CHIP, CCUS, and Other Acronyms: Definition, Implications, and Impact on Practice

Amy E. DeZern, MD, MHS[1]; Luca Malcovati, MD[2]; and Benjamin L. Ebert, MD, PhD[3]

overview

Unexplained blood cytopenias can be a clinical challenge for patients and clinicians alike. The relationship between these cytopenias and myeloid neoplasms like myelodysplastic syndromes (MDS) is currently an area of active research. There have been marked developments in our understanding of clonal hematopoiesis based on findings of somatic mutations in genes known to be associated with MDS. This has led to newer terms to describe precursor states to MDS, such as clonal hematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of undetermined significance (CCUS). These conditions may allow earlier diagnosis, modify surveillance for MDS, and guide additional therapies. This review summarizes recent updates in the field for affected patients.

INTRODUCTION

At the bedside, patients with peripheral blood cytopenias can present a diagnostic and therapeutic challenge. Complicated boundaries and categories now exist as we sort through precursor states or other etiologies of unexplained cytopenia. Refractory anemias, or bilineage and trilineage cytopenia in the context of normocellular or hypercellular bone marrows (BMs), in particular, can often present with concern for myelodysplastic syndrome (MDS) and related disorders, especially in individuals as they advance in years.[1] Specifically, MDS is a heterogeneous collection of clonal hematopoietic malignancies characterized by poor overall survival as a result of ineffective hematopoiesis, progressive cytopenias, and transformation to acute myeloid leukemia (AML).[2] MDS remain a diagnosis most are zealous to rule in or out, as the clinical course and survival can vary widely for an individual; it is this precision clinicians strive for in patients. We are now in an era when next-generation sequencing (NGS) technology permits additional diagnostic information to provide important clinical insights that may or may not allow earlier identification of predisposition states to MDS as well as more accurate capacity to diagnosis MDS.[3,4]

Specifically, patients with unexplained cytopenia are increasingly undergoing these NGS molecular genetic tests of peripheral blood or BM for diagnostic purposes. It has been recognized that these tests have the ability to detect genetic mutations that do not have definitive morphologic correlates to MDS. As such, new entities have been recently defined to lessen the risk of incorrect diagnoses of MDS as well as increase our knowledge about precursor states that may evolve to frank MDS.[5-7] Therefore, it is important to define and explain these conditions in more detail and to define them for clinical practice with as much precision as possible but also with appropriate patient relevance.

CURRENT DIAGNOSTIC APPROACH TO PATIENTS WITH PERIPHERAL BLOOD CYTOPENIA

Uni- or multilineage peripheral blood cytopenia often results from a wide range of hematologic or nonhematologic disorders, including viral infections, autoimmune disorders, liver diseases, iatrogenic toxicity, and vitamin deficiencies, along with hematologic cancers and BM failure syndromes.[8] The term unexplained cytopenia is used to define a condition characterized by peripheral blood cytopenia in which origin is not attributable to causes detectable with conventional tests or to any concomitant diseases.[9]

A critical step in the diagnostic approach to patients with cytopenia is to distinguish reactive causes of cytopenia from clonal hematologic disorders, especially myeloid neoplasms. In the context of myeloid malignancies, peripheral blood cytopenia is a typical manifestation of myelodysplasia, a condition characterized by ineffective differentiation and maturation, associated with morphologic abnormalities, in one or more of the hematopoietic lineages.[10] Myelodysplasia is a typical feature of MDS, but it can be found also in other myeloid neoplasms of the World Health Organization (WHO) classification, including myelodysplastic/myeloproliferative neoplasms (MDS/MPNs) and secondary AML.[11] In addition, emerging evidence suggests that primary myelofibrosis, currently included in the category of MPNs by the WHO classification of hematopoietic tumors, shares mixed myeloproliferative and myelodysplastic features at both molecular and

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on May 17, 2019 and published at ascopubs.org on May 17, 2019: DOI https://doi.org/10.1200/EDBK_239083

PRACTICAL APPLICATIONS

- In the context of unexplained cytopenia, there is diagnostic value of a somatic mutation analysis on DNA from peripheral blood cells for patients, which can lead to identification of CHIP or CCUS.
- Knowledge of this biologic information can allow earlier diagnoses, modify the surveillance, and possibly guide earlier therapy (when clinically appropriate).
- Transformation to overt malignancy from CHIP or CCUS generally requires the sequential acquisition of multiple mutations.
- Individuals with CHIP have an approximately 10-fold increased risk of developing a hematologic malignancy, with the risk increasing with the size of the clone and overall risk estimated at 0.5% to 1% per year.
- CHIP is associated with increased overall mortality as well as an increased risk of cardiovascular disease.

clinical levels.[12] Recognizing or excluding a myeloid neoplasm may be even more relevant in individuals previously exposed to chemotherapy or radiotherapy for nonmyeloid malignancies, including either solid tumors or lymphomas, who are at higher risk of developing therapy-related myeloid neoplasms.[11]

The current diagnostic approach to a suspect myeloid neoplasm with myelodysplasia includes morphologic studies of peripheral blood and BM aspirate smears, BM biopsy, and cytogenetic or molecular genetic studies aimed at identifying selected chromosomal abnormalities or genetic lesions recognized of diagnostic value by the WHO classification.[8,13]

Targeted sequencing of a limited number of genes can detect mutations in almost any patient with a myeloid neoplasm with myelodysplasia,[14-16] and a screening for somatic mutations on peripheral blood cells promises to be a valuable complement to the current diagnostic work-up of unexplained cytopenia by providing candidate biomarkers for identifying individuals with, or at high risk of developing, a myeloid neoplasm. However, the interpretation of mutation profiles in patients with cytopenia has been challenging, as some of these genes are commonly detected in elderly adults showing a normal blood count as well as in individuals with nonmalignant BM failure syndromes.[5,7,17]

