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## The genetics of myelodysplastic syndrome: from clonal hematopoiesis to secondary leukemia

Adam S. Sperling<sup>1,2</sup>, Christopher J. Gibson<sup>1,2</sup>, and Benjamin L. Ebert<sup>1,2</sup>

<sup>1</sup>Department of Medical Oncology, Dana-Farber Cancer Institute and Brigham and Women's Hospital, Boston, MA 02115

<sup>2</sup>Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115

### Abstract

Myelodysplastic syndrome (MDS) is a clonal disease that arises from the expansion of mutated hematopoietic stem cells. In a spectrum of myeloid disorders ranging from clonal hematopoiesis of indeterminate potential (CHIP) to secondary acute myeloid leukemia (sAML), MDS is distinguished by the presence of peripheral blood cytopenias, dysplastic hematopoietic differentiation, and the absence of features that define acute leukemia. Over 50 recurrently mutated genes are involved in the pathogenesis of MDS, including genes that encode proteins involved in pre-mRNA splicing, epigenetic regulation, and transcription. In this review we discuss the molecular processes that lead to CHIP and further clonal evolution to MDS and sAML. We also highlight the ways in which these insights are shaping the clinical management of MDS, including classification schemata, prognostic scoring systems, and therapeutic approaches.

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Myelodysplastic syndrome (MDS) is driven by a complex combination of genetic mutations that result in heterogeneity in both clinical phenotype and disease outcome, as is the case for most cancers. The World Health Organization (WHO) classifies MDS as a clonal diseases characterized by morphologic dysplasia, ineffective hematopoiesis leading to cytopenias [G], and a risk of transformation to acute myeloid leukemia (AML)<sup>1</sup>. It is now appreciated that most of the clinical and pathologic features of MDS are the direct result of recurrent acquired somatic genetic lesions (Figure 1). While MDS and related myeloproliferative neoplasms (MPNs) are defined by distinct clinical and morphological criteria, they share many of the same genetic mutations, and the composite genotype of individual cases – including the specific genes mutated, the order in which they were mutated, and the interactions between clones and subclones – is likely to underlie these phenotypic differences (Box 1). In addition, there are a number of rare familial syndromes associated with a predisposition to early onset MDS<sup>2</sup> (Box 2).

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Correspondence: bebert@partners.org.

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**Box 1****The spectrum of myeloid neoplasms**

Myelodysplastic syndrome (MDS) is a hematologic disorder within the larger spectrum of myeloid neoplasms. Other myeloid neoplasms include the myeloproliferative neoplasms (MPNs), acute myeloid leukemia (AML), and MDS/MPN overlap syndromes. Although these were previously felt to be biologically distinct entities, it is now appreciated that there is a considerable degree of genetic overlap between them.

MPNs include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia (CNL), and systemic mastocytosis (SM)<sup>1</sup>. Each has specific pathologic characteristics, but all are distinguished from MDS by the absence of morphologic dysplasia and normal, or more often increased, production of blood cells. All MPNs have mutations that constitutively activate signaling cascades and cytokine-independent proliferation. These include mutations in Janus kinase 2 (*JAK2*) (PV, ET, MF), calreticulin (*CALR*) (ET, MF), thrombopoietin receptor (*MPL*) (ET, MF), colony stimulating factor 3 receptor (*CSF3R*) (CNL), *KIT* (SM), and *BCR-ABL* rearrangements (CML)<sup>115-119,163</sup>.

Some myeloid neoplasms display both features of MPNs (elevated peripheral blood cell counts or bone marrow fibrosis) and MDS (morphologic dysplasia) and are termed **MDS/MPN overlap syndromes**. These include chronic myelomonocytic leukemia (CMML), which typically presents with peripheral blood monocytosis and dysplasia in the bone marrow; atypical CML, which clinically resembles CML but lacks a *BCR-ABL* rearrangement; and unclassifiable MDS/MPN overlap syndromes<sup>1,164</sup>. Overlap syndromes frequently harbor concomitant MDS-associated mutations (e.g. serine and arginine rich splicing factor 2 (*SRSF2*) and additional sex combs-like 1 (*ASXL1*)) and MPN-associated mutations (e.g. *JAK2*). They may also harbor mutations not specific for any disorder (e.g. ten-eleven translocation 2 (*TET2*) and DNA methyltransferase 3A (*DNMT3A*)), as well as frequent mutations activating the RAS pathway (e.g. *NRAS*, *KRAS*, *CBL* and protein tyrosine phosphatase, non-receptor type 11 (*PTPN11*))<sup>165</sup>.

While not classified as myeloid neoplasms, **paroxysmal nocturnal hemoglobinuria [G]** (PNH) and **aplastic anemia [G]** (AA) can overlap with and transform into MDS and AML. PNH is defined by clonal, somatic mutations in phosphatidylinositol glycan anchor biosynthesis class A (*PIGA*). In AA, a subset of patients have clonal mutations in genes specifically associated with myeloid malignancy, including *DNMT3A*, *ASXL1*, *JAK2*, and *TP53*, at a relative frequency very similar to that seen in clonal hematopoiesis of indeterminate potential (CHIP)<sup>45,166,167</sup>. In addition, mutations in *PIGA* and *BCL6* corepressor (*BCOR*) are more common in AA than in CHIP, and are associated with a better response to immunosuppressive therapy and improved overall survival<sup>45</sup>. Mutations in CHIP and myeloid malignancy genes, such as *DNMT3A*, *ASXL1* or *TP53*, were associated with worse overall survival<sup>45</sup>. It is unclear how specific clonal somatic aberrations affect disease pathogenesis and evolution in AA and PNH and whether they

can be used clinically to help differentiate AA from the morphologically related hypoplastic MDS [G].

## Box 2

### Inherited bone marrow failure and MDS

While most myelodysplastic syndrome (MDS) cases are caused by somatic mutations, a few are due to inherited mutations that lead to increased risk of bone marrow failure<sup>1,2</sup>. These typically affect patients in adolescence or early adulthood and include:

- Diamond-Blackfan anemia: characterized by absent erythroid precursors due to mutations in ribosomal protein genes, causing abnormal ribosome biogenesis, and rarely MDS or AML<sup>168</sup>.
- Telomerase complex disorders: due to mutations in genes encoding members of the telomerase complex (dyskerin pseudouridine synthase 1 (*DKCI*), telomerase reverse transcriptase (*TERT*), telomerase RNA component (*TERC*), GAR1 ribonucleoprotein (*GARI*)<sup>169–172</sup>. The best known is dyskeratosis congenita (DKC), characterized by bone marrow failure, pulmonary fibrosis, oral leukoplakia, dystrophic nails, hyperpigmentation, and cirrhosis.
- Familial platelet disorder with propensity to myeloid malignancy: characterized by childhood thrombocytopenia and a propensity to progress to MDS. It is caused by mutations in runt related transcription factor 1 (*RUNXI*), which encodes a component of the core binding factor transcriptional activator complex<sup>101</sup>.
- Familial thrombocytopenia and malignancy: characterized by dominantly-inherited thrombocytopenia, an elevated **mean corpuscular volume (MCV)** [G], and a propensity to hematologic malignancies, including MDS and acute myeloid leukemia (AML). It is caused by mutations in ETS variant 6 (*ETV6*)<sup>99,173</sup>.
- Fanconi anemia: an early-onset aplastic anemia often associated with dysmorphic features, short stature, and limb defects, caused by mutations in any of more than 15 genes involved in DNA repair<sup>168</sup>.
- Li-Fraumeni syndrome: caused by loss-of-function mutations in *TP53*, leading to an increased predisposition to many cancers, including MDS and AML<sup>174</sup>.
- Inherited GATA binding protein 2 (*GATA2*) mutations: a heterogeneous group of disorders, including MonoMAC syndrome (monocytosis with increased susceptibility to mycobacterial infections)<sup>175</sup> and Emberger syndrome (familial lymphedema and deafness)<sup>176</sup>, both of which also carry a risk of early-onset MDS.

- Inherited DEAD-box helicase 41 (*DDX41*) mutations: associated with mild monocytosis and an increased risk of MDS and AML, with a longer latency to disease initiation (average age of 62) than other genetic syndromes discussed here<sup>138,177</sup>.
- Shwachman-Diamond syndrome (SDS): characterized by skeletal defects, pancreatic insufficiency and bone marrow failure, with risk of transformation to MDS. It is caused by mutations in the *SBDS* gene, which is required for normal ribosome maturation<sup>178</sup>.

MDS is among the most common of the hematologic malignancies, with current estimates placing its incidence in the United States between 5.3 and 13.1 cases per 100,000 persons<sup>3</sup>. In adults, advanced age is the predominant risk factor for developing MDS, with a median age at diagnosis of 71–76 years<sup>4,5</sup>. Precise enumeration of the incidence of MDS has been challenging because it was not independently recorded in the National Cancer Institute's Survey, Epidemiology and End Results (SEER) cancer databases until 2001; the true prevalence is likely underestimated since a bone marrow biopsy is required for diagnosis and many older patients with mild cytopenias do not undergo marrow evaluation. The limited data available suggest that the incidence is increasing over time, which may be due to an aging population, improving survival following treatments for other neoplasms that place patients at risk for subsequent development of therapy-related MDS (t-MDS), and increased awareness of MDS among general practitioners<sup>5</sup>.

