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The need for additional genetic markers for MDS stratification: what does the future hold for prognostication?

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Abstract

Myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal hematopoietic disorders. Metaphase cytogenetics (MC) has been the gold standard for genetic testing in MDS, but it can detect clonal cytogenetic abnormalities in only 50% of cases. New karyotyping tests include fluorescence *in situ* hybridization (FISH), array-based comparative genomic hybridization (aCGH), and single nucleotide polymorphism arrays (SNP-A). These techniques have increased the detected genetic abnormalities in MDS, many of which confer prognostic significance to overall and leukemia-free survival. This has eventually increased our understanding of MDS genetics. With the help of new technologies, we anticipate that the existing prognostic scoring systems will incorporate mutational data into their parameters. This review discusses the progress in MDS diagnosis through the use of array-based technologies. We also discuss the recently investigated genetic mutation in MDS, and revisit the MDS classification and prognostic scoring systems.

Keywords

myelodysplastic syndromes; cytogenetics; new genetic markers; classification; prognosis; review

Introduction

Myelodysplastic syndromes (MDS) constitute a diverse and heterogeneous group of clonal hematopoietic disorders characterized by ineffective erythropoiesis, dysplastic changes, clonal chromosomal abnormalities, and increased likelihood of progression to acute myeloid leukemia (AML) [1-2]. MDS generally arise *de novo* (primary) especially in older patients or, less often, are a consequence of prior cytotoxic chemotherapy or radiotherapy for a distinct disease (secondary; treatment-related) [2]. The incidence rate of MDS in the United States is approximately 3.4 per 100,000 people, accounting for more than 10,000 new diagnoses yearly [3], and more recently has been reported to be 4.5 per 100,000 people [101]. This incidence rate is comparable to that reported in western European countries [4-7]. Typically, patients with MDS are diagnosed in their 70s. MDS is more frequent in

males than females [7-8]. Common risk factors for developing MDS include advanced age, male gender, family history of hematopoietic cancers, smoking, and exposure to solvents and agricultural chemicals [9-10]. Treatment-related MDS account for 10% of cases [11].

The clinical phenotypes of patients with MDS are diverse with respect to the number and severity of blood cytopenias, cellularity and blast count in the bone marrow, susceptibility to leukemic transformation, survival, and response to treatment. Much of this phenotypic heterogeneity is likely driven by the multitude of somatic mutations and cytogenetic aberrations that contribute to disease pathogenesis [12]. In an attempt to adjust for the diversity of MDS and its various classifications schemas, MDS patients can be stratified into lower-risk and higher-risk. Patients who have refractory cytopenias but <5% blasts and International Prognostic Scoring System (IPSS) scores of 1.0 (low and intermediate-1) are considered lower-risk, while those with excess blasts and IPSS scores of 1.5 (intermediate-2 and high) are the higher-risk group [11]. Lower-risk MDS cases are approximately twice as common as higher-risk MDS. Lower-risk MDS patients have a median survival of 3.5-5.7 years, while higher-risk patients have a dismal prognosis, with a median survival of 0.4-1.2 years. Thus, higher-risk MDS patients often are treated immediately.

Hematopoietic stem cell transplantation (HSCT) remains the only potential curative treatment for MDS. However, less than 5% of patients are considered for this option [13] even in the era of reduced intensity transplantation. For the remaining 95% of patients, treatment approaches are based on risk stratification, and thus its importance. Therapeutic approaches for lower-risk disease focus on improving transfusion needs and quality of life, commonly through the use of growth factors and erythropoiesis-stimulating agents (ESAs) [11,14-15], or by abrogating the effects of proapoptotic and proinflammatory cytokines by the use of a drug such as lenalidomide, particularly in patients with deletion of 5q [16-17]. Some patients with immunologically-mediated disease will respond to immunosuppressive therapy [18]. Non-responders can be considered for clinical trials [11]. For higher-risk MDS, the DNA methyltransferase inhibitors azacitidine and decitabine are standard. Azacitidine can improve survival compared to conventional care regimens [19], while decitabine improves response, including hematologic improvements and transfusion independence, compared to best supportive care [20-21]. Future MDS treatments rest in combination therapies [22].

