

## Unraveling the Molecular Pathophysiology of Myelodysplastic Syndromes

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### ABSTRACT

Somatically acquired genetic abnormalities lead to the salient features that define myelodysplastic syndromes (MDS): clonal hematopoiesis, aberrant differentiation, peripheral cytopenias, and risk of progression to acute myeloid leukemia. Although specific karyotypic abnormalities have been linked to MDS for decades, more recent findings have demonstrated the importance of mutations within individual genes, focal alterations that are not apparent by standard cytogenetics, and aberrant epigenetic regulation of gene expression. The spectrum of genetic abnormalities in MDS implicates a wide range of molecular mechanisms in the pathogenesis of these disorders, including activation of tyrosine kinase signaling, genomic instability, impaired differentiation, altered ribosome function, and changes in the bone marrow microenvironment. Specific alterations present in individual patients with MDS may explain much of the heterogeneity in clinical phenotype associated with this disease and can predict prognosis and response to therapy. Elucidation of the full complement of genetic causes of MDS promises profound insight into the biology of the disease, improved classification and prognostic scoring schemes, and the potential for novel targeted therapies with molecular predictors of response.

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### INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal disorders of hematopoiesis characterized by inefficient hematopoiesis, peripheral blood cytopenias, and risk of progression to acute myeloid leukemia (AML). The clinical phenotype of patients with MDS are diverse with respect to the number and severity of cytopenias, cellularity and blast count in the bone marrow, rate of progression to AML, overall survival, and response to treatment. Much of this phenotypic heterogeneity is likely due to the variety of genetic lesions that contribute to disease pathogenesis. Unraveling the genetic complexity of MDS promises to elucidate the pathophysiology, refine the taxonomy and prognostic scoring systems, and provide novel therapeutic targets.

The known set of genetic lesions that cause MDS include copy number changes (genetic amplifications or deletions), mutations that alter the sequence or expression of individual genes, and epigenetic abnormalities. Although balanced translocations are rare, chromosomal abnormalities evident by standard karyotypic analysis are present in approximately half of patients with MDS. The most common of these are loss of 5q (5q-), loss of 7 or 7q (-7/7q-), trisomy 8 (+8), loss of 20q (20q-), and loss

of Y (-Y; Table 1).<sup>1</sup> More sensitive technologies such as single nucleotide polymorphism microarrays can detect copy number changes or acquired uniparental disomy in as many as 75% of patients with MDS.<sup>2,3</sup> Moreover, the majority of patients have mutations that alter the sequence and function of oncogenes or tumor suppressor genes (Fig 1).<sup>4</sup> In addition, patients with MDS commonly have abnormal epigenetic profiles, resulting in aberrant gene expression.<sup>5</sup>

Molecular lesions are already used to guide the diagnosis, prognosis, and treatment of MDS. The International Prognostic Scoring System (IPSS) incorporates the common karyotypic abnormalities.<sup>6</sup> The use of lenalidomide is guided by the presence of a chromosome 5q deletion because this karyotypic abnormality increases the likelihood of cytogenetic and hematologic responses.<sup>7</sup> However, genetic predictors of response to hypomethylating agents or bone marrow transplantation have not yet been identified and validated. Point mutations, epigenetic states, and gene expression profiles are not currently integrated into diagnostic classifications or prognostic scoring systems.

The catalog of genes that play a role in the pathophysiology of MDS is rapidly expanding, and studies are underway to investigate the biologic and clinical consequences of each genetic lesion. With

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## Unraveling the Molecular Pathophysiology of MDS

**Table 1.** Genetic Abnormalities in MDS

Genetic Abnormality	Frequency (%)	Pathogenic Mechanism	Clinical Consequence
<b>Chromosomal abnormalities</b>			
5q-	15	Haploinsufficiency for <i>RPS14</i> , <i>miR-145/146a</i> , <i>CTNNA1</i> , <i>EGR1</i> , <i>APC</i> , <i>NPM</i>	Better prognosis, high rate of response to lenalidomide
-7/7q-	5-10	Unknown	Poor prognosis, more common in therapy-related MDS
Trisomy 8	5-8	Unknown	Can help predict response to immunosuppression, some evidence as a marker of progression to AML
20q-	2-5	Unknown	Better prognosis
-Y	2-4	Age-related phenomenon that may not be disease related	May be useful as a marker of clonal hematopoiesis
Complex (three or more abnormalities)	10-15	Various; often abnormal chromosome 17 (TP53 locus)	Poor prognosis
-13/13q-, 11q-, 12p-, 9q-, idic(X)(q13), i(17q), t(11;16), t(3;21), t(1;3), t(2;11), inv(3), t(6;9)	Rare	Various, mostly unknown; chromosome 3q26 lesions alter expression of <i>EVI1</i>	Presumptive evidence of MDS in patients with otherwise unexplained refractory cytopenia and no morphologic evidence of dysplasia
<b>Gene mutations</b>			
<i>TET2</i>	Approximately 20	Unknown	Unknown
<i>RUNX1</i>	15-20	Mutations typically alter DNA-binding domain or disrupt protein-binding domain	Increased risk of progression to AML; more common in therapy-related MDS
<i>TP53</i>	5-10	Loss of function of p53 tumor suppressor activity; associated with chromosomal instability	Often complex cytogenetics, relative resistance to therapy, poor prognosis
<i>ASXL1</i>	10-15	Unknown; most mutations are distal heterozygous frame shifts, suggesting a dominant negative function	Unknown
<i>NRAS/KRAS</i>	Approximately 10	Loss of GTPase activity leads to constitutive activation of serine/threonine kinase	Increased risk of progression to AML
<i>EZH2</i>	6	Loss of histone 3 lysine 27 methyltransferase activity	Poor prognosis
<i>CBL/CBLB</i>	Rare	Loss of ubiquitin ligase activity; mutants can inhibit wild-type enzymatic function	Unknown; increased risk of progression to leukemia in MPN and in CMML where this mutation is more common
<i>JAK2</i>	5% of RA, 50% of RARS-T	Constitutive activation of tyrosine kinase	Unknown; does not appear to alter prognosis
<i>MPL</i>	5% of RARS-T	Constitutive activation of tyrosine kinase	Unknown
<i>ATRX</i>	Rare	Loss of function leads to decreased alpha-globin expression, likely through epigenetic dysregulation	Acquired alpha-thalassemia, often with very severe anemia
<i>NPM1</i>	Rare	Terminal frame shift disrupts nucleolar localization signal leading to cytoplasmic redistribution of protein	Unknown; this mutation is very common in AML with normal cytogenetics
<i>IDH1, IDH2</i>	Rare	Missense mutations alter catalytic function to convert $\alpha$ -ketoglutarate into 2-hydroxyglutarate while consuming NADPH	Associated with more advanced disease and progression to AML
<i>CEBPA</i>	Rare	Loss of function known to impair granulopoiesis	Unknown; germline mutations associated with risk of AML, not MDS
<i>WT1</i>	Very rare	Impairment of transcription factor activity	Unknown; mutations are more common in AML and associated with poor outcomes
<i>PTPN11</i>	Very rare	Alters function of gene product SHP2, an adaptor protein with tyrosine phosphatase activity	More common in JMML, rare in CMML
<i>FLT3, CSF1R, CKIT</i>	Very rare	Constitutive activation of tyrosine kinase	Associated with more advanced disease and progression to AML

