Myelodysplastic Syndromes

Olatoyosi Odenike, M.D.[a], **John Anastasi, M.D.**[b], and **Michelle M. Le Beau, Ph.D.**[c,d]

[a]Assistant Professor of Medicine, Section of Hematology/Oncology, and the Comprehensive Cancer Center, University of Chicago

[b]Associate Professor of Pathology, Hematopathology and Clinical Hematology Laboratory, and the Comprehensive Cancer Center, University of Chicago

[c,d]Professor of Medicine, Section of Hematology/Oncology, and the Comprehensive Cancer Center, University of Chicago

Synopsis

The myelodysplastic syndromes are a diverse group of clonal stem cell disorders characterized by ineffective hematopoiesis, peripheral cytopenias, and an increased propensity to evolve to acute myeloid leukemia. The molecular pathogenesis of these disorders is poorly understood, but recurring chromosomal abnormalities occur in ~50% of cases, and are the focus of much investigation. The availability of newer molecular techniques has allowed the identification of additional genetic aberrations, including mutations and epigenetic changes of prognostic and potential therapeutic importance. This review will focus on the key role of cytogenetic analysis in MDS in the context of the diagnosis, prognosis, and pathogenesis of these disorders.

Keywords

myelodysplastic syndromes; cytogenetics; molecular genetics; diagnosis; prognosis; classification

Introduction

The myelodysplastic syndromes (MDS) include a large spectrum of clonal hematopoietic stem cell disorders that are characterized by peripheral cytopenia(s), morphologic dysplasia, ineffective hematopoiesis, and a variable propensity to transform to acute myeloid leukemia (AML).[1,2] The cytopenia can be limited to a single cell line resulting in anemia,

[c]Corresponding Author: Michelle M. Le Beau, Ph.D., Section of Hematology/Oncology, 5841 S. Maryland, MC2115, Chicago, Illinois, USA, 60637, TEL: (773) 702-0795; FAX: (773) 702-9311, mlebeau@bsd.uchicago.edu.
[a,b]Co-Authors addresses: Olatoyosi Odenike, M.D., Section of Hematology/Oncology, 5841 S. Maryland, MC2115, Chicago, Illinois, USA, 60637, TEL: (773) 702-3354; FAX: (773) 702-3163, todenike@bsd.uchicago.edu, John Anastasi, M.D., Department of Pathology, 5841 S. Maryland, MC0008, Chicago, Illinois, USA, 60637, TEL: (773) 702-6196; FAX: (773) 702-1200, john.anastasi@uchospitals.edu

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thrombocytopenia, or neutropenia, and can be chronic and somewhat indolent, or profound involving all three lineages with life-threatening consequences. The morphologic dysplasia associated with the ineffective hematopoiesis may be subtle and difficult to recognize but, in some cases, it can be impressive and evident in both the bone marrow and peripheral blood. The variable increase in blasts relates, in part, to the risk for transformation to AML, although progression is not solely dependent on the blast percentage. MDS is a disease of older adults with a median age at diagnosis of ~70 years[3]. Other risk factors for the development of MDS include tobacco use and exposure to solvents, such as benzene and agricultural chemicals. In ~10–15% of cases, the disease arises as a late complication of cytotoxic therapy (radiotherapy and/or chemotherapy) for a prior disorder, and is referred to as a therapy-related myeloid neoplasm (t-MN). MDS is frequently associated with clonal cytogenetic abnormalities, of significant prognostic[4,5] and emerging therapeutic importance. An in-depth analysis of some of these is providing significant insights into underlying molecular alterations, which may hold clues to unraveling the pathogenesis of these disorders. This review will focus on the key role of cytogenetic analysis in MDS, in the context of the diagnosis, prognosis and molecular pathobiology of these disorders.

Diagnostic Considerations

MDS Classification

The earliest recognition of myelodysplastic disorders came with the identification of an anemia that was long-standing and refractory, followed by the recognition that these were sometimes preleukemic.[6] The classic myelodysplastic syndrome is exemplified in Figure 1, which illustrates pancytopenia with multilineage dysplasia. Although such a case is classic, it belies the wide pathologic spectrum of MDS, which includes cases that are diagnostically challenging and difficult to distinguish on one hand from other benign causes of cytopenia and, on the other hand, from AML and other more aggressive clonal myeloid neoplasms.

The disease spectrum was expanded with the first classification proposed by the French American British (FAB) group in 1982.[7] In this classification scheme the myelodysplastic disorders were divided into 4 subtypes with increasing blast percentage, or as chronic myelomonocytic leukemia (CMML). The 4 entities included refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), refractory anemia with excess of blasts (RAEB), and refractory anemia with excess of blasts in transformation (RAEB-T). These entities differed mainly by the percentage of blasts seen in the bone marrow (Table 1). In the FAB scheme, AML was defined by the presence of   30% blasts in the blood or marrow.

CMML was included by the FAB in the MDSs, although it was well recognized that CMML differed in that it had a proliferative component with increased circulating and marrow monocytes.[8] At times, the peripheral leukocytosis was particularly elevated in the so-called myeloproliferative type, whereas the process was considered to be a dysplastic type and included in the MDS category when the count was less than 13K/uL.[9]

The classification of MDS presented by the WHO committee in 2001 resulted in significant changes to the classification of both MDS and AML (Table 1).[10] Most notable was the reduction in the blast percentage required for a diagnosis of AML from 30% to 20% leading

to the elimination of RAEB-T. The new classification also included a new subtype of MDS that, despite the lack of increased blasts (less than 5%), had a more aggressive course, probably owing to the presence of more pronounced multi-lineage dysplasia.[11] This category was called refractory cytopenia with multi-lineage dysplasia (RCMD), and it comprised a substantial proportion of cases previously grouped in the low grade RA and RARS categories. Although the recognition of RCMD as a relatively more aggressive MDS served to de-emphasize the importance of the blast percentage for prognosis, the new classification sub-divided the RAEB category into 2 types, with 5–9% blasts (RAEB-1) and 10–19% blasts (RAEB-2), paradoxically emphasizing the prognostic significance of blast percentage in this category.[12]

A further significant change in the WHO 2001 classification scheme for MDS included the exclusion of CMML from the MDS category, and the development of a separate nosologic group for CMML and other diseases in which there were features of both myelodysplasia and myeloproliferation.[13] These "overlap" disorders are called myelodysplastic syndromes/ myeloproliferative neoplasms (MDS/MPN) and include CMML, "atypical CML", and a unclassifiable category, which includes a provisional entity called RARS-T, a disorder that resembles RARS, but has associated thrombocytosis.

Further refinements in the WHO classification scheme for MDS were made most recently in 2008 (Table 1).[14,15] These included expanding low grade MDSs from refractory anemia to refractory cytopenia with uni-lineage dysplasia (RCUD), in which there is involvement of only one cell line. The 2008 WHO classification scheme also placed emphasis on the key role of cytogenetic analysis in the diagnosis of MDS, particularly in cases where there is otherwise insufficient morphologic evidence to substantiate a diagnosis of MDS. This is reflected in the inclusion of the subtype, myelodysplastic syndrome unclassified (MDS-U), defined by the presence of cytopenia, less than 1% peripheral blasts, less than 10% dysplasia, less than 5% bone marrow blasts with the presence of a cytogenetic abnormality commonly seen in MDS (Table 2). In addition, the WHO 2008 classification now includes "MDS with an isolated del(5q)" as a separate entity. This entity, sometimes referred to as "the 5q- syndrome" [16], had been well known for some time, and is characterized typically by its presentation in middle-aged women with macrocytic anemia, splenomegaly, normal to elevated platelet counts, hypo-lobated megakaryocytes in the bone marrow, and an isolated del(5q).