This review focuses on the progress in MDS classification through the use of array-based technologies and next generation sequencing. We also revisit established MDS classification and prognostic scoring systems, and discuss recently identified genetic mutations in MDS.

Cytogenetic classification of MDS

Clonal cytogenetic abnormalities are detected in about 30-50% of *de novo* MDS cases, while more than 80% of therapy-related cases harbor these abnormalities [23]. The karyotype of bone marrow cells remains the most important prognostic marker for MDS and is a key component in all MDS scoring systems [24-26]. Metaphase cytogenetics (MC) has been the gold standard for genetic testing in MDS [27]. Twenty metaphases are considered typical for MC analyses [28]. A clonal abnormality as defined by the International System for Human

Cytogenetic Nomenclature (ISCN) 2009 [29] consists of two or more cells with the same chromosome gain or structural rearrangement, or three cells with the same chromosome loss. MC can detect balanced chromosomal changes, including translocations or inversions, and unbalanced chromosomal changes, including trisomies, duplications, and deletions.

Cytogenetic abnormalities are essential in determining the prognosis of patients with MDS [30]. The most widely accepted risk assessment system in MDS is the IPSS which, in addition to cytogenetic grouping, assesses marrow blasts and blood cytopenias [24]. The IPSS classifies MDS cases into three cytogenetic prognostic subgroups: low, intermediate, and high. Nevertheless, this system classifies only 86% of all cytogenetic findings, while the remaining 14% have unknown prognosis based on their cytogenetic abnormalities. In addition, the prognostic classification of many of the rare cytogenetic abnormalities, which are frequent in various patterns of complex karyotypes, remains a challenge [30-32]. As a result, the previously existing cytogenetic subtyping was refined to incorporate the broad variability of abnormalities, thus, improving the prognostic value of cytogenetics in MDS. Schanz and colleagues have proposed a new comprehensive cytogenetic scoring system which includes five prognostic subgroups: very good, good, intermediate, poor, and very poor [33]. Compared with the IPSS, the cytogenetic scoring system developed by Schanz *et al* was based on 2,902 patients; 2,801 (97%) of these patients were evaluable according to the ISCN [29], and 1,258 (45%) had clonal abnormalities. A total of 19 cytogenetic categories were defined and then classified according to the five prognostic subgroups (Table 1). In addition to rare lesions, the new system identified and weighed double and complex abnormalities [33]. This new cytogenetic scoring system provided the foundation for the Revised IPSS (IPSS-R) [26].

While the new cytogenetic scoring system is likely to become the new gold standard in MDS, it does have limitations. While the median number of metaphases analyzed in the project was 20, there was a wide individual variation (range, 2 to 94 metaphases), which might have introduced confounding in the analysis. In addition, there was significant karyotypic heterogeneity in the cases, which might have affected the major goal of this system, i.e. defining the prognostic impact of rare, single cytogenetic abnormalities [34]. The impact of this new system in the setting of recently discovered mutations in MDS, which are proving to be prognostically relevant, has yet to be determined [35-36].

Progress in diagnosis of MDS

Accurate diagnoses of MDS require bone marrow morphologic and cytogenetic analyses. While MC has been the standard genetic test in routine use for MDS diagnosis [24], this method is time consuming and technically demanding. In addition, MC depends on the analysis of cultured mononuclear cells, which may not grow or may provide noninformative karyotypes in MDS samples.

As a result, MC detects karyotypic abnormalities in approximately 50% of MDS cases [37] – a gross underestimate of the true genetic underpinnings of the disease, as many cases harbor submicroscopic amplifications or deletions, acquired uniparental disomy, or point mutations that might have prognostic implications. As a result, even when karyotypic abnormalities are detected by MC, patients with identical chromosomal abnormalities are

often clinically and phenotypically heterogeneous [30,38], implying that the presence of undetected clonal chromosomal abnormalities are causing this variability. These limitations of MC have led several investigators to try other existing and new karyotyping tests with higher resolution and independence on dividing cells, including fluorescence *in situ* hybridization (FISH), array-based comparative genomic hybridization (aCGH), and single nucleotide polymorphism arrays (SNP-A) [36-37,39]. **Table 2** summarizes the technical advantages and limitations of the different methods of karyotyping.

