The myelodysplastic syndromes (MDS) are associated with a diverse set of acquired somatic genetic abnormalities. Bone marrow karyotyping provides important diagnostic and prognostic information and should be attempted in all patients who are suspected of having MDS. Fluorescent in situ hybridization (FISH) studies on blood or marrow may also be valuable in selected cases, such as patients who may have 5q− syndrome or those who have undergone hematopoietic stem cell transplantation. The MDS-associated cytogenetic abnormalities that have been defined by karyotyping and FISH studies have already contributed substantially to our current understanding of the biology of malignant myeloid disorders, but the pathobiological meaning of common, recurrent chromosomal lesions such as del(5q), del(20q), and monosomy 7 is still unknown. The great diversity of the cytogenetic findings described in MDS highlights the molecular heterogeneity of this cluster of diseases. We review the common and pathophysiologically interesting genetic abnormalities associated with MDS, focusing on the clinical utility of conventional cytogenetic assays and selected FISH studies. In addition, we discuss a series of well-defined MDS-associated point mutations and outline the potential for further insights from newer techniques such as global gene expression profiling and array-based comparative genomic hybridization.


The term myelodysplastic syndromes (MDS) describes a diverse group of acquired hematopoietic stem cell malignancies unified by a common functional consequence—ineffective production of mature blood cells.1,2 In MDS, cytologic dysplasia of blood and marrow cells is associated with functional defects in neutrophils and platelets, which contribute to infection and bleeding complications, respectively, even in the absence of substantial deficits in blood cell production. The variable risk of progression to acute myeloid leukemia (AML) that is characteristic of MDS appears to be driven by somatic genomic instability and epigenetic genomic modification. Evidence for this includes allelic imbalance arising from structural or numerical chromosome aberrations, defects in DNA repair,3,4 and linkage to genotoxic exposures including alkylating agents, topoisomerase II inhibitors, environmental poisons, or radiation.1,2

This overview highlights the genetic and molecular abnormalities in adults with MDS, methods to detect these abnormalities, and their potential importance for diagnosis, prognosis, and therapy (Table 1). The inherited and acquired MDS in pediatric patients display genetic and clinical features distinct from those in adults5 and will not be addressed in this review.

Most adult patients with MDS present to their physicians with signs and symptoms related to anemia or pancytopenia, or their illness is discovered incidentally when a blood cell count is performed for another purpose. The diagnosis of MDS is established from the characteristic appearance of a bone marrow aspirate, but in some cases cytologic features may be subtle, creating challenges with respect to excluding nonneoplastic causes of cellular dysplasia. In such cases, genetic testing provides an alternative means of diagnosis confirmation and offers further insight into prognosis and management considerations.

The 1976 French-American-British (FAB) Co-operative Group classification scheme for MDS, revised and expanded in 1982, provided the first widely used tool for disease classification and prognostication.5 The FAB classification is based on the proportion of immature blast cells in the blood and marrow and on the presence or absence of ringed sideroblasts or peripherial blood monocytes. In 1999, a group of hematopathologists and clinicians operating under the auspices of the World Health Organization (WHO) created a new classification system for MDS based on the FAB framework but incorporated newer morphologic insights and, to a more limited extent, cytogenetic findings (Table 2).7,8

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### TABLE 1. Genetic Abnormalities Characteristic of MDS and Their Consequences*

<table>
<thead>
<tr>
<th>Genetic or molecular abnormality</th>
<th>Karyotypic findings</th>
<th>Functional consequence</th>
<th>Importance in MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del(20q), isolated del(5q), –Y, normal karyotype</td>
<td>Unknown</td>
<td>3q21 translocations are associated with overexpression of MDS1/EVI1 gene; 11q23 translocations rearrange MLL gene</td>
<td>IPSS good-risk markers; del(5q31.1) is associated with specific 5q− syndrome (female predominance, dysplastic micromegakaryocytes, thrombocytosis)</td>
</tr>
<tr>
<td>Complex karyotype, abnormalities of chromosome 7</td>
<td>Unknown</td>
<td>t(8;21)(q22;q22), Leukemogenic rearrangements of core-binding factors</td>
<td>IPSS poor-risk markers</td>
</tr>
<tr>
<td>Other karyotypes not listed above</td>
<td>Unknown</td>
<td>t(15;17)(q22;q22), and be consistent with MDS</td>
<td>IPSS intermediate-risk markers, likely to be subdivided in the future as IPSS is revised</td>
</tr>
<tr>
<td>Trisomy 8</td>
<td>Unknown</td>
<td>Rearranges MDS1/EVI1 gene at 3q26 in many cases; GATA2 at 3q21 is overexpressed in some cases</td>
<td>Increased risk of leukemic transformation (single-institution studies); male predominance; oral ulceration (rare)</td>
</tr>
<tr>
<td>Abnormalities of chromosome 1q, 3q21, and 11q23 Del(12p)</td>
<td>Unknown</td>
<td>Rearranges MDS1/EVI1 gene at 3q26 in many cases; GATA2 at 3q21 is overexpressed in some cases</td>
<td>Decreased survival (single-institution studies)</td>
</tr>
<tr>
<td>t(8;21)(q22;q22), inv(16)(p13q22) or t(16;16)(p13q22), and (t;15;17)(q22;q12)</td>
<td>Unknown</td>
<td>ABCB7 at 3q12-13 plays a critical role in iron homeostasis</td>
<td>Relatively benign prognosis (single-institution studies)</td>
</tr>
<tr>
<td>Isoentric X</td>
<td>Unknown</td>
<td>Rearranges MDS1/EVI1 gene at 3q26 in many cases; GATA2 at 3q21 is overexpressed in some cases</td>
<td>WHO classifies cases with these translocations as AML by definition, even if they would otherwise be consistent with MDS</td>
</tr>
<tr>
<td>t(3;3)(q21;q26) or inv(3)(q21q26.2) Deleted chromosome 3 such as 3q21, and 11q23 Isolated isochromosome (17q)</td>
<td>Unknown</td>
<td>PDGFR-β subunit constitutive activation; TEL at 12p13 is a common fusion partner, but many other partners have been described</td>
<td>Iron accumulation in elderly women</td>
</tr>
<tr>
<td>Isolated isochromosome (17q)</td>
<td>Unknown</td>
<td>Thrombocytosis with megakaryocyte dysplasia</td>
<td>Occasional myeloproliferative features; rapid transformation to leukemia and poor response to therapy</td>
</tr>
<tr>
<td>Rearrangements of 5q33 such as t(5;12)(q33:p13)</td>
<td>Unknown</td>
<td>Occasional myeloproliferative features; rapid transformation to leukemia and poor response to therapy</td>
<td>CMML with eosinophilia; frequent clinical responses to imatinib or other PDGF inhibitors; other molecularly defined myelodysplastic-myeloproliferative overlap syndromes exist, including rearrangements of PDGFR-α subunit</td>
</tr>
<tr>
<td>All metaphases abnormal Marker of clonality</td>
<td>Unknown</td>
<td>Marker of clonality</td>
<td>Poorer prognosis than patients with mixture of abnormal and normal metaphases</td>
</tr>
</tbody>
</table>