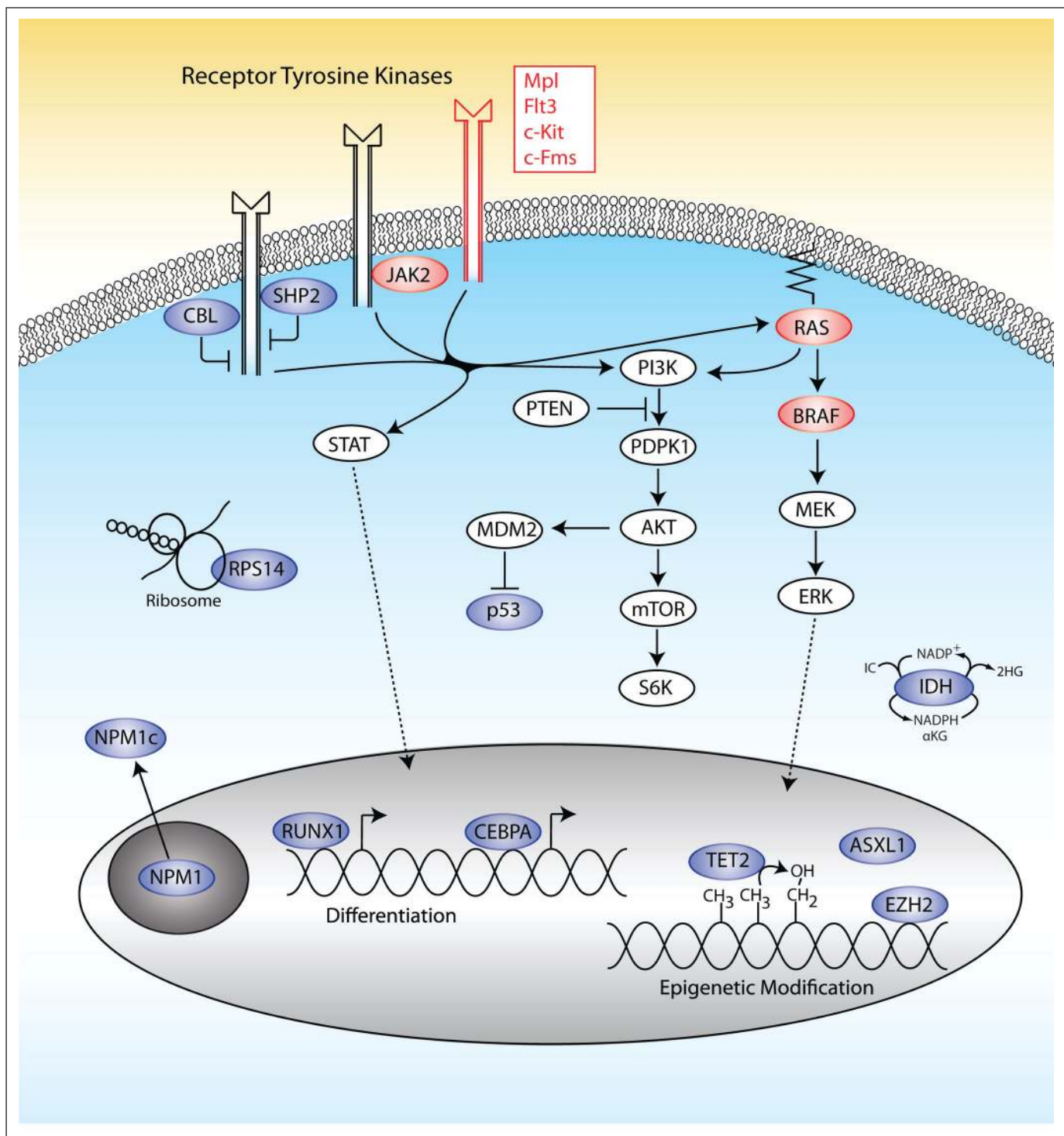
NOTE. Rare mutations are present in 2% to 5% of patients; very rare refers to individual reports or < 2% of patients.

Abbreviations: MDS, myelodysplastic syndromes; *CTNNA1*, alpha-catenin 1; *EGR1*, early growth response 1; *APC*, adenomatous polyposis coli; *NPM1*, nucleophosmin 1; AML, acute myeloid leukemia; GTPase, guanosine triphosphatase; MPN, myeloproliferative neoplasms; CMML, chronic myelomonocytic leukemia; NADPH, nicotinamide adenine dinucleotide phosphate; JMML, juvenile myelomonocytic leukemia.

the advent of whole-genome sequencing in human malignancies, the rate of discovery is likely to accelerate. A more complete genetic characterization of MDS has great potential to elucidate the molecular basis for the clinical heterogeneity of these disorders and identify disease subtypes with shared outcomes and responses to therapy.