a- Fluorescence *in situ* hybridization - FISH—FISH, using a panel of different probes to identify specific genomic deletions or amplifications, is now in widespread use in MDS. It improves the likelihood of identifying specific gene rearrangements common in MDS [40-41]. FISH can be performed on mitotic as well as on interphase cells, which overcomes this limitation of MC; thus, it can be quickly performed with high sensitivity and specificity [41]. An important application of FISH has been to identify cryptic defects in MDS patients with a normal cytogenetic pattern. Using FISH, cryptic clonal defects have been identified in 15-18% of MDS patients, which consist of submicroscopic deletions [41-44]. Nevertheless, FISH also has its limitations; it is a targeted technique which allows identifying cytogenetic changes that are indicated by defined molecular probes, such as del(5q), del(20q), and chromosome 7 abnormalities, ignoring less common lesions. Due to the lower precision of FISH, the clinical significance of detecting unbalanced defects is not well defined [45].

b- Array-based comparative genomic hybridization - aCGH—Array-based comparative genomic hybridization (aCGH) has frequently been used to overcome the limitations of MC in studying hematologic malignancies. a-CGH relies on the difference in the copy number between differentially labelled test and reference DNA samples. a-CGH does not require a dividing cell population and can be performed on archived DNA isolated [46]. In addition, analysis of a-CGH is objective, amenable to automation and can be performed with some training or equipment. Consequently, this technology has enhanced the diagnostic yield in MDS, especially for cryptic chromosomal lesions [47-49]. In one study of 38 MDS patients, aCGH was capable of detecting new karyotypic abnormalities in 87% of patients [47]. In another study of 30 patients, submicroscopic copy number alterations (CNAs) identified by aCGH were concordant with the cytogenetic/FISH results in 25 (83%) of 30 samples tested. Moreover, aCGH revealed new CNAs in 14 (47%) of the 30 samples, including 28 submicroscopic or hidden aberrations verified by FISH [50].

A larger study using aCGH detected genomic imbalances in 42 MDS cases out of 107 (39%) samples with normal karyotype by MC [48]. Several recurrent cryptic deletions overlapping with known cytogenetic aberrations or sites of known tumor-associated loci were identified, including small 4q24 deletions (spanning *TET2*); deletions in 21q22 (spanning *RUNX1*); and interstitial 5q31.2 and 7q22.1 deletions [48]. An investigation of lower-risk MDS patients using aCGH identified cryptic CNAs in 36 out of 44 patients. Moreover, 20 out of 25 patients with a normal karyotype had at least one cryptic CNA identified. More importantly, these findings were prognostic for overall and leukemia-free survival [51]. The ability of aCGH to compare the hybridization signals of test and control DNA provides high precision and exclusion of DNA artifacts, which is an obvious advantage of aCGH over SNP-A. In

contrast to SNP-A, aCGH enables a targeted distribution of probes, including areas of known copy number variants [52], though it does not allow for the detection of acquired somatic uniparental disomy (AS-UPD).

c- Single nucleotide polymorphism arrays - SNP-A—High-resolution single nucleotide polymorphisms arrays (SNP-A) can be applied in karyotypic testing. SNP-A does not depend upon the availability of dividing cells, and consequently can be informative when routine MC is not. Rather, SNP-A rely on oligonucleotide probes corresponding to the allelic variants of selected SNPs. Moreover, due to the higher resolution of SNP-A, smaller and previously cryptic deletions and duplications can be detected. A major advantage of SNP-A over MC and aCGH is the ability to detect copy-neutral loss of heterozygosity (CN-LOH), which is caused by uniparental disomy [45,53]. Given this advantage, several groups have tested MDS genomes in an effort to find new disease-associated genes and to extract prognostic information from cryptic CNAs and areas of CN-LOH. In a study of 72 MDS genomes using a SNP 50K array, areas of LOH were reported in 33% of cases, with overall chromosomal defects found in 82% of MDS patients using SNP arrays versus 50% by MC. Moreover, lesions were present in 68% of patients with normal cytogenetics [54]. Again, when this group of investigators analyzed patients with myeloid malignancies, including 94 MDS patients, using a higher resolution platform (SNP 250K), CN-LOH was detected in 20% of MDS genomes, with overall chromosomal abnormalities found in 78% of MDS genomes versus 59% using MC [37].