While patients with MDS can be asymptomatic at diagnosis, identified only by the incidental discovery of cytopenias, many patients present with clinical symptoms such as fatigue, often related to anemia; bleeding due to thrombocytopenia; and fevers or recurrent infections as a result of neutropenia. The clinical course is variable: some patients live for many years with minimal therapy, while others rapidly progress to AML. Morbidity and mortality in MDS is related primarily to complications arising from cytopenias and transformation to AML. The risk of either event can be assessed using one of several prognostic systems, including the International Prognostic Scoring System (IPSS), revised IPSS (IPSS-R), the WHO-based Prognostic Scoring System (WPSS), and the MD Anderson Comprehensive Scoring System (MDA-CSS)<sup>6–8</sup>. While the IPSS and, more recently, the IPSS-R have become the most widely utilized, these scoring systems are largely interchangeable and all take into account some combination of the degree of cytopenias, the proportion of bone marrow blasts [**G**], and the karyotype. These prognostic scoring systems do not include information about somatic mutations in individual genes, though this genetic information can predict prognosis independent of each of the prognostic scoring systems<sup>9,10</sup>.

In this review we discuss the molecular and genetic basis of MDS beginning with initiating mutations in hematopoietic stem cells (HSCs) that lead to the development of clonal hematopoiesis. This initiating event is followed by the accumulation of additional cooperating mutations and eventual progression to overt clinical disease including MDS and secondary AML [**G**] (sAML). Finally, we discuss how our evolving understanding of the genetics of MDS provides insights into the clinical course, prognosis and treatment of patients with MDS.

## The Molecular Genetics of MDS

### Cell of origin

A central challenge in understanding the development of MDS, as in other malignancies, has been identifying the cell of origin. Recent work has demonstrated that distinct MDS stem cells bearing the immunophenotype of normal HSCs (Lineage<sup>low</sup> [G], CD34+, CD38-, CD90+ (also known as THY1+), CD45RA- (also known as PTPRC-) are able to sustain the generation of myeloid progenitors *in vitro* and *in vivo*, whereas other early myeloid progenitors are unable to do so<sup>11</sup>.

Although the founding genetic event in MDS pathogenesis has long been assumed to occur in a myeloid-biased HSC, patients with clonal hematopoiesis have an increased risk of developing both lymphoid and myeloid malignancies, perhaps suggesting mutational overlap or a common early precursor stem cell<sup>12</sup>. Many of the mutations commonly found in myeloid malignancies such as ten-eleven translocation 2 (*TET2*) and splicing factor 3b subunit 1 (*SF3B1*) have also been identified in lymphoid cells or have been described in a variety of lymphomas<sup>13-17</sup>.

While most patients with MDS follow a course dominated by cytopenias and their consequences, about one third progress to high-risk MDS and sAML<sup>18</sup>. The genetic mutations in MDS appear to be initiated in a hematopoietic stem cell. Sequencing of sequential samples from individual patients demonstrated that in the 5q<sup>-</sup> syndrome [G] clinical stability was associated with mutational stability<sup>11</sup>. Conversely, those patients who progressed to AML developed multiple new mutations within the leukemic stem cell compartment, coupled with new myeloid progenitor (i.e. non-HSC) populations that had gained self-renewal potential<sup>11</sup>. The expansion of self-renewal activity outside the stem cell compartment, and expansion of the population of cells with proliferative potential, are other key steps in the transition from MDS to AML. Eradicating the mutated MDS stem cells, capable of maintaining the disease indefinitely, is likely to be essential to curing the disease.

### Clonal hematopoiesis: initiators of disease

The presence of initiating mutations leading to clonal expansion, and thus a pre-malignant state, has long been suspected to precede the development of most malignancies (Figure 2). Initial studies of healthy women demonstrated skewing of X-chromosome inactivation in almost 40% of women over 60, and a subset of these women were later found to harbor mutations in *TET2*, suggestive of clonal hematopoiesis driven by a somatic mutation<sup>19</sup>. More recently, exome sequencing of peripheral blood samples from over thirty thousand patients without known hematologic malignancies demonstrated recurrent somatic myeloid malignancy-associated mutations in up to 10% of patients over the age of 65 and more than 20% of patients over the age of 90<sup>12,20,21</sup>. This phenomenon has subsequently been termed clonal hematopoiesis of indeterminate potential (CHIP) [G]<sup>22</sup>. The most common recurrently mutated genes were DNA methyltransferase 3A (*DNMT3A*), *TET2*, additional sex combs-like 1 (*ASXL1*), *TP53* (which encodes p53), Janus kinase 2 (*JAK2*) and *SF3B1*, all of which are also mutated in MDS (Figure 1)<sup>12,21</sup>.

In these studies, the presence of CHIP was a strong predictor of the development of subsequent hematologic malignancy (hazard ratio [G] of 11.1), with an annual risk [G] of approximately 0.5–1%, and decreased overall survival (hazard ratio for all cause mortality of 1.4) as compared to age-matched controls<sup>12</sup>. In two patients with CHIP who later developed AML, sequencing of bone marrow-derived DNA demonstrated that the leukemias were clonally derived from the previously identified CHIP<sup>21</sup>. Importantly, however, the absolute risk of transformation to overt malignancy in patients with CHIP was low during the time periods under study, likely reflective of the need to acquire additional mutations in a relatively small pool of potential cooperating genes. In fact, the most significant driver of decreased survival in association with CHIP was an increased propensity for thrombosis, coronary artery disease, and stroke, the reason for which is unclear and remains under active investigation<sup>12</sup>. Despite these effects on survival, there are currently no data to suggest that screening of asymptomatic patients for the presence of CHIP is clinically indicated, especially in the absence of an intervention that could restore polyclonal hematopoiesis.

### From CHIP to MDS: a blurred distinction

In a model of clonal evolution beginning with CHIP and ending in frank hematologic malignancy, the transition to MDS likely involves a complex interplay between epigenetic alterations within the HSC, a dysfunctional bone marrow microenvironment (Box 3), and the stepwise acquisition of additional driver mutations (Figure 2). The clinical diagnosis of MDS, as currently defined, does not incorporate somatic mutations, but is instead based on the morphology of hematopoietic cells in the bone marrow, the finding of cytogenetic abnormalities, and the development of cytopenias in the peripheral blood<sup>1</sup>. As currently defined, a diagnosis of CHIP requires the presence of a somatic mutation with a mutant allele fraction of at least 2% in the peripheral blood and no other evidence of a hematologic malignancy<sup>22</sup>. On the other hand, the presence of a mutation and otherwise unexplained cytopenias or borderline dysplasia is suggestive of, but does not confirm, progression to MDS, since the formal diagnosis still requires the fulfillment of specific morphologic criteria<sup>23,24</sup>. Approximately 35% of patients with idiopathic cytopenias of undetermined significance (ICUS) [G] have somatic mutations characteristic of MDS, though whether all such patients go on to develop morphologic dysplasia, or have clinical courses similar to MDS patients even in the absence of dysplasia, requires further study<sup>24</sup>. Conversely, since the full complement of mutations involved in MDS has yet to be defined, the absence of a known somatic mutation also does not exclude the diagnosis of MDS<sup>22</sup>. On the whole, however, MDS is a more genetically complex disease than CHIP, with the majority of patients harboring at least two, and sometimes many more, somatic mutations in recurrent driver genes, often with a high mutant allele fraction (>10%), at the time of diagnosis<sup>25,26</sup>.

#### Box 3

##### Aberrant hematopoiesis, the bone marrow microenvironment and MDS

The somatic mutations that drive myelodysplastic syndrome (MDS) increase self-renewal, aberrant differentiation and morphological abnormalities in hematopoietic stem cells (HSCs). The effects of MDS mutations on HSCs are apparent in some mouse models<sup>179</sup>. For example, mutations in or loss of *Dnmt3a* or *Tet2* expand the mutant HSC



clone and increase HSC function at the expense of normal polyclonal hematopoiesis<sup>75,77,180</sup>.

Asymptomatic patients with CHIP, by definition, have normal blood counts, but many have an elevated mean red cell distribution width [G], suggesting some dyserythropoiesis even with a single mutation<sup>12</sup>. Certain mutations are associated with specific abnormalities in differentiation, such as the formation of ring sideroblasts in cases with *SF3B1* mutations and impaired erythropoiesis and hypolobated micromegakaryocytes in patients with 5q<sup>-</sup> syndrome<sup>54,181</sup>.

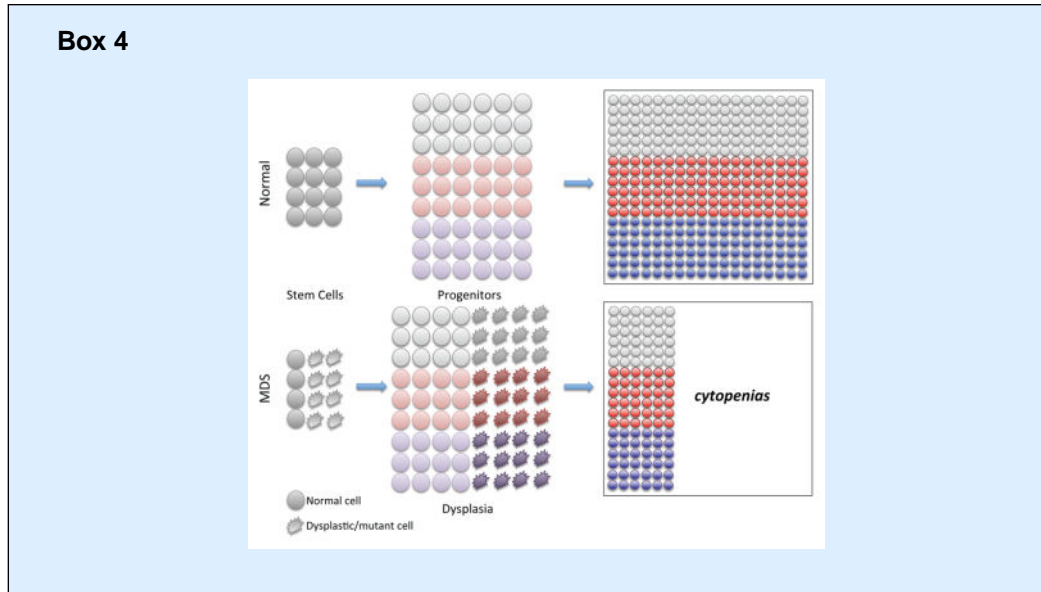
The ineffective hematopoiesis and cytopenias that define MDS may seem paradoxical, as the genetic lesions in MDS give HSCs a clonal advantage, and generally expand progenitor cells leading to a hypercellular bone marrow. Terminal maturation, however, is impaired, leading to increased apoptosis of differentiating cells and thus peripheral cytopenias (see the figure).

