HemaSphere
Powered by EHA

EHA EUROPEAN HEMATOLOGY ASSOCIATION

Myelodysplastic syndromes - Section 2

Molecular genetics in clinical decision making for patients with myelodysplastic syndromes or related disorders

Mario Cazzola[1,2]

[1]Department of Molecular Medicine, University of Pavia; [2]Division of Hematology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy

Take Home Messages

- Mutation profiling has a high predictive value for identifying individuals with, or at high risk of developing, a myelodysplastic syndrome.
- Targeted gene sequencing may improve prognostication and prediction of response to treatment in patients with a myelodysplastic syndrome or a related disorder.
- Combining gene mutation with gene expression may improve outcome prediction in MDS.

Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell disorders characterized by cytopenia, dysplasia in one or more of the major myeloid cell lines, ineffective hematopoiesis, recurrent genetic abnormalities, and increased risk of progression to acute myeloid leukemia (AML).[1]

The clonal nature of myelodysplastic hematopoiesis can be documented by the presence of chromosomal abnormalities and/or discrete gene rearrangements in hematopoietic cells with the capacity to differentiate into mature myeloid cells.[2] While recurrent chromosomal abnormalities are detected in only about half of cases,[3] up to 90% of MDS patients carry one or more oncogenic mutations, and most of these are found in individuals with normal karyotype.[4,5] Driver mutant genes include those of RNA splicing, DNA methylation, chromatin modification, transcription regulation, DNA repair, signal transduction, and cohesin complex. Only 6 genes (*TET2*, *SF3B1*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*) are consistently mutated in about 10% or more MDS patients, while a long tail of about 40 genes are mutated less frequently.[4]

Although a complete bone marrow karyotype will remain a critical test in any newly diagnosed MDS case, molecular characterization of the patient's genome has the potential of becoming a very useful tool for clinical decision making in MDS.

Current state-of-the-art

Molecular genetics in the diagnosis of myelodysplastic syndromes

According to the WHO classification, current clinical diagnosis of MDS requires complete blood count for assessment of cytopenia(s), morphological examination of peripheral blood smear and bone marrow aspirate for evaluation of dysplasia and enumeration of blasts, and conventional bone marrow karyotype analysis.[1] The only gene included in the diagnostic criteria is *SF3B1*, whose mutations can be used for diagnosis of the myelodysplastic syndrome with ring sideroblasts.[1] The current WHO definition of MDS does not yet incorporate somatic mutations because those found in MDS patients can occur also in apparently healthy individuals with the so-called age-related clonal hematopoiesis or clonal hematopoiesis of indeterminate potential (CHIP),[6] a condition associated with increased risk of hematologic neoplasms and coronary heart disease.[7,8]

Two important studies have shown that a large proportion of patients with idiopathic cytopenia of undetermined significance (ICUS) or preclinical MDS, not meeting the current diagnostic criteria for MDS, carry somatic mutations in genes that are recurrently mutated in MDS.[9,10] The variant allele fractions (VAF) observed in this condition, which has been defined as clonal cytopenia of undetermined significance (CCUS), are comparable to those observed in MDS and significantly higher than those detected in healthy individuals with CHIP.

We recently studied individuals with unexplained cytopenia undergoing a comprehensive diagnostic workup.[11] Carrying a somatic mutation with a variant allele frequency ≥0.10, or carrying 2 or more mutations, had a positive predictive value for diagnosis of myeloid neoplasm equal to 0.86 and 0.88, respectively. Spliceosome gene mutations and comutation patterns involving *TET2*, *DNMT3A*, or *ASXL1* had positive predictive values for myeloid neoplasm ranging from 0.86 to 1.0. Within subjects with inconclusive diagnostic findings, carrying 1 or more somatic mutations was associated with a high probability of developing a myeloid neoplasm during follow-up.[11]

Thus, while the presence of MDS-associated somatic mutations alone cannot be considered as diagnostic of MDS, their presence at high VAF (≥0.10) in patients with unexplained cytopenia may allow to diagnose CCUS, a condition at high risk of progression to overt MDS, or MDS, depending on the absence or presence of significant dysplasia.[10,11]