TABLE 1. Major Driver Genes in Myeloid Neoplasms With Myelodysplasia

Pathway/Function	Genes
DNA Methylation	*DNMT3A, TET2, IDH1, IDH2*
Chromatin Modification	*ASXL1, EZH2, KDM6A, JARID2, PHF6*
RNA Splicing	*SF3B1, SRSF2, U2AF1, ZRSR2, SF1, PRPF8*
Transcription Regulation	*RUNX1, ETV6, GATA2, BCOR, BCORL1, CUX1, IRF1*
Signaling	*NRAS, KRAS, CBL, NF1, PTPN11, GNAS, PTEN, KIT, FLT3, JAK2, MPL, CALR*
Cell Cycle	*TP53, PPM1D, CDKN2A*
Others	*NPM1, SETBP1*

GENETIC LANDSCAPE OF MYELOID NEOPLASMS WITH MYELODYSPLASIA

To appreciate the utility of these panels in peripheral cytopenia, we will first describe their relevance in true myeloid neoplasms or MDS. In the past decade, comprehensive analyses of known or candidate genes relevant in myeloid neoplasms using massive parallel sequencing showed that more than 90% of patients with myeloid malignancy with myelodysplasia carry one or more oncogenic mutations (Table 1).[14-16]

A high prevalence of somatic mutations in genes that control DNA methylation (*TET2*, *DNMT3A*, *IDH1*, *IDH2*) or histone modification (*ASXL1*, *EZH2*) has been reported in patients with myeloid neoplasms.[18-21] Mutations in these genes are common drivers of age-related clonal hematopoiesis in individuals without hematologic phenotype, and consistent clinical and experimental evidence supports the notion that these lesions are common drivers of premalignant clones occurring frequently during aging as well as after exposure to chemoradiotherapy for solid tumors.[5,7,22-24]

More than 50% of patients suffering from a myeloid neoplasm with myelodysplasia carry somatic mutations in genes encoding splicing factors, mainly *SF3B1*, *SRSF2*, and *U2AF1*.[25-27] Mutations of the RNA splicing machinery are most often early events in these malignancies, with a variant allele frequency (VAF) typically between 40% and 50%. Genotype-phenotype correlation studies consistently show strong associations between spliceosome mutations and myelodysplasia. In fact, *SF3B1* mutations were proven highly predictive of ring sideroblasts, and a direct link between abnormal splicing of target genes and disease pathophysiology was proven.[28,29] Likewise, *SRSF2* mutations were detected in approximately half of patients with MDS/MPN with monocytosis.[26,30,31]

TP53 mutations are consistently detected in almost all subtypes of myeloid neoplasm, mainly associated with complex karyotype and unfavorable clinical outcome, and are greatly enriched in patients with therapy-related myeloid neoplasms.[32,33] Recent studies of clonal hematopoiesis have confirmed an enrichment of mutations in *TP53* in cohorts of patients previously exposed to cytotoxic therapy relative to their frequency in unselected cohorts.[23,34,35] This

is true to an even greater degree with mutations in *PPM1D*, a phosphatase that negatively regulates multiple components of the DNA damage response pathway. Cells with *PPM1D* mutations have a powerful competitive advantage under the selective pressure of chemotherapy, as occurs in the development of therapy-related myeloid neoplasms.[36,37]

Additional driver mutant genes in myeloid neoplasm with myelodysplasia include transcription regulation (*RUNX1*, *CUX1*), signal transduction (*NRAS*, *KRAS*, *CBL*) and cohesin complex (*STAG2*, *RAD21*). In most cases, these are the subclonal genetic events driving disease progression.[14-16]

The presence of mutations in splicing factors, epigenetic regulators, or cohesin complex also defines a core set of mutations that is highly specific to cases of secondary AML with an antecedent MDS phase.[38] Notably, mutations in the same genes are also a typical finding in the majority of primary myelofibrosis occurrences, either in association or not with MPN-restricted driver genes. In this disorder, the presence of these mutated genes increases the myelodysplastic features and the severity of the disease.[12]

CLINICAL SIGNIFICANCE OF SOMATIC MUTATION IN UNEXPLAINED BLOOD CYTOPENIA

Despite the potential ambiguities in the interpretation of mutation profiles in the context of unexplained cytopenia, recent studies explored the diagnostic value of a somatic mutation analysis on DNA from peripheral blood cells in patients with cytopenia, providing useful hints for their disentangling.[4,39]

A recent study applying targeted sequencing of genes recurrently mutated in myeloid malignancies on DNA from peripheral blood cells to a prospective cohort of individuals with unexplained cytopenia undergoing a comprehensive diagnostic work-up showed that both the number of somatic mutations per patient and the size of the mutant clone, as defined by the VAF, had clinically meaningful predictive value to recognize or rule out a diagnosis of myeloid neoplasm. In fact, having two or more mutations or having a somatic mutation with VAF greater than 10% was highly predictive of a diagnosis of myeloid neoplasm. Conversely, the absence of mutations in the set of genes studied had a high negative predictive value for such malignancies. In addition, considerable differences were found between different mutant genes. In agreement with previous biologic and clinical observations, mutations in spliceosome genes showed the highest predictive value. By contrast, the predictive value of isolated mutations in genes frequently mutated in age-related clonal hematopoiesis, like *TET2*, *DNMT3A*, and *ASXL1*, was low, whereas the concomitant detection of concurrent mutations was highly predictive of a diagnosis of myeloid neoplasm. Notably, the positive predictive values of the number of somatic mutations were comparable using different sets of 10 to 40 genes, whereas the negative predictive value tended to increase with the number of genes analyzed.[4]

A more recent study examined the use of mutational analysis in the investigation of patients presenting with a monocytosis in most cases in the context of uni- or multilineage peripheral blood cytopenia. Somatic mutations were detected in 79% of patients, being invariably identified in those with a confirmed diagnosis of myeloid neoplasm though also in 57% of patients with nondiagnostic BM features. Objective outcome measures, including overall survival and longitudinal blood counts, indicated that the presence of a mutation was associated with outcome indistinguishable from that of patients with myeloid neoplasm and notably worse than patients without a mutation. Furthermore, the presence of a mutation was associated with a progressive fall in hemoglobin and platelet levels and increasing monocyte counts compared with patients without a mutation.[40]

