquired in either the HSC or downstream in the myeloid progenitors, resulting in a proliferative advantage and an impaired differentiation capacity. These mutated cells cause an increase in immature blood cells (i.e., blast cells) and loss of mature circulating leukocytes from one or more lineages (i.e., Cytopenia) due to this differentiation block (Table 1 and Figure 1) [6]. Lastly, MDS can convert to AML when there is a leukemic transformation, drastically increasing HSCs, progenitors, and blast cells; circulating leukocytes; and reducing blood cell differentiation [7]. The factors that drive the acquisition of MDS-related genetic mutations are incompletely understood [8]. Conventional chemoradiotherapy has been shown to cause a therapy- or treatment-related MDS (t-MDS/t-AML) in patients receiving treatment for other malignancies [9–11]. However, for the vast majority of patients, the cause of genetic abnormalities driving MDS is less understood [8,12].

Table 1. MDS, CCUS and CHIP diagnostic criteria.

Diagnostic Criteria for MDS and Pre-Cursor Conditions (Defined by the WHO) [1,7]	
MDS	Persistent cytopenia in one or more peripheral-blood cell lineages and morphologic dysplasia (>10% dysplastic cells) in one or more bone marrow lineages on the basis of morphologic and cytogenetic abnormalities
MDS categories	<p>MDS with:</p> <ul style="list-style-type: none"> • Single-lineage dysplasia • Multilineage dysplasia <ul style="list-style-type: none"> • Ring sideroblasts and single-lineage dysplasia or multilineage dysplasia <ul style="list-style-type: none"> • Isolated del (5q) • Excess blasts type 1 or type 2 <ul style="list-style-type: none"> • Unclassifiable
CCUS	Unexplained cytopenia in one or more peripheral blood cell lineages; a somatic mutation at a variant allele frequency of at least 20% in one or more genes that are recurrently mutated in myeloid neoplasms and insufficient dysplasia (<10%) for an MDS diagnosis
CHIP	Normal peripheral blood cell counts with a somatic mutation at a variant allele frequency of at least 2% in a gene that is recurrently mutated in myeloid neoplasms

2.1. MDS Precursor Conditions

2.1.1. CHIP

Clonal hematopoiesis of indeterminate potential (CHIP) is characterized by the acquisition of somatic mutations in HSCs which provide a competitive advantage over healthy HSCs, leading to an increase in the number of mutated HSCs, progenitors and their mature progeny (Figure 1B) [13–18]. While there is no change in total circulating white blood cells in CHIP, it results in a progressive clonal-specific expansion of WBCs, and ultimately an increased risk of hematological cancers. Additionally, there is significant overlap between mutations identified in CHIP and MDS. The most common genetic mutations affect DNA methyltransferase 3A (DNMT3A), Tet methylcytosine dioxygenase 2 (TET2) and additional sex combs like 1 (ASXL1). Due to the high mutational overlap between CHIP and MDS, CHIP is considered to be a precursor to MDS.

Age is the most clearly defined risk factor for the development of CHIP, with less than 5% of people under the age of 60 carrying a CHIP mutation, and up to 18% of people over the age of 90 testing positive for a CHIP mutation [17,19]. Interestingly, people with CHIP can remain stable with a low variant allele frequency (VAF) and with no clinical symptoms. People with CHIP may go on to develop a myeloid neoplasm such as MDS or AML, or may not develop any malignant disease. However, what drives the transformation from CHIP to MDS/AML is not well-known, but certain mutations have been linked with stronger chance of malignant transformation than others. These are mentioned in this review. Interestingly, whilst mutations in CHIP are strongly linked to hematological cancers

such as MDS/AML, it was recently described that people with CHIP were also at increased risk of death due to atherosclerotic CVD [14,17,19,20].

2.1.2. CCUS and ICUS

When a CHIP mutation is associated with blood cytopenia without, or with very little, change in BM blast numbers or BM dysplasia, it is termed clonal cytopenia of undetermined significance (CCUS; Figure 1B and Table 1) [1]. VAF in CCUS tends to be extremely high, usually greater than 30%, and has also been linked to a higher risk of MDS and AML progression [21,22]. One study calculated a 70% chance of malignant progression in patients with CCUS within 4 years of diagnosis [22]. Furthermore, idiopathic cytopenia of undetermined significance (ICUS) refers to situations when cytopenia is the only symptom identified without evidence of clonal cells or MDS morphology. This is also considered a pre-MDS condition but has a relatively low risk of MDS progression compared to CHIP and CCUS [22]. Therefore, diagnosing these conditions requires multiple parameters, and just the presence of a mutation in the peripheral blood alone cannot determine whether it is CHIP/CCUS (pre-MDS) or MDS without also assessing blood counts and BM characteristics (Table 1).

3. Genetic Abnormalities in MDS Development

MDS is defined as hematological abnormalities that result in increased immature cells (blast cells) and cytopenia in one or more lineages (Figure 1C and Table 1). Multiple genetic mutations and chromosomal abnormalities have been identified to cause MDS, and this diversity in driver mutations is responsible for the phenotypic heterogeneity observed in MDS (Table 2). The genes that are most commonly mutated in MDS are involved in a variety of cellular regulatory functions, including DNA methylation, RNA-splicing, DNA transcription, histone modification, signal transduction and the cohesion complex subunits [7,12,23,24]. Generally, mutations in epigenetic and RNA-splicing processes are termed “driver mutations”, as it is these mutations that tend to dictate what type of clone will outgrow and outcompete (also termed “clonal dominance”) [25–27]. This is combined by other mutations that contribute to clonal proliferation and progression, which eventually lead to MDS and/or AML.

Table 2. Summary of the generic causes of MDS discussed in this review.

Summary of the Genetic Causes of MDS	
Somatic Mutations	
Epigenetic Regulators	<i>TET2, DNMT3A, ASXL1, EZH2</i> genes
RNA Spliceosome	<i>SF3B1, SRSF2, U2AF1</i> genes
DNA Transcription	<i>RUNX1, TP53</i> genes
Signal Transduction Pathways	<i>KRAS, NRAS, JAK2</i> genes
Cohesion complex	<i>SMC3, SMC1A, RAD21, STAG2</i> genes
Germline Mutations	
<i>CEBPA, DDX41, ETV6, GATA2, RUNX1</i> genes	
Inherited Disorders	
Fanconi Anemia	<i>FANC</i> genes
Shwachman-Diamond Syndrome	<i>SBDS</i> genes
Li-Fraumeni Syndrome	<i>TP53</i> gene
Diamond-Blackfan Anemia	<i>GATA1/RPS19</i> genes
Dyskeratosis congenita	Telomerase complex disorder
Chromosomal Abnormalities	
Chromosomal Deletion	del(5q), del(7q), del(20q), del(17q)
Mosaicism syndromes	Trisomy 8

3.1. Somatic Mutations

At the onset of the clinical disease, MDS patients will exhibit an average of two or three mutations. As many as 10 additional genetic mutations can be acquired as the disease progresses [28]. While MDS can occur from alterations in over a hundred genes, mutations in only six genes account for the majority of MDS cases: *TET2*, *ASXL1*, *DNMT3A*, the splicing factor 3b, subunit 1 (*SF3B1*), the serine and arginine-rich splicing factor 2 (*SRSF2*) and the RUNX family transcription factor 1 (*RUNX1*) [6,26,29]. Interestingly, somatic mutations in differing genes are associated with diverse MDS stages, disease progression and clinical outcome [30,31]. For example, mutations in epigenetic regulators, e.g., *TET2* or *DNMT3A*, can remain in the CHIP phase for many years, while modifications in spliceosome genes (e.g., *SF3B1* and *SRSF2*) have a more rapid transformation to MDS [22].

3.1.1. Epigenetic Regulators

DNMT3A, *TET2*, *ASXL1* and the enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*) are the most frequently mutated epigenetic regulators in MDS [24,32–34]. *DNMT3A* and *TET2* regulate DNA methylation/demethylation respectively, which is particularly important in influencing stem-cell renewal, function and differentiation [20,35,36]. *DNMT3A* methylates DNA by transferring methyl groups to specific CpG regions in DNA. Mutations in *DNMT3A* are found in up to 13% of all MDS subtypes, and while mutations occur across the gene, there is strong enrichment for the R882 mutation which affects the catalytic domain of the protein [24,32]. *DNMT3A* R882 mutations tend to occur early in disease progression (i.e., in CHIP) and are associated with a rapid progression to AML and decreased survival [24,32]. On the other hand, mutations in *TET2*, which demethylates DNA by converting 5-methylcytosine (5 mc) to 5-hydroxy-methylcytosine (5 hmc), are associated with a mild clinical prognosis [31,33,34]. Interestingly, if a *TET2* mutation is acquired as the second or later mutation in the development of MDS, it does not appear to stimulate MDS progression further, but does promote monocytic differentiation [33]. *TET2* mutations can occur throughout the entire gene and almost always result in a loss-of-function [35]. It is found in up to 25% of all MDS cases and is also the most commonly mutated gene in low-risk MDS [31]. However, mutations in *ASXL1*, which modulates post-translational histone modifications, are strongly associated with disease progression, poor prognosis and lower survival rates [31,37,38]. Similarly, mutations in *EZH2*, a histone methyltransferase, are also linked with poor clinical outcome [31,38,39].

3.1.2. RNA Spliceosome

The most common RNA-splicing genes mutated in MDS are *SF3B1*, *SRSF2* and U2-complex auxiliary factor 1 (*U2AF1*); and zinc finger CCCH-type RNA binding motif and serine/arginine-rich 2 (*ZRSR2*) [12,23,40,41]. The most common *SF3B1* mutation observed is a lysine to glutamic acid substitution at codon 700 (K700E); however, mutations in the conserved amino acids 622, 625, 662 and 666 are also documented to a lesser degree [23,42,43]. Interestingly, *SF3B1* mutations are more commonly associated with a low-risk type of MDS called refractory anemia with ring sideroblasts (RARS) [23,42]. In contrast, somatic mutations in *U2AF1*, most frequently mutated in sites S34 and Q157, are associated with MDS to AML progression and worse clinical outcome [23,31]. Additionally, *SRSF2* mutations, which nearly exclusively occur at proline 95 (P95H), are also linked with poor survival outcome [6,23,44]. Indeed, *SRSF2* mutations are found in 14% of MDS patients and up to 47% of patients with another form of leukemia, chronic myelomonocytic leukemia (CMML) [6,44]. Additionally, *U2AF1* and *SRSF2* mutations also lead to a downregulation of *EZH2* which further drives MDS and AML disease progression [39]. Interestingly, while mutations in *U2AF1* and *SRSF2* are both common in MDS, they are mutually exclusively suggestive of a lethal interaction [41].