### Point mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Functional consequence</th>
<th>Importance in MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>Mutations result in loss of function of tumor suppressor p53, “the guardian of the genome”—an essential regulator of cell cycle, specifically transition from G0 to G1</td>
<td>Mutated in 5%-10% of patients with MDS, more commonly mutated in secondary MDS, may be associated with progression to AML</td>
</tr>
<tr>
<td>NRAS</td>
<td>Constitutively activates oncogene by preventing normal GTP recycling, resulting in uncontrolled growth signalling</td>
<td>Codon 12 mutation is most common in MDS and found in 10%-15% of cases; acquisition of mutation commonly associated with progression to AML</td>
</tr>
<tr>
<td>FLT3</td>
<td>Internal tandem duplications of juxtamembrane domain or point mutations result in constitutive activation and uncontrolled growth signalling</td>
<td>Found in up to 10% of MDS cases; acquisition of mutation commonly associated with progression to AML</td>
</tr>
<tr>
<td>RUNX1/AML1</td>
<td>Point mutations result in loss of normal trans-activating potential of the key RUNX1 core-binding factor subunit and consequent changes in gene expression</td>
<td>Most common point mutation described to date in MDS—in up to 25% of cases; associated with high risk of progression to AML, previous radiation exposure, and chromosome 7 abnormalities</td>
</tr>
<tr>
<td>c-FMS</td>
<td>Mutations constitutively activate the receptor for colony-stimulating factor 1, a cytokine that controls the production, differentiation, and function of monocyte-macrophages</td>
<td>Occasionally associated with CMML</td>
</tr>
<tr>
<td>PTPN11</td>
<td>Mutations alter function of the SHP2 that has diverse roles in cell signalling, including modulation of PDGFR phosphorylation and STAT activity</td>
<td>Rarely associated with CMML or other MDS, commonly associated with JMML</td>
</tr>
<tr>
<td>ATRX</td>
<td>Point mutations result in loss of function of ATRX multiprotein complex with consequent changes in gene expression, including down-regulation of α globin cluster, probably by epigenetic mechanisms</td>
<td>Acquired α-thalassemia; no apparent effect on proliferative potential of thalassemic clone</td>
</tr>
</tbody>
</table>

*Gene expression changes that have been documented via microarray analyses are not included because their relevance has not yet been established. AML = acute myeloid leukemia; ATRX = α-thalassemia mental retardation X-linked; c-FMS = McDonough feline sarcoma virus oncogene homolog; CMML = chronic myelomonocytic leukemia; FLT3 = fms-related tyrosine kinase 3; GTP = guanosine triphosphate; IPSS = International Prognostic Scoring System; JMML = juvenile myelomonocytic leukemia; MDS = myelodysplastic syndromes; NRAS = neuroblastoma viral rat sarcoma oncogene homolog; PDGFR = platelet-derived growth factor receptor; PTPN11 = protein tyrosine phosphatase, nonreceptor type 1; RUNX1 = runt-related transcription factor 1; SHP2 = SH2 domain-containing protein tyrosine phosphatase 2; STAT = signal transducer and activator of transcription; TP53 = tumor protein p53; WHO = World Health Organization.
### TABLE 2. World Health Organization Classification of the Myelodysplastic Syndromes*

<table>
<thead>
<tr>
<th>Disease subtype</th>
<th>Blood findings</th>
<th>Bone marrow findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory anemia</td>
<td>Anemia</td>
<td>Erythroid dysplasia only—ie, not myeloid or megakaryocytic dysplasia</td>
</tr>
<tr>
<td></td>
<td>No blasts or rare (&lt;1%) blasts</td>
<td>No blasts</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>&lt;1 × 10^9/L monocytes</td>
<td>&lt;15% ringed sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with ringed sideroblasts</td>
<td>Anemia</td>
<td>Erythroid dysplasia only</td>
</tr>
<tr>
<td></td>
<td>No blasts</td>
<td>≥15% ringed sideroblasts</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>&lt;1 × 10^9/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia</td>
<td>Cytopenias (bicytopenia or pancytopenia)</td>
<td>Dysplasia in ≥10% of cells in 2 or more myeloid cell lines</td>
</tr>
<tr>
<td></td>
<td>No or rare (&lt;1%) blasts</td>
<td>&lt;5% blasts in marrow</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>&lt;15% ringed sideroblasts</td>
</tr>
<tr>
<td></td>
<td>&lt;1 × 10^9/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia and ringed sideroblasts</td>
<td>Cytopenias (bicytopenia or pancytopenia)</td>
<td>Dysplasia in ≥10% of cells in 2 or more myeloid cell lines</td>
</tr>
<tr>
<td></td>
<td>No or rare (&lt;1%) blasts</td>
<td>≥15% ringed sideroblasts</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>&lt;1 × 10^9/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Refractory anemia with excess blasts 1</td>
<td>Cytopenias</td>
<td>Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td>&lt;5% blasts</td>
<td>5% to 9% blasts</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>No Auer rods</td>
</tr>
<tr>
<td></td>
<td>&lt;1 × 10^9/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Refractory anemia with excess blasts 2</td>
<td>Cytopenias</td>
<td>Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td>5%-19% blasts</td>
<td>10%-19% blasts</td>
</tr>
<tr>
<td></td>
<td>Auer rods may be present</td>
<td>Auer rods may be present</td>
</tr>
<tr>
<td></td>
<td>&lt;1 × 10^9/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Myelodysplastic syndrome, unclassified</td>
<td>Cytopenias</td>
<td>Unilineage dysplasia in granulocytes</td>
</tr>
<tr>
<td></td>
<td>No or rare blasts</td>
<td>or megakaryocytes but no erythroid dysplasia</td>
</tr>
<tr>
<td></td>
<td>No Auer rods</td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>&lt;1 × 10^9/L monocytes</td>
<td>No Auer rods</td>
</tr>
<tr>
<td>Myelodysplastic syndrome associated with isolated del(5q)</td>
<td>Anemia; other cytopenias</td>
<td>Normal to increased megakaryocytes</td>
</tr>
<tr>
<td></td>
<td>possible</td>
<td>with hypolobated nuclei</td>
</tr>
<tr>
<td></td>
<td>&lt;5% blasts</td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>Platelets normal or increased</td>
<td>No Auer rods</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isolated del(5q)</td>
</tr>
</tbody>
</table>