Myeloid neoplasms with germ line predisposition

Myeloid neoplasms with germ line predisposition include: (i) conditions without a preexisting disorder or organ dysfunction(*CEBPA* or *DDX41* mutation), (ii) conditions associated with a platelet disorder (*RUNX1*, *ANKRD26*, or *ETV6* mutation), and (iii) conditions associated with organ dysfunction (*GATA2* mutation, bone marrow failure syndromes, and telomere biology disorders).[1] Identifying the inherited predisposition is important not only for genetic counseling, but also for clinical decision making in the individual patient. In a recent study on allogeneic stem cell transplantation, 4% of relatively young MDS patients had compound heterozygous mutations in the gene *SBDS* (associated with the Shwachman-Diamond syndrome) with concurrent *TP53* mutations and a poor prognosis.[12]
Detailed recommendations for management and surveillance of patients with genetic predisposition to hematologic malignancies have been recently published.[13] Defining a genetic predisposition is of fundamental importance in MDS patients undergoing allogeneic stem cell transplantation from a familial donor.

Molecular genetics in the prognostication of MDS patients

The IPSS-R represents a very useful tool for risk assessment in a clinical setting.[14] However, IPSS-R does not consider gene mutations. A study conducted by the International Working Group for Prognosis in MDS (IWG-PM) has first shown that IPSS-R risk groups were strongly associated with overall survival in 2173 MDS patients.[15] More interestingly, the study has identified 6 adverse genes (*TP53*, *CBL*, *EZH2*, *RUNX1*, *U2AF1*, *ASXL1*) that retained independent association with shorter survival after adjusting the hazard ratio of death for IPSS-R risk groups.[15]
In patients with normal karyotype, *SF3B1* mutation identifies a distinct subset of MDS characterized by ring sideroblasts and isolated erythroid dysplasia that is unlikely to develop detrimental subclonal mutations and is associated with indolent clinical course and favorable outcome.[16] By contrast, MDS with ring sideroblasts and wild-type *SF3B1* is mainly characterized by multilineage dysplasia and unfavorable prognosis.[16]
A few studies have evaluated the utility of using both genomic and transcriptomic data for prognostication in MDS patients.

Table 1. **Potential uses of molecular genetics in clinical decision making for patients with myelodysplastic syndromes or related disorders.**

Clinical procedure or setting, genetic analysis of interest	Usefulness for clinical decision making
Diagnostic approach Massive parallel sequencing of a panel of 20-40 MDS-associated genes in patients with unexplained cytopenia undergoing a comprehensive diagnostic workup	Comprehensive mutational analysis is recommended for: - diagnosis of clonal cytopenia of undetermined significance (CCUS), a condition at high risk of progression to overt MDS, even in the absence of significant dysplasia (different mutation patterns define different risks) - diagnosis of MDS. Molecular genetics allows the dentification of a clonal marker in about 90% of patients with MDS (chromosomal abnormalities are found in only about 50% of cases), thus reinforcing considerably diagnosis of this condition
Diagnostic approach in patients under the age of 60 years and/or with evidence of familial disease Gene panel or targeted gene sequencing (whole exome sequencing in selected patients)	Molecular genetics is required and recommended for diagnosis of myeloid neoplasms with germ line predisposition, that is: - conditions without a preexisting disorder or organ dysfunction (*CEBPA* or *DDX41* mutation) - conditions associated with a platelet disorder (*RUNX1*, *ANKRD26*, or *ETV6* mutation) - conditions associated with organ dysfunction (*GATA2* mutation, bone marrow failure syndromes like Shwachman-Diamond syndrome, and telomere biology disorders associated with germline mutations in *TERT*, *TERC*, *DKC1*, or *RTEL1*)
Prognostication Gene panel or targeted gene sequencing	Molecular genetics is currently recommended for: - diagnosis of the MDS with ring sideroblasts associated with *SF3B1* mutation and normal karyotype, a condition with indolent clinical course - identification of *TP53* mutation, which is almost invariably associated with poor clinical outcome, particularly when associated with complex karyotype - assessment of the number of oncogenic mutations: the higher the number, the worse the clinical outcome Evaluation of gene expression through transcriptomic analysis may be useful for defining the risk of leukemic transformation in MDS but is an investigational procedure at present
Prediction of response to treatment Gene panel or targeted gene sequencing	Molecular genetics is currently recommended for the identification of *TP53* mutation in patients with MDS del(5q) who are candidates for lenalidomide treatment: a concomitant *TP53* mutation predicts poor response to lenalidomide and high risk of leukemic transformation The following uses of molecular genetics for predicting response to treatment should be currently considered as investigational procedures: - high VAF, heterozygous *TET2* mutations predict response to hypomethylating agents, particularly in patients with wild-type *ASXL1* - in lower-risk MDS patients, *SF3B1* mutation predicts better erythroid response to luspatercept - high-risk MDS patients carrying a *TP53* mutation may have clinical response and significant mutation clearance with decitabine treatment
Transplantation setting Gene panel or targeted gene sequencing	Molecular genetics is currently recommended for the identification of *TP53* mutation, which invariably predicts poor transplantation outcome Molecular genetics is currently recommended for the identification of a potential genetic predisposition in patients under the age of 60 years and/or with evidence of familial disease when allogeneic stem cell transplantation involves a familial donor