Taken together, these findings suggest that mutation profiling on peripheral blood cells may be a valuable complement to the current diagnostic work-up of unexplained cytopenia.[13,41] The number of mutations identified in each patient, the size of the mutant clone as defined by the VAF, and mutation profiles have high predictive values for identifying individuals with a myeloid neoplasm. Furthermore, the absence of somatic mutations has a high negative predictive value for an underlying myeloid malignancy. Optimization of the sequencing design to detect clinically relevant copy number alterations by targeting single nucleotide polymorphisms is likely to further improve the sensitivity of mutation analysis, enabling simultaneous detection of both gene mutations and cytogenetic abnormalities in a single assay.[15] The implementation of noninvasive diagnostic procedures are in turn expected to improve compliance to diagnostic tests, in particular in the frail population of elderly individuals, and overall the diagnostic accuracy of myeloid neoplasms.

ICUS, CHIP, AND CCUS: DEFINITIONS AND CLINICAL CORRELATES

During the past decade, an alphabet soup of acronyms has been evolving with the goal of further subdividing patients and their hematologic conditions for clinical management in the setting of what is known about somatic mutations. It has been intended for clarity but has actually added layers of complexity at the bedside for patients and clinicians alike. These newly coined acronyms represent idiopathic cytopenia of undetermined significance (ICUS), clonal hematopoiesis of indeterminate potential (CHIP), and clonal cytopenia of undetermined significance (CCUS; Sidebar 1).[6,42] Additional acronyms used less often include IDUS (i.e., idiopathic dysplasia of unknown significance) and

ARCH (i.e., age-related clonal hematopoiesis). These precursor diagnoses go beyond simple clonal hematopoiesis but do not include frank MDS WHO criteria,[43] as they lack increased blasts, cytogenetic abnormalities, or morphologic dysplasia in at least 10% of cell lines. It is worth noting that all hematopoiesis is clonally derived, but diseases or clinical problems can arise when hematopoiesis is abnormal or marked by acquired mutations, and this is the clinical benefit of following these precursor conditions. These newer entities may be initially classified in a clinician's mind in various ways, though the nuances are a challenge to describe to patients and do still lack some precision for therapy. For understanding, there are those conditions with preserved blood counts (i.e., CHIP) compared with those with irregular blood counts (i.e., ICUS and CCUS). There are the disorders with no mutations (i.e., ICUS) and those with somatic mutations confirmed by NGS (i.e., CHIP and CCUS). These broader categories can be helpful for longitudinal follow-up and for relay of information to patients. In any patient in whom one of these states has been diagnosed, the concern of evolution to MDS dictates that proper monitoring should be pursued. This includes further studies and markers such as flow cytometry, cytogenetics, molecular, and immunohistochemical studies in order to confirm or exclude definitively the MDS diagnosis.

ICUS

ICUS is defined as peripheral cytopenia lacking fulfillment of the formal diagnostic criteria for MDS.[44] Specifically, ICUS characterized by persistent cytopenia in one or more cell lineages (leukocytes, erythrocytes, or platelets) of any degree (Sidebar 1). In patients with ICUS, the cytopenic state cannot be explained by any other (hematologic or nonhematologic) etiology.[45] In the pathology literature, ICUS has been further subdivided into ICUS-anemia, ICUS-neutropenia, ICUS-thrombocytopenia, and ICUS-bi/pancytopenia,[45] but these criteria are not routinely implemented in practice, as the clinical utility or prognostic impact of this categorization remains ambiguous.

CHIP

CHIP is a condition with no peripheral blood cytopenia but documented presence of a somatic mutation in a leukemia-associated driver gene at a VAF greater than or equal to 2%.[6] The diagnosis of CHIP is based on (1) the presence of at least one somatic mutation that is relevant clinically and is otherwise found in MDS (or other myeloid neoplasms), (2) the absence of persistent cytopenia, and (3) the exclusion of MDS and of all other hematopoietic neoplasms (and other diseases) as the causal underlying condition (Sidebar 1).[6] CHIP does require a thorough investigation of the BM to exclude any marrow infiltrative neoplasms. It should be emphasized that the VAF of the detected somatic mutations must be at least 2% to count as a CHIP-defining mutation. In patients with CHIP, mild dysplasia—but, again, no cytopenia—may also be detected. As soon as persistent (≥ 4 months) cytopenia exist in the presence of confirmed dysplasia, the diagnosis will formally meet MDS criteria.[45]

CCUS

CCUS is the presence of a WHO-defined cytopenia as well as a mutation in an MDS-associated gene.[4,42] In CCUS, there is progressive risk relative to the VAF of the mutation and number and type of mutations. This definition implies that a number of examinations must be performed (including a thorough BM investigation) to exclude MDS and to diagnose CCUS. As soon as dysplastic features are present or there is documentation of MDS-related criteria, the

SIDEBAR 1. Definitions of the Precursor States

Clonal Hematopoiesis of Indeterminate Potential (CHIP)
- Presence of at least one somatic mutation that is relevant clinically and is otherwise found in MDS (or other myeloid neoplasms)
- Absence of persistent cytopenia
- Exclusion of MDS and of all other hematopoietic neoplasms (and other diseases) as the causal underlying condition

Idiopathic Cytopenia of Undetermined Significance (ICUS)
- Relevant cytopenia in one or more lineage persistent for at least 6 months
- Not explained by any other disease
- Diagnostic criteria of myeloid neoplasm not fulfilled

Clonal Cytopenia of Undetermined Significance (CCUS)
- One or more somatic mutations otherwise found in patients with myeloid neoplasms detected in bone marrow or peripheral blood cells with an allele burden of ≥ 2%
- Persistent cytopenia (≥ 4 months) in one or more peripheral blood cell lineages
- Diagnostic criteria of myeloid neoplasm not fulfilled
- All other causes of cytopenia and molecular aberration excluded

Abbreviation: MDS, myelodysplastic syndrome.

diagnosis of CCUS changes to MDS. The demarcation between CCUS and MDS may be difficult. For example, patients with CCUS may present with mild dysplasia (< 10%) or rare (not MDS related) molecular or flow cytometric abnormalities. The clinical implications of this specific delineation may ultimately be of little therapeutic importance, as both CCUS and lower-risk MDS require watchful waiting in the initial phases.

