Review

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Laboratory and clinical risk assessment to treat myelodysplatic syndromes

DOI 10.1515/cclm-2015-0789
Received August 14, 2015; accepted November 11, 2015; previously published online January 14, 2016

Abstract: Myelodisplastic syndromes (MDS) are heterogeneous myeloid disorders characterized by peripheral cytopenias and increased risk of transformation into acute myelogenous leukemia (AML). MDS are generally suspected in the presence of cytopenia on routine analysis and the evaluation of bone marrow cells morphology and cellularity leads to correct diagnosis of MDS. The incidence of MDS is approximately five cases per 100,000 people per year in the general population, but it increases up to 50 cases per 100,000 people per year after 60 years of age. Typically MDS affect the elderly, with a median age at diagnosis of 65–70 years. Here the current therapeutic approaches for MDS are evaluated by searching the PubMed database. Establishing the prognosis in MDS patients is a key element of therapy. In fact an accurate estimate of prognosis drives decisions about the choice and timing of the therapeutic options. Therapy is selected based on prognostic risk assessment, cytogenetic pattern, transfusion needs and biological characteristics of the disease, comorbidities and clinical condition of the patients. In lower-risk patients the goals of therapy are different from those in higher-risk patients. In lower-risk patients, the aim of therapy is to reduce transfusion needs and transformation to higher risk disease or AML, improving the quality of life and survival. In higher-risk patients, the main goal of therapy is to prolong survival and to reduce the risk of AML transformation. Current therapies include growth factor support, lenalidomide, immunomodulatory and hypomethylating agents, intensive chemotherapy, and allogenic stem cell transplantation. The challenge when dealing with MDS patients is to select the optimal treatment by balancing efficacy and toxicity.

Keywords: clonal myeloid disorders; hematopoiesis; myelodisplastic syndromes; risk assessment; therapy.

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Introduction

Myelodisplastic syndromes (MDS) are a group of clonal myeloid disorders characterized by progressive cytopenia due to ineffective hematopoiesis, with a variable risk of transformation into acute myeloid leukemia (AML) [1, 2].

The incidence of MDS is estimated to be around five cases per 100,000 people per year in the general population, but after 60 years it increases up to 50 cases per 100,000 people per year. Typically MDS affect elderly people (median age at diagnosis of 65–70 years), while they occur in <10% of patients under 50 years of age [3]. The annual incidence of MDS increases logarithmically after 20 years of age, from <1.0 per million persons to 20 per 100,000 persons in septuagenarians. Males are affected approximately 1.5 times as often as females. The incidence is widely distributed with no ethnic differences, but in the Asian population MDS occur at an earlier age, as compared to the Western population [3].

MDS are generally suspected in the presence of cytopenia on routine analysis of peripheral blood, which triggers bone marrow evaluation. The evaluation of bone marrow cells morphology and cellularity generally shows hypercellular bone marrow, with variable grades of dysplasia, with or without immature blood cells. Parameters such as the percentage of blasts in the bone marrow, the number of cell lines involved in peripheral cytopenia, and karyotype abnormalities can significantly affect the natural course of the disease and its prognosis [4]. The International Prognostic Scoring System (IPSS)

on the basis of the above mentioned parameters identifies four risk groups of patients with different prognosis: low, intermediate-1, intermediate-2, and high risk [5]. The median overall survival of MDS intermediate-2 or high-risk patients is 1.2 and 0.4 years, respectively; these patients are at high risk of developing AML, which means that the aim of the treatment is to modify the natural history of the disease and extend survival. Over the years more accurate prognostic scores, including an evaluation of transfusion requirement and assessment of patients' comorbidities, have been developed, in order to obtain a deeper risk assessment to guide treatment in MDS patients [5–12].

The available therapies range from the treatment of symptomatic cytopenias in the low-risk group of MDS patients, to immunomodulatory agents, chemotherapy or allogenic stem cell transplantation in high-risk patients. The aim of this article is to review the role of oldest and newer laboratory assays in association with clinical and hematological parameters in stratifying the risk of MDS patients.