HSCs exist within a supportive stromal microenvironment comprised of sinusoidal vascular endothelial cells, osteoblasts, adipocytes, and other components, which can also alter HSC function and differentiation<sup>182–184</sup>. In mice, selective disruption of certain genes only in the stroma, such as deletion of  $\beta$ -catenin (*Ctnnb1*), the *Dicer1* ribonuclease or *Sbds*, the gene mutated in the Schwachman-Diamond syndrome (Box 2), can cause abnormal differentiation of HSCs and the development of dysplasia<sup>185,186</sup>. This interaction is bidirectional, as myeloid neoplasms can remodel the bone marrow niche, often leading to increased fibrosis<sup>187</sup>. While in these model systems MDS can be induced purely by stromal defects, it is unclear whether human MDS can be caused by defects isolated to the bone marrow stroma or whether genetic and epigenetic alterations in the HSCs are an essential component of human disease. The fact that MDS can be cured in some cases by transplantation of HSCs alone suggests that the stroma is at least not always the primary disease-initiating element.

Like aplastic anemia, MDS is often associated with immune dysfunction. Inappropriate immune targeting of hematopoietic progenitors may contribute to the cytopenias observed in some cases of MDS and is another example of an abnormal hematopoietic environment contributing to disease phenotype<sup>188</sup>. Up to 30% of patients with MDS show improvements in their cytopenias following treatment with immunosuppressants and patients with trisomy 8 are particularly likely to respond to this form of therapy<sup>189–191</sup>.

The genetic lesions that initiate MDS promote self-renewal, leading to a proliferative advantage over normal HSCs and asymptomatic clonal expansion and eventually to overt disease (Box 4). Mutations that occur early in disease evolution can be detected by calculating allele frequency in bulk sequencing studies, or by single cell sequencing, and these two approaches correlate well with each other<sup>27</sup>. Using these methods, several studies have demonstrated that, as general groups, the splicing factors (*SF3B1*, serine and arginine rich splicing factor 2 (*SRSF2*), U2 small nuclear RNA auxiliary factor 1 (*U2AF1*), and zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2 (*ZRSR2*)) and epigenetic modifiers, especially *DNMT3A* and *TET2*, tend to be mutated early in the evolution of

MDS, while mutations in transcription factors (runt related transcription factor 1 (*RUNX1*), GATA binding protein 2 (*GATA2*), cut like homeobox 1 (*CUX1*)) can be either early or late events<sup>26</sup>.



The genes most frequently mutated in CHIP partially correspond to the initiating mutations in MDS. The two most commonly mutated genes in CHIP, *DNMT3A* and *TET2*, both encode epigenetic regulators that, when mutated in MDS, tend to occur early in disease pathogenesis<sup>26</sup>. On the other hand, mutations in splicing factors are less common in CHIP than would be expected based on their frequency in MDS. This observation suggests that they may be more morphologically deterministic than mutations in epigenetic regulators, and that the patients who acquire splicing factor mutations develop overt dysplasia more rapidly and are thus relatively under-represented in the cohorts of ‘healthy’ adults in whom CHIP was defined. Other genes mutated frequently in CHIP cohorts are either mutated rarely (*CBL*) or not yet assessed (protein phosphatase, Mg<sup>2+</sup>/Mn<sup>2+</sup> dependent 1D (*PPM1D*)) in MDS<sup>12,21</sup>.

### Progression to Leukemia

sAML is a distinct disease as compared to *de novo* AML [G], characterized by a poorer response to induction therapy [G], a significantly higher relapse rate, and an overall inferior prognosis. Mounting evidence suggests that sAML is also biologically distinct from *de novo* disease, reflecting its evolution from MDS. Careful study of rigorously defined cases of both *de novo* AML and sAML has shown that mutations in *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, enhancer of zeste 2 (*EZH2*), BCL6 corepressor (*BCOR*, part of a polycomb repressive complex (PRC) [G]) and stromal antigen 2 (*STAG2*, a component of the cohesin complex [G]) are strongly associated with an antecedent MDS and are thus highly specific for sAML<sup>28</sup>. These mutations define a group of AML patients that behave clinically like sAML even in cases when no pre-existing dysplasia or cytopenias have been documented. In contrast, mutations in nucleophosmin (*NPM1*) and rearrangements involving mixed-lineage leukemia 1 (*MLL1*, also known as KMT2A, located at chromosome 11q23) or genes that



encode components of the core binding factor [G] are primarily restricted to *de novo* AML. Most other mutations, including those in *DNMT3A*, *TET2*, and *RUNX1*, are not unique to either disease entity. *TP53*-mutated AML comprises its own unique category of disease that tends to have fewer cooperating point mutations, which may be functionally replicated by a high frequency of cytogenetic rearrangements that disrupt global chromosomal architecture<sup>9,28</sup>.

Some of the mutations that occur during progression from MDS to AML are found in core hematopoietic transcription factor genes, including *RUNX1*, *GATA2*, and CCAAT/enhancer binding protein  $\alpha$  (*CEBPA*), which abrogate normal differentiation<sup>28</sup>. Activating mutations in signaling pathway components such as fms related tyrosine kinase 3 (FLT3) and RAS family members, which control cellular proliferation, also commonly occur during the progression to sAML, and their subclonal presence in otherwise lower risk MDS is associated with impending transformation<sup>26,29–31</sup>.

### Positive and negative cooperativity between mutations

At the time of diagnosis, most cases of MDS and sAML are clonally and genetically complex, with many clones containing more than three cooperating disease-associated mutations<sup>32</sup>. In MDS, as well as other cancers, certain mutations co-occur at frequencies greater than or less than would be expected by chance. These variances are most likely driven by patterns of functional complementarity, redundancy, and synthetic lethality. Complementarity is most easily appreciated in cases with mutations in genes from different classes of biological function, as for example in a patient with *TET2*, *SRSF2*, and *RUNX1* mutations. On the other hand, certain mutations co-occur much less often than would be expected by chance, which presumably implies either functional redundancy or synthetic lethality, such that co-mutation would have either a neutral or negative consequence. For instance, the individual splicing factors are almost never co-mutated with each other, and neither are cohesin complex genes<sup>33,34</sup>. Other combinations of mutations have a relative paucity of co-occurrence, such as *ASXL1* and *DNMT3A*<sup>25,26,35</sup>. Definitive functional evidence of mutual exclusivity does not exist for most mutation pairs. In the absence of such evidence, it is therefore important not to overstate the biological basis for these associations.

As discussed above, some specific mutations are associated with the initial development of CHIP, whereas subsequent evolution is likely guided by the temporal acquisition of mutations that cooperate to generate overt malignancy. The timing and context of each serial mutation may influence disease phenotype and progression. This concept has been best described in the MPNs, in which mutations in *JAK2* and *TET2* co-occur in approximately 10% of cases. Sequencing of single cell clones isolated from MPN patients showed that those with a *JAK2* mutation occurring first are more likely to develop polycythemia vera [G] and have increased thrombotic risk, whereas those with a *TET2* mutation occurring first are more likely to develop essential thrombocythemia<sup>36</sup>. *JAK2* mutations frequently co-occur with *SF3B1* mutations in the MDS/MPN overlap syndrome (Box 1) MDS/MPN with ring sideroblasts [G] and thrombocytosis (MDS/MPN-RS-T)<sup>1</sup>. As in MPNs, either mutation can occur first, and the original phenotype can subsequently be modified by acquisition of the second mutation<sup>37,38</sup>.

## Evolution in response to therapy

Although MDS undergoes clonal evolution even in untreated patients, the administration of disease-altering therapy, such as hypomethylating agents or lenalidomide, functions as an external selective pressure that can influence the relative proportions of co-existing clones. To date, there are limited genetic data for how MDS evolves with treatment, and much of our understanding is extrapolated from related diseases. Analysis of serial AML samples collected at diagnosis and relapse shows that subclones apparent at diagnosis can become undetectable, while mutations conferring resistance to therapy may evolve over time either through the acquisition of resistance mutations in the dominant subclone or outgrowth of a pre-existing subclone<sup>39,40</sup>. In some cases, these resistant subclones can be detected even in remission samples and provide a source for leukemic relapse<sup>40–42</sup>. In cases of AML in which chemotherapy eliminates leukemic blasts and leaves bone marrow in which there is no morphologic evidence of disease, pre-malignant mutations may still be detectable and able to reinitiate disease<sup>41–43</sup>. It is important to recognize that this post-treatment clonality is an entity distinct from CHIP, carrying a much higher risk of progression to overt leukemia<sup>28,41–43</sup>.

## Recurrently mutated pathways

A number of studies have used targeted sequencing panels built around genes mutated in other myeloid neoplasms to describe the spectrum of somatic mutations in MDS and their clinical and pathological consequences<sup>9,25,26,44</sup>. Between 75% and 90% of patients have a mutation in at least one known, recurrently-mutated gene, and this number will likely increase as additional genes are identified (Figure 1)<sup>25,26</sup>.