Prognosis

The heterogeneity in outcome within the various morphologic categories identified by the FAB and WHO classification systems has led to a proliferation of prognostic tools and scoring systems that attempt to predict outcomes of patients with MDS more accurately. The ability to achieve greater precision in prognostication in MDS is of paramount importance, since therapeutic options vary from supportive care and growth factor use, to more intensive approaches, such as epigenetic modulators, and to approaches associated with significant potential for morbidity and mortality, such as intensive chemotherapeutic strategies and allogeneic stem cell transplantation. At present, the higher intensity approaches are reserved

for patients with high risk disease.[17] All of the contemporary prognostic systems incorporate the cytogenetic pattern as a key element.[18–21]

The most widely used, and validated, prognostic scoring system in current use is the International Prognostic Scoring System (IPSS), which incorporates karyotype, bone marrow blasts and number of cytopenias, and identifies these factors as being the most critical in prognostication (Table 3a) [18]. Nonetheless, there are a number of limitations that have led to the development of other scoring systems, as well as ongoing attempts to refine the IPSS.

Limitations of the IPSS include the fact that it was validated in previously untreated patients with *de novo* MDS, which limits the ability to use this tool to predict outcomes in patients with MDS treated with contemporary treatment approaches. In addition, although the numbers of cytopenias are factored into the IPSS, the severity of cytopenias is not taken into account; in particular, transfusion dependency, which has been identified as a poor prognostic marker in recent scoring systems, is not considered. Furthermore, the IPSS includes a limited number of cytogenetic abnormalities (Table 3b), and there are concerns that high risk cytogenetic aberrations are not accorded sufficient emphasis.

To address some of the concerns associated *with* the IP*SS*, newer systems such as the WHO classification based prognostic scoring system (WPSS) have emerged.[20] The WPSS is dynamic, and can be applied at diagnosis and during follow up, and is now being validated in patients with MDS undergoing contemporary treatment modalities, such as allogeneic stem cell transplantation.[22] Prognostic factors of import included in this model are WHO subtype, transfusion dependency, and karyotype subgroup, as defined by the IPSS. Five risk groups are recognized in the WPSS, with median survivals ranging from 12 months to 103 months.

The MD Anderson Cancer Center (MDACC) prognostic scoring system was also proposed recently to address some of the limitations associated with the IPSS.[19] This model is also dynamic, can be applied at any time during the disease course, and encompasses a broader patient population, including patients with t-MDS and CMML. Four risk categories are identified that predict overall survival ranging from a median of 54 months in low risk patients to 6 months in high risk patients.

Despite the development of newer systems, there continues to be significant heterogeneity in outcome, particularly in patients identified by current models as being in the lower risk category, emphasizing the need for continued refinement of these models based on a broader range of anomalies of emerging prognostic significance.[23] An updated cytogenetic system has been proposed which involves an international collaborative effort using data sets originating from the German-Austrian Working Group, the International Risk Analysis Workshop, the Spanish Cytogenetics Workshop and the International Cytogenetics Working Group of the MDS Foundation. This 4-tiered cytogenetic risk stratification system (Table 4) involves 20 cytogenetic subgroups; median survival ranged from 5.7 months to 50.6 months, and time until 25% of patients evolved to AML ranged from 3.4 months to 71.9 months.[23]

There is also ongoing concern that the IPSS accords more prognostic weight to an increase in bone marrow blasts as compared to poor risk karyotypes, e.g., 2 points are assigned for BM blasts in the 21% to 30% range, and 1.5 points for the 11% to 20% range, whereas poor risk karyotypes are assigned 1 point (Table 3a). In a recent retrospective study of 2,351 patients with MDS, the prognostic impact of a poor risk karyotype, was as unfavorable as the presence of a significant increase in blasts >20% blasts.[24]

In addition to the broad prognostic variables incorporated in the various systems outlined above, it is clear that gene mutations[25] and epigenetic aberrations play a critical role in the pathobiology of MDS [26,27] including the determination of prognosis. These genetic and epigenetic determinants, described in more detail in the following sections, deserve consideration for inclusion in a contemporary prognostic model developed for 21st century use.

Cytogenetic Analysis

Clonal chromosome abnormalities can be detected in marrow cells of 40%-100% of patients with primary MDS at diagnosis (Tables 1 and 2).[5,18] The proportion varies with the risk that a subtype will transform to AML, which is highest for RCMD and RAEB. Most recurring cytogenetic abnormalities found in MDS are unbalanced, most commonly the result of the loss of a whole chromosome, a deletion of part of a chromosome, or an unbalanced translocations (Fig. 2). The most common cytogenetic abnormalities encountered in MDS, del(5q), −7, +8, and del(20q), have been incorporated into the more robust prognostic scoring systems of MDS outlined above. The recurring translocations characteristic of acute leukemia without prior MDS, such as the t(15;17), inv(16) and t(8;21), are almost never seen. With the exception of MDS with isolated del(5q), the chromosome changes show no close association with the morphologic subtypes of MDS. Clones with unrelated abnormalities, one of which typically has +8, are seen at a greater frequency in patients with MDS (5% vs. 1%), than in patients with AML.

Serial cytogenetic evaluations can be informative, particularly when there is a change in the clinical features of a patient. Cytogenetic evolution is the appearance of an abnormal clone where only normal cells have been seen previously, the acquisition of additional abnormalities within an abnormal clone, or the progression from the presence of a single clone to multiple related, or occasionally unrelated, abnormal clones. Karyotypic evolution in MDS is associated with transformation to acute leukemia in about 60% of cases, and reduced survival, particularly for those patients who evolve within a short period of time (less than 100 days).[5,18]

Fluorescence In Situ Hybridization Analysis

In the past decade, molecular cytogenetic techniques, such as fluorescence in situ hybridization (FISH) have become a powerful adjunct to classical cytogenetic analysis.[28] FISH can be performed on marrow or blood smears, or fixed and sectioned tissue, since it does not require dividing cells. Advantages of FISH include (1) the rapid nature of the method and the ability to analyze large numbers of cells; (2) its high sensitivity and specificity; (3) the ability to obtain cytogenetic data from samples with a low mitotic index

or terminally-differentiated cells; and (4) the application to histologically-stained cells allowing a direct correlation of the status of the genetic target within morphologically characterized cells. The major disadvantage is the inability to interrogate more than a few abnormalities, and to identify the full spectrum of abnormalities in each clone, the presence of multiple clones, and clonal evolution.[28] In a clinical setting, cytogenetic analysis could be performed at the time of diagnosis to identify the chromosomal abnormalities in bone marrow cells from an individual with MDS. Thereafter, FISH with the appropriate probes could be used to detect residual disease or early relapse, and to assess the efficacy of therapeutic regimens. Commercially-available probes have been developed for the detection of 11q23/*MLL* translocations, −Y, del(5q)/t(5q), −7/del(7q), +8, del(20q), del(13q), del(11q), and −17/loss of 17p. In MDS, FISH analysis of cases with a normal karyotype by cytogenetic analysis detected recurring abnormalities in 10–15% of cases.[29]