Materials and methods

We reviewed the medical literature for published studies evaluating "Myelodysplastic syndromes and risk assessment, and laboratory evaluation and Therapy and Prognosis". The PubMed electronic database was searched without temporal limits using an English language restriction. The key words used were: myelodysplastic syndromes, risk assessment, laboratory evaluation, therapy and prognosis. References of most recent papers on myelodysplastic syndromes were also cross-referenced to identify potentially relevant papers not captured in our initial literature search. Data of pediatric patients are not considered in the present paper. Search terms were also applied to abstracts from the latest international hematological and oncological congresses.

The reference lists of the trials as well as articles were reviewed for additional publications.

When there was duplication of publications, we reviewed each article and included only the most recent or the complete version of the trial for analysis. In situations in which there was a discrepancy in the data, we considered the safety report from the most recent package insert to be the most accurate and used that report instead of the original publication for our review article.

Clinical and laboratory diagnosis

MDS patients complain about symptoms which are usually consistent with the type and severity of the peripheral blood cytopenias. They commonly report fatigue and decreased exercise tolerance due to anemia. Less often, patients show bleeding, easy bruisability, or recurrent bacterial infections as initial complaint [4]. Hepatomegaly or splenomegaly occur in approximately 5 or 10% of patients, respectively.

Blood and bone marrow examination

Anemia is present in >85% of patients and it is generally macrocytic. Red cell shape abnormalities include oval, elliptical, tear-drop, spherical, and fragmented cells. Reticulocyte counts are usually lower than expected on the basis of the degree of anemia. This latter finding is consistent with ineffective erythropoiesis.

Approximately 50% of patients at the time of diagnosis show neutropenia [13]. The percentage of monocytes is often slightly increased, and monocytosis per se can be the dominant manifestation of the hematopoietic abnormality for months or years [14]. Morphologic abnormalities of neutrophils can occur, sometimes resulting in the acquired Pelger-Huët anomaly. Approximately 25% of patients have mild to moderate thrombocytopenia at the time of diagnosis [4]. Mild thrombocytosis can also occur. Platelets may be abnormally large, may present poor granulation, or have large, fused central granules. Abnormal platelet function, with decreased platelet aggregation in response to collagen or epinephrine, can be responsible for a prolonged bleeding time, or easy bruising [15], Table 1 represents blood and bone marrow findings in MDS [6].

Bone marrow analysis has a pivotal role for the diagnosis of MDS. The bone marrow is generally hypercellular, shows dysplastic features in one or several myeloid series, generally different from those observed in megaloblastic anemia due to vitamin B12 or folate deficiency. The bone marrow blast percentage should be assessed on at least 500 nucleated cells. Ring sideroblasts count, after Prussian blue staining, is mandatory, considering the differential diagnosis with refractory anemia with ringed sideroblasts (RARS). The trephine biopsy is essential when bone marrow fibrosis is suspected, and when a differential diagnosis with aplastic anemia or AML is needed [4]. Table 2 diagnostic approach to myelodysplastic syndromes and their diagnostic significance [6].

Table 1: Peripheral blood and bone marrow findings in myelodysplastic syndromes [6].

	Peripheral blood	Bone marrow examination
Erythropoiesis	Anisocytosis Elliptical red cells Fragmented cells Macrocytic or dismorphic red cells	Erythroid hyperplasia Pathologic sideroblasts Megaloblastoid erythropoiesis Proerythroblasts may be present in excess Ringed sideroblasts
Granulopoiesis	Acquired Pelger-Huët anomaly Neutrophils with condensed chromatin Unilobed or bilobed nuclei Defective primary granules of abnormal size	Granulocytic hyperplasia Hypogranulation Immature myeloid cells Acquired Pelger-Huët anomaly
Thrombopoiesis	Thrombocytopenia Abnormally large platelets Abnormal platelet function (decreased platelet aggregation in response to collagen or epinephrine)	Micromegakaryocytes Megakaryocytes with unilobed or bilobed nuclei

Table 2: Diagnostic approach to myelodysplastic syndromes and their diagnostic significance [6].