## Mutational processes

MDS arises as a consequence of the sequential acquisition of somatic mutations in HSCs. It is estimated that HSCs acquire approximately 0.13 exonic single nucleotide variations (SNVs) per year of life<sup>32</sup>. This calculation assumes a constant mutational rate, but it is possible that initiating mutations alter the mutational milieu of the cell, thus shaping the subsequent evolutionary path<sup>26</sup>. The most frequent base-pair change seen in clonal hematopoiesis and MDS is the C → T transition, a hallmark of methyl-cytidine deamination associated with aging<sup>12,45–47</sup>. Other age-related mutational processes also contribute to the pathogenesis of MDS, including large chromosome rearrangements, the occurrence of small insertions and deletions and progressive telomeric shortening<sup>48</sup>.

## Splicing factors

Alternative splicing of pre-mRNA is a common feature of eukaryotic genes and is one of the most commonly dysregulated processes in cancer<sup>49</sup>. Mutations within components of the spliceosome are the most common recurrent lesions in MDS and are found in up to 60% of cases<sup>50</sup>. The majority are in components of the 3' spliceosome, including *SF3B1*, *SRSF2*, *U2AF1*, and to a lesser extent *ZRSR2*, pre-mRNA processing factor 8 (*PRPF8*), *U2AF2*, LUC7 like 2, pre-mRNA splicing factor (*LUC7L2*) and splicing factor 1 (*SF1*) (Figure 3)<sup>10,25,26,51–53</sup>.

Mutations in *SF3B1*, *SRSF2*, and *U2AF1* occur exclusively as heterozygous missense mutations at defined hotspots, leading to highly recurrent amino acid substitutions that alter the function of the splicing machinery. *SF3B1* mutations, the most common spliceosome alteration in MDS, are highly associated with the presence of ring sideroblasts and a relatively benign prognosis<sup>38,52</sup>. They lead to altered selection of 3' splice sites and aberrant splicing of important genes involved in iron homeostasis that likely mediate the ring sideroblast phenotype<sup>54,55</sup>. *SRSF2* is the second most commonly mutated splicing factor in MDS and is also frequently mutated in MDS/MPN overlap syndromes. Missense mutations alter *SRSF2*'s binding to exonic splice enhancers, which in turn leads to missplicing of a number of important genes, including *EZH2*<sup>56</sup>. Mutations in *U2AF1* occur in 10–15% of MDS cases, are not associated with any particular morphologic phenotype, and promote increased exon skipping<sup>51,57,58</sup>. Neither *SRSF2* nor *U2AF1* mutations confer the favorable prognosis associated with mutations in *SF3B1*<sup>38</sup>. While these studies have shown that mutations in different splicing factor genes lead to distinct patterns of aberrant splicing and alter the abundance or function of independent sets of target genes, no specific alternatively spliced isoform has been demonstrated to directly cause disease. Furthermore, while the data are still limited, there has been little overlap of altered splicing events reported between mice and humans or between individual spliceosomal mutants, raising the possibility that mutations in these genes could promote MDS through some alternative mechanism.

### Epigenetic regulators: DNA methylation and histone modification

Post-translational modifications of DNA and histones are important mechanisms of cellular epigenetic regulation. Mutations in genes involved in these processes are the second most common set of recurrent lesions in MDS.

Methylation of cytosines in repetitive CpG elements in DNA, mediated by the DNA methyltransferases (DNMTs), is one of the most common epigenetic modifications, and functions by altering the accessibility of DNA regulatory regions (Figure 4)<sup>59</sup>. Inactivating mutations in the gene encoding one such enzyme, *DNMT3A*, occur in 10–15% of MDS cases<sup>25,26,60,61</sup>. The opposing process, DNA demethylation, is mediated by the ten-eleven translocation (TET) family of proteins, dioxygenases that catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethyl-cytosine (5hmC) as part of a multistep reaction that eventually leads to DNA demethylation (Figure 4)<sup>62–65</sup>. *TET2* is one of the most commonly altered genes in MDS, with inactivating mutations found in approximately 30% of cases<sup>25,59,66</sup>. These mutations are associated with hypermethylation of cytosines at enhancer sequences and subsequent repression of a number of genes important for myeloid differentiation<sup>67–69</sup>.

Alterations in DNA methylation also occur in patients with mutations in the genes encoding the isocitrate dehydrogenase (IDH) enzymes, *IDH1* and *IDH2*, found in approximately 5% of patients with MDS. These mutations produce a neomorphic enzyme that converts isocitrate to R-2-hydroxyglutarate (2HG) instead of  $\alpha$ -ketoglutarate ( $\alpha$ -KG)<sup>25,70,71</sup>. 2HG acts as an oncometabolite and diffuses to the nucleus, where it promotes neoplasia by, among other things, inhibiting  $\alpha$ -KG-dependent dioxygenases, including *TET2* (Figure 4)<sup>72–74</sup>.

The mechanisms by which changes in methylation contribute to the pathogenesis of MDS are complex, and multiple studies have been unable to identify a clear correlation between methylation and gene expression<sup>60,75</sup>. Although DNMT3A and TET2 are seemingly biochemical opposites, their genes are frequently co-mutated in MDS<sup>25</sup>. Mice deficient in either *Dnmt3a* or *Tet2* have phenotypic similarities and the double mutants show accelerated development of malignancy<sup>75-77</sup>. Interrogation of the global landscape of 5mC and 5hmC in hematopoietic cells has shown that many genes known to be dysregulated in myeloid malignancies lie within ‘canyons’ of sparse methylation, where their expression is regulated by epigenetic histone modifications. *DNMT3A* is important for maintaining the borders of these canyons, but those same borders are also enriched in 5hmC, suggesting synergistic, rather than divergent, roles for *TET2* and *DNMT3A* in this capacity<sup>76,78</sup>.

Genes that encode histone modifying enzymes also contribute to MDS. The covalent modification of histone tails leads to changes in chromatin structure and altered binding of regulatory proteins (Figure 4). The PRCs are two distinct protein complexes (PRC1 and PRC2) that are both required for maintaining the transcriptional silencing of key developmental regulators during differentiation. PRC2 trimethylates histone H3 on lysine 27 (H3K27me3), whereas PRC1 ubiquitinates histone H2A at lysine 119; both alterations lead to chromatin compaction<sup>79-81</sup>. Components of both complexes can be mutated in MDS. *EZH2* encodes a PRC2 catalytic subunit and is mutated in approximately 5% of MDS patients; loss of *Ezh2* promotes the development of MDS in mouse models<sup>25,82</sup>. *BCOR* and *BCORL1* are components of a PRC1 complex known as PRC1.1, are mutated in about 5% of cases of MDS, and are associated with a poor prognosis<sup>25,83-86</sup>.

*ASXL1* is recurrently mutated in approximately 20% of MDS patients<sup>25</sup>. Although not itself a constituent of either PRC, ASXL1 forms a Polycomb repressive deubiquitylase complex with BRCA1-associated protein 1 (BAP1) that physically interacts with PRC2 and deubiquitinates histone H2A<sup>87,88</sup>. Pathogenic *ASXL1* mutations are restricted to exons 11 and 12, and lead to a truncated protein product that increases the deubiquitination activity of BAP1, which is associated with decreased global H3K27me3<sup>88,89</sup>. Mutations in *EZH2*, *ASXL1*, and *BCOR* all lead to dysregulation of a number of important hematopoietic lineage genes, including the homeobox A (HOXA) cluster, possibly explaining their role in promoting dysplasia and cytopenias<sup>82,88,90</sup>.

### Cohesin complex

The cohesins (STAG2, structural maintenance of chromosomes 3 (SMC3), SMC1A and RAD21) form a ring-shaped multi-protein structure that encircles DNA and helps maintain sister chromatid cohesion, which in turn prevents collapse of the replication fork and facilitates homologous recombination-mediated DNA repair. Loss-of-function mutations in cohesin genes occur in approximately 15% of MDS cases<sup>25,26,34,91</sup>. Despite the role of these proteins in sister chromatid cohesion, cohesin mutations in MDS are not associated with aneuploidy or chromosomal aberrations<sup>91</sup>. Cohesins also function to stabilize DNA loops that promote interaction between promoters and distant enhancers (Figure 4)<sup>92</sup>. It is now thought that cohesin mutations primarily drive MDS pathogenesis through dysregulation of long-range chromatin interactions, leading to altered gene expression, rather than through

their roles in replication and homologous recombination, although further work is needed to confirm this hypothesis<sup>93–96</sup>.