3.1.3. DNA Transcription

Mutations in *RUNX1* and the tumor protein P53 (*TP53*) genes, which are involved in DNA transcription, are well-known to be associated with poor prognosis of disease outcome in MDS/AML [31,38,45,46]. *RUNX1*, also known as acute myeloid leukemia 1 (AML1) protein, is well-known to influence HSC differentiation and hematopoiesis, and therefore, a loss-of-function mutation in *RUNX1* drastically increases disease progression and leukemia transformation [31,38,45]. The most common mutation, *D171N*, results in increased HSC self-renewal, impeded differentiation and drastic dysplasia [45]. Similarly, P53 is involved in cell-cycle arrest, proliferation, cell senescence, apoptosis and differentiation [47]. Loss-of-function P53 mutations are commonly associated with the high-risk MDS, and these mutations carry one of the worst disease prognoses in MDS/AML [31,38,46].

3.1.4. Other Genes

MDS patients can also carry mutations in the Kirsten rat sarcoma viral oncogene homolog (KRAS) and the neuroblastoma RAS viral oncogene homolog (NRAS), which are involved in MAPK signal transduction pathway. While they occur in lower frequencies than the previous genes mentioned in this review (~5% of MDS), KRAS/NRAS mutations often occur during the transition of MDS to AML and are associated with reduced survival rates [31,38]. Mutations in Janus Kinase 2 (JAK2) play a crucial role in signal transduction; moreover, protein expression is also found in MDS but at lower frequencies (~5% of MDS). Furthermore, the subunits which comprise the cohesion complex involved in transcriptional co-activation during cell division can also be mutated and are generally also associated with high-risk MDS and AML progression [48]. These include the *SMC3*, *SMC1A*, *RAD21* and *STAG2* genes [48].

3.2. Germline Mutations

While MDS generally develops in the elderly, there is a growing body of evidence showing that a portion of the population appears to have a genetic predisposition to MDS [1,49,50]. In these cases, the germline mutation is the primary mutation and the disease progresses with the accumulation of further somatic mutations. A total of 15% of all MDS cases are thought to arise from an initial germline mutation [49]. While alterations in genes such as *CEBPA*, *DDX41*, *ETV6*, *GATA2* and *RUNX1* are rare, they are also associated with MDS and/or AML development [49]. Additionally, several inherited disorders increase the risk of MDS development [49]. These include Fanconi anemia (*FANC* genes), Shwachman–Diamond syndrome (*SBDS* gene), Li-Fraumeni Syndrome (*TP53*), Diamond–Blackfan anemia (*GATA1/RPS19*), Dyskeratosis congenita and other telomerase complex disorders to name a few [7,12,49]. Patients with Tatton–Brown–Rahmann Syndrome caused by germline mutations in *DNMT3a* may also be at an increased risk of developing MDS/AML; however, this syndrome was only recently described, and there is limited longitudinal data, as all of the patients are still very young [51,52].

3.3. Chromosomal Abnormalities

Chromosomal abnormalities account for about half of all MDS cases and are even more common in t-MDS. These abnormalities include chromosomal loss, amplification, gain and balanced translocations. Additionally, genetic mutations involved in DNA repair or DNA methylation can cause chromosomal instability and consequently also confer a chromosomal defect. Indeed, unbalanced DNA methylation changes can induce chromosomal rearrangements and are frequently observed in a plethora of oncogenic diseases [12,53].

The most common chromosomal abnormality in MDS is the deletion of the long arm of chromosome 5 (5q), del (5q), and can be found in up to 15% of all MDS cases [54,55]. Patients with this sole chromosomal abnormality tend to have a relatively mild case of MDS that does not stimulate malignant transformation to AML. However, if this deletion occurs with either complex karyotypes (that is, at least three chromosomal defects) or with

a TP53 gene mutation, an increased risk of transformation to AML is observed, as well as a poor prognosis, resulting in worse clinical outcomes [54–56].

Other more common chromosomal deletions that can lead to MDS include partial or total loss of chromosome 7; del (7q) and chromosome 20q deletion; del (20q) and chromosome 17 deletion; or del (17q). Del (7q) is more common in t-MDS (~50% of patients), and less so in de novo MDS (~10%), but regardless of MDS type, patients with this chromosomal abnormality have a poor prognosis [12,57]. Similar to del (5q), del (20q) is generally observed in low-risk MDS patients, unless it is coupled with somatic mutations such as ASXL1 and U2AF1 [58]. Comparably, del (17q) is frequently associated with TP53 mutations and is consequently considered a very high-risk type of MDS, but it is also very rare occurring in about ~1 of all MDS cases [55].

In addition to chromosomal deletions, MDS can also be driven by Trisomy 8, complex karyotypes and a list of other rare defects, including chromosomal translocations [54]. Trisomy 8 tends to appear late and is detected commonly in the setting of AML. It is associated with many mutations, including RUNX1, ASXL1 and transcription factor genes. Furthermore, MDS can occur as a consequence of complex karyotypes [54]. Complex karyotypes occur when there are at least three cytogenetic abnormalities and is often associated with gene mutations such as TP53 [54]. Essentially, MDS is an extremely cytogenetically unstable disease that is associated with many chromosomal abnormalities that can be detected across the entire karyotype.

3.4. MDS Progression to AML

AML occurs when there is a leukemic transformation from MDS that results in excessive immature WBC production (Figure 1D). The progression from MDS to AML can either be linear, with the expansion of one mutant clone, or by branching of multiple different mutant clones [28]. Linear expansion can also occur when subclones evolve from the first clone but take over the original clone. Contrarily, one or more clones can outcompete the existing one or more clones to generate an expansion of multiple clones [28]. Clinicians utilize the International Prognostic Scoring System (IPSS), the Revised International Prognostic Scoring System (IPSS-R) and the Low-Risk Prognostic Scoring System (LR-PSS) to stratify the risk of MDS to AML progression [59]. These tools classify MDS as either low risk or high risk, thus allowing clinicians to tailor their patients' therapeutic goals relative to the risk of AML transformation. The risk of transformation to AML is determined by factoring in percentage of BM blasts, any karyotype abnormalities and the number of blood cytopenia's. While these formulas are the best scoring systems at present, they still do not predict AML progression with certainty. Furthermore, evidence suggests that identifying the precise mutations to refine these scoring systems would enhance prognostic accuracy [59]. Interestingly, different mutations in the same gene can also predict differential impact AML on progression and survival. For example, DNMT3A mutations at R882 have been shown to have the most severe AML transformation potential, whereas other DNMT3A mutations are far less likely to progress [60].

4. The Dysfunction of CHIP-Related Genes Alters HSC Function

Somatic mutations in CHIP-related genes predispose individuals to the development of MDS and AML, and, as such, CHIP precedes hematologic neoplasms such as MDS. While patients with CHIP do not display obvious hematological changes, such as changes in absolute WBC levels, these mutated clones have a divergent epigenetic landscape and an altered methylome which can alter HSC lineage priming (i.e., unbalanced expression of myeloid, lymphoid and/or erythroid genes), proliferation frequency, cellular renewal and/or differentiation capacity. Undeniably, changes in DNA methylation have been significantly implicated in all three disease states, namely CHIP, MDS and AML, highlighting the importance of epigenetic regulation in normal hematopoiesis [14,17,19,24]. Indeed, mutations in the epigenetic modifiers *DNMT3a* and *TET2* are the most potent drivers of CHIP

present in around 95% of cases [61,62]. Here we discuss the cellular and hematopoietic consequences of a loss of function of the top five mutated genes in CHIP.

4.1. DNMTs

DNA methylation is catalyzed by a family of DNMT enzymes consisting of *DNMT1*, *DNMT3A* and *DNMT3B*, whereby *DNMT3A* and *DNMT3B* perform de novo methylation in unmethylated DNA [63,64]. While mutations in *DNMT1* are rarely observed in MDS or CHIP, mutations in *DNMT3A* are frequently detected in CHIP.
















Mutant *DNMT3a* is the most commonly identified driver of CHIP, irrespective of age, across multiple studies [61,62,65,66]. Intriguingly, while mutant *DNMT3a* is the most frequently identified gene in patients with CHIP, it does not strongly alter blood-cell composition in people [62]. Mutant *DNMT3a* is pervasive in the blood of middle-aged people who are otherwise completely healthy, making it difficult to determine that pathogenicity of mutant *DNMT3a* driven CHIP [61]. However, not all mutations in *DNMT3a* are made equal, and a recent study has revealed that people with *DNMT3A*-R882-driven CHIP have significantly higher VAF frequencies than people with non-R882 mutations, suggesting that different mutations confer different outcomes [65]. *DNMT3A* has been shown to play a role in HSC differentiation, and this could explain why mutant *DNMT3a* is so abundant in CHIP.

HSCs carrying a *DNMT3A* mutation generally have increased cellular renewal capacity and reduced differentiation (Figure 2A) [36,67,68]. This is evidenced by both in vitro and in vivo observations—a significant decline in differentiation capacity with HSC serial BM transplantations presenting with undifferentiated HSC accumulation in the BM [36], as well as reduced colonies formed with colony forming unit (CFU) replating [68]. Interestingly, the same group recently reported comparing *DNMT3A* or *DNMT3B* single or double knockout (DKO) HSC in serial BM transplantations. They showed that *DNMT3B* plays a critical role in enabling HSC differentiation in the absence of *DNMT3A*. Although loss of *DNMT3A* alone has a more dramatic effect in HSC differentiation and overall the phenotypes of *DNMT3A* KO and DKO HSCs are predominantly similar, suggesting that *DNMT3A* has a more relevant function in regulating hematopoiesis [67].

Regulating DNA methylation is considered to be what shapes the topography of HSC differentiation [69]. Izzo and colleagues showed that *DNMT3A* KO HSCs present with transcriptional priming towards specific hematopoietic lineages in uncommitted HSCs [69]. *DNMT3A* null HSCs display reduced monocytic clusters (i.e., reduced *Ly6c2*, *Prtn2* and *Lyz2* expression) and skewing toward the erythroid lineage, as evidenced by increased *Car1* and *Car2* expression, although RBC levels remained unchanged. Furthermore, changes in DNA methylation also occurred in TF binding sites, evidenced by decreased activity in CpG-rich erythroid TF motifs in *DNMT3A* KO HSCs. Additionally, this study confirmed stem-cell priming from human *DNMT3A*-driven CHIP by isolating circulating CD34+ cells from patients. Sequencing data reconfirmed reduced monocytic priming and increased erythroid priming, as evidenced by increased *GATA-1*+ progenitors. The precise mechanism of HSC priming still remains largely unknown.