International multicenter studies assessed the applicability and prognostic significance of combining MC and SNP-A in MDS karyotyping. In a large cohort of 430 patients with MDS (n=250), MDS/MPN (n=95), and sAML (n=85), the yield of chromosomal abnormalities was significantly increased with the combination of MC and SNP-A [36]. Multivariable analysis showed that the presence of new lesions detected by SNP-A, along with the presence of an increased number of new SNP-A lesions (>2 vs 1 or 2 vs none), were independent predictors of inferior overall survival and event-free survival in patients with MDS and related myeloid malignancies [36]. Consequently, the concurrent use of SNP-A and MC in the initial karyotypic testing was shown to affect outcome prediction and improve prognostic stratification of MDS patients.

Molecular Markers in MDS

Many genes have been investigated in MDS for their prognostic value. These include genes that encode signal transduction proteins, transcription factors and cofactors, components of the RNA splicing machinery, and epigenetic regulators. Here we discuss the most common genetic mutations that have changed our understanding of the pathobiology of MDS, and that confer prognostic significance with respect to overall and leukemia-free survival (**Table 3**).

Ten-eleven translocation 2 (TET2) gene—*TET2* mutations have been found in 20-26% of MDS cases [55-56]. Preliminary results reveal that these lesions are prognostically favorable in MDS. Kosmider *et al* studied the prognostic impact of *TET2* mutation in 96 MDS patients [55]. The 5-year overall survival was 76.9% in mutated versus

18.3% in unmutated patients ($P = 0.005$). The 3-year leukemia-free survival was 89.3% in mutated versus 63.7% in unmutated patients ($P = 0.035$). In multivariate analysis, the presence of *TET2* mutation was an independent favorable prognostic factor irrespective of the MDS subtype [55]. In addition, *TET2* mutations are associated with response to azacitidine in MDS. In a recent study, response to azacitidine was 82% in *TET2* mutant cases versus 45% in WT MDS patients ($P = 0.007$) [57]. Still some investigators have not found any prognostic significance for *TET2* mutations in MDS [58].

Runt-related transcription factor 1 (RUNX1) gene—*RUNX1* point mutations have been identified in MDS and MDS-related AML [59-60]. They are detected in up to 20% of MDS patients, with a higher likelihood of being detected in secondary MDS than in *de novo* disease [60-61]. MDS patients harboring this mutation present with advanced disease, and generally have a worse prognosis compared to those without the mutation [62-63].

ASXL1

ASXL1 was identified in a UPD in chromosome 20 [64]. The frequency of *ASXL1* mutations is approximately 10-15% in MDS [35,65]. *ASXL1* mutations correlate with poor overall survival, and multivariate analyses indicate that *ASXL1* mutations represent a poor prognostic factor for overall survival independent of other established risk factors [35,66]. *ASXL1* was also associated with poor prognosis in chronic myelomonocytic leukemia (CMML) patients, in addition to an increased risk of acute leukemia transformation [67].

EZH2

EZH2 gene mutations located on chromosome 7 were discovered in several myeloid malignancies including MDS [68-70]. Nikoloski *et al* screened 126 patients with MDS and found *EZH2* mutations in 8 patients (6%) [68]. Makishima *et al* identified *EZH2* mutations using SNP-A and next-generation sequencing technologies, detecting these abnormalities in 2 of 25 cases of CMML and 3 of 83 patients with MDS/MPN. Interestingly, MC did not identify a chromosome 7 abnormality in any of the *EZH2* mutant cases, but SNP-A karyotyping detected cryptic LOH 7q in 5 of 6 patients with *EZH2* mutations [69]. Overexpression of *EZH2* gene was generally associated with poor prognosis in MDS [35,70], especially for lower-risk cases [71].