	Clinical utility
Mandatory	
Bone marrow aspirate	Morphological evidence of dysplasia Blast count Ringed sideroblasts count
Conventional cytogenetic analysis	Cytogenetics are of importance to establish clonal haematopoiesis, calculate prognosis of patients and in some subsets of patients to drive specific therapy (e.g. 5q- syndrome and lenalidomide) Bone marrow cellularity Immature CD 34+ myeloid cells
Bone marrow biopsy	Reticulin and collagen fibers evaluation In dry tap or hypoplastic MDS essential to diagnosis
Optional	
Flow cytometry	Can be of help in the identification of abnormal phenotypic patterns and can be of help in cases of minimal dysplasia
Molecular analysis	Assessment of specific genetic abnormalities with diagnostic and prognostic significance
Fluorescent in situ hybridization (FISH)	To establish clonal hematopoiesis, after conventional cytogenetic failure

Cytogenetic findings

Chromosomal abnormalities are described in MDS [16, 17]. Cytogenetic analysis has been shown to be of major prognostic value for MDS, being part of IPSS scoring system. Table 3 describes frequencies of cytogenetic abnormalities and prognostic IPSS-R risk category in MDS [7, 18].

Furthermore, in addition to prognostic value, cytogenetic analysis has a pivotal role in confirming or in ruling out the diagnosis of MDS, when hematological findings

Table 3: Cytogenetic findings in patients with myelodysplastic syndromes, by their prognostic value [7, 18].

IPSS-R risk category	Proportion of patients, %	Karyotype	Median survival, years	Time to 25% acute myeloid leukaemia evolution, years
Very good	4	-Y, del(11q)	5–4	Not reached
Good	72	Normal, del(5q), del(12p), del(20q), double including del(5q)	4–8	9–4
Intermediate	13	del(7q), +8, +19, i(17q), any other single or double independent clones	2–7	2–5
Poor	4	−7, inv(3)/t(3q)/del(3q), double including −7/del(7q); complex: 3 abnormalities	1–5	1–7
Very poor	7	Complex >3 abnormalities	0–7	0–7

are ambiguous. Common abnormalities include an extra chromosome 8; loss of the long arm of the chromosome 5, 7, 9, 20, or 21; and monosomy for chromosomes 7 and 9. Losses of part or all of chromosomes 5 and 7 and complex chromosome aberrations are particularly common in the oligoblastic myelogenous leukemias (and the overt leukemias) associated with prior treatment with cytotoxic drugs, radiation, or exposure to benzene [19]. Categories of cytogenetic abnormalities correlated with median survival have been determined. The more favorable risk category includes a normal karyotype and isolated deletions of 5q32-33.3, 20q, or Y. The poor-risk category includes -5q31.1, -7, del (7q), and complex chromosomal abnormalities. The intermediate-risk group includes other abnormalities. In treatment-induced MDS, complex cytogenetic abnormalities are very common, whereas in de novo MDS abnormalities occur in approximately 15% of cases [20].

Fluorescence in situ hybridization (FISH) is generally considered useful to identify the chromosome involved when low number of mitoses are available [13].

5q syndrome

Abnormalities in chromosome 5 occur in more than 15% of MDS patients, but the incidence of the originally described 5q- syndrome is much less frequent [21, 22].

The 5q- syndrome is characterized by refractory macrocytic anemia, normal or increased platelet counts, increased numbers of megakaryocytes in the bone marrow, and deletion of the long arm of chromosome 5 (5q-) as the unique cytogenetic abnormality. Survival is relatively long, and is characterized by low rate of leukemic transformation. The majority of patients are older women. Iron overload due to transfusion requirement can become a clinical problem in these patients, and iron chelating therapy may be required [22].

Patients with del(5q), either as an isolated abnormality or often as part of a complex karyotype, have a higher rate of concomitant *TP53* mutations. These mutations are associated with diminished response or relapse after treatment with lenalidomide. In these cases, *TP53* mutations may be secondary events and are often present in small subclones that can expand during treatment. More sensitive techniques may be required to identify the presence of sub-clonal, low-abundance *TP53* mutations before treatment [23, 24].

Cytopenias with myelodysplastic changes can be seen in a variety of conditions, some of which are reversible. It is crucial to exclude these reversible causes before giving a patient the diagnosis of myelodysplasia or starting treatment for MDS.