Cytogenetic Findings in MDS

Normal Karyotype

A normal karyotype is found in ~50% of patients with MDS. This group of patients is almost certainly genetically heterogeneous, where technical factors precluded the detection of chromosomally abnormal cells, or where leukemogenic alterations occur at the molecular level and are not detectable with standard cytogenetic methods. Nonetheless, these cases are a standard reference for comparison of outcomes. The IPSS found that patients with a normal karyotype fall within the favorable risk group (Table 3b).[18]

−Y

The clinical and biological significance of the loss of the Y chromosome, −Y, is unknown. Loss of the Y chromosome has been observed in a number of malignant diseases, but is also associated with aging in healthy males.[30] Patients with a hematological disease have a significantly higher percentage of cells with a −Y (52% vs. 37%, p=0.036), and −Y in >75% of metaphase cells accurately predicted a malignant disease.[30] Although loss of a Y chromosome may not be diagnostic of MDS, once the disease is identified by clinical and pathologic means, the IPSS found that −Y as the sole cytogenetic abnormality conferred a favorable outcome.[18]

+8

A gain of chromosome 8 in MDS is observed in all MDS subgroups in ~10% of patients. [18,31,32] Determining the significance of the gain of chromosome 8 in MDS patients is complicated in that +8 is often associated with other recurring abnormalities known to have prognostic significance, e.g., del(5q)t(5q) or −7/del(7q), and may be seen in isolation as a separate clone unrelated to the primary clone in up to 5% of cases. The IPSS[18], as well as the time-dependent WPSS[20] ranked this abnormality in the intermediate risk group; however, several subgroups found that +8 as a sole abnormality had a poorer outcome than expected for an intermediate IPSS risk group.[31,33] Hematopoietic cells with trisomy 8 express higher levels of many genes that localize to chromosome 8, including the *MYC* gene, and antiapoptotic genes

del(20q)

A deletion of the long arm of chromosome 20, del(20q), is seen in approximately 5% of MDS cases and 7% of t-MDS cases.[5,31] Features characterizing MDS patients with a del(20q) include low risk disease (usually RA), low rate of progression to AML, and prolonged survival (median of 45 months vs. 28 months for other MDS patients), and prominent dysplasia in the erythroid and megakaryocytic lineages.[34] The IPSS noted that patients with a del(20q) in the context of a complex karyotype identified a poor-risk group (median survival, 9.6 months), whereas the prognosis for patients with an isolated del(20q) was favorable.[18] Although a commonly deleted segment (CDS) has been identified on 20q, containing 19 genes, there is no definitive evidence linking these genes to the pathogenesis of myeloid neoplasms.[35,36]

Deletions of chromosome 5

A deletion of the long arm of chromosome 5, del(5q), or an unbalanced translocation leading to loss of 5q are observed in 10–20% of MDS patients more commonly, in RAEB 1, 2 in association with a complex karyotype, and 40% of patients with t-MDS (Fig. 3).[37] Abnormalities of 5q are associated with a significant occupational exposure to potential carcinogens, as well as previous exposure to standard and high dose alkylating agent therapy, including use in immunosuppressive regimens.[38,39] Clinically, the patients with del(5q) coupled with other cytogenetic abnormalities have a high frequency of *TP53* mutations, a poor prognosis with early progression to leukemia, resistance to treatment, and short survival.[40,41] MDS with an isolated del(5q) represents a distinct clinical syndrome "the 5q minus syndrome". These patients have a favorable outcome, in fact the best of any MDS subgroup, with low rates of transformation to AML and a relatively long survival of several years duration.[16,18] The immunomodulatory agent, lenalidomide, has been associated with significant responses in IPSS low or intermediate-1 risk MDS with the del(5q), including red cell transfusion independency (67% of patients), and complete cytogenetic responses.[42]

A recent study showed that minor subclones of *TP53*-mutant cells were present in some patients prior to lenalidomide therapy, and were associated with lenalidomide-resistance and trend towards a higher risk of evolution to AML.[43] The molecular mechanisms underlying the clinical effectiveness of lenalidomide in del(5q) MDS remain obscure, but haploinsufficiency of two dual-specificity cell cycle phosphatases encoded by genes on 5q, *CDC25C* and *PP2A* have been implicated in the sensitivity to lenalidomide.[44] However, treatment with lenalidomide is unlikely to be curative since del(5q) malignant stem cells persist in remission, and clinical and cytogenetic progression was associated with recurrence or expansion of the del(5q) clone.[45]

Several groups of investigators have defined a CDS (Fig. 4) on the long arm of chromosome 5, band 5q31.2, predicted to contain a myeloid tumor suppressor gene that is involved in the pathogenesis of the more aggressive forms of MDS and AML.[46,47] A second, distal CDS of 1.5 Mb within 5q33.1 has been identified in MDS with an isolated del(5q).[48] Despite intense efforts, the identification of tumor suppressor genes (TSGs) on chromosomes 5 has been challenging, as a result of the fact that the deletions of 5q are typically large, and encompass both of these regions. Molecular analysis of the 19 candidate genes within the CDS of

5q31.2 and 44 genes in the 5q33.1 CDS did not reveal inactivating mutations in the remaining alleles, nor was there evidence of transcriptional silencing[48–50] [Godley and Le Beau, unpublished data]. Moreover, copy-neutral loss of heterozygosity (also known as acquired uniparental disomy) is not seen on 5q in MDS or AML. These observations are compatible with a haploinsufficiency model in which loss of one allele of the relevant gene(s) on 5q perturbs cell fate, rather than the biallelic inactivation of a tumor suppressor gene.[51] A number of genes and several miRNAs located on 5q, including *RPS14* [52], miRNA-145[53,54], *EGR1* [55], APC[56], *CTNNA1*[57], *HSPA9*[58], and *DIAPH1*[59], have been implicated in the development of myeloid disorders due to a gene dosage effect, and several of these are reviewed below. Together, these studies support a haploinsufficiency model, in which loss of a single allele of <u>more than one gene</u> on 5q act in concert to alter hematopoiesis, promote self-renewal of HSPCs, induce apoptosis of hematopoietic cells, and disrupt differentiation.

RPS14—The gene encoding RPS14, which is required for the processing of 18S pre-rRNA, is located at 5q33.1, and is involved in MDS with an isolated del(5q).[52] Downregulation of *RPS14* in CD34+ bone marrow cells blocks the differentiation and increases apoptosis of erythroid cells via a TP53-dependent mechanism.[60] Of interest, the ribosomal processing defect caused by haploinsufficiency of RPS14 in MDS is highly analogous to the functional ribosomal defect seen in Diamond-Blackfan anemia. Other studies have shown that haploinsufficiency of two micro-RNAs, miR-145 and miR-146a, encoded by sequences near the *RPS14* gene, cooperate with loss of RPS14.[53,54] The Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP) and *FLI1* are targets of these miRNAs. Haploinsufficiency of miR-145 may account for several features of MDS with an isolated del(5q), including megakaryocytic dysplasia; however, neither *RPS14* nor miR-145 haploinsufficiency is predicted to confer clonal dominance.