DNMT3A

DNMT3A mutations were found in up to 8% of *de novo* MDS cases [72]. There is evidence that *DNMT3A* mutations occur early in the course of MDS. Patients with *DNMT3A* mutations have worse overall survival and an increased risk of progression to acute leukemia [72-73]. The discovery of mutations in *DNMT3A* expands the list of MDS genes that appear to be involved in the dysregulation of the epigenetic state, and which appear to have a poor prognostic value in *de novo* MDS.

Casitas B-cell lymphoma (CBL)—Human *c-CBL* gene is located on chromosome 11q23.3 [74]. Makishima *et al* found *CBL* mutations in patients with CMML (5%), sAML (9%), MDS (1%), juvenile myelomonocytic leukemia (19%), and chronic myelogenous leukemia (CML) in blast phase (1%). Patients with *CBL* mutations had inferior overall

survival [75]. Kao *et al* found that *CBL* was not prevalent in MDS but its expression increased dramatically during progression of MDS cases to acute leukemia. However, their study did not show a significant impact of *CBL* mutations on overall survival [76].

IDH1/IDH2

Isocitrate dehydrogenase-1/2 (*IDH1/2*) genes are mutated in approximately 5-10% of MDS patients [35,67,77]. In MDS and other myeloid neoplasms associated with sole del(5q) cytogenetic abnormality, mutant *IDH* has been associated with poor overall and leukemia-free survival [78-79]. Recently, Patnaik *et al* studied the phenotypic and prognostic effects of *IDH1* and *IDH2* mutations among 277 patients with MDS, in the context of the IPSS-R. Their results revealed an adverse prognostic effect of mutant *IDH1* on both overall and leukemia-free survival, while mutant *IDH2* did not affect these parameters [80].

Spliceosomal machinery mutations—The genetic alterations in the major RNA splicing components have been implicated in the pathogenesis of myelodysplasia [81-82]. These splicing components, including U2AF1, SRSF2 and SF3B1, were frequently mutated and highly specific to diseases showing features of myelodysplasia including MDS (43.9%), MDS with increased ring sideroblasts (84.9%), and CMML (54.5%) [81]. Makishima *et al* found that SF3B1 mutations were prevalent in low-risk MDS with ring sideroblasts, whereas U2AF1 and SRSF2 mutations were frequent in CMML and advanced forms of MDS like sAML and refractory anemia (RA) with excess blasts [83]. SF3B1 mutations were associated with a favorable prognosis, while U2AF1 and SRSF2 mutations were predictive of shorter overall survival [83]. Another group found SF3B1 mutations in 20% of patients with MDS, with higher frequency (65%) among patients with ring sideroblasts [84]. Patients with SF3B1 mutations had fewer cytopenias and better event-free survival. On the other hand, other investigators found that SF3B1 mutation had no additional prognostic value in MDS [85]. Recently, experimental research has provided preliminary evidence that SF3B1 haploinsufficiency leads to ring sideroblasts formation in MDS [82].

MDS classification and prognostic scoring systems

Staging and prognostic scoring systems are important to accurately diagnose, predict outcomes, and facilitate selection of treatment of patients [86]. A variety of systems have been developed, and are continuing to be refined to become more valuable in evaluating optimal treatments [87]. No single system is perfect or complete; however, as more is learned about the pathobiology of MDS at the cytogenetic and molecular levels, it is anticipated that these systems will be modified.

French-American-British (FAB) classification—The FAB classification is the oldest scheme for the classification of MDS. It divides MDS into 5 subtypes based bone marrow morphology, including percentage of blasts in the peripheral blood and bone marrow, presence or absence of ring sideroblasts, or increased circulating monocytes [88].