Interestingly, a paper in PNAS reported opposing results utilizing *DNMT3A* R878H mice instead of *DNMT3A* KO mice [70]. These mice mimic human *DNMT3A*-driven CHIP by modelling the most common R882 mutation in CHIP, MDS and AML. The *DNMT3A* R882 mutation has been proposed to act as a dominant negative by most [71–74], but not all groups [75]. The R882 mutation has also been proposed to alter the flanking sequence preference of *DNMT3A* [75,76], as well as sequester WT *DNMT3a* [77]. Using a preclinical model of *DNMT3a*-R882, it was shown that these mice present with a myelomonocytic type of AML, evidenced by increased circulating WBCs, particularly monocytes and platelets, as well as immature hematopoietic cells in the BM, blood and spleen (Figure 2A). Methylated DNA immunoprecipitation sequencing revealed both hypermethylated and hypomethylated DNA segments in mature myeloid leukocytes, which decreased *Gata2*, *Gata3* and *Pax5* and increased *Rpl22*, *Eif4a1* and mTOR expression. LSKs also displayed increased

mTOR, as well as CDK1, which is important in the cell cycle. Indeed, LSK hyperproliferation in DNMT3A R878H mice was attributed CDK1-mediated phosphorylation of EZH2 which inhibited the tri-methylation of histone H2K27 (H2K27me3).

Gene	Genetic Alterations	Haematological Consequences	Potential consequences
A. DNMT3A <i>References:</i> 14, 36, 60, 67, 68, 70, 78	 <i>Dnmt3A</i> KO	↑ HSC Proliferation/self-renewal ✘ HSC differentiation (globally)	• MDS/AML • Atherosclerosis 
	 <i>Dnmt3A</i> ^{R878H}	↑ HSC Proliferation/self-renewal ↑ Myeloid skewing	• Cardiac Dysfunction 
	 CRISPR-DNMT3A editing	⇔ HSC Proliferation/self-renewal ⇔ HSC differentiation (globally)	• Inflammation (IL-6, CXCL1, CXCL2)
B. TET2 <i>References:</i> 13, 14, 17, 20, 68, 78, 79	 <i>Tet2</i> KO or  CRISPR- <i>Tet2</i> editing	↑ HSC Proliferation/self-renewal ↑ Myeloid or lymphoid skewing	• MDS/AML • Atherosclerosis 
	 <i>Tet2</i> mutation in catalytic domain	↑ HSC Proliferation/self-renewal ↑ Myeloid skewing only ✘ Lymphoid skewing	• Cardiac Dysfunction  • Inflammation (IL-1, IL-6)
C. P53 <i>References:</i> 80–86	 <i>P53</i> mutations	↑ HSC Proliferation/self-renewal	• MDS/AML
D. ASXL1 <i>References:</i> 14, 87–91	 <i>Asxl1</i> mutations	↑ HSC Proliferation/self-renewal ✘ HSC differentiation (globally)	• MDS/AML
E. JAK2 <i>References:</i> 14, 86, 92, 93	 <i>Jak2</i> ^{V617F}	↑ Myeloid skewing	• MDS/AML • Atherosclerosis  • Cardiac Dysfunction  • Inflammation (IL-1, IL-6, TNF- α , CCL2)

Legend: ↑ = increases, ✘ = inhibits/reduces, ⇔ = no change.

Abbreviations: HSC; hematopoietic stem cell, MDS; myelodysplasia syndromes, AML; acute myeloid leukemia

Figure 2. Hematopoietic and disease consequences of the top 5 genes mutated in CHIP. (A) DNMT3A: KO, single spot loss-of-function mutation and CRISPR editing studies [14,36,60,67,68,70,78]. (B) TET2: KO, CRISPR editing or mutation in the catalytic domain only studies [13,14,17,20,68,78,79]. (C) P53: studies utilizing P53 loss-of-function mutations [80–86]. (D) ASXL1: studies utilizing loss-of-function mutations [14,87–91]. (E) JAK2: studies reporting the V617F mutation [14,86,92,93]. KO, Knock Out; HSC, hematopoietic stem cell.

Taken together, these contradictory studies highlight the divergent effects of genetic loss of DNMT3a compared with R882 and non-R882 mutations in the blood (Figure 2A). The location of the mutation appears to heavily influence HSC cellular functions, lineage skewing, WBC production and likelihood for leukemic transformations. Indeed, DNMT3A mutations at R882 show the most severe AML transformation potential, whereas other DNMT3A mutations are far less likely to progress [60].

4.2. TET2

TET2 de-methylates DNA and is one of the most commonly mutated genes in both MDS and CHIP, as well as CMML. It was the first gene reported to exhibit somatic mutations in blood cells in CHIP patients (i.e., without leukemia) and more than 130 different TET2 mutations have been reported in cancer-free CHIP patients [13,15–17,19,62,65]. A meta-analysis of six major CHIP studies revealed that 9% of healthy individuals have CHIP and more importantly 11–15% of CHIP is due to TET2 deficiency [34]. Notably, the presence of

ancestral *TET2* mutation (mostly biallelic) is a crucial factor of the MDS pathophysiology, and it is likely derived from *TET2* CHIP [34].

TET2 dysfunction appears to skew hematopoiesis towards the myeloid lineage (Figure 2B) [69]. Indeed, elegant studies using single-cell sequencing by Izzo and colleagues showed that HSCs deficient in *TET2* present with transcriptional priming even in uncommitted HSCs [69]. *TET2*-deficient HSCs displayed an enrichment of monocytic clusters, defined by *Ly6c2*, *Prtn2* and *Lyz2*, which was accompanied by a reduction toward erythroid priming (i.e., reduced *Car1* and *Car2* expression). Interestingly, the methylation changes observed in *TET2* KO HSCs were commonly found in CpG-rich motifs within known transcription factor binding motifs. These data suggest that the HSC hyperproliferation and myeloid skewing in *TET2* deficient cells are due to alterations in transcription factor regulation [69]. Furthermore, Moran-Crusio et al. showed that specific hematopoietic *TET2* loss of function promotes HSC self-renewal, causing a dramatic competitive growth advantage over time [20]. In addition, *TET2* loss-of-function mutations are mostly heterozygous, which result in DNA hypermethylation, HSCs gene dysregulation, and aberrant myeloid-specific proliferation [94,95].

However, it is important to note that most studies describing *TET2* function in hematopoiesis reflect more on the role of *TET2* but not what is actually occurring during CHIP. *TET2*-driven CHIP, as well as MDS, is actually characterized by *TET2* loss-of-function rather than *TET2* protein deletion, and in order to compare these differences, Ito's group created mice carrying a mutation specifically in the catalytic domain (C terminal) of *TET2* and compared these mice to the *TET2* KO mice. They showed that *TET2* catalytic mutant mice exhibited clonal outgrowth solely towards myeloid lineage, whereas *TET2* KO mice presented an increase in either myeloid or lymphoid cells (Figure 2B) [79]. This demonstrates the difference between *TET2* deletion and *TET2* dysfunction, revealing a more translational model of *TET2*-CHIP. Mechanistically, the mutation in the catalytic domain interferes with Fe^{2+} and/or α -ketoglutarate (α -KG) binding, which is essential for *TET2* function, resulting in impaired 5mC oxidation and DNA hypermethylation [96].

Interestingly, an elegant discovery by the Aifantis group showed that Vitamin C can restore *TET2* activity acting as a nutraceutical mimic of *TET2* activation. Specifically, vitamin C operates as a cofactor for Fe^{2+} and α -KG-dependent dioxygenases, activating the catalytic domain and enhancing 5 hmC formation in *TET2*-deficient HSCs [97]. Additionally, another important finding showed that NAD-dependent histone deacetylase-1 (SIRT1) transduces epigenetic changes in *TET2*, enhancing its functionality. Through RNAi screening and proteomics analysis, it was revealed that SIRT1 deacetylates *TET2* at conserved lysine residues in the catalytic domain and enhances *TET2* activity in cells that mimic *TET2* mutant MDS cells [98].

DNMT3A and *TET2* Co-Deletion

Intriguingly, it may be reasonable to assume that the co-deletion of both *DNMT3A* and *TET2* may not provoke further hematopoietic consequences compared to *DNMT3A* KO alone, given the dependence of *TET2* de-methylation on the methyltransferase activity of *DNMT3A*. However, *DNMT3A/TET2* co-deletion in mice had an accumulative effect. The *DNMT3a/TET2* DKO led to an AML-like phenotype, evidenced by increased BM HSCs, myeloid skewing, elevated circulating WBCs, anemia and increased immune cell infiltrate into other organs such as the liver, spleen and lungs [68]. DKO mice all died by 1 year, whereas 70% of either *DNMT3A* KO or *TET2* KO animals were still alive at the 1-year time point. The authors noted increased *Klf1* and *Epor* expression in HSCs of DKO mice, which drove cellular self-renewal by activating the JAK2-STAT5 signaling pathway. They also observed increased *Ikzf1*, *Ebf1*, *Cebpa* and *Cebpe* expression, which was associated with changes in both 5 mc and 5 hmc, but stronger with the latter.

4.3. P53

The TP53 gene, which encodes the tumor suppressor protein p53, ranks in the top five genes mutated in CHIP. Importantly, p53 is a transcription factor that regulates a large number of genes in response to a variety of cellular changes such as oncogene activation, DNA damage and inflammation [99,100]. Over the past decade, somatic TP53 mutations were identified in CHIP, as well as in hematological-malignancies-related therapies such as prior exposure to radiotherapy and chemotherapy [15,17,19,101,102]. In the hematopoietic lineage, p53 plays an important role maintaining HSC quiescence, specifically targeting the melanoma antigen family member Necdin regulating DNA damage response in HSCs [80,81]. Mutant p53 enhances the repopulating potential of HSCs, suggesting that p53 is highly involved in the regulation of HSC self-renewal (Figure 2C) [82–84]. Additionally, Chen and colleagues discovered an epigenetic pathway by which mutant p53 drives CHIP. Mechanistically, they have shown the mutant p53 interacts with the histone-lysine N-methyltransferase EZH2. The interaction enhances EZH2 chromatin association, thereby increasing the levels of H3K27me3, leading to genetic regulation of HSPC self-renewal and differentiation related genes [85].

Interestingly, mutations of EZH2 and p53 are observed in 10% and 20% of patients with MDS, respectively; therefore, the levels of DNA tri-methylation may be compromised in MDS [26,29,38,39,103–105]. Additionally, 30% of patients with t-MDS previously exposed to cancer therapies develop mutations in P53 as mechanism to evade chemotherapy-induced cell death [106,107]. Thus, the literature suggests that P53 mutation has become a potential target in clonal abnormalities (especially CHIP) due to its role in HSC fitness and its frequency in t-MDS [82,108].