*Chronic myelomonocytic leukemia and juvenile myelomonocytic leukemia are classified as myelodysplastic-myeloproliferative overlap syndromes by the World Health Organization.

Modified from Blood.7

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### TREATMENT OVERVIEW

The current spectrum of treatments for MDS is limited; however, encouraging results with novel agents in development offer promise.9 For most patients, management is primarily supportive, relying on transfusions and antimicrobials as dictated by symptoms and infectious complications. Select patients will experience hematologic improvement with the use of recombinant hematopoietic growth factors, including erythropoietin and granulocyte colony-stimulating factor.10,11

With the advent of new therapeutic alternatives, the management strategy for many patients has shifted from sole reliance on supportive measures to more active intervention. The Food and Drug Administration recently approved the first agent with an indication for MDS, 5-azacitidine (Vidaza, Pharmion Corporation, Boulder, Colo), an injectable nucleoside analogue that covalently incorporates into DNA and depletes DNA methyltransferases to promote genome hypomethylation. This may contribute to reactivation of methylation-silenced tumor suppressor genes. In a national cooperative group open-label randomized trial of MDS, 5-azacitidine treatment delayed leukemic transformation and reduced the risk of AML progression or death.12 Most other noninvestigational agents offer limited benefit for the general population with MDS but are reasonable considerations for select individuals. For example, thalidomide, when administered at low doses for an extended schedule, may restore erythropoiesis in some patients; however, neurotoxicity and other adverse effects limit dose escalation and prolonged drug administration, particularly in elderly individuals.13 Antithymocyte...
globin and other immunosuppressants may be effective in patients with hematopoietic inhibitory immune effectors; such patients may be identified through predictive modelling. Old agents such as androgens and pyridoxine are usually ineffective. Aggressive therapy similar to that used for acute leukemia should be reserved for clinical trials; it is rarely curative.

Younger patients are often candidates for curative strategies such as allogeneic hematopoietic stem cell transplantation; however, mortality and morbidity remain high, even in experienced and specialized centers. Autologous transplantation may benefit selected patients without an allogeneic donor, but the potential benefit of this approach appears limited to younger patients. Unfortunately, the vast majority of patients with MDS are not candidates for stem cell transplantation with conventional ablative conditioning regimens because the median age at the time of diagnosis is in the seventh decade of life, and comorbidities are common. Reduced-intensity conditioning or nonmyeloablative approaches promise to expand the upper age limit of candidates for stem cell therapy. The optimal timing of transplantation is a subject of active investigation.

Iron overload remains a clinical challenge, especially in patients who require frequent red blood cell transfusions. Although not specific for MDS, the recurrent chromosomal abnormalities observed in MDS into 3 risk groups: poor risk (including abnormalities of chromosome 7 or complex karyotype [≥3 clonal abnormalities]), good risk (including a normal karyotype, isolated interstitial deletion of the long arm of chromosome 5 [del(5q)], chromosome 20q interstitial deletions [del(20q)], or clonal loss of the Y chromosome, seen in 7.7% of otherwise healthy elderly men), and intermediate risk (including all other abnormalities not defined as good or poor). The corresponding median survival by cytogenetic risk group was 0.8 years (poor risk), 2.4 years (intermediate risk), and 3.8 years (good risk) (Figure 2).

The IPSS clusters the varied chromosomal abnormalities observed in MDS into 3 risk groups: poor risk (including abnormalities of chromosome 7 or complex karyotype [≥3 clonal abnormalities]), good risk (including a normal karyotype, isolated interstitial deletion of the long arm of chromosome 5 [del(5q)], chromosome 20q interstitial deletions [del(20q)], or clonal loss of the Y chromosome, seen in 7.7% of otherwise healthy elderly men), and intermediate risk (including all other abnormalities not defined as good or poor). The corresponding median survival by cytogenetic risk group was 0.8 years (poor risk), 2.4 years (intermediate risk), and 3.8 years (good risk) (Figure 2). The IPSS algorithm has been validated independently.

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the proportion of patients with an abnormal karyotype exceeds 80%.2,28,31,36-38 The relative frequency of specific cytogenetic abnormalities in primary MDS ranges from as high as 30% for del(5q)—either as an isolated anomaly (10%) or as part of a more complex karyotype (20%)—to 20% for trisomy 8 and 15% for monosomy 7.39 Numerous other recurrent abnormalities have been observed, but each accounts for less than 10% of cases of primary MDS.