Excluding or establishing a diagnosis of MDS may be challenging in the absence of robust morphologic changes or specific genetic abnormality. In addition, an increasing number of patients with myeloid neoplasm are diagnosed at an early phase of disease, without prominent dysplasia at the BM examination. To describe patients in whom this diagnosis is possible but not proven, the term ICUS has been introduced.[45,46] Although the rationale underlying a provisional category of cytopenia of undetermined significance is unquestionable, this original definition had intrinsic limitations. In fact, although an adequate period of observation is required, even persistent cytopenia of shorter duration should be carefully considered if a diagnosis is not yet apparent. In addition, it must be acknowledged that, unlike other hematologic conditions of undetermined significance, such as monoclonal gammopathy of undetermined significance or monoclonal B cell lymphocytosis, a diagnosis of ICUS does not require the evidence of a clonal disorder.[47]

Recent studies have shown that a substantial portion of patients with cytopenia of undetermined significance carry MDS-associated somatic mutations,[42,48] and the definition of CCUS has been proposed for describing this condition (Sidebar 1).[42,45] Two studies first reported a high prevalence of somatic mutations in patients with ICUS, with a spectrum of mutated genes largely, but not completely, overlapping with that observed in MDS.[42,48] In a study involving 369 patients with ICUS, one or more somatic mutations were identified in 62% of patients with only rare dysplastic morphology and in 20% of patients with no evidence of dysplasia. Patients with cytopenia of undetermined significance had a lower mean number of mutations per patient than those with MDS and were less likely to have two or more somatic mutations or to harbor mutations of *SF3B1* and other splicing factors.[42]

More recently, in a prospective study involving 154 individuals with ICUS, 36% of patients carried one or more mutations. Patients with CCUS had a probability of developing a myeloid neoplasm that was 14 times higher than that of patients with no evidence of clonal disease. Individuals with CCUS tended to be older than those with unmutated ICUS, with a skewed male-to-female ratio, and had lower hemoglobin levels. Furthermore, patients with selected mutation patterns proven highly specific for a myeloid neoplasm with myelodysplasia, including spliceosome gene mutations or co-mutated patterns involving epigenetic regulators, had survival and risk of disease progression comparable to those with myeloid neoplasm. This observation supports the notion that a fraction of these clonal cytopenias may represent an early manifestation of a myeloid neoplasm rather than a premalignant condition and that selected mutation signatures might provide presumptive evidence of bona fide myeloid neoplasm even in the absence of diagnostic morphologic findings, as acknowledged by the WHO classification for selected cytogenetic abnormalities.[4]

Clonal Hematopoiesis in Acquired BM Failure Syndromes

Targeted deep sequencing studies showed that approximately one-third of patients with acquired aplastic anemia, a BM failure resulting from immune-mediated or toxic destruction of hematopoietic cells, have mutations in genes commonly affected in myeloid neoplasms without having any morphologic evidence of malignancy.[17,49] The prevalence of the mutations increases with age, and the most frequently mutated genes of *DNMT3A*, *ASXL1*, *BCOR* and *BCORL1*, and *RUNX1*, along with *PIGA*, drive paroxysmal nocturnal hemoglobinuria clones. However, a considerable diversity was observed in mutation frequencies compared with myeloid neoplasms; mutations in *PIGA* and *BCOR/BCORL1* were more common in aplastic anemia. In addition, mutations in aplastic anemia showed significantly lower VAFs—on average, less than 10%. Notably, although the size of *DNMT3A*- or *ASXL1*-mutated clones tend to increase over time, that of *BCOR/BCORL1*- or *PIGA*-mutated clones are more likely to decrease or remain stable. Furthermore, mutations in *PIGA* and *BCOR/BCORL1* correlate better response to immunosuppressive therapy and favorable survival, whereas other gene mutations, including *DNMT3A* and *ASXL1*, are associated with worse survival and increased risk of progression to MDS or AML.[17] These specific mutation patterns and clonal dynamics strongly support the concept of selection of restricted cell clones under the pressure of an abnormal BM environment.[50,51]

POTENTIAL IMPLICATIONS OF DIAGNOSIS OF THE PRECURSOR STATES

ICUS, CHIP, and CCUS are all currently considered premalignant conditions that can progress to MDS, AML, or other hematologic malignancies. The main implication for making these diagnoses is that the monitoring and clinical follow-up will change so that malignancy, if it occurs, will be diagnosed efficiently in a patient. Caution is required though, as progression to malignancy is not a foregone conclusion and each precursor state does not carry the same level of risk.[42]

The clinical course of ICUS is variable and unpredictable. In a subset of patients, progression to MDS or AML is observed

after a variable time period.[47] In some patients with ICUS, a smaller clone carrying typical chromosome abnormalities (otherwise found in MDS or AML) is initially detected by a modality such as fluoresence in situ hybridization.[52] These patients who have ICUS may have a higher risk of transformation into an overt MDS or another BM neoplasm compared with patients who have ICUS with any detectable clone that is smaller in size. As soon as the size of the fluoresence in situ hybridization–positive clone increases or one or more MDS-associated somatic aberrations (mutations) are detected in a patient with ICUS, the diagnosis changes to CCUS, provided that diagnostic criteria for the diagnosis MDS are not met. Therefore, it is important to repeat cytogenetic and molecular studies during follow-up in patients with ICUS, idiopathic dysplasia of unkonwn significance, and CCUS, especially when signs of disease manifestation are found. In addition, it is equally important to perform these studies in patients with overt low-risk MDS, as the prognosis may be affected.[53]