4.4. ASXL1

ASXL1 is a mammalian homolog of the *Drosophila* additional sex combs family (1–3) and has a crucial role activating and suppressing the Hox genes (a set of transcription factor genes) [109,110]. Importantly, ASXL1 is mutated in patients with entire spectrum of myeloid malignancies including 21% of MDS and 25% AML patients [87,88]. As mentioned before, DNA methylation plays a pivotal role in hematopoiesis through the regulation of gene expression. Consistent with mutant DNMT3a and TET2, ASXL1 mutations are also involved in disordered hematopoiesis leading to increased HSC self-renewal, impaired differentiation and aberrant proliferation of HSCs (Figure 2D) [89].

Mutant ASXL1 disrupts epigenetic modifications, such as histone methylation or ubiquitination (H2AK199Ub, H3K4me3 and H3K27me3), which could contribute in the long-term to the development of CHIP [89,90,111–113]. The deletion of ASXL1 in mice facilitates aberrant gene expression through histone modifications resulting in myeloid transformation. Mechanistically, ASXL1 mutations disrupt polycomb repressive complex 2 (PRC2) in hematopoietic cells, causing a reduction in genome wide H3K27me3 occupancy. This altered epigenetic landscape leads to dysregulation of specific oncogenic target loci contributing to myeloid transformation and MDS. In fact, EZH2 is a catalytic component of PRC2 which catalyzes the tri-methylation of H3K27me3 and 10% of MDS patients harbor loss-of-function mutations in EZH2 [90,91]. These findings highlight the importance of EZH2 in normal functioning and provide mechanistic insight into how disruptions in ASXL1 contribute to MDS.

4.5. JAK2

JAK2^{V617F} is also a common driver of CHIP; ultra-deep sequencing data detected the mutation in 1% of cancer-free adults over 60 years of age [15,114]. Additionally, the V617F variant has been associated with a number of myeloid proliferative diseases [115]. Recently, Steensma et al. demonstrated that 5% of MDS patients had the JAK2^{V617F} tyrosine kinase mutation and that, importantly, the presence of this mutation is associated with a higher rate of death [86,92]. Mice carrying heterozygous JAK2^{V617F} mutations showed significant increase of red blood cells and expansion of HSCs, as well as myeloid progeni-

tors in BM, suggesting an important role for JAK2^{V617F} in hematopoietic cell regulation (Figure 2E) [116,117].

Interestingly, DNMT3A^{R882} and JAK2^{V617F} mutations drive slow but inexorable clonal expansion, being more common along the process of ageing, but they are the only two mutations also found in young adults (30–39 years of age) [114]. Importantly, in BM transplanted mice carrying JAK2^{V617F} mutations Sano et al. discovered that mutant mice displayed increased expansion of monocytes and neutrophils in the blood [93]. In order to address the effect of JAK2^{V617F} mutations in myeloid cells, they transduced THP-1 (human monocytic cell line) cells with GFP expressing JAK2^{V617F} or JAK2^{WT} and found that mutant cells exhibited activation of STAT1 signaling (phosphorylation at Y701 and S727 sites). Furthermore, JAK2^{V617F} cells upon stimulation with LPS showed significant up-regulation of inflammatory markers such as IL-6, IL-1 β , TNF and CCL2, suggesting that JAK2^{V617F} cells have a heightened inflammatory response, which is linked to an increase in cardiovascular disease [93].

5. CHIP—A Novel Connection between CVD and MDS

Atherosclerotic CVD and leukemia are both responsible for vast global mortality, and while the etiology of these two diseases was historically thought to be mutually exclusive, the recent discovery of CHIP provides genetic evidence that an overlap between the two exists. While it is well-known that CVD is accelerated by metabolic disorders such as diabetes and obesity [118–120], as well as autoimmune diseases such as rheumatoid arthritis [121], there is emerging evidence that CHIP-related mutations found in MDS also contribute to heightened CVD in patients with myeloid neoplasms such as MDS [3,122,123]. Indeed, CVD was recently shown to be the most common non-disease related cause of death in MDS patients [3,122,124]. A matched cohort study of Surveillance, Epidemiology, and End Results (SEER) suggested that, in older adults, MDS is not only an independent risk factor for CVD but also highly associated with mortality in MDS patients [123]. Moreover, MDS diagnosis confers a significant risk especially for myocardial infarction (MI), even in relatively healthy older adults with minimal additional cardiovascular comorbidities. The underlying mechanism behind the increased prevalence of CVD in MDS is not clear; however, recurrent somatic mutations in the CHIP-related genes DNMT3A, TET2, JAK2 and AXSL1 may contribute to the cardiometabolic burden.

Both animal and clinical studies have revealed a causal relationship between CHIP and CVD, with a focus on DNMT3A- and TET2-driven mutations (mainly due to the availability of animal models and these genes accounting for the majority of the annotated mutations) (Figure 2) [13,14,17,18,24,78,79]. One of the hallmarks of the atherosclerotic CVD is the macrophage infiltration in the intimal walls of the aorta, leading to macrophage lipid uptake and transformation into foam cells. Importantly, these cells are derived from circulating monocytes, which are well established to directly increase the risk of CVD [125]. Previous work investigating enhanced hematopoiesis in various inflammatory contexts has largely concluded that the increased number of circulating myeloid cells, namely monocytes, neutrophils and platelets, directly exacerbates atherosclerotic CVD. Conversely, although CHIP enhances CVD, circulating WBC numbers remain largely unchanged [15,17].

In order to understand how these somatic mutations drive CVD, Fuster and colleagues induced murine TET2-driven CHIP in an atherosclerotic mouse model (Figure 2B) [17]. They performed a competitive BM transplant (BMT), using 10% Tet2^{-/-} BM and 90% WT littermate control BM into the atherosclerotic mouse model: the low-density lipoprotein receptor KO mouse (Ldlr^{-/-} mice). In this study, mice with murine TET2-CHIP exhibited almost twice the atheroma size in the descending aorta, as well as increased lesion size and complexity (i.e., increased macrophage infiltration) in the aortic sinus. Notably, the authors attributed these atherogenic consequences in murine CHIP to expanded TET2 mutant clones and macrophage-driven IL-1 β production in lesions [17]. While the importance of IL-1 β in this context is still controversial, it has been suggested that TET2 may be a

negative transcriptional regulator in response to inflammation. Macrophages deficient in TET2 upregulate a multitude of inflammatory chemokines, cytokines and their receptors, and mice with Tet2^{-/-} BM had elevated chemokines important in monocyte adhesion and recruitment such as CXCL1, CXCL2 and CXCL3 [15]. Similarly, an elegant study by Sano et al. used a CRISPR approach to generate HSC gene edited mice carrying TET2 and DNMT3A deficiency to investigate CVD in CHIP. They also used the BMT technique ratio 1:9 to generate murine CHIP and confirmed that Tet2 gene disruption confers a competitive advantage to HSCs leading to cardiac dysfunction via IL-1 β and IL-6 (Figure 2A,B). In parallel, they showed for the first time that DNMT3A gene disruption likewise promotes cardiac dysfunction. However, the nature of the inflammatory response differed, with higher CXCL1 and CXCL2 but not IL-1 β [78].

Interestingly, ~20% of MDS patients harbor TET2 mutations and among other myelo-proliferative disorders this mutation can occasionally predict JAK2 deficiency [126]. In light of the gene overlap between MDS and CHIP, JAK2^{V617F} mutation was another CHIP-MDS driver gene also linked to CVD development, as it is well-known to cause essential thrombocythemia and promote atherothrombotic vascular disease (Figure 2E) [93,125]. Consistent with previous observations of greater cardiometabolic risk in mice carrying Tet2 and Dnmt3a mutations, a recent investigation evaluated the fitness of HSCs expressing the JAK2^{V617F} transgene. The authors observed that JAK2^{V617F} hematopoietic cells had clonal expansion with similar kinetics to Tet2 mutations but much more robust than what was observed in Dnmt3a mutants [13,78]. Additionally, they found that JAK2^{V617F} mutations lead to the expansion of mutant clones specifically towards the myeloid lineage. Furthermore, using a specific system to restrict JAK2^{V617F} expression in myeloid cells in a model of heart failure, mice carrying JAK2^{V617F} cells were more susceptible to cardiometabolic complications [93]. JAK2^{V617F} also activates an inflammatory cascade through STAT1 phosphorylation, which promotes MDS, CHIP and atherogenesis [14,93,127].

Taken together, these data suggest that the most common clonal mutations observed in MDS and CHIP are able to accelerate clonal outgrowth leading to CVD particularly atherosclerosis, MI and heart failure. This is significant as the individuals with CHIP or MDS who may not progress towards a leukemic transformation, should be screened for the risk of developing CV complications. This is particularly important in MDS patients with an existing history of CVD, such as a previous MI, where CHIP mutations are 5 \times more prevalent in these patients [14].

6. Conclusions

MDS is a complex disorder with a plethora of genetic mutations and abnormalities. Recent advances in next-generation sequencing have revealed specific gene mutations that have now been linked to different disease progression rates and diverse clinical outcomes in MDS/AML. Moreover, the recent discovery of the pre-MDS condition, CHIP, highlights that even before circulating leukocyte levels begin to change, these mutations in CHIP initiate HSC priming and alter their activity preceding malignant transformation of hematopoietic cells. While there are no current therapeutic avenues for patients that are diagnosed with CHIP, future work should focus on discovering what factors can drive or suppress clonal outgrowth. The current literature suggests that patients with low VAFs have a low-risk of developing CHIP complications, and thus future therapeutics that prevent mutated HSCs from outgrowing would limit the development of CHIP-related co-morbidities. Furthermore, the acquisition of CHIP mutations has not only been linked to malignant transformations, but also to exacerbated CVD, and therefore patients should also be evaluated for potential CV complications.

Author Contributions: C.B.V. and D.D., writing—original draft preparation; C.B.V., E.M.L., A.J.M., M.J.H. and D.D., writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: A.J.M is supported by a Centenary Award from CSL. C.B.V is supported by an GR scholarship from The University of Melbourne and a Bright Sparks award from the Baker Heart and Diabetes Institute. M.J.H is supported by a fellowship from the Australian National Health and Medical Research Council NHMRC (1156095).