### MODEL OF PATHOGENESIS

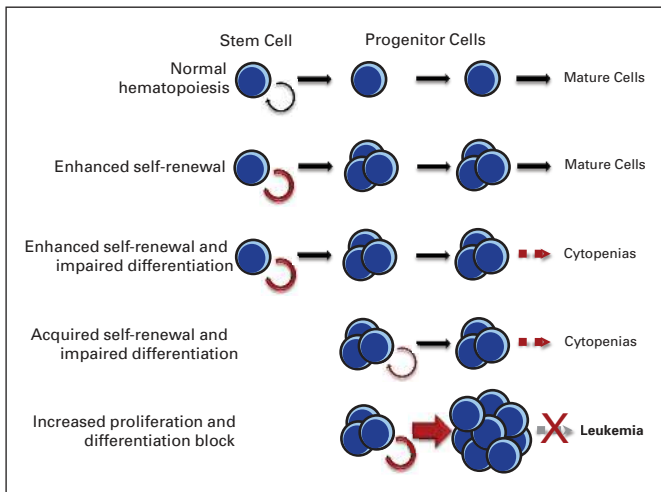
To manifest clinically, an MDS clone must pass several biologic milestones.<sup>8</sup> Individual mutations may contribute to more than



**Fig 1.** Targets of point mutations and deletions in myelodysplastic syndromes. Mutations in multiple pathways have been implicated in the pathogenesis of myelodysplastic syndromes. Features shown in red are targets of activating mutations, whereas mutations or deletions of features in blue result in a loss of function. Mutations that impair function may also cause a gain of function as is believed to be the case for a subset of mutations in *CBL*, *ASXL1*, *NPM1c*, *IDH1/IDH2*, *TP53*, and *RUNX1*.

one step in the transformation process, although most cases of cancer appear to require the acquisition of multiple genetic abnormalities. In addition, each step in the process can be achieved by alterations in one of several genes, providing a multitude of molecular routes to disease development.

Steps associated with the pathogenesis of MDS include (1) enhanced self-renewal of a hematopoietic stem cell or acquisition of self-renewal in a progenitor cell, (2) increased proliferative capacity in the disease-sustaining clone and/or in its more differentiated progeny, (3) impaired or blocked differentiation, (4) genetic and epigenetic



**Fig 2.** Various pathways to transformation. The defining feature of myelodysplastic syndromes is clonal and inefficient hematopoiesis that causes peripheral cytopenias. A heterogeneous set of genetic and epigenetic abnormalities drive the cellular events that give rise to clinical phenotypes. Individual lesions may be responsible for one transformative step alone (eg, enhanced self-renewal or altered apoptosis) and therefore be clinically silent in isolation, or they could result in several abnormalities (eg, acquired self-renewal and impaired differentiation). Cooperation between two or more lesions is likely needed for the full expression of disease.

instability, (5) antiapoptotic mechanisms in the disease-sustaining cell, (6) evasion of the immune system, and (7) suppression of normal hematopoiesis.

The capacity for self-renewal must be present in the MDS disease-initiating cell.<sup>9</sup> This cell may arise from a self-renewing hematopoietic stem cell or it may come from a more differentiated myeloid progenitor that acquires the ability to self-renew. Further clonal expansion may occur through increased proliferation or resistance to apoptosis, and an abnormal bone marrow microenvironment could favor the development of a neoplastic clone. MDS occur when at least one of the molecular lesions present in the dominant clone or in its microenvironment also causes dysplastic differentiation of one or more myeloid lineages giving rise to ineffective hematopoiesis (Fig 2). The degree to which each step is affected can determine how the disease manifests clinically, including the types and degree of cytopenias present and whether the disease is indolent or rapidly progressive.

## CHROMOSOMAL ABNORMALITIES

### Chromosome 5q Deletions

With an incidence of roughly 15%, deletions of chromosome 5q (5q-) are the most common cytogenetic abnormality in patients with MDS, and isolated 5q- is associated with a relatively favorable prognosis. Deletions of 5q are universally heterozygous. Despite extensive sequencing, no mutations have been identified in genes on the remaining intact allele, and uniparental disomy is not seen on 5q in patients with MDS.<sup>10-12</sup> These findings indicate that instead of biallelic inactivation of a tumor suppressor gene, haploinsufficiency for one or more 5q genes is likely pathogenic. Careful analysis of the 5q- breakpoints in multiple patients with MDS localized two distinct commonly deleted regions (CDRs).<sup>13-15</sup> The more distal CDR in 5q33.1 is associated with a clinical phenotype

termed the 5q- syndrome, characterized by severe macrocytic anemia, relative thrombocytopenia, female predominance, and a lower risk of progression to AML.<sup>16</sup> The proximal CDR at 5q31 is associated with therapy-related MDS and a more aggressive MDS and AML phenotype.<sup>13-15</sup>

Multiple genes on 5q have been implicated in the pathogenesis of MDS. *RPS14* was identified as a critical gene for the erythroid phenotype of the 5q- syndrome in a systematic functional screen of the 5q33 CDR.<sup>17</sup> Congenital mutations or deletions causing haploinsufficiency for other ribosomal proteins cause Diamond-Blackfan anemia, a disease also characterized by a severe macrocytic anemia.<sup>18</sup> Since patients with Diamond-Blackfan anemia do not have elevated platelet counts, deletion of *RPS14* alone is unlikely to be responsible for the entire 5q- syndrome phenotype. Recent studies have demonstrated that haploinsufficiency for two microRNAs located on chromosome 5q33, *miR-145* and *miR-146*, can cause elevated platelet counts and may provide a selective advantage to the 5q- clone.<sup>19,20</sup>

The proximal CDR at 5q31 also contains several candidate MDS genes. The early growth response gene (*EGR1*) increases stem-cell self-renewal when one copy is deleted.<sup>21</sup> Alpha catenin, *CTNNA1*, is underexpressed in patients with 5q- syndrome, and hypermethylation of the remaining allele is associated with transformation to AML.<sup>14,22</sup> Deletions of 5q are not limited to the CDRs and often encompass both of these regions and beyond. Other candidate MDS genes that lie outside the CDRs but are often lost with deletions of 5q include *APC* and *NPM1*.<sup>23-25</sup>

Deletion of some 5q genes may not be involved in the development of MDS but could sensitize cells to therapeutic agents. This may explain the robust response that patients with 5q- MDS have to lenalidomide, an analog of thalidomide that is approved by the US Food and Drug Administration for the treatment of 5q- MDS.<sup>26</sup> Although nearly half of patients with low-risk MDS reduce their need for transfusions after lenalidomide therapy, those with 5q- MDS often have complete cytogenetic remissions, demonstrating the unique sensitivity this genetic lesion imparts to these cells. Two cell cycle-regulating phosphatases encoded by the genes on 5q, *CDC25C* and *PP2A*, have been implicated in the favorable response to lenalidomide.<sup>27</sup>