**Cytogenetic Abnormalities in Secondary MDS**

The distribution of chromosome abnormalities is skewed in treatment-related MDS, in which structural or numerical deletions of chromosome 7 (40%) and chromosome 5 (40%) predominate.36,39 Together, abnormalities of chromosome 5 and 7 account for approximately 75% of karyotypic abnormalities in secondary MDS,36 whereas all other karyotypic abnormalities occur with a frequency of 10% or less.39 In addition to the higher frequency of abnormal karyotypes, secondary MDS displays a disproportionate percentage of IPSS poor-risk abnormalities, and as with primary MDS these are associated with an unfavorable prognosis. This cytogenetic pattern is frequently linked to and believed to be induced by prior treatment with alkylating antineoplastics.36

The IPSS good-risk abnormalities occur with sufficient rarity in secondary MDS to raise suspicion that such cases instead represent de novo and treatment-unrelated MDS. Nevertheless, single-institution studies indicate that patients with such karyotypes fare better than their counterparts with secondary disease who have poor-risk karyotype abnormalities.36 When the spectrum of abnormalities associated with primary and secondary MDS is compared, secondary forms more commonly exhibit monosomy 5, monosomy 7, and der(17p) aberrations, whereas in primary MDS, isolated del(5q) and normal karyotype predominate.39 Treatment with topoisomerase II inhibitors has been linked to chromosome 3q inversions or translocations and to balanced translocations involving 11q23 deregulating the *MLL* gene.40,41

**Other Recurrent Cytogenetic Abnormalities in MDS**

Numerous chromosomal abnormalities occur with moderate frequency (ie, 1%-10%) in MDS; however, because of overall low prevalence, their prognostic relevance remains undefined. Limited case series have linked trisomy 8 with an increased risk of AML transformation, and abnormalities of chromosome 1q, 3q21, and 11q23 with an overall inferior survival.30,31,40,42 In contrast, del(12p) as a sole abnormality has been associated with an indolent disease course.31 The prognostic relevance of less common (ie, <1% of cases) yet recurrent chromosomal abnormalities remains uncertain.43-47 Although multiple independent and karyotypically discordant clones may be identified in se-
FIGURE 2. The International Prognostic Scoring System for myelodysplastic syndromes (MDS) predicts survival time (top) and time to leukemic transformation (bottom) based on the patient’s bone marrow karyotype. These Kaplan-Meier curves show that the highest risk of death and leukemia is in patients with MDS with chromosome 7 abnormalities (Chrom 7 abn) or a complex karyotype; the lowest risk is associated with loss of the Y chromosome, isolated interstitial deletion of the long arm of chromosome 5, a normal karyotype, or chromosome 20q interstitial deletions [del(20q)]. Data were derived from a review of 816 patients with primary MDS. AML = acute myeloid leukemia; Misc = miscellaneous. From Blood,29 with permission.

lected cases,46,49 their prognostic importance is believed to be influenced by the highest-risk karyotype. The Mitelman Database of Chromosome Aberrations in Cancer, part of the Cancer Genome Anatomy Project of the National Cancer Institute, includes a comprehensive catalogue of chromosome abnormalities in patients with MDS that can be accessed at: http://cgap.nci.nih.gov/Chromosomes/Mitelman (accessibility verified March 29, 2005).
The karyotypic diversity included in the Mitelman database illustrates that the diagnostic category of MDS contains disorders that phenotypically overlap yet have a molecularly distinct pathobiology. Although the IPSS has proved its clinical usefulness, the IPSS intermediate-risk category includes potentially hundreds of chromosomal abnormalities, suggesting that further refinement of the algorithm will be possible. As therapy for MDS improves, the prognosis associated with particular karyotypes may change. By analogy, before the introduction of retinoic acid therapy for acute promyelocytic leukemia (APL) in the 1980s, the classic t(15;17) APL-associated chromosomal translocation was regarded as an intermediate-risk leukemia karyotype, but with modern drug therapy APL is among the most curable AML subtypes.

Although balanced favorable translocations such as t(15;17) and t(8;21) are relatively common in AML, they are rare in MDS in which deletions and numerical chromosomal abnormalities predominate. Given the responsiveness to conventional leukemia chemotherapy, the WHO categorizes 4 specific rearrangements involving core-binding factor transcriptional repressors [ie, t(8;21)(q22;q22), inv(16)(p13q22) or t(16;16)(p13q22), and t(15;17)(q22;q12)] as AML, regardless of the bone marrow myeloblast percentage. For those exceptional cases that harbor such karyotypic abnormalities with a low myeloblast burden, it remains unclear whether AML therapy impacts survival as it does in more typical AML.

Clonal Cytogenetic Abnormalities in the Absence of Morphologic Evidence of Myelodysplasia
In selected cases in which marrow aspiration is performed for evaluation of cytopenias, routine staging, or follow-up of another malignancy, clonal karyotypic abnormalities may be identified in the absence of cytologic dysplasia that would support a diagnosis of MDS. The constitutional karyotype in nonhematopoietic tissue in such cases has been normal, confirming an acquired somatic cytogenetic abnormality. This constellation of findings may represent a forme fruste of MDS, evidenced by an increased risk of leukemic transformation and subsequent cytopenic mortality. These uncommon cases emphasize the importance of cytogenetic evaluation in the investigation of unexplained cytopenias, even in the absence of overt morphologic evidence of MDS.

Relevance of All Abnormal vs Abnormal-Normal Cytogenetic Pattern
When a chromosomal abnormality is detected by conventional cytogenetics, the abnormality may be present in all examined metaphases (all abnormal [AA]) or in only a fraction of the metaphases (sometimes termed abnormal-normal [AN]). The AA pattern correlates with higher-risk MDS subtypes identified by the FAB MDS pathologic classification, and patients with AA have a poorer overall survival than those with AN. The favorable modifying effect of increasing proportions of normal metaphases has precedent in other hematologic malignancies. The clinical usefulness of the reduction in the number of abnormal metaphases with therapy is perhaps best illustrated by chronic myeloid leukemia (CML). In CML, a “major cytogenetic response” represents an important therapeutic goal because patients who achieve a complete cytogenetic remission experience a superior progression-free survival compared with those patients with persistence of any proportion of metaphases bearing the Philadelphia chromosome. By analogy, an international working group that created standardized response criteria for clinical trials of MDS included reductions in the number of abnormal metaphases as one of these response criteria, although the clinical importance of this measurement had not yet been formally established in MDS. Evidence from recent clinical trials testing new potentially disease-modifying agents supports this notion. In a large phase 2 study of intravenous decitabine in higher-risk MDS, 31% of patients achieved a complete cytogenetic remission, with a corresponding reduction in the relative risk of death (0.38 compared with those in whom the abnormal clone persisted). These preliminary data suggest that a cytogenetic response may also be an important disease-modifying marker in MDS.