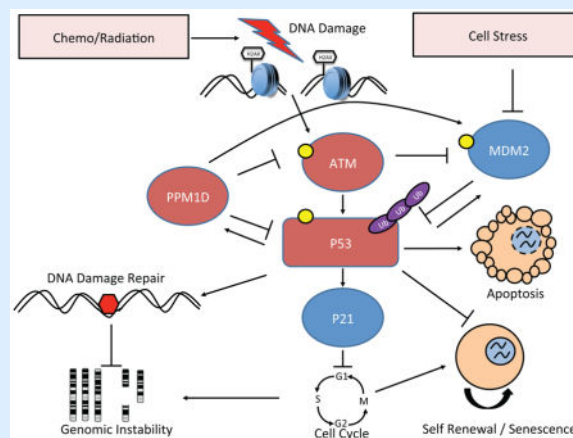
### Transcription factors

A small number of core hematopoietic transcription factors are recurrently mutated in MDS. Germline loss-of-function mutations in *RUNX1*, *GATA2*, and ETS variant 6 (*ETV6*) are associated with inherited bone marrow failure disorders that carry a risk of MDS and AML (Box 2)<sup>97–99</sup>. *RUNX1* is the DNA-binding subunit of the core binding factor, which regulates a number of genes involved in hematopoiesis. In addition to germline mutations, somatic mutations are found in about 10% of cases of MDS, often associated with severe thrombocytopenia<sup>9,25,100,101</sup>. *RUNX1* mutations co-occur with cohesin mutations, as well as in other genetic contexts<sup>26,91</sup>. *GATA2* encodes a zinc finger transcription factor that is highly expressed in hematopoietic stem cells, and is essential for normal hematopoietic differentiation. Like *RUNX1*, both germline and somatic mutations occur in *GATA2*, but somatic mutations are present in only 1–2% of MDS patients<sup>25,102</sup>. A number of other transcription factors are mutated less commonly in MDS. For example, Wilms tumor 1 (*WT1*) encodes a sequence specific DNA-binding transcription factor mutated in <5% of cases of MDS that functions to recruit TET2 to specific genomic loci<sup>103–105</sup>.

### The role of p53 in MDS

*TP53* is the most frequently mutated tumor suppressor gene across all human cancers and is recurrently mutated in MDS (see Figure 1 and Box 5)<sup>49</sup>. Humans born with a single mutant allele of *TP53*, the Li-Fraumeni syndrome, have a dramatically increased risk of many types of cancer, including MDS and AML (Box 2). Somatic disruption of *TP53* in MDS is strongly associated with low platelet levels, a high blast count, complex karyotype, and prior exposure to chemotherapy<sup>9,28</sup>. Many patients with deletion of one *TP53* allele, including cases with del(17p), carry a second inactivating mutation in the other allele of *TP53*<sup>106</sup>. Loss of *TP53* in MDS and AML carries a particularly dismal prognosis<sup>9,107</sup>.

#### Box 5



p53 mediates the response to cellular stress by increasing expression of genes involved in apoptosis and cell cycle arrest<sup>108,109</sup>. This pathway is negatively regulated by the phosphatase PPM1D. Truncating mutations in *PPM1D* have been identified in CHIP and are found at increased frequency in the blood of ovarian cancer patients who have previously been treated with chemotherapy<sup>21,110</sup>. Inappropriate entry into the cell cycle before DNA repair is complete and inadequate activation of the DNA damage repair machinery are believed to contribute to the chemotherapy resistance, accumulation of genomic alterations and chromosomal instability seen in patients with inactivation of the p53 pathway.

While loss of p53 *in vitro* can promote aberrant self-renewal in some assays, HSCs with heterozygous inactivation of *Trp53* do not have an advantage over normal HSCs in competitive mouse transplant models<sup>111,112</sup>. However, if those mice are then exposed to alkylating agents or ionizing radiation, the *Trp53* mutant clone rapidly expands at the expense of normal HSCs<sup>111,113</sup>. Indeed, mutations in *TP53* are present in approximately 5% of MDS cases, but greater than 30% of therapy-related myeloid neoplasms<sup>25,28,44,111,114</sup>. Of note, *TP53* and *PPM1D* are among the genes most frequently found to be mutated in individuals with CHIP, raising the possibility that detection of somatic mutations in patients scheduled to receive chemotherapy for other cancers could identify those most at risk of developing therapy-related myeloid neoplasms<sup>12,21</sup>.

### Abnormal cell signaling

Mutations in signaling pathway components are associated with pro-proliferative states and occur in a range of myeloid malignancies, including AML (*FLT3*), polycythemia vera (*JAK2*), essential thrombocythemia [G] (*JAK2* and the gene encoding the thrombopoietin receptor (*MPL*)), chronic myelomonocytic leukemia (CMML) (*CBL*) and mast cell disorders (*KIT*)<sup>115–119</sup>. Mutations in these genes all occur at a relatively low frequency in MDS as compared to AML, CMML or MPN. Many of these mutations affect the cell through activation of the MAPK pathway, as well as other signaling pathways. Mutations in the MAPK pathway (*NRAS*, *KRAS*, neurofibromin 1 (*NFI*) and protein tyrosine phosphatase, non-receptor type 11 (*PTPNI1*)) are the most frequently found in MDS but still occur in only about 10% of cases overall<sup>25,26</sup>. When such mutations do occur they are typically found in subclonal populations and occur late in disease evolution, often heralding the transition to sAML<sup>30,31</sup>. The majority of signaling pathway mutations are missense mutations or involve small insertions or deletions that lead to constitutive activation. One exception to this is the *CBL* gene, which encodes a tyrosine kinase-associated ubiquitin ligase<sup>120</sup>. *CBL* mutations lead to upregulation of signaling proteins such as FLT3 and MPL<sup>121,122</sup>. While *CBL* mutations are relatively common in CHIP, they occur less frequently in MDS, perhaps due to tropism for myelomonocytic lineages resulting instead in enrichment of MDS/MPN overlap syndromes<sup>123</sup>. When *CBL* mutations do occur in MDS, they are often late events<sup>12,21,26,118</sup>.

### Recurrent cytogenetic rearrangements

Cytogenetic analysis of bone marrow samples from MDS patients is part of routine clinical practice, and large chromosomal rearrangements are seen in approximately half of cases



(Table 1, reviewed in detail elsewhere<sup>124</sup>). Like individual gene mutations, these large-scale copy-number alterations can serve as founding events and drive disease evolution in MDS and AML (reviewed in detail elsewhere<sup>125</sup>). Acquisition of cytogenetic abnormalities during disease evolution is predictive of a poor prognosis<sup>126,127</sup>. Elucidation of the pathogenic genes within large chromosomal deletions has been challenging. Among those that are most common and best understood are isolated deletion of 5q, loss of chromosome 7, and deletion of 17p (discussed above with *TP53*).

### Chromosome 5q deletions

The single most common isolated cytogenetic abnormality in MDS is deletion of chromosome 5q. MDS patients with isolated deletions of chromosome 5q often have a consistent clinical phenotype, termed the 5q<sup>-</sup> syndrome, which is more common in women and has a relatively indolent course<sup>128,129</sup>. Deletion of 5q leads to haploinsufficiency of a small number of genes, including ribosomal protein S14 (*RPS14*), casein kinase 1  $\alpha$ 1 (*CSNK1A1*), adenomatous polyposis coli (*APC*), heat shock protein family A (HSP70) member 9 (*HSPA9*), early growth response 1 (*EGR1*), DEAD-box helicase 41 (*DDX41*), *NPM1*, TRAF-interacting protein with forkhead-associated domain B (*TIFAB*), Diaphanous-related formin 1 (*DIAPH1*), microRNA (miR)-145, and miR-146a, most of which lack evidence of biallelic inactivation<sup>130–136</sup>. A small subset of cases also have point mutations in *CSNK1A1* or *DDX41*, but no other genes have been identified with bi-allelic deletion or mutation<sup>137–139</sup>. Targeted short hairpin RNA (shRNA) screening of the genes within the commonly deleted region in 5q<sup>-</sup> syndrome demonstrated that loss of *RPS14* leads to a block in pre-ribosomal RNA (rRNA) processing and abnormal erythroid differentiation that phenocopies the macrocytosis and anemia seen in the 5q<sup>-</sup> syndrome<sup>140</sup>. Mouse models with conditional heterozygous inactivation of *Rps14* display a similar erythroid defect that is p53-dependent and accompanied by upregulation of components of innate immune signaling<sup>141,142</sup>. This is a similar mechanism to that proposed for Diamond-Blackfan anemia, caused by germline heterozygous inactivation of ribosomal protein genes (Box 2)<sup>143</sup>. An abundance of evidence now indicates that the full clinical phenotype of 5q<sup>-</sup> MDS is caused by combinatorial haploinsufficiency of multiple factors<sup>133,144</sup>.

Another important gene involved in the pathogenesis of 5q<sup>-</sup> syndrome is *CSNK1A1*, which encodes a serine/threonine kinase that when heterozygously deleted leads to upregulation of WNT signaling and stem cell expansion<sup>139,145</sup>. Homozygous deletion of *CSNK1A1*, on the other hand, leads to the accumulation of p53 and loss of the competitive advantage seen in the heterozygous setting. It is this dose dependent effect of *CSNK1A1* that leads to one of the hallmark features of 5q<sup>-</sup> syndrome: its responsiveness to treatment with the thalidomide derivative lenalidomide (Figure 5)<sup>146</sup>. Lenalidomide binds to the substrate recognition component (cereblon, CRBN) of the Cullin-RING E3 ubiquitin ligase CRL4, altering its substrate affinity and leading to the selective degradation of the *CSNK1A1* gene product, CK1 $\alpha$  (Figure 5)<sup>145,147</sup>. Loss of CK1 $\alpha$  leads to activation of p53 and apoptosis, and knockdown of *TP53* abrogates the effect of lenalidomide *in vitro*<sup>139,145</sup>.

### Chromosome 7 deletions

Deletion of 7q and/or monosomy 7 are also common in MDS and are associated with a poor prognosis<sup>6</sup>. Like deletion of 5q, these chromosomal aberrations lead to haploinsufficiency of a number of genes implicated in hematologic malignancies, including *EZH2*; *CUX1*; and *MLL3* gene (also known as *KMT2C*), which encodes a histone H3 lysine 4 (H3K4) methyltransferase<sup>148–150</sup>. In mouse models, *MLL3* haploinsufficiency cooperates with mutations in the RAS pathway and *Trp53* to promote leukemia<sup>148</sup>.