### Chromosome 7 and 7q Deletions

Unlike 5q deletions, monosomy 7 or interstitial loss of 7q is associated with a relatively poor prognosis. Approximately 10% of patients with MDS carry an abnormality of chromosome 7, either in isolation or as part of a complex karyotype.<sup>1</sup> This frequency approaches 50% in patients who have therapy-related MDS from a prior history of treatment with alkylating agents.<sup>28</sup> At least three common deleted regions on 7q have been identified, but the underlying molecular lesions driving the development of MDS in these cases are not well characterized.<sup>29-31</sup> The recent discovery of mutations in *EZH2*, a gene located at 7q36, may partially explain why this chromosomal region is often deleted. Conditional heterozygous deletion of a 2.5-Mb region syntenic to human 7q22 in murine hematopoietic stem cells had no phenotype indicating that this locus may not harbor a critical suppressor.<sup>32</sup> In therapy-related MDS, chromosome 7 abnormalities co-occur with 5q- or mutations in *RUNX1* more often than would be expected if these events were distributed randomly among all patients.<sup>33</sup> This suggests that -7/7q- lesions drive distinct steps in MDS development from 5q deletions or *RUNX1* mutations.



### Trisomy 8 and Response to Immunosuppressive Therapy

Trisomy 8 is the only recurrent chromosomal amplification observed in MDS. It is present as sole abnormality in roughly 8% of patients and is considered to be an intermediate-risk cytogenetic abnormality. MDS patients with +8 have less than half the median expected survival of patients with a normal karyotype (22.0 v 53.4 months).<sup>1</sup> However, a subset of patients with MDS and a sole +8 abnormality respond quite well to immunosuppressive therapy. These patients are more likely to be young, have refractory anemia of short duration, and carry *HLA DR15*.<sup>34</sup> Patients with +8 MDS have been shown to have expansion of V $\beta$ -restricted CD8<sup>+</sup> T cells. After immunosuppressant therapy, V $\beta$  representation in CD8<sup>+</sup> T cells often returns to a more normal polyclonal distribution. Despite improvement in blood counts, examination of the bone marrow in responders demonstrates the persistence or even the expansion of the +8 clone. This suggests that the immune system targets diseased cells and, as a consequence, impairs normal hematopoiesis. Cells with trisomy 8 have been shown to express higher levels of antiapoptotic genes and are resistant to irradiation compared with normal cells. In the setting of autoimmunity, the +8 clone is likely to have a selective advantage over normal hematopoietic precursors. This mechanism of immune-mediated growth advantage and impairment of normal hematopoiesis is not restricted to +8 MDS. Patients without this abnormality, particularly those with low-risk disease, can also benefit from immunosuppression, albeit with a lower frequency of response.<sup>35</sup>

### Chromosome Y and 20q Deletions

MDS patients with isolated 20q- or -Y abnormalities are considered to be in the same favorable cytogenetic risk group as patients with a normal karyotype. The loss of chromosome Y appears to be unrelated to disease pathogenesis.<sup>36</sup> Isolated -Y is found in men without evident hematologic diseases, increasing in incidence with age.<sup>37</sup> Interstitial deletion of 20q, on the other hand, does appear to be pathologic but is not restricted to cases of MDS. It is a common cytogenetic abnormality in myeloproliferative disorders and AML.<sup>38</sup> The CDR of 20q deduced from patients with MDS and AML includes 19 genes, none of which have been conclusively linked to the pathogenesis of myeloid disorders.<sup>39,40</sup> In patients with MDS, the favorable cytogenetic risk associated with 20q- suggests that it does little to modify the course of disease compared with patients with a normal karyotype, although late acquisition of 20q- implies clonal evolution and can precede progression of disease.<sup>41</sup> As with other chromosomal abnormalities, both -Y and 20q- can be useful for confirming the presence of clonal hematopoiesis and for monitoring the response of patients to treatment. However, neither lesion is sufficient to make the diagnosis of MDS.

### Abnormalities Involving Chromosome 3q26

Recurrent translocations and inversions involving 3q26 have been identified in AML and rare cases of MDS in which they are associated with a poorer prognosis.<sup>1,42</sup> Breakpoints typically include the *MDS1-EVII* gene locus (*MECOM*).<sup>43</sup> Alternative splicing can produce the full-length *MDS1-EVII* transcript or just the more distal *EVII* transcript. Chromosomal abnormalities at this locus preferentially activate expression of *EVII* alone.<sup>44</sup> The products of these transcripts have different and potentially antagonistic functions. *EVII* can impair the activity of several transcription factors including *MDS1-EVII*, *GATA1*, *PU.1*, and *RUNX1* leading to impaired

hematopoiesis.<sup>44-47</sup> In mouse models, forced *EVII* expression results in an MDS-like phenotype and increased self-renewal of hematopoietic progenitors.<sup>48,49</sup> The *Mecom* locus is a frequent transforming retroviral insertion site in mice, and retroviral *EVII* activation has been implicated as a cause of MDS in participants in a hematopoietic gene therapy trial.<sup>50-52</sup> Even in patients without 3q26 abnormalities, *EVII* overexpression is associated with a poor outcome.<sup>53,54</sup>

### Other Cytogenetic Abnormalities

Several rare, but recurrent cytogenetic abnormalities have been associated with MDS. In the absence of an alternative diagnosis, these chromosomal lesions can be used as presumptive evidence of MDS in persistently cytopenic patients, even if they have little evidence of dysplasia.<sup>55</sup> These include various abnormalities of chromosome 17 (presumably disrupting *TP53*), isodicentric chromosome Xq13 (often associated with the presence of ring sideroblasts), and the t(6;9)(p23;q34) translocation that generates the *DEK-NUP214* fusion gene (also referred to as *DEK-CAN*). The prognostic implications of these rare karyotypes are not considered independently in common clinical prognostic scoring systems such as the IPSS. These lesions are lumped into the intermediate-risk group when they occur in isolation or with another single abnormality besides -7/7q-. Newer studies that associate outcome with cytogenetic abnormalities are more inclusive of these rarer lesions and may better predict the prognosis of patients with MDS.<sup>42</sup>