World Health Organization (WHO) classification and prognostic scoring system (WPSS)—The WHO classification, which was revised in 2008, is based on the FAB system but better delineates each subtype by adding the criteria of the number of

lineages with dysplasia and incorporating a cytogenetic abnormality [89]. This system has lowered the blast threshold for the diagnosis of AML from 30% to 20%; thus, the refractory anemia with excess blasts in transformation (RAEB-t) FAB category was eliminated. In addition to defining the lower-grade diseases, refractory cytopenia with unilineage dysplasia (RCUD) and refractory anemia with ring sideroblasts (RARS), and the addition of a new subtype, refractory cytopenia with multilineage dysplasia (RCMD), MDS with isolated del(5q) was added. RCUD incorporates patients who exhibit unilineage dysplasia associated with refractory anemia (RA), refractory neutropenia (RN), refractory thrombocytopenia (RT). RAEB-1 with 5-9% marrow blasts and RAEB-2 with 10%-19% marrow blasts were recognized in the WHO classification, as was MDS with isolated 5q- syndrome. In contrast to the FAB system, there is also a category for unclassified MDS (MDS-U) in the WHO system for cases that do not fit into other categories and for atypical presentations of MDS, such as those with extensive fibrosis [89].

Based on the WHO classification, cytogenetic abnormality pattern, and transfusion requirements, the WHO prognostic scoring system (WPSS) was developed, which classifies patients into five risk groups [90]. A refined version of the WPSS was also published based on the degree of anemia [91].

M.D. Anderson Prognostic Scoring System - MPSS—Kantarjian *et al* developed a new MDS risk model, M.D. Anderson Prognostic Scoring System - MPSS, based on a multivariable analysis of prognostic factors in 1,915 MDS patients [25]. They identified the following independent adverse factors: poor performance status, older age, thrombocytopenia, anemia, increased bone marrow blasts, leukocytosis, chromosome 7 or complex (>2) abnormalities, and prior transfusions. The new MDS prognostic system divided patients into four prognostic groups: low, intermediate 1, intermediate 2, and high. One advantage of this system over the IPSS is that it is dynamic – it can be applied to the same patient at different time points within that patient's disease course, and is valid in patients previously treated with therapies.

It is worth mentioning that this same group of investigators has published a prognostic score for patients with lower-risk MDS [92]. Patients with lower-risk MDS were divided into three risk groups based on age, presence of poor cytogenetics, hemoglobin, platelets and percent of blasts in the marrow [93].

IPSS Risk Classification/IPSS-R—The IPSS classification was developed in 1997 when an International MDS Risk Analysis Workshop combined clinical, cytogenetic, and morphological data from seven large previously reported risk-based studies that had generated prognostic systems in an attempt to improve on these systems [24]. The IPSS-R system assigns scores based on the initial cytogenetics, number of cytopenias, and percentage of blasts in bone marrow; these being the most powerful prognostic parameters in MDS. The four IPSS risk groups are low risk (score = 0), intermediate-1 risk (score = 0.5 to 1.0), intermediate-2 risk (score = 1.5 to 2.0), and high risk (score = 2.5). These four risk groups showed significantly different overall survival and risk of acute leukemia transformation. Median survival was 5.7 years for low risk patients, 1.2-3.5 years for intermediate risk, and 0.4 years for those with high risk [24].

The IPSS is of fundamental importance; it is highly reproducible and simple to use. One of its main advantages is that it was calculated using a group of patients who had not received therapy, which allowed for the evaluation of the natural history of the disease. However, this system has several limitations the most important of which is that it does not precisely identify patients with lower-risk disease (IPSS low or intermediate-1 risk). This group accounts for two-thirds of patients with MDS who may benefit from early therapeutic intervention, such as participation in clinical trials. This prompted Garcia-Manero and colleagues to develop the MPSS for lower-risk MDS [92]. Another limitation of the IPSS is that it attributes relatively little weight to cytogenetics. In addition, this system was calculated at initial presentation in patients with *de novo* MDS, which limits the use of this score for later during the evolution of the course of the disease.

A new revised international score (IPSS-R) has recently been refined with multiple statistically weighted clinical features used to generate a prognostic categorization model [26]. Initial cytogenetics, number of cytopenias, and percentage of blasts in bone marrow remained the basis of the revised system. Changes included: five rather than three cytogenetic prognostic subgroups with new classifications of a number of less common cytogenetic abnormalities; splitting the low marrow blast percentage in those with <2% blasts and those with 2%-4% blasts; and depth of cytopenias. This model defined five rather than the four prognostic categories of the IPSS.