APC—APC is a multifunctional tumor suppressor involved in the pathogenesis of colorectal cancer via regulation of the WNT signaling cascade. The *APC* gene is located at 5q22.2, and is deleted in >95% of patients with a del(5q).[47] Conditional inactivation of a single allele of *Apc* in mice leads to the development of severe macrocytic anemia, a block in erythropoiesis at the early stages of differentiation, and an expansion of the short-term and long-term HSCs.[56] *Apc* heterozygous myeloid progenitor cells display an increased frequency of apoptosis, and decreased *in vitro* colony-forming capacity, recapitulating several characteristic features of myeloid neoplasms with a del(5q).

EGR1—The early growth response 1 gene (*EGR1*) encodes a member of the WT-1 family of zinc finger transcription factors, and mediates the cellular response to growth factors, mitogens, and stress stimuli.[61] Recently, Egr1 has been shown to be a direct transcriptional regulator of many known TSGs, e.g., *Tp53, Cdkn1a/p21, Tgfb*, and *Pten*, and acts as a TSG in several human tumors, including breast non-small cell lung cancer. [61] *Egr1-null* mi*ce* show spontaneous mobilization of HSPCs into the periphery, identifying Egr1 as a transcriptional regulator of stem cell migration.[55,62] Moreover, loss of a single allele of *Egr1* cooperates with mutations induced by an alkylating agent in the development of malignant

myeloid diseases (MPD with ineffective erythropoiesis) in mice, indicating that Egr1 is a haploinsufficient myeloid suppressor gene.[55]

Loss of Chromosome 7 or del(7q)

A −7/del(7q) is observed as the sole abnormality in ~5% of adult patients, in ~50% of children with primary MDS, and in ~55% of patients with t-MDS (Fig. 3). [33,37,39,63] As with del(5q), occupational or environmental exposure to mutagens including chemotherapy, radiotherapy, benzene exposure, and smoking, as well as severe aplastic anemia (regularly treated with immunosuppressive agents alone) have been associated with −7/del(7q). [18] The IPSS considers the −7/del(7q) to be a poor prognostic cytogenetic finding.[18] "Monosomy 7 Syndrome" has been described in young children, and is characterized by a preponderance of males (~4:1), hepatosplenomegaly, leukocytosis, thrombocytopenia, and a poor prognosis.[63] Juvenile myelomonocytic leukemia (JMML) is a MDS/MPD disease in the WHO classification, and shares many features with this entity.[63] An emerging paradigm is that −7/del(7q) cooperates with deregulated signaling via the RAS pathway as a result of mutations in the *NRAS* or *KRAS* gene, inactivating mutations in the gene encoding NF1, a negative regulator of RAS proteins, or activating mutations in the gene encoding the PTPN11/SHP2 phosphatase, a positive regulator of RAS proteins, as well as *RUNX1* mutations, and methylation silencing of the *CDKN2B* (*p15INK4B*) gene.[41,64,65]

To date, three CDSs have been identified on 7q; however, the molecular mutations underlying the development of MDS and AML with del(7q) are poorly-understood[66–68] We previously identified two distinct CDSs, a 2.52 Mb CDS within 7q22 spanning the interval containing *LRCC17* and *SRPK2*, and a second, less frequent, region in q32–33.[68] Each of the candidate genes within the CDS at 7q22 has been evaluated for mutations[69]; however, no inactivating mutations have been identified in the remaining allele. Mice with a conditional heterozygous deletion of this region in murine HSPCs had no alterations of hematopoiesis, suggesting that this region does not contain a haploinsufficient myeloid tumor suppressor gene, or that mutations in cooperating genes are required.[70] Recently, Dohner et al. reported the analysis of a large series of patients with abnormalities of 7q using FISH. Whereas most patients had large deletions, they identified an ~2 Mb deleted segment in proximal q22, that overlaps with the proximal portion of the CDS defined by Le Beau et al., but extends more proximally, and includes the *CUTL1, RASA4, EPO*, and *FBXL13* genes in 7q22.1.[71] The recent recognition of *m*utations in *EZH2*, a gene located at 7q36.1, is intriguing; however, myeloid neoplasms with *EZH2* mutations typically do not have −7/del(7q), and the del(7q) does not always result in loss of one *EZH2* allele.[72,73]

Rare Recurring Translocations

In MDS, several recurring translocations have been identified, which result in fusion proteins. Translocations involving the *MLL* gene on 11q23 are noted in 3–5% of MDS and t-MDS cases. The most common translocations are the t(9;11)(p22;q23) and t(11;16) (q23;p13.3). The t(11;16) occurs primarily in t-MDS, but rare cases have presented as t-AML.[74] *MLL* is fused with the *CREBBP* (CREB binding protein) gene on chromosome 16. The MLL protein is a histone methyltransferase that assembles in protein complexes that regulate gene transcription, e.g., *HOX* genes during embryonic development, via chromatin

remodeling. CREBBP is a histone acetyltransferase involved in transcriptional control via histone acetylation.

The t(5;12)(q33.1;p13) is observed in ~1% of patients with CMML, and results in fusion of the protein encoded by the beta chain of the platelet derived growth factor receptor (*PDGFRB*) on 5q, and a novel transcriptional repressor gene, *ETV6* (also known as *TEL*) on 12p. Alterations in the PDGFRB kinase activity and function of the ETV6 repressor contribute to the transformed phenotype. The t(5;12) predicts for a response to imatinib mesylate, a selective inhibitor of the PDGFRB kinase activity.[75] *PDGFRB* participates in several other rare translocations in myeloid neoplasms (reviewed in[76]); a unifying feature is the presence of eosinophilia.

Complex Karyotypes

Complex karyotypes are variably defined, but generally involve the presence of > 3 chromosomal abnormalities. Complex karyotypes are observed in ~20% of patients with primary MDS, and in as many as 90% of patients with t-MDS.[37] Abnormalities involving chromosomes 5, 7, or both are identified in most cases with complex karyotypes. There is general agreement that a complex karyotype carries a poor prognosis.[18,20,31]

Alterations in gene function

A growing body of evidence suggests that mutations of multiple genes mediate the pathogenesis and progression of MDS. The involved genes fall into two main classes, namely, genes encoding hematopoietic transcription factors or proteins that regulate cytokine signaling pathways. There is an increase in the frequency of molecular mutations from low-risk to high- risk MDS, or AML evolving from MDS, emphasizing the role of these mutations in disease progression. A detailed review of these genes is beyond the scope of this chapter, and has been provided elsewhere.[25,76] Table 5 provides a partial list and overview of some of the salient features of genes implicated in the pathogenesis of MDS.

Recently, Bejar et al. demonstrated that the integration of mutation analysis into diagnostic classification and prognostic scoring systems in MDS has the potential to stratify a diverse disease into discrete subsets with more consistent clinical phenotypes and prognosis.[25,77] For example, mutations in *RUNX1, TP53*, and *NRAS* were associated with severe thrombocytopenia and blast percentage. In multivariate analysis, mutations in 5 genes, occurring in one-third of patients, retained independent prognostic significance: *TP53, EZH2, ETV6, RUNX1*, and *ASXL1*, and predicted poor overall survival. Mutations of these genes stratified low- and intermediate-1, and intermediate 2 IPSS risk groups into two risk groups each, identifying patients within these subgroups with a poorer prognosis who may require a more intensive therapeutic approach. The genes most commonly mutated in MDS are *TET2, ASXL1, EZH2, RUNX1*, and *TP53*, which are described briefly below.