## GENE MUTATIONS

### TET2 Mutations

*TET2* is the most frequently mutated gene identified in MDS to date. Mutations of *TET2* are present in nearly 20% of patients with MDS and are also seen in myeloproliferative neoplasms (MPN) (10%), chronic myelomonocytic leukemia (CMML; 30% to 50%), and secondary AML (25%).<sup>56-59</sup> Its homolog, the ten-eleven translocation gene (*TET1*), was named for its role as a fusion partner with the mixed-lineage leukemia (*MLL*) gene in rare cases of AML.<sup>60</sup> *TET1* encodes an alpha ketoglutarate ( $\alpha$ KG)-dependent dioxygenase that converts 5-methylcytosine into 5-hydroxymethylcytosine, thereby altering the epigenetic mark created by DNA methyltransferases (DNMTs).<sup>61</sup> *TET2* shares a high degree of homology with *TET1*, including its catalytic domain, and has been shown to have the same enzymatic capability.<sup>61a</sup>

*TET2* mutations are not tightly associated with other recurrent mutations or cytogenetic abnormalities. The presence of *TET2* mutations in MPN indicates that they do not cause dysplasia since these disorders retain normal differentiation. Nor are *TET2* mutations likely to cause the highly proliferative phenotype of AML and MPN, since they are frequently present in low-risk cases of MDS. Instead, *TET2* mutations may drive a pathogenic step common to all of the myeloid neoplasms in which they are found, such as the establishment or enhancement of clonal dominance in the disease cell of origin.

In some patients with *TET2* and *JAK2 V617F* mutant MPN, a clonal population of cells with mutant *TET2* and unmutated *JAK2* can be found, indicating that the *JAK2* mutation occurred in an already expanded *TET2* mutant clone. In other patients, *TET2* mutations were clearly acquired after *JAK2 V617F* and were associated with

progression of disease.<sup>62</sup> In MDS, the impact of *TET2* remains unclear. Early studies suggested that *TET2* mutations were associated with improved prognosis independent of the IPSS or were more likely to occur in low-risk patients, but more recent studies suggest no impact on overall survival.<sup>58,63,63a</sup> In contrast, *TET2* mutations in AML and CMML are associated with a relatively poor prognosis.<sup>57,64</sup>

### ASXL1 Mutations

Mutations in the additional sex-comb like-1' (*ASXL1*) gene have been described in roughly 10% of MDS and MPN, 17% of AML, and > 40% of patients with CMML.<sup>65-67</sup> *ASXL1* encodes a member of the polycomb family of chromatin-binding proteins and is involved in the epigenetic regulation of gene expression.<sup>68</sup> It contains the plant homeo domain finger and nuclear receptor box domains and functions as a ligand-dependent coactivator of the retinoic acid receptor. It has been shown to mediate its effects through a direct interaction with the histone acyltransferase encoded by *NCOA1* or the histone demethylase encoded by *LSD1*.<sup>69,70</sup> Unlike *TET2*, mutations in *ASXL1* appear to be mutually exclusive of some of the other recurrent genetic abnormalities. For example, *ASXL1* mutations were described in patients with essential thrombocythemia and primary myelofibrosis but only in those with wild-type *JAK2*.<sup>67</sup> In a small study of patients with AML,<sup>71</sup> mutations of *ASXL1* were almost entirely exclusive of the common mutations in the terminal exon of *NPM1*. In contrast, *ASXL1* mutations can co-occur with mutations of *RUNX1* and *TET2*.<sup>65</sup>

Targeted disruption of *Asxl1* in the germline of mice resulted in embryonic/perinatal lethality in a subset of animals. Mice deficient for *Asxl1* who survived to birth had only mild hematopoietic defects with reduced numbers of lymphocytes and modest splenomegaly. However, *Asxl1*-deficient mice had no evidence of myeloid dysplasia, had no detectable progenitor or stem-cell abnormalities, and did not go on to develop leukemia or lymphoma.<sup>72</sup> Unlike in these mice in which *Asxl1* was deleted, most of the *ASXL1* mutations seen in patients occur in the C-terminal portion of the protein. The result is deletion of the plant homeo domain protein interaction domain while sparing its N-terminal motifs, potentially indicating that *ASXL1* mutations generate a dominant-negative protein that could inhibit its wild-type counterpart as well as other members of the polycomb multiprotein complex. The prognostic significance of *ASXL1* mutations in MDS has yet to be determined.

### RUNX1 Mutations

*RUNX1* is a member of the transcriptional core-binding factor gene family (also known as *CBFA2* or *AML1*) and is the second most commonly mutated gene in MDS. It was identified as the translocation partner to *ETO* (now called *RUNX1T1*) in cases of t(8;21) core-binding factor AML. As with other translocations, those involving *RUNX1* are more common in or exclusive to AML compared with MDS. *RUNX1* point mutations, however, are found in MDS, AML, CMML, and more rarely, in MPN. In MDS, they are present in 7% to 15% of de novo patients and at a higher frequency in therapy-related disease.<sup>28</sup> In both MDS and AML, *RUNX1* mutations are markers of poor prognosis.<sup>73-75</sup>

The *RUNX1* protein contains a proximal Runt homology domain, important for DNA binding, and a distal transactivation domain responsible for protein-protein interactions and recruitment of cofactors. Missense mutations of *RUNX1* are clustered in the Runt domain, whereas stop codon mutations and frame shifts are found

throughout the length of the gene and almost always disrupt the transactivation domain.<sup>75,76</sup> This distinction may be physiologically relevant since Runt domain mutants with impaired DNA binding can function as dominant negatives. These mutants would be capable of reducing Runx1 activity much more than simple loss-of-function mutations in one allele.<sup>77,78</sup>