The diagnostic approach recommended by the WHO in the case of a patient with suspected MDS includes the integration of the cytological evaluation of peripheral blood smears, evaluation of bone marrow aspirates and bone marrow biopsy. Data resulting from the analysis of conventional cytogenetics, FISH and immunophenotyping can complete the diagnosis. Diagnosis can be difficult when cytopenias are moderate, especially when a mild bone marrow dysplasia coexist. It is calculated that diagnostic discrepancy can occur at the time of initial presentation in 20%–30% of patients [4].

Classification

The integration of morphological, histopathological and cytogenetic tests allows to define the diagnosis of MDS in accordance with the current classification proposed by the WHO in 2008 [25].

Seven distinct categories have been identified according to the WHO classification: refractory anemia (RA), refractory anemia with ringed sideroblasts, refractory cytopenia with multilineage dysplasia (RCMD), refractory cytopenia and ringed sideroblasts (RCMD-RS), refractory anemia with excess blasts-1 (RAEB I) and refractory anemia with excess blasts-2 (RAEB-2), myelodispastic syndrome unclassified (MDS-U), MDS associated with isolated del(5q). This classification is a useful tool for the definition of different subtypes characterized by different prognosis. Therapy-related myelodysplastic syndromes are separately classified, together with therapy-related AML.

Variants of myelodysplastic syndromes

Therapy-related myelodysplasia

Therapy-related myelodysplasia (t-MDS) currently accounts for ~10% to 15% of MDS cases [26] and it develops in patients who were previously receiving chemotherapy or radiotherapy. Hypocellular MDS and MDS with myelofibrosis are more common in patients with t-MDS. Most patients have a relatively brief myelodysplastic phase and progress to overt leukemia within a few months. There is a high incidence of adverse cytogenetic

abnormalities, particularly abnormalities involving chromosomes 5 and 7.

Hypocellular myelodysplasia

Approximately 10%–15% of patients with myelodysplasia have a hypocellular bone marrow [27]. The challenge is to distinguish this form from aplastic anemia: the presence of striking myelodysplastic changes together with cytogenetic analysis are the key elements for differential diagnosis. A trial of immunosuppressant agents may be warranted.

Risk stratification

The prognostic score based on the French American British (FAB) classification called IPSS includes percentage of blasts, number of cytopenias, and cytogenetics [5]. This system is highly reproducible and very simple to use. In order to overcome the main limitation of the system (imprecise predictor of prognosis in low-risk patients) a new scoring system called IPSS-R has been developed [7]. Bone marrow cytogenetics, bone marrow blast percentage, and cytopenias remain the basis of the new system but novel components are included (five rather than three cytogenetic prognostic subgroups, splitting the low bone marrow blast percentage value and depth of cytopenias). This model defined five rather than the four major prognostic categories that are present in the IPSS. The WHO classification-based prognostic scoring system (WPSS) represents another commonly used scoring system [8]. This system was developed when it was clear that red cell transfusion dependency is an independent predictor of prognosis in MDS. This system was also developed as time-dependent model, meaning that it can be used sequentially at any time during the course of the disease. The WPSS requires WHO classification of the disease and prior information on transfusion needs. Recently, the WPSS score was modified to include hemoglobin levels instead of transfusion needs. Both the IPSS and WPSS were developed in a very specific subset of patients: newly diagnosed patients at the time of initial presentation. Recently the global MD Anderson Cancer Centre (MDACC) model was developed [9]. Poor performance, older age, thrombocytopenia, anemia, increased bone marrow blasts, leukocytosis, chromosome 7 or complex (≥3) abnormalities and prior transfusions are considered the main predictors of prognosis in MDS patients in a multivariate analysis of prognostic factors. The new MDS prognostic model divided patients into four prognostic groups with significantly different outcomes. The model was found applicable to any patient with MDS at any time during the course of MDS. Low-risk MDS patients are challenging due to their heterogeneous prognosis which does not allow to distinguish between longer survival patients and those with intermediate outcome. In a study enrolling 600 MDS patients, the assessment of comorbidities performed with the adult comorbidity evaluation showed that patients with severe diseases had 50% lower survival than did those without co-morbidities, independently of age and IPSS risk group [10]. The impact of comorbidities on prognosis of MDS has been largely underlined also by other groups [11].