An emerging paradigm in MDS is the high frequency of mutations in genes involved in the regulation of transcription via chromatin modifications (*IDH1/2, TET2, EZH2, ASXL1*), and the intriguing observation that mutations often occur in more than one gene in the same patient, implying functional cooperation (note that *IDH1/2* and *TET2* mutations are mutually

exclusive). The most frequently mutated gene in MDS is *TET2* (20%); point mutations are observed in all cytogenetic subsets.[25,77] TET2 converts 5-methylcytosine to 5-hydroxymethylcytosine, thereby altering the epigenetic mark created by DNA methyltransferases.[78] Recent studies suggest no impact of *TET2* mutations on overall survival in MDS.[77,79]

ASXL1 mutations are observed in 10–15% of MDS.[25,80] ASXL1 is a member of the polycomb family of chromatin-binding proteins, and is involved in the epigenetic regulation of gene expression (typically repression). Mutated proteins are predicted to function as dominant negative proteins inhibiting the function of the wild type proteins as well as other members of the polycomb complex. The prognostic significance of ASXL1 mutations in MDS is not yet known.

EZH2 (enhancer of zeste homolog 2) mutations occur in 5% of MDS. [25,72,73] EZH2 encodes a histone methyltransferase that trimethylates histone 3 lysine 27, an epigenetic mark that confers gene silencing. In MDS, the mutations lead to loss of the catalytic activity, and are predicted to increase HSC expansion.[81] Although *EZH2* is located at 7q36.1, loss or mutation of *EZH2* does not appear to be the sole driver of myeloid neoplasms associated with −7/del(7q).

Point mutations in the Runt-related transcription factor 1 (RUNX1) have been reported in AML and MDS (12%), particularly in MDS secondary to treatment with cytotoxic therapy, and increase with the severity of the disease.[82] RUNX1, also known as CBFA2 or AML1, encodes the DNA-binding subunit of the heterodimeric core-binding factor (CBF) complex, which is essential for definitive hematopoiesis.[83] *RUNX1* mutations impair DNA binding and act as dominant negative proteins, and are associated with activating mutations of the RAS pathway, −7/del(7q), and a shorter overall survival.[82] Germline mutations of RUNX1 cause a rare human disease called familial platelet disorder; affected individuals have an MDS-like phenotype with thrombocytopenia, and/or dysfunctional platelets, and a predisposition to progress to AML.[84]

The *TP53* tumor suppressor gene encodes an essential checkpoint protein that monitors the integrity of the genome, and arrests cell cycle progression in response to DNA damage. Mutations of *TP53* (exons 4–8) or loss of an allele, typically as a result of a cytogenetic abnormality of 17p, are observed in MDS (5–10%) and t-MDS (25–30%), particularly in patients who have received alkylating agent therapy.[40] *TP53* mutations may occur as either an early or late event in the course of the disease, and are associated with rapid progression, and a poor outcome. In t-MDS, *TP53* mutations are associated with −5/del(5q) and a complex karyotype.

JAK2^{V617F} is a constitutively active cytoplasmic tyrosine kinase that is able to activate JAK-STAT signalling and mediate transformation to cytokine independent growth in MPN, and has been identified in rare cases of MDS (2–5%) and CMML (3%).[85] An exception is RARS-T, in which 60% of patients have the *JAK2^{V617F}* mutation.[86] RARS-T patients with *JAK2^{V617F}* mutations present with higher WBCs and platelet counts.

The role of epigenetic changes in the pathogenesis and treatment of MDS is becoming increasingly important. Transcriptional silencing via DNA methylation of the *CDKN2B (p15^{INK4B})* gene increases with progression from RA to RAEB-T, and is observed in a high percentage of patients with t-MDS, and is associated with −7/del(7q), and a poor prognosis.[64] Recent genome-wide studies have demonstrated that increases in promoter hypermethylation are predictive of survival in MDS, even when age, sex, and IPSS risk groups are considered. Moreover, increases in promoter methylation are seen during progression to AML.[27] These observations form the rationale for use of demethylating agents in MDS. Similarly, inhibition of histone-modifying enzymes represents another rational target for MDS therapy.

Emerging Technologies

Recent advances in microarray technology have enabled high-resolution genome-wide genotyping using single nucleotide polymorphisms (SNPs) for the identification of disease susceptibility loci, as well as the identification of acquired genetic imbalances, and loss of heterozygosity that occurs without concurrent changes in the gene copy number, which can be attributed to somatic mitotic recombination (referred to as copy-neutral LOH or acquired uniparental disomy). Gondek et al. used high-density 250K arrays to examine 174 patients (94, MDS; 33, AML following MDS; 47, MDS/MPD).[87] Acquired, copy-neutral LOH was identified in 20% of MDS, 23% of MDS->AML, and 35% of MDS/MPD (particularly CMML). Collectively, abnormalities were detected in a higher proportion of cases as compared to conventional cytogenetic analysis (78% vs. 59% for MDS). New lesions detected by microarray analysis included copy-neutral LOH of 6p21.2-pter, 11q13.5-qter, 4q23-qter, 7q11.23-qter, and 7q22.1. When the presence of newly-identified SNP array lesions were factored into the IPSS classification, the survival curves diverged for patients originally classified as IPSS Intermediate- 1, suggesting that SNP arrays provide additional information allowing for better prognostic resolution (median survival 28 vs. 9 mos., p=0.03). Thus, the results of these studies suggest that SNP array analysis may have future diagnostic application, and may complement conventional cytogenetic analysis in risk stratification and the selection of therapy.

SUMMARY

Cytogenetic analysis in MDS remains a critical genetic test for establishing the diagnosis and prognosis, and for therapeutic decision-making. The IPSS and the WHO classification systems incorporate only the most common chromosomal abnormalities. An international effort is underway to develop a comprehensive cytogenetic scoring system for MDS that incorporates rare cytogenetic subsets, which will inform an ongoing revision of the IPSS.[23] With the advent of more sensitive techniques already available in the research setting, including next generation genome and transcriptome sequencing and SNP arrays, the rate of discovery will accelerate, and the compendium of genetic alterations in MDS will undoubtedly expand. Defining the genetic complexity of MDS holds tremendous promise for elucidating the pathogenesis of these diseases, refining the prognostic scoring systems, and identifying novel therapeutic targets.[88]