Conclusion: What does the future hold for MDS prognostication?

MDS disorders carry stereotypic chromosomal abnormalities and genetic mutations, which remain the most powerful prognostic factors in this heterogeneous group of disorders. Our understanding of MDS genetics has grown enormously with the efforts of many researchers. The list of gene mutations that confer prognosis is growing with the advancement of new technologies. The use of array-based technologies (aCGH and SNP-A) and whole exome sequencing can identify amplifications and deletions in a group of MDS patients that probably contain pathogenic genes. This is particularly significant in MDS patients who have identical karyotypes by metaphase cytogenetics but are phenotypically and clinically different. The finding of new mutations in specific genes explains this heterogeneity of MDS, and it would improve the prediction of prognosis in these patients. The recently investigated genes encode signal transduction proteins (*CBL*), transcription factors and cofactors (*RUNX1*), components of the RNA splicing machinery (*SF3B1*, *SRSF2*, and *U2AF1*), and epigenetic regulators (*ASXL1*, *DNMT3A*, *EZH2*, *IDH1* and *IDH2*, and *TET2*).

Various classification and risk scoring systems that take cytogenetics into account have been developed and are used to stratify patients into distinct categories. These systems include the IPSS, WPSS, and MPSS. Recurrent cytogenetic abnormalities that are incorporated in these systems cannot entirely account for the pathogenesis of MDS, as they are detected only in 50% of patients. Single gene mutations are not currently used in MDS prognostic scoring systems. Each of these mutations most likely affects the biologic characteristics of MDS in specific ways. A more accurate prediction of prognosis might require the integration of these genetic defects, which are detected by high-resolution SNP-A, Sanger sequencing, and

whole exome sequencing, into the already existing prognostic classification and scoring systems.

Will these additional cytogenetic abnormalities and mutational lesions alter the existing prognostic scoring systems of MDS? We believe this is definitely going to happen. Tiu *et al* assessed the effect of additional SNP-A on outcome parameters within IPSS risk groups. Overall survival was worse within the lower-risk group after incorporating additional SNP-A [36]. The same was applied to the MD Anderson Lower-Risk Prognostic Scoring System; *EZH2* mutations retained prognostic significance which demonstrated the value of integrating additional genetic information in the calculation of this scoring system [71]. So as these chromosomal defects are assigned prognostic effect, they will eventually be incorporated into the prognostic schemes and scoring systems of MDS.

Expert commentary

Myelodysplastic syndromes (MDS) constitute a diverse and heterogeneous group of clonal hematopoietic disorders that is evolving in its diagnostic and prognostic schemes. Metaphase cytogenetics (MC) has been the gold standard for genetic testing in MDS, and it can detect balanced chromosomal changes, including translocations or inversions, and unbalanced chromosomal changes, including trisomies, duplications, and deletions. However, clonal cytogenetic abnormalities are detected in only 50% of *de novo* MDS cases by MC. In addition, MC has its limitations in being time consuming, technically demanding, and requiring dividing cells. Many times MC provides noninformative karyotypes in MDS samples.

These limitations have led several investigators to try other karyotyping tests including fluorescence *in situ* hybridization (FISH), array-based comparative genomic hybridization (aCGH), and single nucleotide polymorphism arrays (SNP-A). These karyotyping techniques have increased the detected genetic abnormalities in MDS, many of which confer prognostic significance in overall and leukemia-free survival. This has eventually increased our understanding of MDS genetics. The recently investigated genes in MDS encode signal transduction proteins (*CBL*, *JAK2*, *KRAS*, *NRAS* and *PTPN11*), transcription factors and cofactors (*NPM1*, *RUNX1* and *TP53*), components of the RNA splicing machinery (*SF3A1*, *SF3B1*, *SRSF2*, and *U2AF1*), and epigenetic regulators (*ASXL1*, *DNMT3A*, *EZH2*, *IDH1* and *IDH2*, and *TET2*).