Patients with CCUS have the highest rates of progression to MDS of the precursor states, especially if they have mutations such as a *U2AF1*, *ZRSR2*, *SRSF2*, *JAK2*, or *RUNX1*.[4] This risk may be as high as 80%–90% at 5 years with some of these mutations or in the presence of multiple (greater than two) mutations. The risk of progression to MDS is currently thought to be lower (50% at 5 years) in the presence of single mutations of *TET2*, *DNMT3A*, or *ASXL1*.[4]

Risk of Hematologic Malignancy

CHIP diagnoses have recently had two unique implications for patients (Fig. 1). Toward our oncology thoughts, the concept of CHIP is in keeping with the multiple hit theory in cancer evolution.[54] However, individuals with CHIP generally have a single somatic mutation and do not, by definition, have an overt malignancy. The mutations found in CHIP are commonly mutated in myeloid malignancies, including AML, MDS, and MPN as well as some lymphomas.[55] Transformation to malignancy, in most cases, requires the

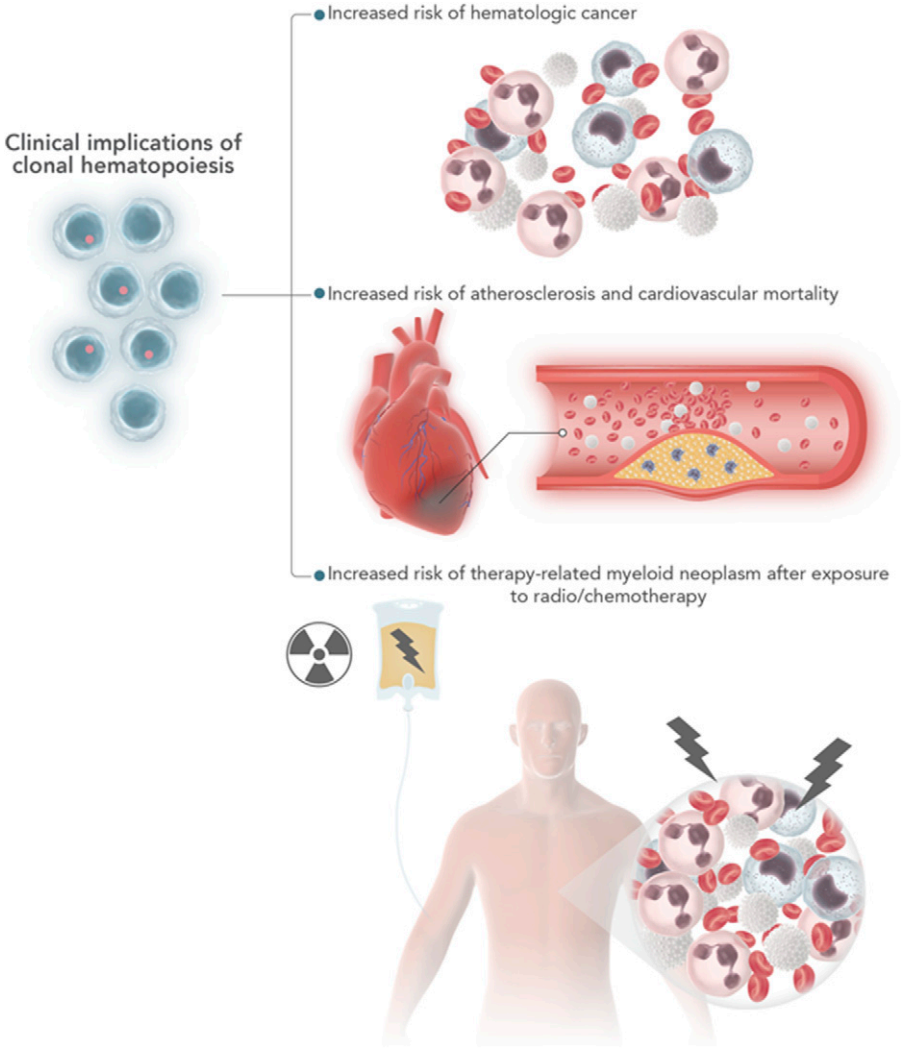

FIGURE 1. Clinical Consequences of CHIP

Individuals with CHIP have an approximately 10-fold increased risk of developing a hematologic malignancy, with a cumulative risk of transformation to malignancy of approximately 0.5% to 1% per year. CHIP is also associated with increased of myocardial infarction, playing a direct functional role in the pathogenesis of atherosclerosis. Finally, individuals with CHIP who are treated for solid tumors have an elevated risk of therapy-related myeloid neoplasms and increased overall mortality.

sequential acquisition of multiple mutations. Individuals with CHIP have an approximately 10-fold increased risk of developing a hematologic malignancy, with the risk increasing with the size of the clone (Fig. 2).[5,7] Overall, the risk of transformation to malignancy is approximately 0.5% to 1% per year, roughly the same as the risk of transformation of monoclonal gammopathy of undetermined significance to multiple myeloma.

Risk of transformation to AML has been evaluated specifically in large prospective cohort studies. Specific features predicted a three- to five-fold increased risk of developing AML. In particular, mutations in *TP53* and genes encoding splicing factors were associated with an elevated risk of developing leukemia.[56,57]

Therapy-related myeloid neoplasms represent a unique clinical scenario in which chemotherapy or radiation may select for a mutant hematopoietic stem cell clone, increasing the risk that this clone will acquire additional mutations and progress to malignancy.[33,58] In particular, mutations in *TP53* and *PPM1D* gain a selective advantage in response to radiation or chemotherapy.[23,33,36,37,59] Individuals with CHIP who are treated for solid tumors have an elevated risk of *t*-MNs and increased overall mortality.[34,35,59]