Data Availability Statement: No new data were created or analyzed in this study. Data sharing not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Arber, D.A.; Orazi, A.; Hasserjian, R.; Thiele, J.; Borowitz, M.J.; Le Beau, M.M.; Bloomfield, C.D.; Cazzola, M.; Vardiman, J.W. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* **2016**, *127*, 2391–2405. [[CrossRef](#)] [[PubMed](#)]
2. Hasserjian, R.P. Myelodysplastic Syndrome Updated. *Pathobiol. J. Immunopathol. Mol. Cell. Biol.* **2019**, *86*, 7–13. [[CrossRef](#)] [[PubMed](#)]
3. Brunner, A.M.; Blonquist, T.M.; Hobbs, G.S.; Amrein, P.C.; Neuberg, D.S.; Steensma, D.P.; Abel, G.A.; Fathi, A.T. Risk and timing of cardiovascular death among patients with myelodysplastic syndromes. *Blood Adv.* **2017**, *1*, 2032–2040. [[CrossRef](#)] [[PubMed](#)]
4. Steensma, D.P. Graphical representation of clinical outcomes for patients with myelodysplastic syndromes. *Leuk. Lymphoma* **2016**, *57*, 17–20. [[CrossRef](#)]
5. Reya, T.; Morrison, S.J.; Clarke, M.F.; Weissman, I.L. Stem cells, cancer, and cancer stem cells. *Nature* **2001**, *414*, 105–111. [[CrossRef](#)]
6. Cazzola, M.; Della Porta, M.G.; Malcovati, L. The genetic basis of myelodysplasia and its clinical relevance. *Blood* **2013**, *122*, 4021–4034. [[CrossRef](#)]
7. Cazzola, M. Myelodysplastic Syndromes. *N. Engl. J. Med.* **2020**, *383*, 1358–1374. [[CrossRef](#)]
8. Hasserjian, R.P. Controversies in the recent (2016) World Health Organization classification of acute myeloid leukemia. *Best Pr. Res. Clin. Haematol.* **2021**, *34*, 101249. [[CrossRef](#)]
9. Andersen, M.K.; Christiansen, D.H.; Pedersen-Bjergaard, J. Centromeric breakage and highly rearranged chromosome derivatives associated with mutations of TP53 are common in therapy-related MDS and AML after therapy with alkylating agents: An M-FISH study. *Genes Chromosomes Cancer* **2005**, *42*, 358–371. [[CrossRef](#)]
10. Smith, S.M.; Le Beau, M.M.; Huo, D.; Karrison, T.; Sobecks, R.M.; Anastasi, J.; Vardiman, J.W.; Rowley, J.D.; Larson, R.A. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: The University of Chicago series. *Blood* **2003**, *102*, 43–52. [[CrossRef](#)]
11. Kuendgen, A.; Nomdedeu, M.; Tuechler, H.; Garcia-Manero, G.; Komrokji, R.S.; Sekeres, M.A.; Della Porta, M.G.; Cazzola, M.; DeZern, A.E.; Roboz, G.J.; et al. Therapy-related myelodysplastic syndromes deserve specific diagnostic sub-classification and risk-stratification—an approach to classification of patients with t-MDS. *Leukemia* **2021**, *35*, 835–849. [[CrossRef](#)]
12. Awada, H.; Thapa, B.; Visconte, V. The Genomics of Myelodysplastic Syndromes: Origins of Disease Evolution, Biological Pathways, and Prognostic Implications. *Cells* **2020**, *9*, 2512. [[CrossRef](#)]
13. Fuster, J.J.; MacLauchlan, S.; Zuriaga, M.A.; Polackal, M.N.; Ostriker, A.C.; Chakraborty, R.; Wu, C.L.; Sano, S.; Muralidharan, S.; Rius, C.; et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* **2017**, *355*, 842–847. [[CrossRef](#)]
14. Jaiswal, S.; Natarajan, P.; Silver, A.J.; Gibson, C.J.; Bick, A.G.; Shvartz, E.; McConkey, M.; Gupta, N.; Gabriel, S.; Ardissino, D.; et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N. Engl. J. Med.* **2017**, *377*, 111–121. [[CrossRef](#)]
15. Genovese, G.; Kähler, A.K.; Handsaker, R.E.; Lindberg, J.; Rose, S.A.; Bakhoum, S.F.; Chambert, K.; Mick, E.; Neale, B.M.; Fromer, M.; et al. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *N. Engl. J. Med.* **2014**, *371*, 2477–2487. [[CrossRef](#)]
16. Zink, F.; Stacey, S.N.; Norddahl, G.L.; Frigge, M.L.; Magnusson, O.T.; Jonsdottir, I.; Thorgeirsson, T.E.; Sigurdsson, A.; Gudjonsson, S.A.; Gudmundsson, J.; et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* **2017**, *130*, 742–752. [[CrossRef](#)] [[PubMed](#)]
17. Jaiswal, S.; Fontanillas, P.; Flannick, J.; Manning, A.; Grauman, P.V.; Mar, B.G.; Lindsley, R.C.; Mermel, C.H.; Burt, N.; Chavez, A.; et al. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. *N. Engl. J. Med.* **2014**, *371*, 2488–2498. [[CrossRef](#)]
18. Dragoljevic, D.; Westerterp, M.; Veiga, C.B.; Nagareddy, P.; Murphy, A.J. Disordered haematopoiesis and cardiovascular disease: A focus on myelopoiesis. *Clin. Sci.* **2018**, *132*, 1889–1899. [[CrossRef](#)] [[PubMed](#)]
19. Xie, M.; Lu, C.; Wang, J.; McLellan, M.D.; Johnson, K.J.; Wendl, M.C.; McMichael, J.F.; Schmidt, H.K.; Yellapantula, V.; Miller, C.A.; et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat. Med.* **2014**, *20*, 1472–1478. [[CrossRef](#)] [[PubMed](#)]
20. Moran-Crusio, K.; Reavie, L.; Shih, A.; Abdel-Wahab, O.; Ndiaye-Lobry, D.; Lobry, C.; Figueroa, M.E.; Vasanthakumar, A.; Patel, J.; Zhao, X.; et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* **2011**, *20*, 11–24. [[CrossRef](#)]
21. Bejar, R. Implications of molecular genetic diversity in myelodysplastic syndromes. *Curr. Opin. Hematol.* **2017**, *24*, 73–78. [[CrossRef](#)]

22. Malcovati, L.; Galli, A.; Travaglino, E.; Ambaglio, I.; Rizzo, E.; Molteni, E.; Elena, C.; Ferretti, V.V.; Catricalà, S.; Bono, E.; et al. Clinical significance of somatic mutation in unexplained blood cytopenia. *Blood* **2017**, *129*, 3371–3378. [[CrossRef](#)]
23. Makishima, H.; Visconte, V.; Sakaguchi, H.; Jankowska, A.M.; Abu Kar, S.; Jerez, A.; Przychodzen, B.; Bupathi, M.; Guinta, K.; Afable, M.G.; et al. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. *Blood* **2012**, *119*, 3203–3210. [[CrossRef](#)]
24. Walter, M.J.; Ding, L.; Shen, D.; Shao, J.; Grillot, M.; McLellan, M.; Fulton, R.; Schmidt, H.; Kalicki-Veizer, J.; O’Laughlin, M.; et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. *Leukemia* **2011**, *25*, 1153–1158. [[CrossRef](#)]
25. Lindsley, R.C.; Mar, B.G.; Mazzola, E.; Grauman, P.V.; Shareef, S.; Allen, S.L.; Pigneux, A.; Wetzler, M.; Stuart, R.K.; Erba, H.P.; et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood* **2015**, *125*, 1367–1376. [[CrossRef](#)]
26. Papaemmanuil, E.; Gerstung, M.; Malcovati, L.; Tauro, S.; Gundem, G.; Van Loo, P.; Yoon, C.J.; Ellis, P.; Wedge, D.C.; Pellagatti, A.; et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* **2013**, *122*, 3616–3699. [[CrossRef](#)]
27. Yoshida, K.; Sanada, M.; Shiraishi, Y.; Nowak, D.; Nagata, Y.; Yamamoto, R.; Sato, Y.; Sato-Otsubo, A.; Kon, A.; Nagasaki, M.; et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* **2011**, *478*, 64–69. [[CrossRef](#)]
28. Makishima, H.; Yoshizato, T.; Yoshida, K.; Sekeres, M.A.; Radivoyevitch, T.; Suzuki, H.; Przychodzen, B.; Nagata, Y.; Meggendorfer, M.; Sanada, M.; et al. Dynamics of clonal evolution in myelodysplastic syndromes. *Nat. Genet.* **2017**, *49*, 204–212. [[CrossRef](#)]
29. Haferlach, T.; Nagata, Y.; Grossmann, V.; Okuno, Y.; Bacher, U.; Nagae, G.; Schnittger, S.; Sanada, M.; Kon, A.; Alpermann, T.; et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* **2014**, *28*, 241–247. [[CrossRef](#)]
30. Nagata, Y.; Makishima, H.; Kerr, C.M.; Przychodzen, B.P.; Aly, M.; Goyal, A.; Awada, H.; Asad, M.F.; Kuzmanovic, T.; Suzuki, H.; et al. Invariant patterns of clonal succession determine specific clinical features of myelodysplastic syndromes. *Nat. Commun.* **2019**, *10*, 5386. [[CrossRef](#)]
31. Bejar, R.; Stevenson, K.E.; Caughey, B.A.; Abdel-Wahab, O.; Steensma, D.P.; Galili, N.; Raza, A.; Kantarjian, H.; Levine, R.L.; Neuberg, D.; et al. Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. *J. Clin. Oncol.* **2012**, *30*, 3376–3382. [[CrossRef](#)] [[PubMed](#)]
32. Ley, T.J.; Ding, L.; Walter, M.J.; McLellan, M.D.; Lamprecht, T.; Larson, D.E.; Kandoth, C.; Payton, J.E.; Baty, J.; Welch, J.; et al. DNMT3A mutations in acute myeloid leukemia. *N. Engl. J. Med.* **2010**, *363*, 2424–2433. [[CrossRef](#)] [[PubMed](#)]
33. Awada, H.; Nagata, Y.; Goyal, A.; Asad, M.F.; Patel, B.; Hirsch, C.M.; Kuzmanovic, T.; Guan, Y.; Przychodzen, B.P.; Aly, M.; et al. Invariant phenotype and molecular association of biallelic TET2 mutant myeloid neoplasia. *Blood Adv.* **2019**, *3*, 339–349. [[CrossRef](#)] [[PubMed](#)]
34. Hirsch, C.M.; Nazha, A.; Kneen, K.; Abazeed, M.E.; Meggendorfer, M.; Przychodzen, B.P.; Nadarajah, N.; Adema, V.; Nagata, Y.; Goyal, A.; et al. Consequences of mutant TET2 on clonality and subclonal hierarchy. *Leukemia* **2018**, *32*, 1751–1761. [[CrossRef](#)]
35. Ko, M.; Huang, Y.; Jankowska, A.M.; Pape, U.J.; Tahilian, M.; Bandukwala, H.S.; An, J.; Lamperti, E.D.; Koh, K.P.; Ganetzky, R.; et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature* **2010**, *468*, 839–843. [[CrossRef](#)]
36. Challen, G.A.; Sun, D.; Jeong, M.; Luo, M.; Jelinek, J.; Berg, J.S.; Bock, C.; Vasanthakumar, A.; Gu, H.; Xi, Y.; et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet.* **2012**, *44*, 23–31. [[CrossRef](#)]
37. Thol, F.; Friesen, I.; Damm, F.; Yun, H.; Weissinger, E.M.; Krauter, J.; Wagner, K.; Chaturvedi, A.; Sharma, A.; Wichmann, M.; et al. Prognostic significance of ASXL1 mutations in patients with myelodysplastic syndromes. *J. Clin. Oncol.* **2011**, *29*, 2499–2506. [[CrossRef](#)]
38. Bejar, R.; Stevenson, K.; Abdelwahab, O.; Galili, N.; Nilsson, B.; Garcia-Manero, G.; Kantarjian, H.; Raza, A.; Levine, R.; Neuberg, D.; et al. Clinical effect of point mutations in myelodysplastic syndromes. *N. Engl. J. Med.* **2011**, *364*, 2496–2506. [[CrossRef](#)]
39. Ernst, T.; Chase, A.J.; Score, J.; Hidalgo-Curtis, C.E.; Bryant, C.; Jones, A.V.; Waghorn, K.; Zoi, K.; Ross, F.M.; Reiter, A.; et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat. Genet.* **2010**, *42*, 722–726. [[CrossRef](#)]
40. Graubert, T.A.; Shen, D.; Ding, L.; Okeyo-Owuor, T.; Lunn, C.L.; Shao, J.; Krysiak, K.; Harris, C.C.; Koboldt, D.C.; Larson, D.E.; et al. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. *Nat. Genet.* **2011**, *44*, 53–57. [[CrossRef](#)]
41. Lee, S.C.; North, K.; Kim, E.; Jang, E.; Obeng, E.; Lu, S.X.; Liu, B.; Inoue, D.; Yoshimi, A.; Ki, M.; et al. Synthetic Lethal and Convergent Biological Effects of Cancer-Associated Spliceosomal Gene Mutations. *Cancer Cell* **2018**, *34*, 225–241.e228. [[CrossRef](#)]
42. Malcovati, L.; Karimi, M.; Papaemmanuil, E.; Ambaglio, I.; Jädersten, M.; Jansson, M.; Elena, C.; Galli, A.; Walldin, G.; Della Porta, M.G.; et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. *Blood* **2015**, *126*, 233–241. [[CrossRef](#)]
43. Obeng, E.A.; Chappell, R.J.; Seiler, M.; Chen, M.C.; Campagna, D.R.; Schmidt, P.J.; Schneider, R.K.; Lord, A.M.; Wang, L.; Gambe, R.G.; et al. Physiologic Expression of Sf3b1(K700E) Causes Impaired Erythropoiesis, Aberrant Splicing, and Sensitivity to Therapeutic Spliceosome Modulation. *Cancer Cell* **2016**, *30*, 404–417. [[CrossRef](#)]
44. Liang, Y.; Tebaldi, T.; Rejeski, K.; Joshi, P.; Stefani, G.; Taylor, A.; Song, Y.; Vasic, R.; Maziarsz, J.; Balasubramanian, K.; et al. SRSF2 mutations drive oncogenesis by activating a global program of aberrant alternative splicing in hematopoietic cells. *Leukemia* **2018**, *32*, 2659–2671. [[CrossRef](#)]
45. Harada, Y.; Inoue, D.; Ding, Y.; Imagawa, J.; Doki, N.; Matsui, H.; Yahata, T.; Matsushita, H.; Ando, K.; Sashida, G.; et al. RUNX1/AML1 mutant collaborates with BMI1 overexpression in the development of human and murine myelodysplastic syndromes. *Blood* **2013**, *121*, 3434–3446. [[CrossRef](#)]