### Genetics and MDS management

The standard backbone of therapy for MDS involves supportive measures such as transfusion, infection control, growth factor support and iron chelation. The use of disease-altering therapy, including lenalidomide, 5-azacitidine, decitabine, or allogeneic HSC transplantation (alloHSCT), is based upon careful evaluation of the individual patient, including age, prognostic risk stratification, and genetics<sup>18</sup>.

### Genetic predictors of response to therapy

The hypomethylating agent 5-azacitidine and its derivative 5-aza-2'-deoxycytidine (decitabine) inhibit DNMTs and thus lead to global hypomethylation. Both drugs are active in MDS, and 5-azacitidine improves overall survival when compared to standard supportive care in higher risk patients as defined by the IPSS<sup>151,152</sup>. Molecular analysis may help predict which patients will benefit the most from these therapies. Loss of *TET2* across the entire MDS clone predicts improved response to hypomethylating agents, which may be related to the finding that these patients often have low risk disease. The presence of additional mutations, such as in *ASXL1*, are associated with a poorer prognosis, and subclonal *TET2* mutations do not similarly predict for response to treatment<sup>103,153</sup>.

Isolated deletion of chromosome 5q predicts response to treatment with lenalidomide, which leads to complete cytogenetic remissions in over half of patients and a reduced need for transfusions in 75% of those treated<sup>146</sup>. As discussed above, lenalidomide functions via selective degradation of CK1 $\alpha$ , leading to activation of p53. *TP53*-mutant subclones can often be detected in pre-treatment samples and expand following treatment with lenalidomide, leading to the development of resistance<sup>154</sup>. This also may explain the lack of efficacy of lenalidomide in patients with 5q deletion in the setting of complex karyotypes or in AML, as many of these patients likely carry concomitant mutations in *TP53*. The molecular correlates of response to lenalidomide in non-5q<sup>-</sup> syndrome patients (approximately 25% become transfusion independent in this context) are not known<sup>155</sup>.

### Allogeneic bone marrow transplantation

The only curative treatment for MDS is alloHSCT. Although some patients receive myeloablative conditioning regimens [G], recent studies showing equivalent success rates with reduced-intensity regimens suggest that the primary mechanism of action is immunological elimination of the MDS stem cell clone<sup>156</sup>. The efficacy is far from complete, however, and specific mutations have been associated with poor survival following alloHSCT, including *TP53*, owing to increased rates of post-transplant

relapse<sup>35,157</sup>. *TP53* mutation is a powerful marker of poor survival after transplant: in a retrospective analysis of 87 patients, not one of the 18 with a *TP53* mutation was still alive five years following alloHSCT with reduced intensity conditioning regimens<sup>35</sup>. Interestingly, the rare patients with a complex karyotype who lack a concomitant *TP53* mutation have a similar prognosis to patients with a normal karyotype<sup>35</sup>.

### Clinical implications of molecular genetics

A more complete understanding of MDS genetics can inform multiple aspects of our clinical practice, including diagnosis, prognosis and prediction of response to therapy. First, although morphologic analysis is still required to diagnose MDS, it is evident that morphology often directly relates to the underlying genetic lesions. It is likely that genetics will play an increasing role in the diagnosis of MDS in coming years as accumulating evidence strengthens the association between specific mutations and clinicopathologic features of the disease. It is also clear that certain mutations influence the prognosis of MDS patients. Initial attempts to integrate mutational data into the IPSS have demonstrated improved accuracy in predicting overall survival, and large-scale international collaborations are now underway to fully incorporate molecular genetics into the next generation of prognostic scoring systems<sup>33</sup>.

As discussed above, specific mutations may predict response to standard therapies such as hypomethylating agents, lenalidomide, and alloHSCT, and prospective studies are needed to validate these findings. Finally, understanding the molecular underpinnings of MDS can aid in the development of new targeted therapeutics. Targeting epigenetic modifiers and splicing factors is an attractive option as mutations in these are often founding events. For example, small molecule inhibitors of neomorphic IDH1 and IDH2 enzymes have shown initial promising results and induce differentiation of primary AML cells *in vitro*<sup>158,159</sup>. IDH mutations also lead to increased sensitivity to pro-apoptotic BH3 mimetics, such as the small molecule BCL-2 inhibitor venetoclax, likely due to altered mitochondrial function related to elevated 2-HG<sup>160</sup>. Initial attempts to target epigenetic regulation with histone deacetylase inhibitors have thus far not shown clinical efficacy<sup>161</sup>. MDS cells carrying mutations in splicing factors may be uniquely sensitive to further inhibition of splicing via mechanisms probably analogous to haploinsufficiency, and this observation has led to the development of small molecule splicing inhibitors<sup>162</sup>. Clinical trials of these agents are expected to begin soon.

### Conclusions

The rapid accumulation of genetic data over the last decade has provided a molecular taxonomy of MDS, a guide to the genetic progression of CHIP to MDS and MDS to sAML, and the identification of core molecular processes that are functionally disrupted through somatic mutations. CHIP, a premalignant condition, is common in older adults and is associated with a specific subset of mutations, most commonly in *DNMT3A*, *TET2*, *ASXL1*, *TP53*, and *SF3B1*, suggesting that mutations in these key genes are important for initiating disease. In contrast, mutations in hematopoietic transcription factors and activated signaling pathways tend to occur during disease progression to high risk MDS and sAML.

Patterns of cooperativity and mutual exclusivity act to define the evolutionary path from disease initiation to leukemia, and it is now clear that clinical phenotype, prognosis, and response to therapy in MDS are influenced by combinations of genetic lesions and the order and context in which they occur. Further understanding these relationships will enable refined prognostic staging models, the identification of patients most likely to respond to therapy, and the development of new targeted therapeutics.

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## Glossary

### **5q<sup>-</sup> syndrome**

MDS associated with isolated deletion of chromosome 5q and characterized by a macrocytic anemia, normal or elevated platelet count, a low marrow blast count, and a relatively indolent course.

### **Annual risk**

The probability of acquiring a condition over the course of one year.

### **Aplastic anemia (AA)**

Pancytopenia in the setting of aplastic bone marrow caused by immune-mediated destruction of hematopoietic progenitors. It is often difficult to distinguish morphologically from hypoplastic MDS.

### **Blasts**

Immature, hypofunctional leukemic cells found in the peripheral blood or bone marrow.

### **Clonal hematopoiesis of indeterminate potential (CHIP)**

The presence of a somatic mutation associated with hematological malignancy at a variant allele fraction of at least 2% and the absence of morphological evidence of malignancy or diagnostic criteria for paroxysmal nocturnal hemoglobinuria, monoclonal gammopathy of undetermined significance or monoclonal B-lymphocytosis.

### **Cohesin complex**

A multisubunit protein complex that forms a ring structure capable of encircling two chromosomal strands of DNA and required for sister chromatid cohesion during mitosis.

### **Conditioning regimen**

High dose preparative chemotherapy regimen given prior to stem cell transplant. Can be either myeloablative (doses sufficient to completely ablate the bone marrow) or non-myeloablative.

### **Core Binding Factor**

A core hematopoietic transcription factor complex, mutations of which are associated with AML in younger patients and a relatively good prognosis.

**Cytopenias**

Decreased blood counts of any kind (white cells, red cells or platelets).

***de novo* AML**

AML that arises without a pre-existing myeloid neoplasm or a history of cytotoxic therapy. More common in younger patients and associated with an overall better prognosis.

**Essential thrombocythemia (ET)**

Myeloproliferative neoplasm characterized by elevated platelet count, associated with mutations in *JAK2*, *CALR* or *MPL*.

**Hazard ratio**

A statistical measure that corresponds to the probability of a particular outcome attributable to a given variable as compared to normal controls.

**Hypoplastic MDS**

While most patients with MDS have normal or increased bone marrow cellularity, some patients have low cellularity. Can be difficult to distinguish from aplastic anemia due to the small number of cells available to evaluate for morphologic dysplasia.

**Idiopathic cytopenias of undetermined significance (ICUS)**

Cytopenias that remain unexplained after thorough evaluation and do not meet WHO criteria for a hematologic neoplasm.

**Induction therapy**

High-dose intensive chemotherapy directed at inducing remission in acute leukemias.

**Lineage**

A collection of cell surface markers that defines mature blood cells including B-cells, T-cells, monocytes, granulocytes and red blood cells.

**Mean corpuscular volume (MCV)**

A measure of the average volume of red blood cells. Increased size is associated with abnormal or delayed red blood cell differentiation.

**Paroxysmal nocturnal hemoglobinuria (PNH)**

Caused by mutations in the *PIGA* gene leading to the loss of glycosylphosphatidylinositol (GPI), a chemical linker that functions to anchor a number of proteins to blood cell membranes including those that block complement mediated hemolysis.

**Polycomb repressive complex (PRC)**

Multiprotein complex involved in epigenetic repression of gene transcription.

**Polycythemia vera (PV)**

Myeloproliferative neoplasm characterized by an elevated red blood cell count, and almost exclusively associated with activating mutations in *JAK2*.

**Red cell distribution width (RDW)**

A measure of the distribution of red blood cell sizes, and indicates the degree of variation within a sample.

### Ring sideroblasts

Early red cell precursors containing aberrant mitochondrial iron staining; associated with mutations in splicing factors, most commonly *SF3B1*.

### Secondary AML (sAML)

AML that arises out of a pre-existing myeloid neoplasm such as MDS or MPN.

Distinguished from MDS by the presence of 20% or more blasts in the bone marrow or peripheral blood.