Amongst other prognostic factors, age is an important predictor of poor prognosis and bone-marrow fibrosis has been shown to be independently associated with poorer prognosis, in both lower-risk and higher-risk MDS patients [28]. Recurrent mutations in genes encoding components of the splicing machinery, including *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*, have also been reported in myelodysplastic syndromes [29, 30] *SF3B1* mutations are strictly linked with ring sideroblastic subtypes of myelodysplastic syndromes; directly contributing to formation of ringed sideroblasts and abnormal iron retention. Furthermore, *SF3B1* mutations seem to be predictors of favorable clinical outcome [31].

Somatic mutations also strongly affect survival. The presence of any of the mutations *TP53*, *RUNX1*, *EZH2*, or *ETV6* worsens outcome, independently of IPSS [32, 33]. *ASXL1* and *SRSF2* mutations are also associated with a poorer outcome according to the literature [33, 34]. These genes can be divided into four main groups: (1) transcription factors (*TP53, RUNX1, ETV6*); (2) epigenetic regulators and chromatin-remodeling factors (*TET2, DNMT3A, ASXL1, EZH2, IDH1/2*); (3) pre-mRNA splicing factors (*SF3B1, SRSF2, U2AF1, ZRSR2*); and (4) signaling molecules (*NRAS, CBL, JAK2, SETBP1*). The most frequently mutated genes were *TET2, SF3B1, ASXL1, DNMT3A, SRSF2, RUNX1, TP53, U2AF1, EZH2, ZRSR2, STAG2, CBL* and *NRAS*, although no single mutated gene was found in more than a third of patients. Several of these gene mutations are associated with adverse clinical features, such as complex karyotypes (*TP53*), excess bone marrow blast proportion (*RUNX1, NRAS*, and *TP53*) and severe thrombocytopenia (*RUNX1, NRAS*, and *TP53*). Despite associations with clinical features considered by prognostic scoring systems, mutations in several genes hold independent prognostic value as shown in Table 4. Mutations of *TP53, EZH2, ETV6, RUNX1*, and *ASXL1* have been shown to predict decreased OS in multivariable models adjusted for IPSS

Table 4: Reported frequency of genetic lesions in MDS [27, 30, 34–36].

Gene	Frequency, %	Location	Function	Prognosis
SF3BI	28	2q33	Splicing factor	Favorable
TET2	21	4q24	Control of cytosine hydroxymethylation	Neutral
ASXL1	14	20q11	Epigenic regulator	Unfavorable
SRSF2	12	17q25	Splicing factor	Neutral
RUNX1	9	21q22	Transcription factor	Unfavorable
TP53	8	17q13	Transcription factor	Unfavorable
U2AF1	7	21q22	Splicing factor	Unfavorable
EZH2	6	7q36	Polycomb group protein	Unfavorable
NRAS	4	Ip13	Signal transduction	Unfavorable
JAK2	3	9p24	Tyrosine Kinase	Favorable
ETV6	3	12p13	Transcription factor	Unfavorable
CBL	2	11q23	Signal transduction	Unknown
IDH2	2	15q26	Cell metabolism, epigenetic regulation	Unfavorable
NPM1	2	5q35	Phosphoprotein	Unknown
IDH1	1	2Q33	As IDH2	Unfavorable
KRAS	<1	12q12	Signal transduction	Unfavorable
GNAS	<1	20q13	G protein	Unknown
PTPN	<1	12q24	Protein phospatase	Unknown
BRAF	<1	7q34	Raf Kinase	Unknown
PTEN11	<1	10q23	Phosphatase	Unknown
CDKN2A	<1	9q21	Cell cycle control	Unknown

or IPSS-R risk groups in several studies. Within IPSS risk groups, a mutation in one or more of these genes identifies patients whose risk resembles that of patients in the next highest IPSS risk group (e.g. the survival curve for INT-1-risk patients with an adverse gene mutation was similar to that of patients assigned to the INT-2-risk group by the IPSS) [35].

When applied to patients stratified by the IPSS-R, the presence of a mutation in one or more of these five genes was associated with shorter OS for patients in the low- and intermediate-risk groups. Other mutated genes have been associated with decreased OS, including *DNMT3A, U2AF1, SRSF2, CBL, PRPF8, SETBP1*, and *KRAS*.