46. Bernard, E.; Nannya, Y.; Hasserjian, R.P.; Devlin, S.M.; Tuechler, H.; Medina-Martinez, J.S.; Yoshizato, T.; Shiozawa, Y.; Saiki, R.; Malcovati, L.; et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. *Nat. Med.* **2020**, *26*, 1549–1556. [[CrossRef](#)]
47. Lieschke, E.; Wang, Z.; Kelly, G.L.; Strasser, A. Discussion of some ‘knowns’ and some ‘unknowns’ about the tumour suppressor p53. *J. Mol. Cell Biol.* **2018**, *11*, 212–223. [[CrossRef](#)]
48. Kon, A.; Shih, L.-Y.; Minamino, M.; Sanada, M.; Shiraishi, Y.; Nagata, Y.; Yoshida, K.; Okuno, Y.; Bando, M.; Nakato, R.; et al. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. *Nat. Genet.* **2013**, *45*, 1232–1237. [[CrossRef](#)]
49. Kennedy, A.L.; Shimamura, A. Genetic predisposition to MDS: Clinical features and clonal evolution. *Blood* **2019**, *133*, 1071–1085. [[CrossRef](#)]
50. Churpek, J.E.; Pyrtel, K.; Kanchi, K.L.; Shao, J.; Koboldt, D.; Miller, C.A.; Shen, D.; Fulton, R.; O’Laughlin, M.; Fronick, C.; et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood* **2015**, *126*, 2484–2490. [[CrossRef](#)]
51. Tatton-Brown, K.; Zachariou, A.; Loveday, C.; Renwick, A.; Mahamdallie, S.; Aksglaede, L.; Baralle, D.; Barge-Schaapveld, D.; Blyth, M.; Bouma, M.; et al. The Tatton-Brown-Rahman Syndrome: A clinical study of 55 individuals with de novo constitutive DNMT3A variants. *Wellcome Open Res.* **2018**, *3*, 46. [[CrossRef](#)]
52. Hollink, I.; van den Ouweland, A.M.W.; Beverloo, H.B.; Arentsen-Peters, S.; Zwaan, C.M.; Wagner, A. Acute myeloid leukaemia in a case with Tatton-Brown-Rahman syndrome: The peculiar DNMT3A R882 mutation. *J. Med. Genet.* **2017**, *54*, 805–808. [[CrossRef](#)]
53. Jacobs, R.H.; Cornbleet, M.A.; Vardiman, J.W.; Larson, R.A.; Le Beau, M.M.; Rowley, J.D. Prognostic implications of morphology and karyotype in primary myelodysplastic syndromes. *Blood* **1986**, *67*, 1765–1772. [[CrossRef](#)]
54. Haase, D.; Stevenson, K.E.; Neubergh, D.; Maciejewski, J.P.; Nazha, A.; Sekeres, M.A.; Ebert, B.L.; Garcia-Manero, G.; Haferlach, C.; Haferlach, T.; et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. *Leukemia* **2019**, *33*, 1747–1758. [[CrossRef](#)]
55. Sebaa, A.; Ades, L.; Baran-Marzack, F.; Mozziconacci, M.J.; Penther, D.; Dobbelstein, S.; Stamatoullas, A.; Récher, C.; Prebet, T.; Moulessehou, S.; et al. Incidence of 17p deletions and TP53 mutation in myelodysplastic syndrome and acute myeloid leukemia with 5q deletion. *Genes Chromosomes Cancer* **2012**, *51*, 1086–1092. [[CrossRef](#)]
56. Jädersten, M.; Saft, L.; Smith, A.; Kulasekararaj, A.; Pomplun, S.; Göhring, G.; Hedlund, A.; Hast, R.; Schlegelberger, B.; Porwit, A.; et al. TP53 Mutations in Low-Risk Myelodysplastic Syndromes With del(5q) Predict Disease Progression. *J. Clin. Oncol.* **2011**, *29*, 1971–1979. [[CrossRef](#)]
57. Kere, J.; Ruutu, T.; de la Chapelle, A. Monosomy 7 in Granulocytes and Monocytes in Myelodysplastic Syndrome. *N. Engl. J. Med.* **1987**, *316*, 499–503. [[CrossRef](#)]
58. Shiseki, M.; Ishii, M.; Okada, M.; Ohwashi, M.; Wang, Y.H.; Osanai, S.; Yoshinaga, K.; Mori, N.; Motoji, T.; Tanaka, J. Expression analysis of genes located within the common deleted region of del(20q) in patients with myelodysplastic syndromes. *Leuk. Res.* **2019**, *84*, 106175. [[CrossRef](#)]
59. Caponetti, G.C.; Bagg, A. Mutations in myelodysplastic syndromes: Core abnormalities and CHIPping away at the edges. *Int. J. Lab. Hematol.* **2020**, *42*, 671–684. [[CrossRef](#)]
60. Balasubramanian, S.K.; Aly, M.; Nagata, Y.; Bat, T.; Przychodzen, B.P.; Hirsch, C.M.; Adema, V.; Visconte, V.; Kuzmanovic, T.; Radivoyevitch, T.; et al. Distinct clinical and biological implications of various DNMT3A mutations in myeloid neoplasms. *Leukemia* **2018**, *32*, 550–553. [[CrossRef](#)]
61. Young, A.L.; Challen, G.A.; Birmann, B.M.; Druley, T.E. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat. Commun.* **2016**, *7*, 12484. [[CrossRef](#)] [[PubMed](#)]
62. Buscarlet, M.; Provost, S.; Zada, Y.F.; Barhdadi, A.; Bourgoin, V.; Lépine, G.; Mollica, L.; Szuber, N.; Dubé, M.P.; Busque, L. DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. *Blood* **2017**, *130*, 753–762. [[CrossRef](#)] [[PubMed](#)]
63. Chen, T.; Ueda, Y.; Dodge, J.E.; Wang, Z.; Li, E. Establishment and Maintenance of Genomic Methylation Patterns in Mouse Embryonic Stem Cells by Dnmt3a and Dnmt3b. *Mol. Cell Biol.* **2003**, *23*, 5594–5605. [[CrossRef](#)] [[PubMed](#)]
64. Li, E.; Bestor, T.H.; Jaenisch, R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **1992**, *69*, 915–926. [[CrossRef](#)]
65. Acuna-Hidalgo, R.; Sengul, H.; Steehouwer, M.; van de Vorst, M.; Vermeulen, S.H.; Kiemeneij, L.; Veltman, J.A.; Gilissen, C.; Hoischen, A. Ultra-sensitive Sequencing Identifies High Prevalence of Clonal Hematopoiesis-Associated Mutations throughout Adult Life. *Am. J. Hum. Genet.* **2017**, *101*, 50–64. [[CrossRef](#)]
66. Guermouche, H.; Ravalet, N.; Gallay, N.; Deswarte, C.; Foucault, A.; Beaud, J.; Rault, E.; Saindoy, E.; Lachot, S.; Martignoles, J.-A.; et al. High prevalence of clonal hematopoiesis in the blood and bone marrow of healthy volunteers. *Blood Adv.* **2020**, *4*, 3550–3557. [[CrossRef](#)]
67. Challen, G.A.; Sun, D.; Mayle, A.; Jeong, M.; Luo, M.; Rodriguez, B.; Mallaney, C.; Celik, H.; Yang, L.; Xia, Z.; et al. Dnmt3a and Dnmt3b Have Overlapping and Distinct Functions in Hematopoietic Stem Cells. *Cell Stem Cell* **2014**, *15*, 350–364. [[CrossRef](#)]
68. Zhang, X.; Su, J.; Jeong, M.; Ko, M.; Huang, Y.; Park, H.J.; Guzman, A.; Lei, Y.; Huang, Y.H.; Rao, A.; et al. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. *Nat. Genet.* **2016**, *48*, 1014–1023. [[CrossRef](#)]