Bone Marrow vs Peripheral Blood Cytogenetic Studies in MDS
Most chromosomal studies in patients with MDS are performed on bone marrow cells. Peripheral blood karyotyping has a higher failure rate than marrow studies and rarely adds useful information beyond that available from the marrow study. Unlike CML, in which tracking the proportion of cells containing the Philadelphia chromosome or its molecular equivalent is critical for monitoring therapy and the results may prompt management changes in the absence of the need for an additional marrow aspiration, there is currently little compelling clinical indication for frequent assessment of chromosomal status in MDS. In special situations in which chromosomal information is desirable but a marrow examination is not required, such as measuring donor/host chimerism after stem cell transplantation, fluorescent in situ hybridization (FISH) assays (discussed subsequently) may be preferable.

Indications for Cytogenetic Testing in MDS
When should a karyotype be obtained for patients with MDS? Given the importance of the IPSS risk assessment, a
chromosomal study is an essential part of the initial evaluation (Table 3). Acquisition of new chromosome abnormalities (ie, cytogenetic evolution) portends disease progression and therefore justifies cytogenetic evaluation in the routine management of MDS. Some clinicians obtain a cytogenetic study every time a marrow aspiration is performed during the care of patients with MDS, whereas others obtain such studies only in relationship to planned therapeutic trials or when there are signs of a change in the patient’s condition. No evidence base exists to help guide this practice, and therefore such decisions are best left to the discretion of the individual treating clinician. After transplantation, karyotypic analysis may not only help in early determination of relapse (ie, before morphologic evidence of recurrent disease is present) but also is useful in assessment of donor-host chimerism in the case of sex mismatches between marrow donor and host.

### MOLECULAR CYTOGENETICS IN MDS

Delineating the pathogenetically relevant alterations in MDS at the gene level, even for recurrent cytogenetic abnormalities, has proved challenging. The so-called 5q– syndrome is perhaps the best example of the difficulties faced. First delineated in the early 1970s, 5q– syndrome is a form of refractory anemia characterized by female predominance, atypical megakaryocytes, erythroid hypoplasia with red blood cell transfusion dependence, and a low risk of leukemic transformation with prolonged survival. These clinical and pathological features are consistently associated with an isolated 5q31.1 interstitial deletion in the absence of other cytogenetic abnormalities, a low bone marrow blast percentage, and no history of genotoxic therapy.62

The WHO’s most recent classification schema for malignant hematopoietic diseases formally recognizes 5q– syndrome as a discrete entity within the general category of MDS.7 Although no other specific MDS-associated chromosome aberration has been recognized by the WHO as a distinct pathologic and clinical syndrome, other recurrent chromosome abnormalities have been linked with specific disease features. For example, isolated isochromosome 17q may be associated with myeloproliferative features and poor response to therapy,63 isodicentric X has been linked to iron overload in older women,64 trisomy 8 displays a male predominance,65-67 and inv(3)(q21q26.2) is associated with thrombocytosis with megakaryocytic dysplasia and poor therapeutic response.42,68

Boulton and colleagues at Oxford have carefully defined the commonly deleted region (CDR) in patients with 5q– syndrome using FISH and other molecular mapping techniques, and they have cloned several novel genes in the process.69-72 The defined CDR spans 1.5 megabase pairs at 5q31.1.71 This CDR is relatively gene rich, containing no less than 40 discrete genes, 33 of which are expressed in early hematopoietic cells.71 Several of these genes are members of the interleukin family or have other potential promoting roles in hematopoiesis. A number of genes within the CDR are not well characterized, and their function is as yet unknown. To date, systematic sequencing of these genes in patients with 5q– syndrome has not revealed pathogenetic mutations, and thus it is now believed that the pathophysiology of 5q– syndrome may relate to gene dosage rather than mutational gene deregulation in the CDR.
Other groups are investigating additional recurrent MDS-associated chromosome abnormalities in an attempt to identify critical gene targets, including del(20q), trisomy 8, del(7q), and del(9q). In selected cases, study of patients with parallel inherited disorders may provide insight. For example, patients with constitutional trisomy 8 mosaicism appear to have a high risk of developing myeloid malignancies including MDS and leukemia.\(^7^3\) Chromosome 7 may be particularly challenging because there are multiple CDRs, including 7q22 and several in the region 7q31–7q35.\(^2^4^–2^6\)

Perhaps the most important molecular cytogenetic discovery in recent years with immediate therapeutic implication is the reciprocal chromosome translocation involving chromosome 5q33, where the gene encoding the β subunit of the platelet-derived growth factor receptor (PDGFR-β) is located. Although a number of chromosomes and genes may partner with 5q33, the clinical phenotype is distinct and is now recognized by the WHO classification as chronic myelomonocytic leukemia (CMML) with eosinophilia (CMML-Eos). (The WHO classification removed all CMML subtypes from the MDS group, where they had been placed in the widely used 1982 FAB MDS classification, and placed them in a separate myelodysplastic/myeloproliferative overlap category. This was done because patients with CMML frequently exhibit prominent myeloproliferative features, unlike patients with other subtypes of MDS.\(^6^,7^7\)) The CMML-Eos phenotype arises from the generation of novel fusion transcripts with constitutive activation of the PDGFR-β receptor tyrosine kinase.\(^7^8^–8^2\) Transgenic mouse models have shown that these novel receptor tyrosine kinase fusion genes are singularly responsible for the generation of these myelodysplastic/myeloproliferative disorders and are selectively responsive to PDGFR kinase inhibitors such as imatinib (Gleevec, Novartis, Basel, Switzerland).\(^7^9^–8^3,8^4\) Several patients with CMML-Eos have been reported to achieve rapid hematologic control and sustained cytogenetic remission with imatinib monotherapy\(^8^5\) or treatment with SU11657, an experimental agent that also inhibits PDGF signalling.\(^8^6\)