Acknowledgments

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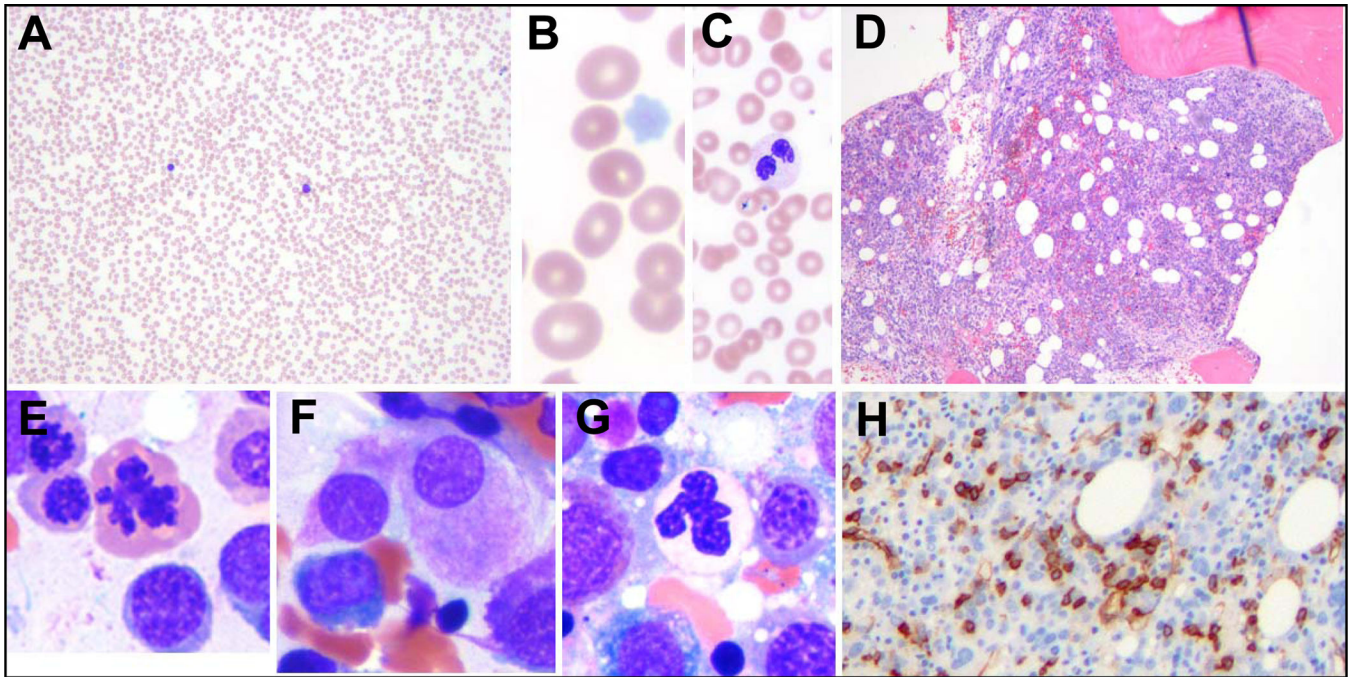
Fig 1.
Morphological features of a typical case of MDS. (A–C) The peripheral smear shows severe pancytopenia (A), with a macrocytic anemia (B), neutropenia with dysplastic neutrophils and thrombocytopenia with pale platelets (C). (D) The marrow is typically hypercellular, indicating ineffective hematopoiesis. (E–H) The marrow cytology reveals dysplasia in the erythroid (E), megakaryocytic (F), and granulocytic lineage (G), as well as increased blasts, which can sometimes be detected by CD34 immunostaining (H).

Primary MDS

t-MN
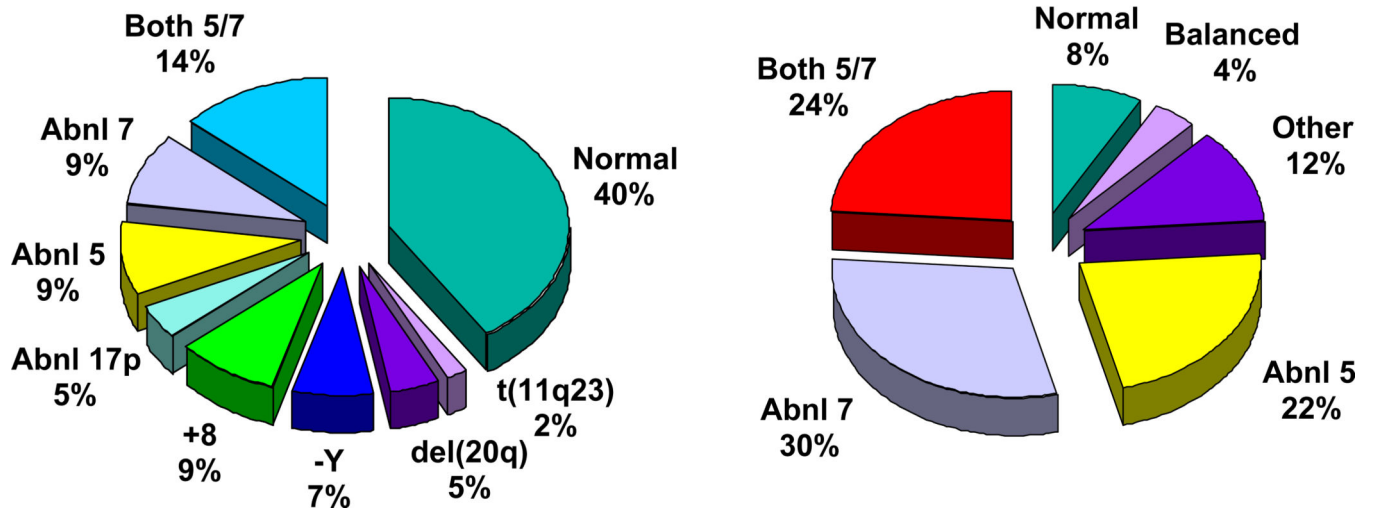
Fig. 2.
Recurring chromosomal abnormalities in MDS. Relative frequencies are depicted in the pie chart.
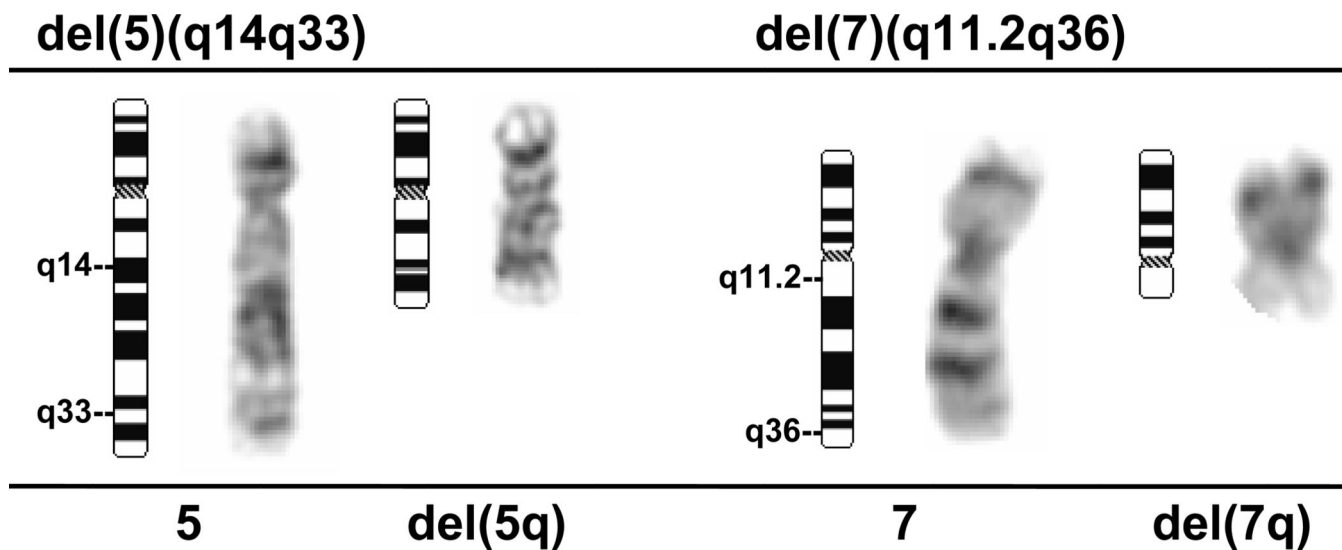
Fig. 3.
Deletions of 5q and 7q in myeloid neoplasms. In this del(5q), breakpoints occur in q14 and q33 resulting in interstitial loss of the intervening chromosomal material. In this del(7q), breakpoints occur in q11.2 and q36. In both cases, the critical commonly deleted segments are lost. Normal chromosome 5 and 7 homologs are shown for comparison.