Mutations of *SF3B1* have been associated with a more favorable prognosis, but this may not be an independent risk factor.

For example, SF3B1 mutated patients are likely to present reduced hemoglobin levels leading to a higher transfusion dependence, while patients harboring SRSF2 mutations clustered in RAEB-1 and RAEB-2 subtypes and had pronounced thrombocytopenias [36]. Table 4 reports the frequency of genetic mutations in MDS [4, 27, 30, 34–38].

Hyperferritininemia and high levels of lactate dehydrogenase are associated with poorer prognosis and higher cardiac or extra hematological mortality [39].

The introduction of new therapies that can modify the clinical course of MDS revealed new factors able to predict the response to these treatments, in addition to the traditional ones.

For example, patients with low levels of blood erythropoietin (EPO) respond better to erythroid stimulating agents (ESA) and an early resistance to ESA seems to be associated with a worse outcome [39–41]. Response to hypomethylating agents can be predicted by performance status, karyotype, erythrocyte transfusion requirements, presence of circulating immature bone marrow elements or *TP53* mutations [42, 43]. Although screening for such molecular defects on a routine basis cannot currently be recommended, the spread of massive genotyping technology will allow clinicians to detect a broad range of genetic aberrations in peripheral blood at a reasonable cost in the near future, making it easier to confirm the diagnosis in patients with suspected MDS.

Treatment strategies

In the recent years, despite the improvement of treatment strategies, MDS remain challenging diseases. New drugs such as lenalidomide, demethylating agents and iron chelators, helped slowing the natural history of the disease and improving the quality of life of patients who are not eligible for transplantation [44, 45]. Many factors make identification of an univocal treatment strategy in

MDS patients very difficult. First of all, MDS are a heterogeneous group of hematological diseases, with different clinical and prognostic features but they are all linked by a clonal disorder of stem cells, ineffective haematopoiesis and a variable risk of transformation into AML. In addition, the complexity of cytogenetic and molecular abnormalities (except for 5q-) does not allow to identify targeted therapies. Most patients with MDS are old and affected by many comorbidities, therefore less able to tolerate aggressive therapies. Some patients with MDS may have prolonged survival; older patients with low-grade myelodysplasia may be more likely to die of illnesses other than MDS.

Establishing the prognosis for patients with MDS is a key step of their management. In fact, possible benefit from therapy has to be carefully balanced against the risks of complications. Treatment for myelodysplasia has to be highly individualized. An accurate estimate of prognosis drives decisions about timing and choice of the therapeutic options. In low-risk and unfit patients control of symptoms is the primary goal of therapy. In younger or healthier patients with high-risk MDS aggressive therapy in attempt to achieve cure might be warranted.

Treatment strategies in low-risk/intermediate-1 MDS (IPSS), very low, low, intermediate MDS (IPSS-R), very low, low, intermediate MDS (WPSS)

The approach for treatment of low-risk MDS is aimed at correcting cytopenias. Chronic anemia adversely affects the quality of life of MDS patients and the clinical course of disease while transfusion dependence leads to reduced survival [46, 47]. Erythropoietic stimulating agents (ESAs) increase the hemoglobin level in approximately 15%–25% of MDS patients. The multivariate analysis in several studies confirmed that predictive factors of major erythroid response after treatment with ESAs were baseline serum levels of EPO <100 IU/L, favorable cytogenetics, low number of blasts, no or low transfusion requirement [46–48]. Anti-apoptotic effects on erythroid progenitors are probably the most important mechanism of action of ESAs. However, in responding patients the median duration of response is only approximately 2 years [47]. A recent study from our group has shown that biosimilar epoetin-α is effective for the treatment of anemia in MDS patients with comparable efficacy to that of other ESAs [49]. Several studies have shown that ESAs have no effects on the risk of progression to AML [50–52].