69. Izzo, F.; Lee, S.C.; Poran, A.; Chaligine, R.; Gaiti, F.; Gross, B.; Murali, R.R.; Deochand, S.D.; Ang, C.; Jones, P.W.; et al. DNA methylation disruption reshapes the hematopoietic differentiation landscape. *Nat. Genet.* **2020**, *52*, 378–387. [[CrossRef](#)]
70. Dai, Y.J.; Wang, Y.Y.; Huang, J.Y.; Xia, L.; Shi, X.D.; Xu, J.; Lu, J.; Su, X.B.; Yang, Y.; Zhang, W.N.; et al. Conditional knockin of Dnmt3a R878H initiates acute myeloid leukemia with mTOR pathway involvement. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 5237–5242. [[CrossRef](#)]
71. Kim, M.S.; Kim, Y.R.; Yoo, N.J.; Lee, S.H. Mutational analysis of DNMT3A gene in acute leukemias and common solid cancers. *APMIS Acta Pathol. Microbiol. Immunol. Scand.* **2013**, *121*, 85–94. [[CrossRef](#)]
72. Nguyen, T.V.; Yao, S.; Wang, Y.; Rolfe, A.; Selvaraj, A.; Darman, R.; Ke, J.; Warmuth, M.; Smith, P.G.; Larsen, N.A.; et al. The R882H DNMT3A hot spot mutation stabilizes the formation of large DNMT3A oligomers with low DNA methyltransferase activity. *J. Biol. Chem.* **2019**, *294*, 16966–16977. [[CrossRef](#)]
73. Russler-Germain, D.A.; Spencer, D.H.; Young, M.A.; Lamprecht, T.L.; Miller, C.A.; Fulton, R.; Meyer, M.R.; Erdmann-Gilmore, P.; Townsend, R.R.; Wilson, R.K.; et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. *Cancer Cell* **2014**, *25*, 442–454. [[CrossRef](#)]
74. Lu, R.; Wang, J.; Ren, Z.; Yin, J.; Wang, Y.; Cai, L.; Wang, G.G. A Model System for Studying the DNMT3A Hotspot Mutation (DNMT3AR882) Demonstrates a Causal Relationship between Its Dominant-Negative Effect and Leukemogenesis. *Cancer Res.* **2019**, *79*, 3583. [[CrossRef](#)]
75. Emperle, M.; Dukatz, M.; Kunert, S.; Holzer, K.; Rajavelu, A.; Jurkowska, R.Z.; Jeltsch, A. The DNMT3A R882H mutation does not cause dominant negative effects in purified mixed DNMT3A/R882H complexes. *Sci. Rep.* **2018**, *8*, 13242. [[CrossRef](#)]
76. Emperle, M.; Adam, S.; Kunert, S.; Dukatz, M.; Baude, A.; Plass, C.; Rathert, P.; Bashtrykov, P.; Jeltsch, A. Mutations of R882 change flanking sequence preferences of the DNA methyltransferase DNMT3A and cellular methylation patterns. *Nucleic Acids Res.* **2019**, *47*, 11355–11367. [[CrossRef](#)]
77. Holz-Schietinger, C.; Matje, D.M.; Reich, N.O. Mutations in DNA methyltransferase (DNMT3A) observed in acute myeloid leukemia patients disrupt processive methylation. *J. Biol. Chem.* **2012**, *287*, 30941–30951. [[CrossRef](#)]
78. 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. [[CrossRef](#)]
79. Ito, K.; Lee, J.; Chrysanthou, S.; Zhao, Y.; Josephs, K.; Sato, H.; Teruya-Feldstein, J.; Zheng, D.; Dawlaty, M.M.; Ito, K. Non-catalytic Roles of Tet2 Are Essential to Regulate Hematopoietic Stem and Progenitor Cell Homeostasis. *Cell Rep.* **2019**, *28*, 2480–2490. [[CrossRef](#)]
80. Liu, Y.; Elf, S.E.; Miyata, Y.; Sashida, G.; Liu, Y.; Huang, G.; Di Giandomenico, S.; Lee, J.M.; Deblasio, A.; Menendez, S.; et al. p53 regulates hematopoietic stem cell quiescence. *Cell Stem Cell* **2009**, *4*, 37–48. [[CrossRef](#)]
81. Asai, T.; Liu, Y.; Di Giandomenico, S.; Bae, N.; Ndiaye-Lobry, D.; Deblasio, A.; Menendez, S.; Antipin, Y.; Reva, B.; Wevrick, R.; et al. Necdin, a p53 target gene, regulates the quiescence and response to genotoxic stress of hematopoietic stem/progenitor cells. *Blood* **2012**, *120*, 1601–1612. [[CrossRef](#)] [[PubMed](#)]
82. Chen, S.; Gao, R.; Yao, C.; Kobayashi, M.; Liu, S.Z.; Yoder, M.C.; Broxmeyer, H.; Kapur, R.; Boswell, H.S.; Mayo, L.D.; et al. Genotoxic stresses promote clonal expansion of hematopoietic stem cells expressing mutant p53. *Leukemia* **2018**, *32*, 850–854. [[CrossRef](#)] [[PubMed](#)]
83. Bondar, T.; Medzhitov, R. p53-Mediated Hematopoietic Stem and Progenitor Cell Competition. *Cell Stem Cell* **2010**, *6*, 309–322. [[CrossRef](#)] [[PubMed](#)]
84. Marusyk, A.; Porter, C.C.; Zaberezhnyy, V.; DeGregori, J. Irradiation Selects for p53-Deficient Hematopoietic Progenitors. *PLoS Biol.* **2010**, *8*, e1000324. [[CrossRef](#)]
85. Chen, S.; Wang, Q.; Yu, H.; Capitano, M.L.; Vemula, S.; Nabinger, S.C.; Gao, R.; Yao, C.; Kobayashi, M.; Geng, Z.; et al. Mutant p53 drives clonal hematopoiesis through modulating epigenetic pathway. *Nat. Commun.* **2019**, *10*, 5649. [[CrossRef](#)]
86. Lindsley, R.C.; Saber, W.; Mar, B.G.; Redd, R.; Wang, T.; Haagenson, M.D.; Grauman, P.V.; Hu, Z.-H.; Spellman, S.R.; Lee, S.J.; et al. Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation. *N. Engl. J. Med.* **2017**, *376*, 536–547. [[CrossRef](#)]
87. Rocquain, J.; Carbuccia, N.; Trouplin, V.; Raynaud, S.; Murati, A.; Nezri, M.; Tadrist, Z.; Olschwang, S.; Vey, N.; Birnbaum, D.; et al. Combined mutations of ASXL1, CBL, FLT3, IDH1, IDH2, JAK2, KRAS, NPM1, NRAS, RUNX1, TET2 and WT1 genes in myelodysplastic syndromes and acute myeloid leukemias. *BMC Cancer* **2010**, *10*, 401. [[CrossRef](#)]
88. Boulwood, J.; Perry, J.; Pellagatti, A.; Fernandez-Mercado, M.; Fernandez-Santamaria, C.; Calasanz, M.J.; Larrayoz, M.J.; Garcia-Delgado, M.; Giagounidis, A.; Malcovati, L.; et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. *Leukemia* **2010**, *24*, 1062–1065. [[CrossRef](#)]
89. Nagase, R.; Inoue, D.; Pastore, A.; Fujino, T.; Hou, H.-A.; Yamasaki, N.; Goyama, S.; Saika, M.; Kanai, A.; Sera, Y.; et al. Expression of mutant Asxl1 perturbs hematopoiesis and promotes susceptibility to leukemic transformation. *J. Exp. Med.* **2018**, *215*, 1729–1747. [[CrossRef](#)]
90. Abdel-Wahab, O.; Adli, M.; LaFave, L.M.; Gao, J.; Hricik, T.; Shih, A.H.; Pandey, S.; Patel, J.P.; Chung, Y.R.; Koche, R.; et al. ASXL1 Mutations Promote Myeloid Transformation through Loss of PRC2-Mediated Gene Repression. *Cancer Cell* **2012**, *22*, 180–193. [[CrossRef](#)]
91. Jani, K.S.; Jain, S.U.; Ge, E.J.; Diehl, K.L.; Lundgren, S.M.; Müller, M.M.; Lewis, P.W.; Muir, T.W. Histone H3 tail binds a unique sensing pocket in EZH2 to activate the PRC2 methyltransferase. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 8295–8300. [[CrossRef](#)]