**Fluorescent In Situ Hybridization**

Although conventional cytogenetic analysis remains the standard for purposes of diagnosis and prognosis in MDS, interest is increasing in the application of more sensitive techniques such as FISH. Reviews of FISH applications in hematologic malignancies have been published recently.\(^2^7^,8^7\) Briefly, FISH involves hybridization of patient genetic material with a fluorescently labelled DNA probe designed to anneal either to specific DNA sequences or to chromosomal features such as centromeres. The advantages of FISH include its applicability to either peripheral blood or bone marrow, the opportunity to analyze interphase cells instead of only dividing cells, and the capacity to analyze substantially more cells than is possible by conventional cytogenetic analysis. When chromosomal rearrangements are present in only a small subset of neoplastic cells, a common situation in MDS, FISH can offer increased sensitivity over conventional cytogenetics. For purposes of investigation of specific MDS-associated chromosomal deletions, FISH probes are extremely useful for narrowing the common CDRs,\(^7^1,7^4,7^6,8^8–8^9\) and they can also identify the precise types of cells involved in the neoplastic process.\(^9^0,9^2–9^8\)

Several groups have developed FISH “panels” that are designed to detect the chromosome abnormalities most commonly identified in patients with MDS, such as those involving chromosomes 5, 7, 8, 11, 13, and 20.\(^9^9,1^0^0\) Such FISH analyses in MDS occasionally reveal cryptic cytogenetic abnormalities that are not recognized on metaphase analysis.\(^1^0^1^–1^0^3\) However, conventional cytogenetics and FISH should be viewed as complementary because there are also abnormalities better detected by conventional cytogenetics than FISH.\(^1^0^4^,1^0^5^–1^0^5\)

Many of the abnormalities detected by FISH that are not always recognized by conventional cytogenetics are among those included in the IPSS (eg, monosomy 7\(^5^0^9\)) or those found by other groups to have prognostic importance in MDS (eg, trisomy 8\(^1^0^2,1^0^6\) and 12p rearrangements\(^1^0^1\)). Chromosome aberrations detected only by FISH have not yet been shown to have the same prognostic importance as those revealed by conventional cytogenetics, and thus at present there is no compelling clinical reason to perform FISH at the time of diagnosis of MDS if the karyotype is successful. However, because the critical molecular defect is likely to be the same regardless of how the chromosomal breakpoint is detected, specific high-yield FISH assays will probably be incorporated eventually into prognostic and treatment-response algorithms,\(^1^0^7\) as in CML.\(^2^6,1^0^8\) For example, in the European clinical trial of decitabine for MDS, 2 patients had chromosomal responses detected by FISH that correlated with clinical and cytogenetic response.\(^5^9\)

If standard karyotyping is unsuccessful because of a fibrotic marrow, failure of aspirated cells to grow in culture, or other technical problems, it is reasonable to resort to FISH using a panel designed to detect abnormalities with recognized prognostic relevance. Additionally, in view of the promising recent findings by List et al\(^1^0^3\) of a high rate of complete hematologic and cytogenetic response to lenalidomide therapy in patients with MDS with 5q31.1 deletions, it may be especially valuable to perform FISH directed at detection of loss of the CDR on chromosome 5, particularly when the marrow morphologic features suggest 5q– syndrome. Patients with cryptic 5q deletions might then be referred for appropriate clinical trials. Over time, as the presence of other cytogenetic abnormali-
ties is correlated with response to novel therapies, the role of specific FISH tests in MDS is likely to expand.

**Multicolor FISH and Spectral Karyotyping**

“Chromosome painting” methods such as multicolor FISH (M-FISH) or spectral karyotyping (SKY) offer the ability to analyze all human autosomes and sex chromosomes at the same time by labeling metaphase cells with band-selective color probes (Figure 3). These methods offer greater sensitivity than conventional karyotyping and are useful for characterizing cryptic chromosomal rearrangements and the origin of structurally ill-defined chromosomal material such as “marker” chromosomes, in which the normal counterpart is not readily identified because of an ambiguous banding pattern.

Despite the heightened power of discrimination that M-FISH and SKY technologies may offer, their clinical usefulness in MDS has yet to be proved. For example, in patients with MDS with a normal karyotype by conventional cytogenetics, unrecognized cryptic abnormalities are rarely identified by M-FISH or SKY. In contrast, additional rearrangements are often detected when chromosome painting methods are performed in patients with MDS with abnormal karyotypes; however, the clinical relevance of these abnormalities is as yet undefined.

**Comparative Genomic Hybridization**

Comparative genomic hybridization (CGH) is a new technique that applies fluorescent hybridization to detect differences in DNA copy number between a patient sample and a normal control, giving insight into loss or gain of chromosomal segments. The ratio of the signal strength from the patient DNA to the reference DNA is measured in each chromosomal region. Until recently, the resolution of CGH was limited and only allowed detection of chromosomal gains or losses of 20 megabase pairs or greater. Despite this, even first-generation CGH probes provided sufficient sensitivity to discover cryptic rearrangements in patients with myeloid diseases including MDS, and soon the resolution had improved to almost 1 megabase pair. Newer array-based CGH methods, including a recently described tiling array that maps the entire human genome with more than 30,000 clones, promise to improve sensitivity sufficient to resolve even minute DNA alterations.

**Point Mutations in MDS**

Conventional cytogenetic studies and FISH probes detect large chromosomal rearrangements. However, isolated base pair changes or so-called point mutations may also inactivate or result in constitutive gain of function of the affected gene, with important biological consequences. Discovery of these mutations is one of the major open frontiers in MDS molecular pathology, with initial investigations focusing on recognized tumor suppressor genes.

**TP53**

The tumor suppressor gene TP53, a critical cell-cycle checkpoint regulator, was the first gene evaluated in MDS because of its high frequency of inactivation in solid tumors. In myeloid malignancies such as MDS and AML, TP53 mutations are uncommon, detected in no more than 15% of patients with primary MDS, with a higher frequency in atomic bomb survivors and patients treated previously with chemotherapy or radiotherapy. Such mutations are generally demonstrable at the time of diagnosis and may have independent prognostic value.