Commonly Deleted Segments of 5q
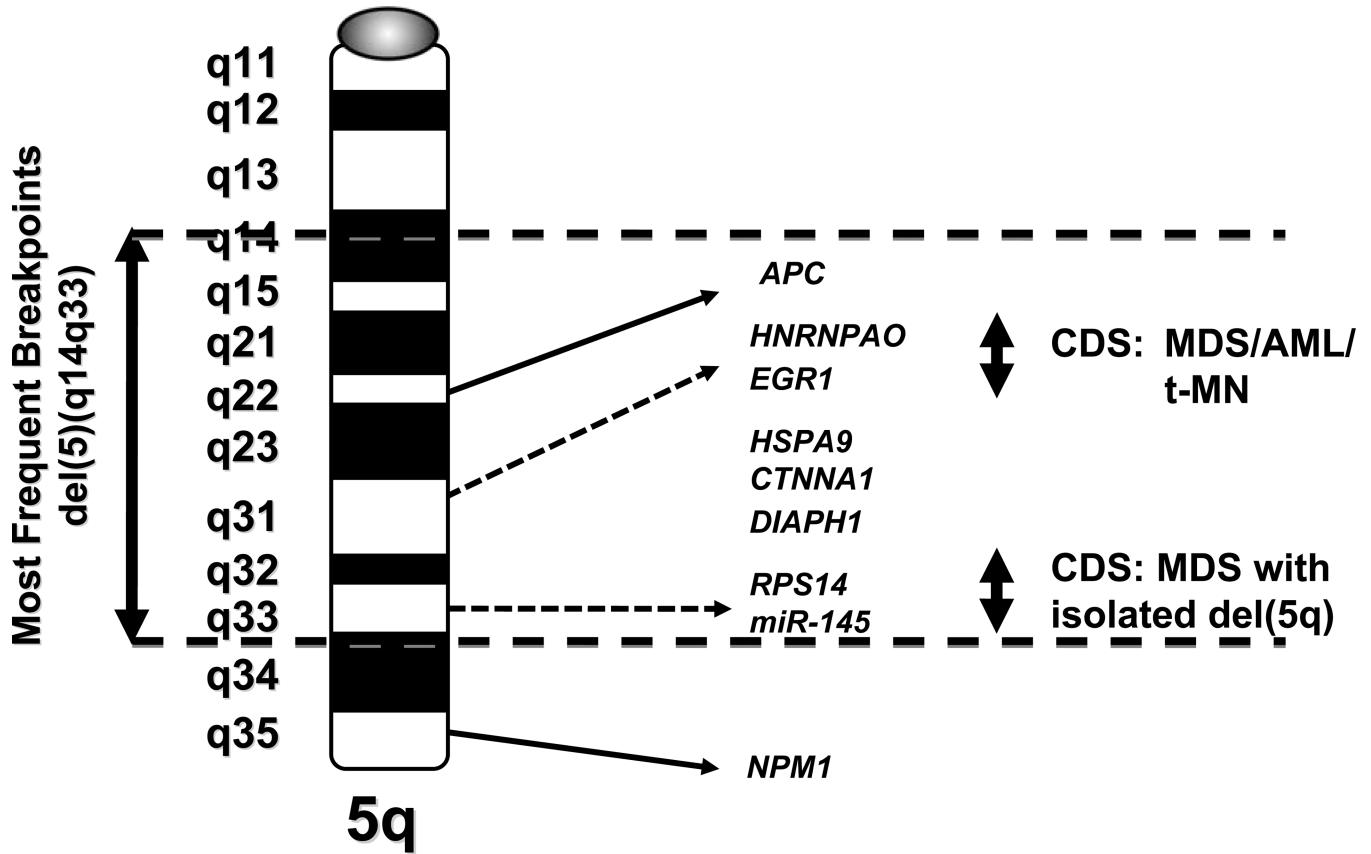
Fig. 4.
Ideogram of the long arm of chromosome 5 showing candidate genes within the commonly deleted segments (CDSs) as reported by various investigators. The proximal CDS in 5q31.2 was identified in MDS, AML and t-MN, whereas the distal CDS in 5q33.1 was identified in MDS with isolated del(5q).

Table 1

The Evolving Classification of MDS

FAB 1982	WHO 2001	WHO 2008	Blasts	Other Findings	Cytogenetic abnormalities
RA	RA	**RCUD** **RA** **RN** **RT**	<1% PB**, <5% BM	Uni or bi-lineage dysplasia *, <15% RS	25%
RARS	RARS	**RARS**	None in PB, <15% BM	At least 15% RS	10%
	RCMD RCMD-RS	**RCMD (–RS)**	<1% PB***, <5% BM	Dysplasia >10% in 2 cell lines, +/– RS, no AR****	50%
RAEB	RAEB-1	**RAEB-1**	<5% PB, 5–9% BM	Cytopenia, <1K/ul mono; dysplasia in 1 or more lines, no AR*****	50–70%
	RAEB-2	**RAEB-2**	5–19%, 10–19 BM	Cytopenia, <1K/uL mono, +/– AR; dysplasia in 1 or more lines	50–70%
		MDS-U	<1% PB; <5% BM	Cytopenia, <10% dysplasia; cytogenetic abnormalities present	50%
		MDS with 5q	<1% PB, <5% BM	Anemia, nml or incr. platelets, isolated del(5q), hypo-lobated megakaryocytes, no AR	100%
RAEB-T			20–29% BM		
CMML					

Stipulations:

*
If pancytopenia present, change to MDS-U;

**
If 1% blasts present, change to MDS-U;

***
if 2–4% PB blasts present, upgrade to RAEB-1;

****
if AR present, upgrade to RAEB-2.

Abbreviations: **FAB**, French American British classification; **WHO**, World Health Organization classification; **RA**, Refractory Anemia; **RARS**, Refractory Anemia with Ring Sideroblasts; **RAEB**, Refractory Anemia with Excess Blasts; **RAEB-T**, Refractory Anemia with Excess Blasts in Transformation; **CMML**, Chronic Myelomonocytic Leukemia; **AML**, Acute Myeloid Leukemia; **RCMD**, Refractory Cytopenia with Multi-lineage Dysplasia; **RCUD**, Refractory Cytopenia with Uni-lineage Dysplasia; **MDS-U**, Myelodysplastic syndrome-unclassified; **AR**, Auer Rods; **PB**, peripheral blood; **BM**, bone marrow; **RS**, ring sideroblasts (% indicates percent RS of total nucleated erythroid precursors).

Bold text indicates the current classification.