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## Biographies

Adam Sperling completed his MD, PhD at the University of California, Los Angeles in the laboratory of Michael Grunstein where he studied epigenetic mechanisms of gene regulation. He is currently pursuing a fellowship in medical oncology at the Dana-Farber Cancer Institute and is performing post-doctoral research on basic mechanisms of myeloid disease in Benjamin Ebert's lab.

Christopher Gibson received his MD from Yale University, New Haven, CT, USA and is completing his fellowship training in hematology/oncology at the Dana-Farber Cancer Institute in Benjamin Ebert's lab. His research interests focus on incorporating translational genomics into the care of patients with hematologic malignancies.

Benjamin Ebert is an Associate Professor of Medicine at Harvard Medical School, Boston, MA, USA; an Institute Member of the Broad Institute, Leader of the Leukemia Program for the Dana-Farber/Harvard Cancer Center, and co-Director of the Cancer Program at the Harvard Stem Cell Institute. His laboratory has a long-standing interest in the genetic lesions that drive myeloid malignancies, with a particular focus on myelodysplastic syndrome.

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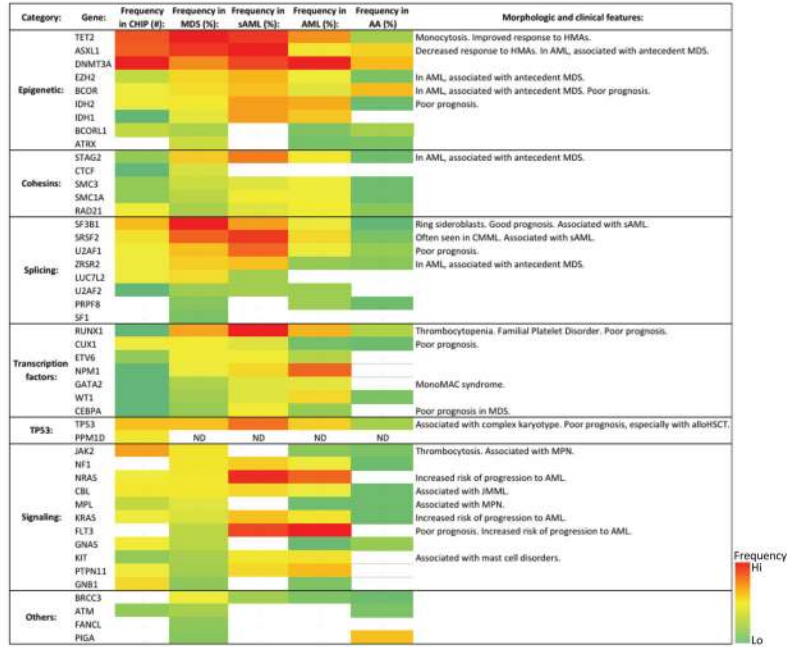
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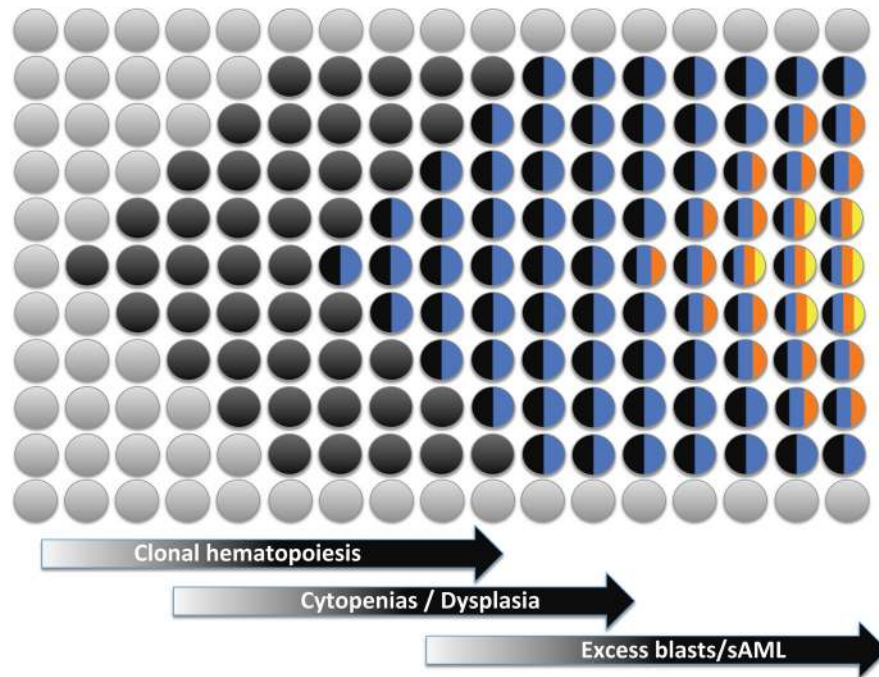
### Key Points

- Myelodysplastic syndrome (MDS) is one of the most common hematologic malignancies and is associated with increased age and exposure to prior chemotherapy and radiation. It is characterized by cytopenias, morphologic dysplasia and a propensity to transform to AML.
- Clonal hematopoiesis of indeterminate potential (CHIP) is a condition in which a substantial percentage of hematopoietic cells bear a somatic mutation in a gene that is recurrently mutated in hematologic malignancies, including MDS. CHIP is strongly associated with age and an increased risk of hematologic malignancy.
- Over 50 recurrently mutated genes have been identified in MDS, many of which occur in genes encoding RNA splicing factors, epigenetic regulators, hematopoietic transcription factors, and kinase signaling pathways.
- Individual mutations in MDS are associated with specific morphologic findings, have independent prognostic significance, and can predict response to therapy in some cases.
- AML that arises out of a pre-existing MDS can be distinguished from *de novo* AML by the presence of specific mutations, such as those in the splicing factors and certain epigenetic regulators.
- Some mutations are associated with increased sensitivity or resistance to standard therapeutic interventions, providing new targets for the development of novel therapeutic agents.
- Currently, allogeneic hematopoietic stem cell transplantation is the only known curative treatment for MDS.



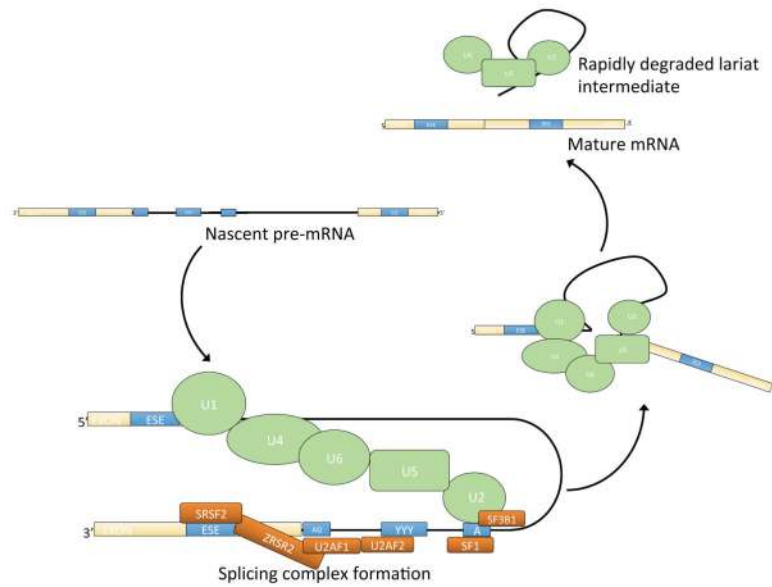


**Figure 1. Recurrent mutations in CHIP and MDS**  
 Mutations are sorted by their frequency in MDS within functional categories. Mutation percentages (%) shown for all categories except CHIP, where the absolute mutation count is shown (#). CHIP: Clonal hematopoiesis of indeterminate potential<sup>12,21</sup>. MDS: Myelodysplastic syndrome<sup>9,25,26,35</sup>. AML: Acute myeloid leukemia<sup>192-194</sup>. sAML: Secondary acute myeloid leukemia<sup>28</sup>. AA: Aplastic anemia<sup>45</sup>. AlloHSCT, allogeneic hematopoietic stem cell transplantation; HMAs, hypomethylating agents; MonoMAC, monocytosis with increased susceptibility to mycobacterial infections; MPN: Myeloproliferative neoplasm. CMML: Chronic myelomonocytic leukemia. JMML: Juvenile myelomonocytic leukemia.



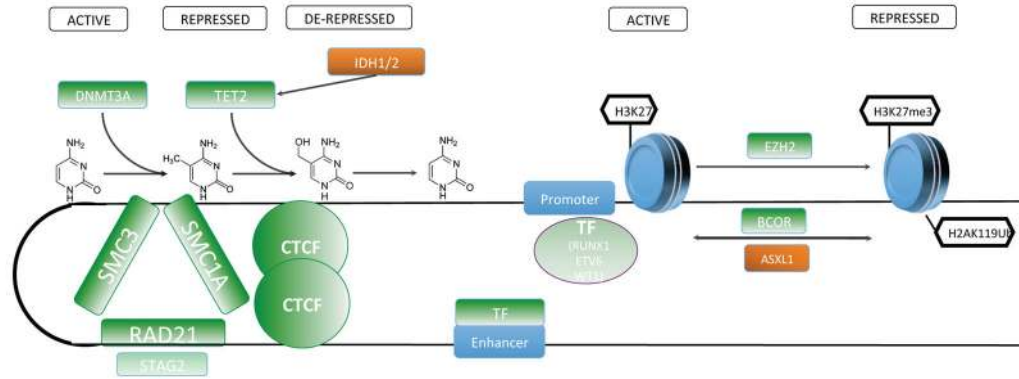
**Figure 2. Clonal expansion in MDS**

Early mutations tend to lead to increased hematopoietic stem cell (HSC) self-renewal, clonal expansion and the development of clonal hematopoiesis of indeterminate potential (CHIP). As the mutant clone continues to enlarge, it gives rise to an expanding population of cells in which acquisition of additional genetic or epigenetic lesions can promote progression to overt malignancy. These secondary subclonal events tend to lead to the development of overt dysplasia, myelodysplastic syndrome (MDS) and eventually secondary acute myeloid leukemia (sAML).