Even in tumor types in which TP53 mutations are common, TP53 mutation testing is rarely performed in clinical practice, unless a germline mutation leading to a general cancer susceptibility syndrome (the Li-Fraumeni syndrome) is suspected. This is primarily because patients with TP53-mutant neoplasias have not yet been shown to benefit from differential clinical management. For both inherited and acquired mutations, direct genetic analysis of TP53 by sequencing is required because immunohistochemical results do not correlate well with mutation status. Newer high-throughput technology may change the cost-benefit analysis of TP53 mutation screening and make this assay more accessible to clinicians (although its usefulness remains to be determined). For instance, denaturing high-performance liquid chromatography, which separates wild-type and mutant DNA on the basis of the differential affinity of DNA heteroduplexes and homoduplexes on polystyrene beads coated with a gradient of organic solvents, has shown promise as a more rapid TP53 screening method in MDS and other tumors (D.P.S., unpublished data, 2004).

**NRAS and FLT3**

The RAS proto-oncogene superfamily encodes guanosine triphosphate hydrolases (GTPase) that are critical regulators of cellular growth-related signals. Three RAS proto-oncogenes (H, N, and K) encode four 21-kd G proteins, including 2 alternatively spliced K-Ras products that are posttranslationally modified before incorporation into the inner leaflet of the plasma membrane. RAS mutations occur at critical regulatory sites (eg, codons 12, 13, and 61) that inactivate the GTPase response normally stimulated by the binding of GTPase-activating proteins, thereby extending the half-life and signalling activity of the Ras-GTP bound mutant. In myeloid malignancies, NRAS mutations predominate, especially a common mutation in codon 12; nonetheless, the frequency of activating RAS mutations in
unselected cases of MDS is relatively low, ranging from 10% to 15% of MDS. In contrast, in CMML, RAS mutations are found in 40% to 60% of patients and are believed to contribute to growth factor hypersensitivity of myelomonocytic progenitors.

Internal tandem duplications and other constitutively activating mutations of the receptor tyrosine kinase FLT3 are common in AML but are relatively rare in MDS. Acquisition of NRAS and/or FLT3 mutations has been associated with progression of MDS to AML.

RUNX1/AML1
The most common point mutations detected in MDS to date are those involving the core-binding–factor subunit RUNX1/AML1. In leukemia, this transcription factor and oncogene is involved in common balanced translocations such as t(8;21), which leads to formation of aberrantly active chimeric fusion proteins and uncontrolled cell growth. Although early reports estimated that RUNX1/AML1 mutations were rare in MDS, more recent studies have described these mutations in as many as 25% of patients, and single-institution studies have associated RUNX1/AML1 mutation status with chromosome 7q abnormalities, more advanced forms of MDS, and a high risk of progression to overt leukemia.

ATRX
Specific gene mutations may be associated with unique MDS phenotypes. For instance, the rare complication of acquired α-thalassemia (hemoglobin H disease) arising in the context of MDS was recently linked to mutations in the ATRX gene at Xq13.1. Inherited mutations in ATRX cause a mild form of α-thalassemia and severe syndromic X-linked mental retardation, whereas acquired somatic ATRX mutations create a severe imbalance in globin synthesis, a large proportion of hemoglobin H-containing cells, and the unusual finding of microcytic, hypochromic red cell indices. The distinct behavior of mutations like ATRX when they are acquired in the context of MDS, rather than inherited in the germline, may offer important insights into MDS pathobiology.

Mutations Found Predominantly in CMML
In addition to having unique clinical features and unique chromosomal translocations [such as the t(5;12) PDGFRβ-TEL fusion], CMML has several point mutations that appear with great frequency but are uncommon in other MDS subtypes. Patients with CMML have a high frequency of activating RAS mutations and a disproportionately higher frequency of c-FMS point mutations, although the latter are rare overall in myeloid disorders. Likewise, mutations in the protein tyrosine phosphate gene PTPN11 (which cause a form of Noonan syndrome when inherited) are extremely uncommon in MDS in general, more common in CMML, and especially common (ie, 34% frequency) in the pediatric syndrome juvenile myelomonocytic leukemia, which shares some features with CMML.
PROSPECTS FOR A MOLECULAR CLASSIFICATION OF MDS

Although the association of unique disease subtypes with specific point mutations promises molecular clarity for the perplexing heterogeneity of MDS, the prospect of a molecular classification to replace the current morphologic-and cytogenetics-based WHO scheme threatens to be unwieldy. For example, one of us (D.P.S.) recently detected acquired, clonally restricted point mutations in the GATA1 gene (chromosome Xp11.23) and RUNX1/AML1 gene (chromosome 21q22.3) as well as a cancer-associated germline polymorphism in TP53 (chromosome 17p13.1) in a single patient with MDS with acquired thalassemia but without a demonstrable ATRX mutation—a phenotype seemingly unrelated to the patient’s karyotype of trisomy 8 and del(20q) (Figure 4).

The frequencies of specific point mutations described previously are likely to be underestimates. Unlike AML, in which the marrow is almost completely replaced by neoplastic cells, blood and marrow in MDS include a heterogeneous mix of neoplastic and residual normal cells, and direct sequencing may be sensitive only to mutant DNA encompassing at least 20% to 30% of the tested sample. Techniques such as denaturing high-performance liquid chromatography, denaturing gradient gel electrophoresis, and polymerase chain reaction–single-strand conformational polymorphism are more sensitive to mosaicism than direct sequencing. In addition, for a more accurate estimate of mutation frequency, it is important not only to assay genes of interest at the genomic DNA (gDNA) level but also the gene message since messenger RNA splicing variants are common causes of human disease.

Mutations that affect splicing can be missed if sequencing of gDNA is limited only to the coding region (exons) because splicing abnormalities frequently arise from mutations residing in introns, both within the consensus splice donor and acceptor sites and outside of these areas (ie, in intronic splice enhancer or inhibitor sites). In the case of ATRX, patients with MDS with clonally restricted splicing abnormalities predicted to result in loss of critical ATRX protein domains have been described, but in all of these cases the causative mutation has not yet been identified at the gDNA level. A mutation in a cryptic splice enhancer or inhibitor element at some distance from the site of the splicing abnormality is likely responsible. The expensive and time-consuming search for splicing mutations and missense mutations as well as the need to use a technique sensitive to mosaicism illustrates why point mutation detection in MDS has not yet become mainstream.