92. Steensma, D.P.; Dewald, G.W.; Lasho, T.L.; Powell, H.L.; McClure, R.F.; Levine, R.L.; Gilliland, D.G.; Tefferi, A. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both “atypical” myeloproliferative disorders and myelodysplastic syndromes. *Blood* **2005**, *106*, 1207–1209. [[CrossRef](#)]
93. Sano, S.; Wang, Y.; Yura, Y.; Sano, M.; Oshima, K.; Yang, Y.; Katanasaka, Y.; Min, K.-D.; Matsuura, S.; Ravid, K.; et al. JAK2 (V617F)-Mediated Clonal Hematopoiesis Accelerates Pathological Remodeling in Murine Heart Failure. *JACC Basic Transl. Sci.* **2019**, *4*, 684–697. [[CrossRef](#)]
94. Ko, M.; Rao, A. TET2: Epigenetic safeguard for HSC. *Blood* **2011**, *118*, 4501–4503. [[CrossRef](#)]
95. Tung-Liang, L.; Yasunobu, N.; Hsiao-Wen, K.; Masashi, S.; Yusuke, O.; Chein-Fuang, H.; Der-Cherng, L.; Ming-Chung, K.; Chang-Liang, L.; En-Hui, L.; et al. Clonal leukemic evolution in myelodysplastic syndromes with TET2 and IDH1/2 mutations. *Haematologica* **2014**, *99*, 28–36. [[CrossRef](#)]
96. Pastor, W.A.; Aravind, L.; Rao, A. TETonic shift: Biological roles of TET proteins in DNA demethylation and transcription. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 341–356. [[CrossRef](#)]
97. Cimmino, L.; Dolgalev, I.; Wang, Y.; Yoshimi, A.; Martin, G.H.; Wang, J.; Ng, V.; Xia, B.; Witkowski, M.T.; Mitchell-Flack, M.; et al. Restoration of TET2 Function Blocks Aberrant Self-Renewal and Leukemia Progression. *Cell* **2017**, *170*, 1079–1095.e1020. [[CrossRef](#)]
98. Sun, J.; He, X.; Zhu, Y.; Ding, Z.; Dong, H.; Feng, Y.; Du, J.; Wang, H.; Wu, X.; Zhang, L.; et al. SIRT1 Activation Disrupts Maintenance of Myelodysplastic Syndrome Stem and Progenitor Cells by Restoring TET2 Function. *Cell Stem Cell* **2018**, *23*, 355–369.e359. [[CrossRef](#)]
99. Levine, A.J.; Oren, M. The first 30 years of p53: Growing ever more complex. *Nat. Rev. Cancer* **2009**, *9*, 749–758. [[CrossRef](#)]
100. Brosh, R.; Rotter, V. When mutants gain new powers: News from the mutant p53 field. *Nat. Rev. Cancer* **2009**, *9*, 701–713. [[CrossRef](#)]
101. Coombs, C.C.; Zehir, A.; Devlin, S.M.; Kishtagari, A.; Syed, A.; Jonsson, P.; Hyman, D.M.; Solit, D.B.; Robson, M.E.; Baselga, J.; 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.e374. [[CrossRef](#)]
102. Wong, T.N.; Miller, C.A.; Jotte, M.R.M.; Bagegni, N.; Baty, J.D.; Schmidt, A.P.; Cashen, A.F.; Duncavage, E.J.; Helton, N.M.; Fiala, M.; et al. Cellular stressors contribute to the expansion of hematopoietic clones of varying leukemic potential. *Nat. Commun.* **2018**, *9*, 455. [[CrossRef](#)]
103. Nikoloski, G.; Langemeijer, S.M.C.; Kuiper, R.P.; Knops, R.; Massop, M.; Tönnissen, E.R.L.T.M.; van der Heijden, A.; Scheele, T.N.; Vandenbergh, P.; de Witte, T.; et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat. Genet.* **2010**, *42*, 665–667. [[CrossRef](#)]
104. Takahashi, K.; Patel, K.; Bueso-Ramos, C.; Zhang, J.; Gumbs, C.; Jabbour, E.; Kadia, T.; Andreff, M.; Konopleva, M.; DiNardo, C.; et al. Clinical implications of TP53 mutations in myelodysplastic syndromes treated with hypomethylating agents. *Oncotarget* **2016**, *7*, 14172–14187. [[CrossRef](#)]
105. Kulasekararaj, A.G.; Smith, A.E.; Mian, S.A.; Mohamedali, A.M.; Krishnamurthy, P.; Lea, N.C.; Gäken, J.; Pennaneach, C.; Ireland, R.; Czepulkowski, B.; et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. *Br. J. Haematol.* **2013**, *160*, 660–672. [[CrossRef](#)]
106. Sperling, A.S.; Gibson, C.J.; Ebert, B.L. The genetics of myelodysplastic syndrome: From clonal haematopoiesis to secondary leukaemia. *Nat. Rev. Cancer* **2017**, *17*, 5–19. [[CrossRef](#)]
107. Lindsley, R.C.; Ebert, B.L. Molecular Pathophysiology of Myelodysplastic Syndromes. *Annu. Rev. Pathol. Mech. Dis.* **2013**, *8*, 21–47. [[CrossRef](#)] [[PubMed](#)]
108. Sallman, D.A.; McLemore, A.F.; Aldrich, A.L.; Komrokji, R.S.; McGraw, K.L.; Dhawan, A.; Geyer, S.; Hou, H.A.; Eksioğlu, E.A.; Sullivan, A.; et al. TP53 mutations in myelodysplastic syndromes and secondary AML confer an immunosuppressive phenotype. *Blood* **2020**, *136*, 2812–2823. [[CrossRef](#)] [[PubMed](#)]
109. Park, U.-H.; Yoon, S.K.; Park, T.; Kim, E.-J.; Um, S.-J. Additional sex comb-like (ASXL) proteins 1 and 2 play opposite roles in adipogenesis via reciprocal regulation of peroxisome proliferator-activated receptor γ . *J. Biol. Chem.* **2011**, *286*, 1354–1363. [[CrossRef](#)] [[PubMed](#)]
110. Guo, S.; Bai, H.; Megyola, C.M.; Halene, S.; Krause, D.S.; Scadden, D.T.; Lu, J. Complex oncogene dependence in microRNA-125a-induced myeloproliferative neoplasms. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 16636–16641. [[CrossRef](#)]
111. Wang, J.; Li, Z.; He, Y.; Pan, F.; Chen, S.; Rhodes, S.; Nguyen, L.; Yuan, J.; Jiang, L.; Yang, X.; et al. Loss of Asxl1 leads to myelodysplastic syndrome-like disease in mice. *Blood* **2014**, *123*, 541–553. [[CrossRef](#)]
112. Abdel-Wahab, O.; Gao, J.; Adli, M.; Dey, A.; Trimarchi, T.; Chung, Y.R.; Kuscu, C.; Hricik, T.; Ndiaye-Lobry, D.; Lafave, L.M.; et al. Deletion of Asxl1 results in myelodysplasia and severe developmental defects in vivo. *J. Exp. Med.* **2013**, *210*, 2641–2659. [[CrossRef](#)]
113. Inoue, D.; Kitaura, J.; Togami, K.; Nishimura, K.; Enomoto, Y.; Uchida, T.; Kagiya, Y.; Kawabata, K.C.; Nakahara, F.; Izawa, K.; et al. Myelodysplastic syndromes are induced by histone methylation-altering ASXL1 mutations. *J. Clin. Investig.* **2013**, *123*, 4627–4640. [[CrossRef](#)]
114. McKerrell, T.; Park, N.; Moreno, T.; Grove, C.S.; Ponstingl, H.; Stephens, J.; Group, U.S.S.; Crawley, C.; Craig, J.; Scott, M.A.; et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep.* **2015**, *10*, 1239–1245. [[CrossRef](#)]

115. Ihle, J.N.; Kerr, I.M. Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends Genet.* **1995**, *11*, 69–74. [[CrossRef](#)]
116. Marty, C.; Lacout, C.; Martin, A.; Hasan, S.; Jacquot, S.; Birling, M.-C.; Vainchenker, W.; Villeval, J.-L. Myeloproliferative neoplasm induced by constitutive expression of JAK2V617F in knock-in mice. *Blood* **2010**, *116*, 783–787. [[CrossRef](#)]
117. Akada, H.; Yan, D.; Zou, H.; Fiering, S.; Hutchison, R.E.; Mohi, M.G. Conditional expression of heterozygous or homozygous Jak2V617F from its endogenous promoter induces a polycythemia vera-like disease. *Blood* **2010**, *115*, 3589–3597. [[CrossRef](#)]
118. Flynn, M.C.; Kraakman, M.J.; Tikellis, C.; Lee, M.K.S.; Hanssen, N.M.J.; Kammoun, H.L.; Pickering, R.J.; Dragoljevic, D.; Al-Sharea, A.; Barrett, T.J.; et al. Transient Intermittent Hyperglycemia Accelerates Atherosclerosis by Promoting Myelopoiesis. *Circ. Res.* **2020**, *127*, 877–892. [[CrossRef](#)]
119. Nagareddy, P.R.; Murphy, A.J.; Stirzaker, R.A.; Hu, Y.; Yu, S.; Miller, R.G.; Ramkhalawon, B.; Distel, E.; Westerterp, M.; Huang, L.-S.; et al. Hyperglycemia promotes myelopoiesis and impairs the resolution of atherosclerosis. *Cell Metab.* **2013**, *17*, 695–708. [[CrossRef](#)]
120. Nagareddy, P.R.; Kraakman, M.; Masters, S.L.; Stirzaker, R.A.; Gorman, D.J.; Grant, R.W.; Dragoljevic, D.; Hong, E.S.; Abdel-Latif, A.; Smyth, S.S.; et al. Adipose tissue macrophages promote myelopoiesis and monocytosis in obesity. *Cell Metab.* **2014**, *19*, 821–835. [[CrossRef](#)]
121. Dragoljevic, D.; Kraakman, M.J.; Nagareddy, P.R.; Ngo, D.; Shihata, W.; Kammoun, H.L.; Whillas, A.; Lee, M.K.S.; Al-Sharea, A.; Pernes, G.; et al. Defective cholesterol metabolism in haematopoietic stem cells promotes monocyte-driven atherosclerosis in rheumatoid arthritis. *Eur. Heart J.* **2018**, *39*, 2158–2167. [[CrossRef](#)] [[PubMed](#)]
122. Dayyani, F.; Conley, A.P.; Strom, S.S.; Stevenson, W.; Cortes, J.E.; Borthakur, G.; Faderl, S.; O'Brien, S.; Pierce, S.; Kantarjian, H.; et al. Cause of death in patients with lower-risk myelodysplastic syndrome. *Cancer* **2010**, *116*, 2174–2179. [[CrossRef](#)] [[PubMed](#)]
123. Adrianzen Herrera, D.; Pradhan, K.; Snyder, R.; Karanth, S.; Janakiram, M.; Mantzaris, I.; Braunschweig, I.; Budhathoki, A.; Shah, U.A.; Verma, A.K.; et al. Myelodysplastic syndromes and the risk of cardiovascular disease in older adults: A SEER-medicare analysis. *Leukemia* **2020**, *34*, 1689–1693. [[CrossRef](#)]
124. World Health Organization. *Cardiovascular Diseases*; World Health Organization: Geneva, Switzerland, 2017.
125. Murphy, A.J.; Tall, A.R. Disordered haematopoiesis and athero-thrombosis. *Eur. Heart J.* **2016**, *37*, 1113–1121. [[CrossRef](#)]
126. Delhommeau, F.; Dupont, S.; Valle, V.D.; James, C.; Trannoy, S.; Massé, A.; Kosmider, O.; Le Couedic, J.-P.; Robert, F.; Alberdi, A.; et al. Mutation in TET2 in Myeloid Cancers. *N. Engl. J. Med.* **2009**, *360*, 2289–2301. [[CrossRef](#)]
127. Sallman, D.A.; List, A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. *Blood* **2019**, *133*, 1039–1048. [[CrossRef](#)]