Combining gene mutation with gene expression has been shown to improve outcome prediction in MDS, with a significant contribution from the transcriptome.[17] More recently, through a comprehensive transcriptomic analysis, we discovered 2 major subgroups of MDS defined by gene expression profiles.[18] The first subgroup was characterized by increased expression of genes related to erythroid/megakaryocytic (EMK) lineages, whereas the second subgroup showed upregulation of genes related to immature progenitor (IMP) cells. The IMP subgroup was associated with a significantly higher risk of leukemic transformation and a shorter survival.

Clinical use of genetic data in patients who are candidates for allogeneic stem cell transplantation

Allogeneic stem cell transplantation still represents the only curative treatment for patients with myelodysplastic syndromes.[19] High IPSS-R risk and monosomal karyotype are independently associated with relapse and poor survival after transplantation.[20]

Recent investigations have assessed the impact of somatic genes mutations on transplantation outcome.[12,21,22] The GITMO[21] and CIBMTR studies[12] have clearly shown that *TP53* mutation is the most important molecular predictor of poor transplantation outcome. In the Japanese study,[22] the negative prognostic significance of *TP53* mutation was mainly observed in patients who also had complex karyotype. The CIBMTR study[12] also showed that in patients aged ≥40 years without *TP53* mutation, RAS pathway mutation predicted worse outcome. In the Japanese study,[22] the negative effect of RAS-pathway mutations was confined to patients with myelodysplastic/myeloproliferative neoplasms, mainly chronic myelomonocytic leukemia. The GITMO study[21] showed that not only *TP53*, but also *ASXL1* and *RUNX1* mutations had a negative impact on posttransplantation survival that was independent of the IPSS-R.

Future perspectives

The available evidence indicates that massive parallel sequencing, now feasible in clinical laboratories, has the potential of considerably improving clinical decision making for patients with MDS (Table 1).

Facing a patient with cytopenia and mild or no dysplasia, targeted gene sequencing may be of value in distinguishing between MDS, nonclonal cytopenia, and CCUS. In any case, mutation profiling has a high predictive value for identifying individuals with, or at high risk of developing, a myelodysplastic syndrome.[11] On the other hand, molecular genetics is of fundamental important in the diagnosis of myeloid neoplasms with germline predisposition.[1]

As discussed above, a few mutant genes have independent prognostic significance in MDS, in particular, *SF3B1* (favorable outcome) and *TP53* (poor outcome). A number of oncogenic mutations >2 represents an additional negative prognostic factor *per se*.[4] Transcriptomic analysis may be useful for defining the risk of leukemic transformation.[18]

Molecular genetics can also provide predictors of response to therapy. High-VAF *TET2* mutations are associated with increased response to hypomethylating agents, particularly in subjects with wild-type *ASXL1*.[15] Lenalidomide is effective in most patients with MDS del(5q): however, a concomitant subclonal *TP53* mutation predicts poor response to lenalidomide and leukemic transformation.[23] In lower-risk MDS patients treated with luspatercept, the presence of *SF3B1* mutation is associated with higher likehood of response.[24] Patients with high-risk MDS carrying *TP53* mutations may have clinical response and significant mutation clearance with decitabine treatment.[25]

Ongoing studies of the IWG-PM are likely to provide additional information on the use of molecular genetics in clinical decision making for patients with myelodysplastic syndromes or related disorders.