Table 2

Recurring Chromosomal Abnormalities in the Myelodysplastic Syndromes

Disease[*]	Chromosome abnormality¶	Frequency	Involved genes[+]		Consequence
MDS Unbalanced					
	+8	10%			
	−7/del(7q)¶	10%			
	del(5q)/t(5q)¶	10%			
	del(20q)	5–8%			
	−Y	5%			
	i(17q)/t(17p)¶	3–5%	TP53		Loss of function, DNA damage response
	−13/del(13q)¶	3%			
	del(11q)¶	3%			
	del(12p)/t(12p)¶	3%			
	del(9q)¶	1–2%			
	idic(X)(q13)¶	1–2%			
Balanced	t(1;3)(p36.3;q21.2)¶	1%	MMEL1	RPN1	Deregulation of MMEL1- Transcriptional activation?
	t(2;11)(p21;q23)/t(11q23)¶	1%		MLL	MLL fusion protein - altered transcriptional regulation
	inv(3)(q21q26.2)/t(3;3)(q21;q26.2)¶	1%	RPN1	MDS1/EVI1	Altered transcriptional regulation by EVI1
	t(6;9)(p23;q34)¶	1%	DEK	NUP214	Fusion protein - nuclear pore protein
Therapy related MDS	−7/del(7q)¶	50%			
	del(5q)/t(5q)¶	40–45%			
	dic(5;17)(q11.1–13;p11.1–13)¶	5%		TP53	Loss of function, DNA damage response
	der(1;7)(q10;p10)¶	3%			

Disease [*]	Chromosome abnormality[¶]	Frequency	Involved genes[+]			Consequence
	t(3;21)(q26.2;q22.1) [¶]	3%	RPL22L1	RUNX1		RUNX1 fusion protein - altered transcriptional regulation
	t(11;16)(q23;p13.3)/t(11q23) [¶]	2%	MLL	CREBBP		MLL fusion protein – altered transcriptional regulation
CMML	t(5;12)(q33.1;p13)	~2%	PDGFRB	ETV6/TEL		Fusion protein – altered signaling pathway

[*]
MDS, Myelodysplastic Syndrome; CMML, chronic myelomonocytic leukemia.

[¶]
Cytogenetic abnormalities considered in the WHO 2008 Classification as presumptive evidence of MDS in patients with persistent cytopenias(s), but with no dysplasia or increased blasts.

[+]
Genes are listed in order of citation in the karyotype, e.g., for the t(11;16), MLL is at 11q23 and CREBBP at 16p13.3.

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Table 3

a: International Prognostic Scoring system

Prognostic Variable	0	0.5	1.0	1.5	2.0
BM Blasts (%)	<5	5–10	–	11–20	21–30
Karyotype	Good	Intermediate	Poor		
Cytopenias*	0/1	2/3			

Risk group	IPSS score	Median Survival (yrs)	Time until 25% of patients developed AML (yrs)
Low	0	5.7	9.4
Intermediate-1	0.5–1.0	3.5	3.3
Intermediate-2	1.5–2.0	1.2	1.1
High	2.5	0.4	0.2

b. Cytogenetic Abnormalities in the International Prognosis Scoring System

	Cytogenetic Abnormalities	Time until 25% of pts developed AML (mos)	Median Survival
Favorable risk	normal karyotype	5.6 years	3.8 years
	isolated del(5q)		
	isolated del(20q)		
	isolated –Y		
Intermediate risk	other abnormalities	1.6 years	2.4 years
Poor risk	–7/del(7q)	0.9 years	0.8 years
	complex kayotypes		

*
Cytopenias were defined as: Hemoglobin < 10 g/dL, ANC < 1500/uL, Platelet count < 100,000/ L.

Table 4

Proposed 4-Tier Cytogenetic Risk Stratification System

Risk group	Karyotype	Median Survival (mos)	Time until 25% of patients developed AML (mos)
Good	Normal, del(5q), double abnormality including del(5q), der(1;7)(q10;p10)/t(1;7), del(11q), del(12p), +19, del(20q), −Y	50.6	71.9
Intermediate-1	any other double abnormality (not including del(5q) or −7/del(7q), +8, i(17)(q10), +21, any **7** other single abnormality	25.7	14.7
Intermediate-2	Double abnormality including −7/del(7q), t(3q26.2), complex with 3 abnormalities	16	9.8
High	Complex (>3 abnormalities)	5.7	3.4

Table 5

Frequency and Significance of Mutated Genes in MDS.

Mutated Gene	Frequency*	Biological Features and Clinical Significance
ASXL1	10–15%	Polycomb group protein involved in transcriptional regulation Prevalence of frameshift mutations suggest a dominant-negative function Clinical significance unknown
ATRX	Rare	Involved in epigenetic modifications of DNA Loss of expression leads to decreased expression of alpha-globin Associated with acquired alpha-thalassemia, often with severe anemia
CEBPA	2–5%	TF involved in hematopoiesis, loss of function impairs granulopoiesis Bi-allelic (N-terminal and C-terminal mutations) No apparent effect on time to progression to AML or overall survival
CSF1R	2–5%	Constitutive activation of the macrophage colony-stimulating factor receptor tyrosine kinase Karyotype predominantly normal Associated with advanced MDS and progression to AML
EZH2	5%	Encodes a histone 3 lysine 27 methyltransferase regulating transcription Mutations result in loss of function Associated with a poor prognosis
FLT3 ITD	5–10%	Rector tyrosine kinase involved in cytokine signaling, critical in hematopoiesis Associated with progression to AML and a poor prognosis
IDH1/2	Rare	Metabolic enzymes catalyzing oxidative decarboxylation of isocitrate to alpha-ketoglutarate. Missense mutations alter catalytic function converting alpha-KG to 2 hydroxyglutarate, while consuming NAPDH Associated with advanced MDS and progression to AML
JAK2	5% of RA 60% of RARS-T	Encodes a tyrosine kinase component of various cytokine signaling pathways Mutations result in constitutive activation of the tyrosine kinase Mutated in 60% of RARS with thrombocytosis, an unclassified MDS/MPD Clinical significance unknown, does not appear to alter prognosis
MPL	5% of RARS-T	Encodes the thrombopoietin receptor Mutations result in constitutive activation of the tyrosine kinase, and are associated with dysmegakaryocytopoiesis Higher expression in advanced MDS is associated with a poor prognosis
NPM1	Rare	Nuclear-cytoplasmic shuttling protein, with pleiotropic functions Terminal frameshift mutations disrupt the nuclear localization signal leading to redistribution to the cytoplasm Unknown clinical significance in MDS
NRAS/KRAS	5%	Encodes a GTPase component of multiple cytokine signaling pathways Activating mutations result in constitutive signal transduction Increased risk of progression to AML
PTPN11	Rare	Encodes the non-receptor SHP2 tyrosine phosphatase, a positive regulator of RAS proteins. Mutations result in protein activation Mutated in 30% of JMML Mutations in NRAS/KRAS, NF1, and PTPN11 are mutually exclusive
RUNX1	10–15%	Encodes the DNA-binding subunit of the heterodimeric core binding factor transcription factor required for hematopoiesis Point mutations in the RUNT (DNA-binding) domain result in loss of function, and a dominant negative effect Associated with mutations of the RAS pathway and −7/del(7q) Increased risk of progression to AML
TET2	20%	Epigenetic regulator Clinical significance unknown
TP53	5–10% (25% in t-MDS)	Encodes a checkpoint protein which monitors integrity of the genome, arrests the cell cycle in response to DNA damage Associated with chromosomal instability, del(5q), loss of 17p, and complex karyotypes Associated with rapid progression and poor outcome Significantly differentiates worse prognosis within each IPSS subgroup

*Rare mutations occur at a frequency of less than 2%.