MITOCHONDRIAL GENE MUTATIONS IN MDS

A recent report of mutations in the mitochondrial genome in patients with MDS, including frequent mutations in the cytochrome-c oxidase genes, generated some excitement because it offered an alternative genomic source potentially contributing to the MDS phenotype. Mitochondria are a logical target for MDS mutation analysis, given the presence of ringed sideroblasts—pathological iron-laden mitochondria—in many patients, which suggests a possible common metabolic disturbance in mitochondrial enzymes such as cytochrome. Mitochondrial enzymes are encoded by both the 16.6 kilobase pair mitochondrial genome and the nuclear genome. However, another group of investigators sequenced the entire mitochondrial genome in 10 patients with MDS and were unable to reproduce the findings.

Functional Importance of Point Mutations in MDS

In each of the genetic mutations described previously, functional consequences arise from modification of the protein encoded. For instance, RUNX1/AML1 point mutations alter DNA binding and transactivating potential of the mutant RUNX1/AML1. In light of the inherent genomic instability in MDS, “innocent bystander” mutations, ie, those with no known functional consequence, may occur with equal or greater frequency. Patients with MDS sometimes have clonally restricted gene mutations that do not result in amino acid substitutions and have no obvious effect on transcript splicing (Figure 4). If there is no detrimental effect on cell survival, darwinian pressure in the marrow will not select against hematopoietic clones bearing this class of mutation. ATRX mutations may be an example of a neutral mutation with a readily discernible phenotype (ie, defective α globin production in red cells).

A vast number of point mutations likely await discovery in MDS. The task of determining which of these are important in the pathobiology of the disease will be challenging.

GENE EXPRESSION PROFILING

A gene need not be altered structurally to modify its expression and affect normal cellular homeostasis. One of the principal ways in which gene expression can be altered is through epigenetic modification, including changes in the way DNA and its associated histones are packaged into chromatin, and, as a consequence, the accessibility of that DNA for transcription. The term epigenetic refers to heri-
table alterations in the pattern of gene expression that are not a consequence of changes in the nucleotide sequence of a gene.\textsuperscript{169} Such changes are of interest in cancer, in part because abnormal epigenetic silencing of genes has been shown to be associated with neoplasia development and progression.\textsuperscript{170} For instance, 5' methylation of cytosine nucleotides in the promoter region of tumor suppressor genes can lead to silencing of those genes.\textsuperscript{171} In MDS, the first gene found to be commonly hypermethylated and silenced was the \textit{P15INK4B} gene that encodes a cyclin-dependent kinase inhibitor.\textsuperscript{172-177} Methylation silencing of p15 is rare in patients with low leukemic cell burden; however, it is detected in more than 75\% of cases with excess blasts and occurs uniformly with AML transformation, suggesting a pathogenetic role in disease progression. Epigenetic gene silencing in human neoplasia has swiftly gained importance as a therapeutic target because of its potential reversibility with pharmacological agents.\textsuperscript{169,178} The clinically beneficial nucleoside analogue 5-azacitidine has dual therapeutic effects resulting from direct cytotoxicity as a nucleoside analogue and from inhibition of genomic methylation.\textsuperscript{12,179} Treatment with decitabine, the active metabolite of 5-azacitidine, has been associated with demethylation of hypermethylated \textit{P15INK4B} in MDS, al-

FIGURE 4. Analysis of exon 6 of GATA1 in archival blood and marrow samples from a group of patients with myelodysplastic syndromes. Top, Chromatogram from denaturing high-performance liquid chromatography Transgenomic WAVE mutation detection system. Most patient samples exhibit only a single peak from homoduplexed wild-type DNA (right peak), but DNA derived from a peripheral blood buffy coat from one patient exhibits a second peak (left peak, arrow), indicating the presence of a DNA heteroduplex formed from a mix of wild-type and mutant DNA. Bottom, Sequencing of the patient's GATA1 gene revealed an acquired c.1066C>T mutation (described in terms of Genbank Accession NM_002049.2) (arrow), which was not present in DNA derived from an Epstein-Barr virus–transformed lymphoblastoid cell line from the patient or from DNA derived from a wild-type (normal) individual. Surprisingly, this clonally restricted mutation did not obviously result in an amino acid change. RNA was unavailable to determine whether the mutation might affect splicing.
though not consistently. The addition of histone deacetylase inhibitors and other epigenetic modifiers to demethylating agents holds considerable promise for powerful chromatin remodelling strategies.187

Until recently, molecular investigations focused on expression of 1 or more genes of interest at a time. For example, in a few patients with MDS with intact TP53 genes (ie, no coding mutation), expression of the gene has been found to be decreased.188 This down-regulation may result in loss of p53 function with biological consequences equivalent to those resulting from mutational inactivation. The advent of complementary DNA microarray technology offers the ability to survey the expression pattern of thousands of genes concurrently.189 The results of MDS microarray studies have not yet yielded many pathophysiological insights, although some leads have been generated.183-186 For instance, the first ATRX mutation was identified after a microarray study of purified neutrophils showed dramatic down-regulation of the gene compared with normal controls (ultimately found to be the consequence of a point mutation in a consensus splice site).182

Another group has detected overexpression of DLK1 (Delta-like homolog), a gene of unknown function with homology to epidermal growth factor that was first identified as a regulator of adipocyte differentiation, in patients with MDS but not in patients with AML.187 If microarray studies finally fulfill their promise, it is hoped that they will lead to more accurate MDS diagnosis, classification, and prognostic discrimination and provide more insight into the disease pathobiology. Recent microarray-generated advancements in the understanding of diffuse large B-cell lymphoma187 and breast cancer188 illustrate the tremendous power of this technology.

CONCLUSION

Genetic testing in MDS is currently an essential component of clinical care of patients, and testing options are likely to evolve in the near future. At present, conventional cytogenetic studies remain a critical part of the diagnostic evaluation of MDS, and the results offer valuable prognostic information. Detection of specific MDS-associated point mutations and measurement of gene expression patterns are not yet part of routine clinical care, but they promise to expand clinicians’ ability to diagnose, prognosticate, and ultimately treat patients with MDS.

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