Germline mutations of *RUNX1* cause a rare human disease called familial platelet disorder with propensity to leukemia.<sup>79</sup> Patients with this partially penetrant, genetically dominant disease have an MDS-like phenotype with thrombocytopenia and/or dysfunctional platelets.<sup>80</sup> Roughly a third of affected patients with familial platelet disorder will develop AML, often after acquiring a second *RUNX1* mutation.<sup>81</sup> The long latency to leukemia in these individuals (median age of onset is 33 years) and in mice carrying mutant *RUNX1* genes indicates that secondary mutations are required for progression to AML. In patients with MDS, *RUNX1* mutations are often accompanied by activation of the Ras pathway or mutations in these genes.<sup>82,83</sup>

The genetic manipulation of *Runx1* in mouse models has helped explain how acquired *RUNX1* mutations might promote the development of MDS. *Runx1* knockout mice are not viable and die as embryos with no evidence of definitive hematopoiesis.<sup>84-86</sup> If *Runx1* is excised in the adult hematopoietic compartment, mice develop extramedullary hematopoiesis, lymphoid defects, expansion of the myeloid progenitor pool, and inefficient platelet production, but do not progress to AML.<sup>87</sup> In mice transplanted with bone marrow cells that overexpress a Runt domain mutant form of *RUNX1*, the phenotype is different. The mice have increased numbers of bone marrow blasts and splenomegaly, and they often succumb to an AML-like disease. If instead, *RUNX1* with a more distal frame shift mutation sparing the Runt domain is used, the phenotype more closely resembles MDS with marked erythroid dysplasia, pancytopenia, and fewer cases of AML.<sup>88</sup> These findings support a gain of function for *RUNX1* mutations that affect the Runt domain.

### IDH1 and IDH2 Mutations

Two isocitrate dehydrogenase genes (*IDH1* and *IDH2*) were identified as mutated oncogenes in a high percentage of glioma and secondary glioblastoma samples.<sup>89</sup> Analysis of nearly 500 solid tumor samples from tissues outside the CNS revealed no mutations in *IDH1* and *IDH2*, suggesting that these were tissue-specific oncogenes. Therefore, it was unexpected when whole-genome sequencing of an AML sample led to the discovery of recurring mutations of *IDH1* in 16 of 188 patients with AML.<sup>90</sup> Mutations of *IDH1* and *IDH2* have been confirmed in AML, MPN at the time of leukemic transformation, and in rare cases of MDS.<sup>91-95</sup>

*IDH1* and *IDH2* are homodimeric, nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>) dependent enzymes that convert isocitrate to  $\alpha$ KG and reside in the cytoplasm and mitochondria, respectively. The mutations identified in these genes are specific missense mutations of conserved codons. No frame shift or early termination mutations are seen, and all mutations appear to be heterozygous to an intact wild-type allele. This suggests that *IDH* mutations cause either a dominant negative phenotype, in which the mutant protein inhibits the wild-type enzyme, or a gain of function, in which the mutant protein acts in a way that the wild-type form does not. Recent evidence suggests the latter. Metabolic profiling of cell lines expressing mutant *IDH1* were found to contain high levels of 2-hydroxyglutarate (2HG).<sup>96</sup> This abnormal metabolite can also be

detected in primary leukemic cells harboring *IDH1* or *IDH2* mutations.<sup>97</sup> It appears that mutant *IDH* enzymes have altered substrate specificity. Instead of generating a NADPH molecule by catalyzing the conversion of isocitrate to  $\alpha$ KG, mutant *IDH* consumes NADPH while converting  $\alpha$ KG into 2HG. Whether 2HG is directly oncogenic or whether NADPH and  $\alpha$ KG depletion are drivers of leukemogenesis is not yet known.

### Tyrosine Kinases, RAS Genes, and CBL Mutations

Members of the tyrosine kinase (TK) signaling pathways are mutated commonly in myeloid malignancies but infrequently in MDS. For example, mutations of *FLT3* and *KIT* occur frequently in AML, and *MPL* mutations and platelet-derived growth factor receptor (*PDGFR*) rearrangements are found in various MPN. In MDS, rare mutations of *CSF1R* have been described in addition to rare mutations in the TKs more commonly mutated in other myeloid neoplasms. Patients with MDS are more commonly characterized by activating mutations of the downstream *RAS* genes. *NRAS* mutations are present in 10% to 15% of patients with another 1% to 2% having *KRAS* mutations.<sup>98,99</sup> Considered in isolation, these mutations are associated with a poor prognosis and progression of MDS to AML.<sup>100,101</sup> Other members of this signaling pathway that are mutated in rare cases of MDS include *BRAF*, *PTPN11*, and *CBL*.<sup>99,102-104</sup>

The *CBL* gene product is a TK-associated ubiquitin ligase that negatively regulates signaling through these receptors by targeting them for degradation. It is mutated in roughly 15% of CMML and in patients with juvenile myelomonocytic leukemia but is found in fewer than 5% of MDS. *CBL* mutations result in increased receptor TK levels and phosphorylation of STAT5, which are believed to mediate the hypersensitivity of these mutant cells to a wide variety of growth factors and cytokines.<sup>104-106</sup>

Mutations of *CBL* are often biallelic, suggesting that *CBL* is a tumor suppressor gene and that loss of wild-type function is advantageous. However, frame shift and early truncation mutants are uncommon. Instead, *CBL* mutations cluster in exons 8 and 9 and usually leave the rest of the gene intact, suggesting a gain-of-function effect of these mutations. The common *CBL* mutations have been shown to encode a dominant negative protein that inhibits the ubiquitin ligase activity of the wild-type gene product as well as the product of its homolog, *CBLB*.<sup>104</sup> Mouse cells deficient for *Cbl* show modest cytokine hypersensitivity.<sup>107</sup> This is greatly enhanced by the introduction of a mutated *CBL* gene into these cells, perhaps through the inhibition of partially compensatory *CBLB* activity.

Across myeloid disorders, *CBL* mutations appear to be mutually exclusive of several other commonly mutated genes including *FLT3*, *KIT*, *NPM1*, *CEBPA*, *PTPN11*, and *NRAS*.<sup>108,109</sup> In AML, they are more frequently associated with the t(8;21) abnormality, suggesting a cooperative advantage to this pairing.<sup>110</sup> If *CBL* mutations are equivalent to *RAS* mutations in MDS, then they will likely be associated with a negative prognosis, although this has yet to be described.