The various classification and risk scoring systems of MDS have been evolving with years with the advancement in diagnostic techniques. Now these systems include IPSS, WPSS, and MPSS. They incorporate regular cytogenetics. The topics discussed in this article focus on the progress in MDS diagnosis through the use of array-based technologies. We also discuss the recently investigated genetic mutations in MDS. We also revisit the MDS classification and prognostic scoring systems.

Five-year view

With the help of new technologies, we are identifying new genetic lesions that affect the disease course in MDS. A more accurate prediction of prognosis might require the

integration of these genetic defects into the already existing prognostic classification and scoring systems. We anticipate that the existing prognostic scoring systems will evolve in the direction of incorporating mutational data into their parameters.

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Key issues

- Myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal hematopoietic disorders characterized by ineffective erythropoiesis, dysplastic features, clonal chromosomal abnormalities, and increased risk of acute myeloid leukemia (AML) progression.
- The clinical phenotypes of patients with MDS are diverse but are generally stratified into lower-risk and higher-risk.
- Metaphase cytogenetics (MC) has been the gold standard for genetic testing in MDS and it can detect cytogenetic abnormalities in up to 50% of *de novo* MDS cases.
- Cytogenetic abnormalities are essential in determining the prognosis of patients with MDS and they are incorporated in the most commonly used MDS risk assessment system, the International Prognostic Scoring System (IPSS).
- Due to the limitations of MC, investigators have used other existing and new karyotyping tests including FISH, aCGH, and SNP-A to increase the yield of detected genetic lesions in MDS.
- Recently investigated genes in MDS include genes that encode signal transduction proteins (*CBL*), transcription factors (*RUNX1*), components of the RNA splicing machinery (*SF3B1*, *SRSF2*, and *U2AF1*), and epigenetic regulators (*ASXL1*, *DNMT3A*, *EZH2*, *IDH1* and *IDH2*, and *TET2*).
- A variety of prognostic and scoring systems for MDS have been developed and these include FAB classification, WHO classification and prognostic scoring system (WPSS), M.D. Anderson Prognostic Scoring System (MPSS), IPSS, and very recently the revised IPSS (IPSS-R).
- A more accurate prediction of prognosis in MDS might require the integration of recently discovered genetic mutations into the already existing prognostic classification and scoring systems.

Table 1

New cytogenetic scoring system for myelodysplastic syndromes

Cytogenetic abnormality			
Subgroup	Single	Double	Complex
Very good	Del(11q), -Y	-	-
Good	Normal, del(5q), del(12p), del(20q)	Any including del(5q)	
Intermediate	Del(7q), +8, i(17q), +19, any other independent clones	Any other	-
Poor	Inv(3)/t(3q)/del(3q)	Including - 7/del(7q)	3
Very poor	-	-	>3

Table 2

Comparison of technical advantages and limitations among the different methods of karyotyping

Properties	Metaphase cytogenetics	FISH	aCGH	SNP-A
Needs dividing cells	Yes	No	No	No
Resolution	Low	Low	High	High
Sensitivity	10%	High	2-30%	2-30%
Detects balanced chromosomal abnormalities	Yes	No	No	No
Distinguishes individual clones	Yes	Yes	No	No
Detects AS-UPD	No	No	No	Yes

FISH, fluorescence *in situ* hybridization; aCGH, array-based comparative genomic hybridization; SNP-A, single nucleotide polymorphism arrays; AS-UPD, acquired somatic uniparental disomy

Table 3

Summary of mutational abnormalities in MDS and their clinical significance

Mutation	Chromosome location	Frequency	Clinical significance in mutated cases	Reference
TET2	4q	20-26%	Inconsistent impact on survival; improved response to azacitidine	[55-58]
RUNX1	21q	Up to 20%	Decreased survival	[60-63]
ASXL1	20q	10-15%	Decreased survival	[35,64-66,94]
EZH2	7q	2-6%	Decreased survival	[68-71]
DNMT3A	2p	Up to 8%	Decreased survival and increased risk of sAML	[72-73]
CBL	11q	1%	Unknown	[74-76]
IDH1/IDH2	2q/15q	5-10%	Decreased survival (unknown for IDH2)	[35,67,77-80]