Acknowledgments

The studies on the genetic basis of myeloid neoplasms conducted at the Division of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, and the Department of Molecular Medicine, University of Pavia, are supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC), Special Program "Molecular Clinical Oncology 5 per mille", project #1005, and by Fondazione Regionale Ricerca Biomedica (FRRB) project no. 2015-0042.

References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391-405.
2. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. Blood 2013;122:4021-34.
3. Haase D, Germing U, Schanz J, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood 2007;110:4385-95.
4. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 2013;122:3616-27.
5. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 2014;28:241-7.
6. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood 2015;126:9-16.
7. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371:2488-98.
8. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017;377:111-21.
9. Kwok B, Hall JM, Witte JS, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood 2015;126:2355-61.
*10. Cargo CA, Rowbotham N, Evans PA, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. Blood 2015;126:2362-5.
Defines clonal cytopenia of undetermined significance (CCUS) as a preclinical myelodysplastic syndrome.
*11. Malcovati L, Galli A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood 2017;129:3371-8.
Defines the relevance of mutation analysis for clinical decision making in patients with unexplained blood cytopenia
*12. Lindsley RC, Saber W, Mar BG, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. N Engl J Med 2017;376:536-47.
Defines the prognostic significance of somatic mutations in myelodysplastic syndrome after stem-cell transplantation
*13. Godley LA, Shimamura A. Genetic predisposition to hematologic malignancies: management and surveillance. Blood 2017;130:424-32.
Provides detailed recommendations for management and surveillance of patients with genetic predisposition to hematologic malignancies.
14. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood 2012;120:2454-65.
15. Bejar R, Papaemmanuil E, Haferlach C, et al. Somatic mutations in

MDS patients are associated with clinical features and predict prognosis independent of the IPSS-R: Analysis of combined datasets from the International Working Group for Prognosis in MDS-Molecular Committee (abstract). Blood 2015;126: abstract 907.
16. Malcovati L, Karimi M, Papaemmanuil E, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood 2015;126:233-41.
17. Gerstung M, Pellagatti A, Malcovati L, et al. Combining gene mutation with gene expression data improves outcome prediction in myelodysplastic syndromes. Nat Commun 2015;6:5901.
18. Shiozawa Y, Malcovati L, Galli A, et al. Gene expression and risk of leukemic transformation in myelodysplasia. Blood 2017;130:2642-53.
19. de Witte T, Bowen D, Robin M, et al. Allogeneic hematopoietic stem cell transplantation for MDS and CMML: recommendations from an international expert panel. Blood 2017;129:1753-62.
20. Della Porta MG, Alessandrino EP, Bacigalupo A, et al. Predictive factors for the outcome of allogeneic transplantation in patients with MDS stratified according to the revised IPSS-R. Blood 2014;123:2333-42.
21. Della Porta MG, Galli A, Bacigalupo A, et al. Clinical effects of driver somatic mutations on the outcomes of patients with myelodysplastic syndromes treated with allogeneic hematopoietic stem-cell transplantation. J Clin Oncol 2016;34:3627-37.
22. Yoshizato T, Nannya Y, Atsuta Y, et al. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. Blood 2017;129:2347-58.
23. Jadersten M, Saft L, Pellagatti A, et al. Clonal heterogeneity in the 5q- syndrome: p53 expressing progenitors prevail during lenalidomide treatment and expand at disease progression. Haematologica 2009;94:1762-6.
24. Platzbecker U, Kiewe P, Germing U, et al. Mutational profile and analysis of lower-risk myelodysplastic syndromes (MDS) patients treated with luspatercept: Phase 2 PACE-MDS study (abstract). Blood 2017;130: abstract 2982.
*25. Welch J, Petti AA, Miller CA, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. N Engl J Med 2016;375:2023-36.
Shows that clinical responses to decitabine correlate strongly with the presence of karyotypes associated with unfavorable risk and the presence of TP53 mutations.