Activating mutations of *JAK2* form part of the diagnostic criteria for polycythemia vera and are found in roughly half of patients with essential thrombocythemia and primary myelofibrosis. Only the most common mutation, V617F, has been described in MDS. It is present in just 5% of unselected patients and does not appear to have prognostic significance.<sup>111</sup> The MDS subtype defined by refractory anemia with ring sideroblasts and thrombocythemia (RARS-T) is the exception. *JAK2* V617F mutations are present in 50% of these patients, as are rare

mutations of *MPL*, another MPN-associated gene.<sup>112,113</sup> This prompts the possibility that RARS-T is actually a frustrated form of MPN and not a subtype of MDS.<sup>111,114</sup> However, cases of *JAK2* wild-type RARS have been described in which thrombocythemia occurs only at the time that the V617F mutation is acquired.<sup>115</sup> Given the high rate of *JAK2* mutation in RARS-T, there would appear to be a cooperative advantage between those abnormalities responsible for the ring sideroblast phenotype and the constitutive activation of *JAK2*. The nature of this interaction is not yet understood.

### TP53 Activation and Mutation

The *TP53* gene, located on chromosome 17p, is a prototypical tumor suppressor gene. Its gene product, *p53*, is activated by a variety of cellular stressors and can cause cell cycle arrest, induce the DNA repair response, and drive cells into apoptosis. In cases of 5q- MDS, activation of *p53* appears to be essential for the erythropoietic defect associated with haploinsufficiency for *RPS14*.<sup>116</sup>

*TP53* mutations have been found in nearly every tumor type and are often associated with genomic instability. In MDS, *TP53* is mutated in 5% to 15% of de novo patients and more frequently in patients who have had prior exposure to alkylating agents or radiation.<sup>33,117</sup> Loss of wild-type *p53*, either through point mutations or larger abnormalities of 17p, is associated with advanced disease, complex karyotype, and resistance to treatment.<sup>118,119</sup> Mutations of *TP53* are among the few single-gene mutations to have been associated with a poor prognosis in MDS after adjusting IPSS risk group.<sup>120,121</sup> The detection of *TP53* mutations in small subclones of MDS may prove to be important, because this treatment-resistant population could drive progression or relapse despite the presence of otherwise favorable prognostic markers.<sup>122</sup>

## EPIGENETICS IN THE PATHOGENESIS AND TREATMENT OF MDS

The term "epigenetic" refers to the heritable component of cellular phenotypes that are not mediated by changes to the genomic DNA sequence. Since most cells in an organism share an identical genetic code, it is the epigenetic state of each cell and its interaction with the environment that are the greatest determinants its behavior. The most relevant molecular mediators of the epigenetic state in MDS are gene-expression patterns maintained by methylation of cytosine residues in DNA and covalent modification of histones.

DNA methyl transferases convert cytosine bases into 5-methylcytosines, particularly when they form the first base in a CpG dinucleotide. These CpG islands are commonly found clustered in and around gene promoters, consistent with their role in the regulation of gene expression. Methylation of CpG islands can alter their interaction with DNA-binding proteins, such as transcription factors and histone-modifying enzymes. Typically, methylation of CpG islands in promoters leads to silencing of neighboring genes and represents a mechanism for loss of tumor suppressor gene expression. In MDS and AML, several genes have been described as targets of DNA hypermethylation. These include the cell cycle regulators *CDKN2A* (p14 and p16) and *CDKN2B* (p15), *CTNNA1*, E-cadherin (*CDH1*), and many others. Genome-wide increases in promoter hypermethylation predict survival, even after taking into consideration age, sex, and IPSS risk group, and are seen during progression to AML.<sup>123,124</sup>



These observations provide a rationale for the use of demethylating agents in MDS. The nucleoside analog azacitidine and its 2'-deoxy counterpart decitabine are inhibitors of DNMTs and have been approved for the treatment of MDS in the United States. Both medications have good response rates in MDS with 30% to 73% of patients experiencing a 50% or better decrease in transfusion dependence. Low-risk patients have response rates comparable to those of higher-risk patients, and no cytogenetic or molecular marker has been validated as a predictor of response in MDS. Surprisingly, the degree of promoter hypermethylation does not predict response to DNMT inhibitors. In practice, decitabine or azacitidine are offered mainly to higher-risk patients, particularly since azacitidine has been shown to prolong survival in this group.<sup>124a</sup>

Inhibition of histone-modifying enzymes represents another potential epigenetic target for MDS therapy. Histones are protein multimers associated with DNA that help form chromatin structure. They can be covalently modified in several ways that alter how they interact with DNA and with other chromatin-binding proteins. Taken together, these modifications represent the histone code, which influences the level of transcription of nearby genes. The acetylation of lysine residues on histones is associated with an open chromatin conformation and increased transcription. Histone deacetylases (HDACs) can remove these acetyl groups and silence nearby genes. This is a critical mechanism for the regulation of gene expression, but when gone awry, can lead to changes that promote tumor development. As with DNMT inhibitors, drugs that inhibit HDACs theoretically work by allowing for the re-expression of inappropriately silenced tumor suppressor genes. This explanation is certainly too simplistic since these drugs and their targets have multiple effects, and dramatic changes in gene expression are not observed after treatment with HDAC inhibitors. Given the success of DNMT-targeted therapy and the interaction between DNA methylation and histone modification, HDAC inhibitors have been considered promising for the treatment of myeloid malignancies.

### ROLE OF THE BONE MARROW MICROENVIRONMENT

Abnormalities of the bone marrow microenvironment are well documented in MDS and other myeloid malignancies. Levels of vascular endothelial growth factor and several inflammatory cytokines are elevated in the bone marrow of patients with MDS.<sup>125,126</sup> These changes are thought to be the result of the complex interplay between the abnormal hematopoietic cells and the adaptive immune response they instigate: activation of the innate immune system and autocrine or cell contact-mediated interactions with the stroma. Such alterations to the microenvironment can negatively impact normal hematopoiesis, providing a potential explanation for why cytopenias can occur even when MDS cells occupy only a fraction of the bone marrow.