Nonmalignant Consequences of CHIP

CHIP is associated with increased overall mortality.[5,7] The increased risk of hematologic malignancies does not explain this mortality risk, as blood cancers are relatively rare. In human genetic studies, CHIP was surprisingly associated with myocardial infarction, with a hazard ratio greater than many of the established risk factors for cardiovascular disease, such as blood pressure, cholesterol levels, and smoking.[7] Validation studies have confirmed this association, with CHIP approximately doubling the risk of myocardial infarction.[60] Consistent with this finding, patients with MDS have an elevated risk of cardiovascular mortality.[61] In addition, patients with heart failure and CHIP have a particularly poor prognosis.[62]

Atherosclerosis is mediated to a large extent by blood cells, including monocytes, macrophages, and neutrophils. Two groups demonstrated functionally that *TET2* inactivation in murine blood cells, following BM transplantation, is sufficient to accelerate atherosclerosis in low density lipoprotein receptor–knockout mice.[60,63] In addition, *JAK2* mutations can enhance atherosclerosis in a murine model.[64] These studies indicate that the CHIP plays a direct functional role in the pathogenesis of atherosclerosis.

MPNs driven by *JAK2* mutations are associated with a striking risk of venous thrombosis. *JAK2* mutations are associated with an increased propensity of neutrophils to undergo NETosis. Individuals with *JAK2*-mutant CHIP, who do not have MPNs, have an approximately 12-fold increased risk of thrombosis.[65] *JAK2*-mutant CHIP is associated both with venous thrombosis and myocardial infarction.[60,65]

TET2 mutations are associated with the elaboration of inflammatory cytokines from macrophages.[60,63,66,67] Similarly, *DNMT3A* mutations have been linked to aberrant expression of a number of inflammatory cytokines.[68] Altered inflammatory response in the blood cells of patients with CHIP has the potential to influence a wide range of human

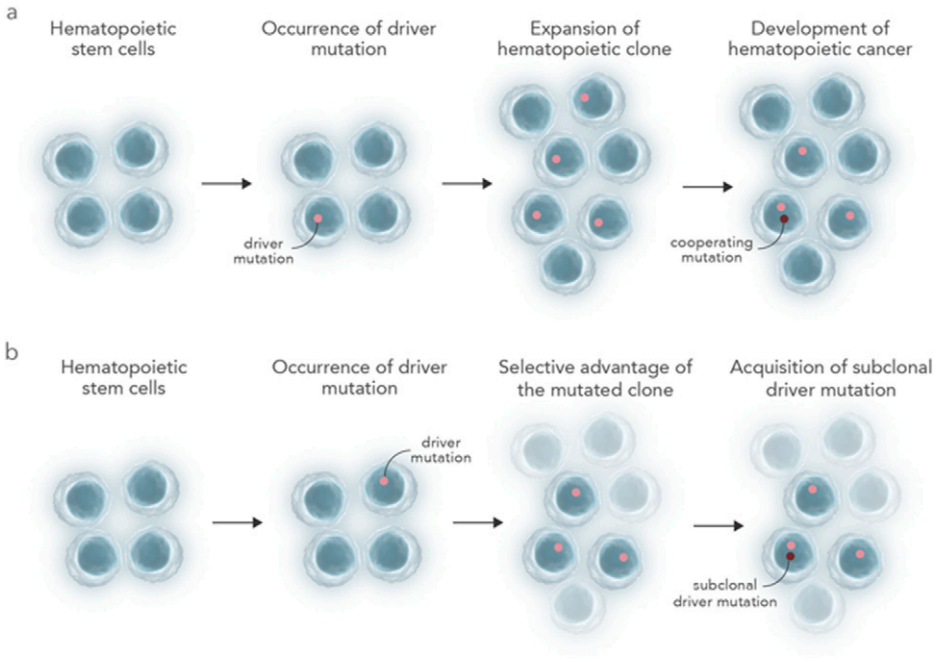

FIGURE 2. Models for the Expansion of Hematopoietic Clones and the Progression Into a Hematologic Cancer

(A) Expansion of a hematopoietic stem cell into a clonal population under the influence of a driver somatic mutation conferring a growth advantage, and progression into a myeloid neoplasm through the acquisition of subsequent subclonal driver mutations. (B) Selection of a hematopoietic stem cell carrying a somatic mutation under the pressure of hematopoietic environment. The mutated clone is more fit to survive in an abnormal microenvironment than in normal stem cells and may expand sustaining a clonal hematopoiesis and eventually progress through the acquisition of subsequent subclonal driver mutations.

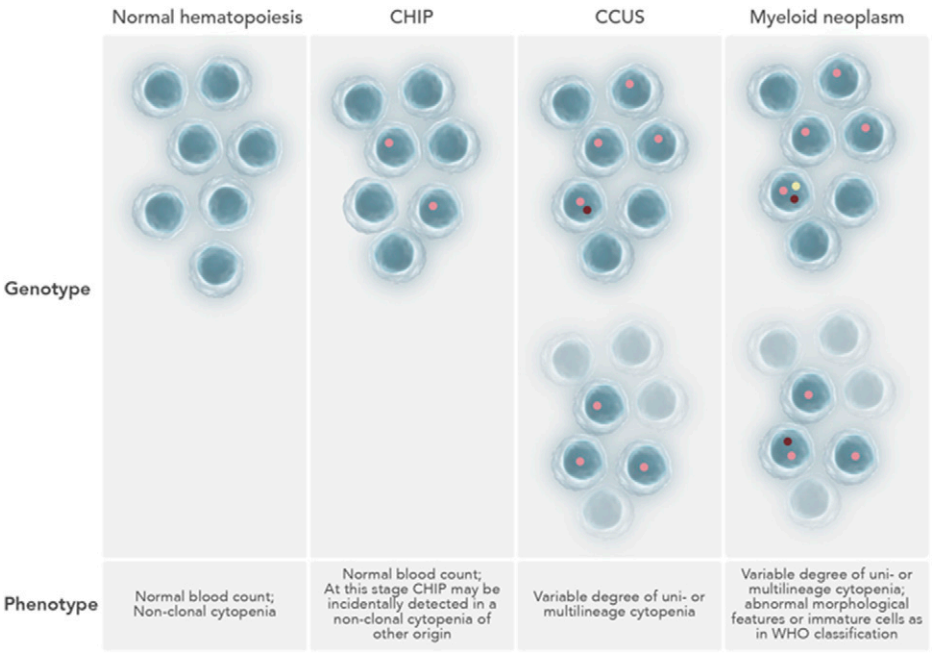

FIGURE 3. Correlations Between Genotype and Clinical Phenotype in Clonal Precursors States