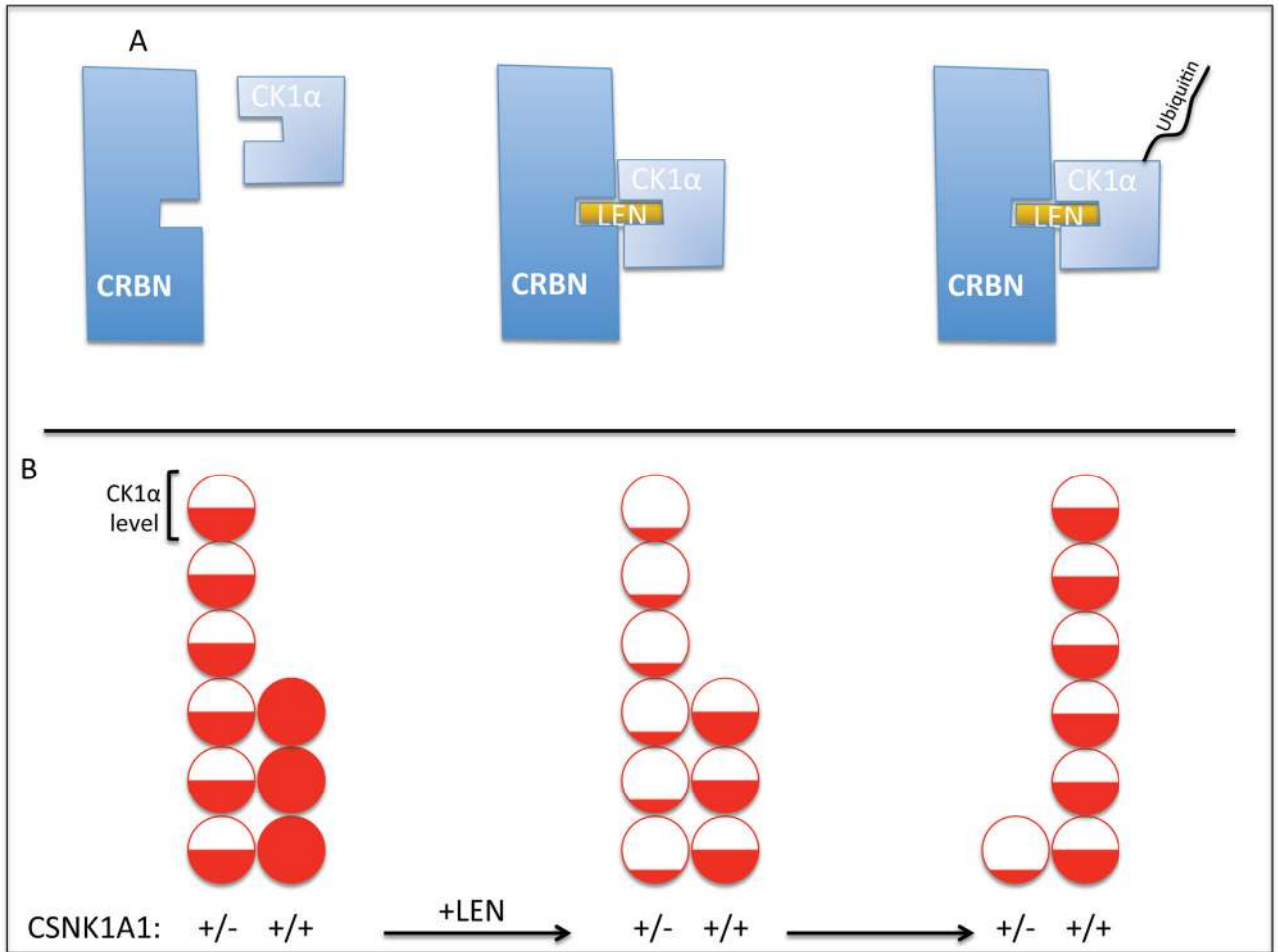
### Figure 3. Splicing factor mutations in myeloid neoplasms

Shown is a simplified schematic of the steps involved in mRNA splicing. Splicing factor mutations cluster within components of the 3' spliceosome. Genes mutated in MDS are denoted in red. YYY: Polypyrimidine tract. A: Branch site. AG: Splice acceptor site. ESE: Exonic splicing enhancer. SF1, splicing factor 1; SF3B1, splicing factor 3b subunit 1; SRSF2, serine and arginine rich splicing factor 2; U2AF1, U2 small nuclear RNA auxiliary factor 1; ZRSR2, zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2. Modified with permission from Boulton et al<sup>195</sup>.



**Figure 4. Multiple steps in gene expression are recurrently disrupted in MDS**

Shown is a prototypical gene promoter with chromosomal looping facilitated by CCCTC-binding factor (CTCF) and the cohesin complex, allowing transcription factors (TFs) bound at distant enhancers to interact with the promoter. Alterations in epigenetic marks such as DNA methylation and histone post-translational modifications function to regulate the transcription of genes. Green signifies loss of function (or dominant negative function) mutations. Red signifies gain of function mutations. C, cytosine; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethyl-cytosine; H2AK119Ub, histone H2A lysine 119 ubiquitylation; H3K27, histone H3 lysine 27; H3K27me3, H3K27 trimethylation; IDH, isocitrate dehydrogenase; DNMT3A, DNA methyltransferase 3A; TET2, ten-eleven translocation 2; EZH2, enhancer of zeste 2; BCOR, BCL6 corepressor; ASXL1, additional sex combs-like 1; RUNX1, runt related transcription factor 1; ETV6, ETS variant 6; WT1, Wilms tumor 1; SMC1A, structural maintenance of chromosomes 1A; STAG2, stromal antigen 2.



**Figure 5. Mechanism of lenalidomide efficacy in 5q<sup>-</sup> syndrome**

(A) Lenalidomide (LEN) functions through modulation of the substrate binding specificity of cereblon (CRBN), a component of an E3 ubiquitin ligase, for casein kinase 1α (CK1α, encoded by *CSNK1A1*). In the absence of LEN, CRBN has low affinity for CK1α. Binding of LEN, however, induces a conformational change in CRBN that significantly increases this affinity, thereby catalyzing efficient ubiquitination and degradation of CK1α. (B)

Hematopoietic stem cells (HSCs) harboring 5q deletion (5q<sup>-</sup> syndrome), which lack one copy of *CSNK1A1* and have a lower CK1α level have a clonal advantage over wildtype cells at baseline. Treatment with LEN selectively depletes CK1α in all HSCs; in 5q<sup>-</sup> cells this pushes the CK1α level below a critical threshold and triggers cell death, whereas wildtype *CSNK1A1* cells retain enough CK1α for survival and can eventually repopulate the bone marrow.

**Table 1**

Recurrent cytogenetic abnormalities in MDS.

Chromosomal abnormality	Key genes deleted*	IPSS-R risk category <sup>6</sup>	Clinical features
Normal	--	Good	
del(5q)	<i>CSNK1A1, RPS14, EGR1, APC, DDX41, HSPA9, miR-145, miR-146a</i> <sup>130–133,137–140</sup>	Good	Sensitive to lenalidomide <sup>196</sup> .
Monosomy 7 or del(7q)	<i>EZH2, MLL3, CUX1</i> <sup>148–150</sup>	Poor	Monosomy 7 may have a worse prognosis than del(7q) <sup>197</sup> .
Trisomy 8	Unknown	Intermediate	High response rate to immunosuppression <sup>190</sup> .
Trisomy 19	Unknown	Intermediate	
del(20q)	<i>MYBL2, TP53RK, TP53TG5</i> <sup>198</sup>	Good	Often associated with mutations in splicing factors <sup>198</sup> .
del(17p)	<i>TP53</i> <sup>109</sup>	N/A	Poor response to alloHSCT <sup>35</sup> .
Complex <sup>‡</sup> and monosomal <sup>§</sup>	<i>TP53</i> <sup>109</sup>	Poor to very poor	Associated with <i>TP53</i> mutation <sup>35</sup> .
del(11q)	<i>MLL, ATM</i> <sup>199</sup>	Very good	
“-Y”	Unknown	Very good	May not be pathogenic, but instead may be lost during normal aging <sup>200</sup> .

\* Other genes have been implicated in some studies.

<sup>‡</sup>Complex: ≥3 abnormalities.

<sup>§</sup>Monosomal: >2 monosomies.

N/A: Not included in the IPSS-R. alloHSCT, allogeneic hematopoietic stem cell transplantation; del, deletion; IPSS-R, Revised International Prognostic Scoring System. *EZH2*, enhancer of zeste 2; *CUX1*, cut like homeobox 1; *CSNK1A1*, casein kinase 1  $\alpha$  1; *RPS14*, ribosomal protein S14; *EGR1*, early growth response 1; *APC*, adenomatous polyposis coli; *DDX41*, DEAD-box helicase 41; *HSPA9*, heat shock protein family A (HSP70) member 9; *miR*, microRNA; *MYBL2*, v-myb avian myeloblastosis viral oncogene homolog-like 2; *TP53RK*, TP53 regulating kinase; *TP53TG5*, TP53-target gene 5; *MLL*, mixed lineage leukemia; *ATM*, ataxia telangiectasia mutated.