Stromal changes need not always be a consequence of abnormal hematopoiesis, however. A recent study has demonstrated how a primary stromal cell abnormality can create dysplasia in normal hematopoietic cells and even drive the clonal evolution of these cells into a malignant state.<sup>127</sup> Selective deletion of the microRNA processing gene, *dicer*, in osteoprogenitor cells of mice causes a phenotype that closely resembles MDS. These mice have cytopenias, multilineage dysplasia, bone marrow hypercellularity, and increased apoptosis. Af-

ter a long latency, three mice developed an AML-like disease characterized by clonal chromosomal abnormalities. Additional support for primary stromal changes in MDS comes from studies that have identified acquired chromosomal abnormalities in nonhematopoietic bone marrow cells from patients with MDS.<sup>128</sup> These lesions were distinct from those seen in the MDS clones. Whether genetic abnormalities in mesenchymal cells provide a clonal advantage that allows them to outgrow normal stroma is not known.

### THE CLINICAL ROLE OF GENETIC TESTING IN MDS

Metaphase karyotyping is the sole genetic analysis currently performed in routine clinical practice for the diagnosis of MDS. Fluorescent in situ hybridization with markers that detect common cytogenetic abnormalities need only be performed if a bone marrow aspirate cannot be obtained or if metaphase karyotyping is unsuccessful. MDS-specific fluorescent in situ hybridization panels are available commercially and are often performed in addition to metaphase karyotyping. In practice, however, these tests rarely add clinically relevant information beyond that obtained with a standard karyotype.<sup>129</sup> More sensitive techniques are available in the research setting, including spectral karyotyping and single nucleotide polymorphism array genotyping. However, the clinical significance of small amplifications, deletions, or loss of heterozygosity that are missed by standard karyotyping has not been determined.

Assays for single-gene mutations are becoming increasingly available but are not yet clinically used in MDS. However, experience with these types of tests in other myeloid neoplasms suggests that they could become routine. In AML, for example, assays for *FLT3* and *NPM1* mutations have prognostic relevance and can inform the decision to proceed to transplant in first complete remission.<sup>130</sup> In chronic myeloid leukemia and acute promyelocytic leukemia, quantitative detection of the translocation gene product is used to measure response to treatment and to monitor for early recurrence, and *JAK2/MPL* mutations are an important component of the diagnostic evaluation of MPN. In the future, the evaluation of individual genes for mutations will likely be useful for diagnosis, prediction of prognosis, and selection of therapy in patients with MDS.

### DISCUSSION

The clinical heterogeneity of MDS is a reflection of the various pathogenic mechanisms responsible for their development. Single genetic lesions are unlikely to be the sole disease-causing abnormalities in these disorders. Instead, a combination of two or more mutations may be needed in cooperation with more global changes in epigenetic states and cellular environment. In a reductionist model of myeloid leukemogenesis, cooperation has been postulated between type I mutations that drive proliferation and survival and type II mutations that impair differentiation.<sup>131</sup> This model provides a framework in which one can interpret the observation that in AML, type I and type II mutations are often mutually exclusive of other mutations within their own type. Some genes, however, have more complex effects on self-renewal, clonal expansion, and differentiation that may be difficult to assign to this binary classification. Given the emerging complexity of genetic abnormalities in MDS, it may be



clearer to consider how each mutation contributes to the pathogenesis of these disorders and how they cooperate with other mutations and microenvironmental changes. Mutations of *TP53*, for example, are difficult to categorize as type I or type II abnormalities since they do not appear to be mutually exclusive with either type in AML and often occur in isolation in MDS.<sup>33</sup> Instead, we can describe *TP53* mutations as contributing to genomic instability and antiapoptotic features of advanced MDS. Similarly, *TET2* mutations have not been found to be mutually exclusive of any other abnormality and thus are difficult to categorize. They do not severely alter differentiation in MPN and do not appear to generate a proliferative phenotype in MDS. This suggests that *TET2* mutations drive other pathogenic mechanisms such as epigenetic dysregulation or a clonal advantage for the disease-initiating cell.

Detailed analyses of a large number of MDS patient samples are needed to define the range of potential mutations and the frequency with which they co-occur. The identification of mutually exclusive lesions suggests either that these are alternative means of activating a common pathogenic mechanism or that the combination of these mutations leads to a more advanced, non-MDS phenotype such as AML. Finding frequently co-occurring mutated genes or pathways implies the activation of synergistic pathologic mechanisms, each driving different steps in the transformation of a normal hematopoietic cell into a diseased clone.

In conclusion, a complete definition of the genetic abnormalities associated with MDS will do more than provide us with a molecular taxonomy of these disorders. Characteristic mutations will help diagnose MDS in patients with refractory cytopenias and ambiguous morphologic features. The ability to detect these mutations reliably in peripheral blood will be useful when clinicians have a patient they are reluctant to biopsy or as a tool to monitor response to therapy or disease progression. Associations with clinical features will identify mutations that are prognostic and potentially predictive of therapeutic responses. The application of unbiased whole-genome assays may uncover additional mutations that point to novel therapeutic targets, increasing the range of treatment options for patients with MDS. With a more complete catalog of pathogenic mutations and their clinical consequences, genetic tests may play a growing role in the diagnosis, prediction of prognosis, and selection of therapies for patients with MDS.

### Note Added in Proof

***EZH2* Mutations.** The recent discovery of mutations in the enhancer of zeste homolog 2 (*EZH2*) gene expands the list of MDS genes

that are involved in the regulation of epigenetic state. *EZH2* resides on the distal portion of chromosome 7q and encodes a histone methyltransferase that serves as the catalytic subunit of the polycomb repressive complex 2 (PRC2). Trimethylation of lysine 27 on histone 3 by PRC2 is an epigenetic mark that is associated with gene silencing. In solid tumors, increased *EZH2* expression has been linked to more aggressive disease and worse outcomes. However, in hematologic malignancies, *EZH2* is often mutated, which leads to a loss of catalytic activity.<sup>132-135</sup> Loss of PRC2 function has been shown to increase hematopoietic stem cell activity and expansion, which may explain how *EZH2* mutations promote the development of myeloid neoplasms.<sup>136</sup> *EZH2* mutations have been reported in 6% of MDS cases and over 10% of MDS/MPN overlap disorders. These abnormalities seem to confer a poor prognosis. Despite its chromosomal location, loss of *EZH2* does not seem to be the sole driver for the deletion of chromosome 7q. Several patients with UPD at 7q did not have an apparent *EZH2* mutation, which suggests that duplication of a distinct lesion in that region has yet to be found.<sup>132,133</sup>

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