CHIP is characterized by the absence of persistent cytopenia; however, a passenger CHIP may be incidentally detected in a nonclonal cytopenia of other origin. Conversely, CCUS is typically characterized by a persistent cytopenia in one or more peripheral blood cell lineages without evidence of dysplasia or associated with very mild dysplasia. Thorough investigations, including bone marrow examinations, are required to exclude a diagnosis of MDS or other myeloid neoplasm.

Abbreviations: CCUS, clonal cytopenia of undetermined significance; CHIP, clonal hematopoiesis of indeterminate potential; MDS, myelodysplastic syndrome; WHO, World Health Organization.

pathology, particularly in diseases of aging that are linked to inflammation.

IMPACT ON CLINICAL PRACTICE

Clinicians value the ability to put symptoms or diseases (and the patients in whom these affect) into categories to aid in clinical care. The aforementioned acronyms aid this goal and have the added utility of predicting with greater accuracy patients who will develop MDS (Fig. 3).[4] Additionally, these entities in many ways justify the use of NGS. This molecular genetic testing helps confirm a clonal disorder in a patient with unexplained cytopenia. Furthermore, a negative test result can also influence diagnostic assessmentbecause of the high negative predictive value of a normal result for a disorder like MDS.[41] In some of these patients, overt MDS may be diagnosed in the follow-up. The relative frequencies of mutations in these entities are delineated in an ongoing fashion in the literature,[5,7,69] and the acronyms likely are in a bit of evolution as well.

In clinical practice, use of molecular genetic testing is complicated at the individual patient level, even more so when the acronyms are applied to form categories and standards for follow-up.[41,70] During the past few years, the emerging concept of pre-MDS conditions has received attention and acceptance from the medical community, because the clinical implications of such conditions are solidifying. Overall, the recommendation is to follow pre-MDS conditions in the same way as low-risk MDS, but a few questions remain. For example, must a pre-MDS condition be factored into decisions if a patient with CHIP requires chemotherapy for another malignancy? Care should be taken to avoid prescription of medications that could predispose to the acquisition of additional somatic mutations that could escalate evolution to AML. Additionally, given the cardiovascular associations, proactive management of lipids, smoking cessation, diabetes, and other comorbid illnesses is also prudent, but we must learn whethersuch attention will alter the rates of atherosclerotic events.

CONCLUSIONS

We are increasingly fortunate in medicine to have evolving techniques that add precision to diagnoses and better refine prognoses and clinical care. As delineated in this article, the utility of somatic mutational analysis in patients with cytopenia has been demonstrated. Some of these mutational states confer a high likelihood of disease progression and may allow a provisional diagnosis of a precursor state to MDS, even if morphologic dysplasia and other diagnostic criteria are absent. There remain unanswered questions about the implications of the precursor states, and clinicians may vary in their comfort with interpreting NGS results to diagnose these states in patients. Additional studies will be forthcoming to better refine these entities.

AFFILIATIONS

[1]Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD
[2]Department of Molecular Medicine, University of Pavia, and Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

CORRESPONDING AUTHOR

Amy E. DeZern, MD, MHS, Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins,1650 Orleans St., CRBI Room 3M87, Baltimore, MD 21287-0013; email: adezern1@jhmi.edu.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/EDBK_239083.

REFERENCES

1. DeZern AE, Sekeres MA. The challenging world of cytopenias: distinguishing myelodysplastic syndromes from other disorders of marrow failure. Oncologist. 2014;19:735-745.
2. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114:937-951.
3. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol. 2011;29:504-515.
4. Malcovati L, Gallì A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood. 2017;129:3371-3378.
5. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371:2477-2487.
6. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9-16.
7. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488-2498.
8. Malcovati L, Hellström-Lindberg E, Bowen D, et al; European Leukemia Net. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. 2013;122:2943-2964.
9. Guralnik JM, Eisenstaedt RS, Ferrucci L, et al. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 2004;104:2263-2268.
10. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. Blood. 2013;122:4021-4034.
11. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391-2405.
12. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017;129:667-679.
13. Greenberg PL, Stone RM, Al-Kali A, et al. Myelodysplastic Syndromes, Version 2.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017;15:60-87.
14. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28:241-247.
15. Papaemmanuil E, Gerstung M, Malcovati L, et al; Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122:3616-3627, quiz 3699.
16. Walter MJ, Shen D, Shao J, et al. Clonal diversity of recurrently mutated genes in myelodysplastic syndromes. Leukemia. 2013;27:1275-1282.
17. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373:35-47.
18. Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360:2289-2301.
19. Langemeijer SM, Kuiper RP, Berends M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet. 2009;41:838-842.
20. Ley TJ, Ding L, Walter MJ, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010;363:2424-2433.
21. Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med. 2009;361:1058-1066.
22. Shlush LI, Zandi S, Mitchell A, et al; HALT Pan-Leukemia Gene Panel Consortium. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia [published correction appears in *Nature*. 2014:508:420]. Nature. 2014;506:328-333.
23. Coombs CC, Zehir A, Devlin SM, 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.
24. Akpek G, McAneny D, Weintraub L. Comparative response to splenectomy in Coombs-positive autoimmune hemolytic anemia with or without associated disease. Am J Hematol. 1999;61:98-102.
25. Papaemmanuil E, Cazzola M, Boultwood J, et al; Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med. 2011;365:1384-1395.

26. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478:64-69.
27. Graubert TA, Shen D, Ding L, et al. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. Nat Genet. 2011;44:53-57.
28. Malcovati L, Karimi M, Papaemmanuil E, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015; 126:233-241.
29. Malcovati L, Papaemmanuil E, Bowen DT, et al; Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium and of the Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011;118:6239-6246.
30. Mughal TI, Cross NC, Padron E, et al. An International MDS/MPN Working Group's perspective and recommendations on molecular pathogenesis, diagnosis and clinical characterization of myelodysplastic/myeloproliferative neoplasms. Haematologica. 2015;100:1117-1130.
31. Meggendorfer M, Roller A, Haferlach T, et al. SRSF2 mutations in 275 cases with chronic myelomonocytic leukemia (CMML). Blood. 2012;120:3080-3088.
32. Haase D, Stevenson KE, Neuberg D, et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. Leukemia. Epub on 2019 Jan 11.
33. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015; 518:552-555.
34. Gillis NK, Ball M, Zhang Q, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. Lancet Oncol. 2017;18:112-121.
35. Takahashi K, Wang F, Kantarjian H, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. Lancet Oncol. 2017;18:100-111.
36. Kahn JD, Miller PG, Silver AJ, et al. *PPM1D*-truncating mutations confer resistance to chemotherapy and sensitivity to PPM1D inhibition in hematopoietic cells. Blood. 2018;132:1095-1105.
37. Hsu JI, Dayaram T, Tovy A, et al. PPM1D mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. Cell Stem Cell. 2018;23:700-713.
38. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125:1367-1376.
39. Baer C, Pohlkamp C, Haferlach C, et al. Molecular patterns in cytopenia patients with or without evidence of myeloid neoplasm-a comparison of 756 cases. Leukemia. 2018;32:2295-2298.
40. Cargo C, Cullen M, Taylor J, et al. The use of targeted sequencing and flow cytometry to identify patients with a clinically significant monocytosis. Blood. 2019; 133:1325-1334.
41. Steensma DP. How I use molecular genetic tests to evaluate patients who have or may have myelodysplastic syndromes. Blood. 2018;132:1657-1663.
42. Kwok B, Hall JM, Witte JS, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. 2015;126:2355-2361.
43. Arber DA, Hasserjian RP. Reclassifying myelodysplastic syndromes: what's where in the new WHO and why. Hematology Am Soc Hematol Educ Program. 2015; 2015:294-298.
44. Valent P, Bain BJ, Bennett JM, et al. Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS. Leuk Res. 2012;36:1-5.
45. Valent P, Orazi A, Steensma DP, et al. Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions. Oncotarget. 2017;8:73483-73500.
46. Valent P, Horny HP, Bennett JM, et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. Leuk Res. 2007;31:727-736.
47. Malcovati L, Cazzola M.The shadowlands of MDS: idiopathic cytopenias of undetermined significance (ICUS) and clonal hematopoiesis of indeterminate potential (CHIP). Hematology Am Soc Hematol Educ Program. 2015;2015:299-307.
48. Cargo CA, Rowbotham N, Evans PA, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. Blood. 2015; 126:2362-2365.
49. Kulasekararaj AG, Jiang J, Smith AE, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. Blood. 2014;124:2698-2704.
50. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. Blood. 2016;128:337-347.
51. Cooper JN, Young NS. Clonality in context: hematopoietic clones in their marrow environment. Blood. 2017;130:2363-2372.
52. Petrova-Drus K, Hasserjian R, Pozdnyakova O, et al. Clinicopathologic evaluation of cytopenic patients with isolated trisomy 8: a detailed comparison between idiopathic cytopenia of unknown significance and low-grade myelodysplastic syndrome. Leuk Lymphoma. 2017;58:569-577.
53. Neukirchen J, Lauseker M, Hildebrandt B, et al. Cytogenetic clonal evolution in myelodysplastic syndromes is associated with inferior prognosis. Cancer. 2017; 123:4608-4616.
54. Ghobrial IM, Detappe A, Anderson KC, et al. The bone-marrow niche in MDS and MGUS: implications for AML and MM. Nat Rev Clin Oncol. 2018;15:219-233.
55. Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. Nat Rev Cancer. 2017; 17:5-19.
56. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559:400-404.

57. Desai P, Mencia-Trinchant N, Savenkov O, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med. 2018;24:1015-1023.
58. Walter MJ, Shen D, Ding L, et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med. 2012;366:1090-1098.
59. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J Clin Oncol. 2017;35:1598-1605.
60. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med. 2017;377:111-121.
61. Brunner AM, Blonquist TM, Hobbs GS, et al. Risk and timing of cardiovascular death among patients with myelodysplastic syndromes. Blood Adv. 2017; 1:2032-2040.
62. Dorsheimer L, Assmus B, Rasper T, et al. Association of mutations contributing to clonal hematopoiesis with prognosis in chronic ischemic heart failure. JAMA Cardiol. 2019;4:25-33.
63. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science. 2017;355:842-847.
64. Wang W, Liu W, Fidler T, et al. Macrophage inflammation, erythrophagocytosis, and accelerated atherosclerosis in Jak2 ^{V617F} mice. Circ Res. 2018;123:e35-e47.
65. Wolach O, Sellar RS, Martinod K, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci Transl Med. 2018;10:eaan8292.
66. Zhang Q, Zhao K, Shen Q, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. Nature. 2015;525:389-393.
67. Cull AH, Snetsinger B, Buckstein R, et al. Tet2 restrains inflammatory gene expression in macrophages. Exp Hematol. 2017;55:56-70.
68. Leoni C, Montagner S, Rinaldi A, et al. *Dnmt3a* restrains mast cell inflammatory responses. Proc Natl Acad Sci USA. 2017;114:E1490-E1499.
69. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood. 2017;130:742-752.
70. Brunner AM, Steensma DP. Recent advances in the cellular and molecular understanding of myelodysplastic syndromes: implications for new therapeutic approaches. Clin Adv Hematol Oncol. 2018;16:56-66.