In non-responding MDS patients there is some evidence that adding low-dose granulocyte colony stimulating factors (G-CSFs) has a synergistic effect with erythropoiesis stimulating agents (ESAs) [48, 53, 54]. No randomized study has shown improvement in survival for patients with MDS treated with epoetin-α plus G-CSF and further randomized trials are needed to optimize dosing. Greenberg et al. [48] showed that combination of G-CSFs (initial dose 1 μg/kg per day SQ) plus epoetin-α (150–300 units/kg per day SQ) results in a substantial erythroid response (i.e. decreased transfusion requirements and increased hemoglobin concentrations) in approximately 40%–47% of patients.

Lenalidomide directly acts on del5q- clones, inducing hematopoiesis and erythropoiesis stimulation, whereas other effects including immunomodulation, anti-inflammatory activity, and angiogenesis inhibition [55, 56] are similar to those of thalidomide. Based on this evidence lenalidomide has been licensed in the USA and in Europe for transfusion-dependent anemia in MDS patients with documented 5q-abnormality. Cytogenetic responses are reported in 50%–70% of the treated population [57–59]. List et al. [60] showed that lenalidomide was effective in inducing an erythroid response as well as reversing cytological and cytogenetic abnormalities in 148 MDS patients with del5q31 and transfusion-dependency. The response to lenalidomide was rapid and long-lasting, and the median duration of transfusion independence had not been reached after a median of 104 weeks of follow-up. Moderate-to-severe neutropenia (in 55% of the patients) and thrombocytopenia (in 44%) were the most frequent reasons for interrupting treatment or adjusting the dose of lenalidomide. Fenaux et al. [56] carried out a randomized double-blind study of the efficacy and safety of lenalidomide in 205 patients with MDS who were RBC transfusion-dependent and at IPSS low-risk or intermediate-1 risk and carried del5q31. The researchers concluded that lenalidomide is beneficial and has an acceptable safety profile in transfusion-dependent 5q- patients who were with low to intermediate-1-risk. Lenalidomide reverses transfusion dependence in 25%–30% of lower-risk MDS patients resistant to ESAs [56] but it is not approved for this indication outside clinical trials. Preliminary results suggest that the combination of lenalidomide and ESAs can lead to high rates of independence from erythrocyte transfusion in patients resistant to ESAs alone [61, 62]. Treatment of anemia in patients resistant

to ESAs (or lenalidomide in patients with deleted 5q) or after relapse remains challenging [21]. Hypomethylating agents in low risk MDS patients [63] are effective and are shown to lead to transfusion independence in about 40% of patients. However, despite these treatments, many low-risk MDS patients eventually need erythrocyte transfusion to control anemia.

In selected younger patients with low-risk MDS, with limited erythrocyte transfusion history, normal karyotype (or possibly trisomy 8), no excess marrow blasts or *HLA DR15* genotype, no deleted 5q, limited exposure to previous treatments, and possibly hypocellular marrow, antithymocyte globulins, with or without ciclosporin, can be used to obtain an erythroid response, with response of other cytopenias (especially thrombocytopenia) in 25%–40% of MDS patients [64–67].

The experience gained with thalassemic patients suggests the use of chelation therapy in patients with MDS undergoing transfusion therapy for which prolonged life expectancy is not already affected by leukemic transformation. In lower-risk myelodysplastic patients, receiving more than 20–40 red-blood-cell concentrates, or when serum ferritin rises over 1000 ng/mL [68], iron chelation therapy is considered, in order to prevent iron overload. However, clinically significant iron overload associated with heart failure is quite frequent in MDS patients, especially when elderly, receiving 100 or more red blood cell concentrates. Other papers suggest that potential benefits of iron chelation should be lowering of infection risk, improvement of the outcome of allogeneic hematopoietic stem cell transplantation and delay of leukemic transformation [69]. Deferasirox, an oral iron chelator, has shown efficacy and acceptable tolerability in MDS setting and has also been shown to improve peripheral cytopenia in 10%–20% of MDS patients [70, 71]. However, desferasirox has a potential renal toxicity and is contraindicated in patients with renal failure. According to literature data, we suggest a starting dose of 10–20 mg/kg/per day with dose escalation up to 40 mg/kg. Young MDS patients suitable for bone marrow transplantation need to be especially controlled for iron overload. Several studies have shown that iron overload can affect the outcome of bone marrow transplantation [72–74].

Although MDS patients have a high incidence of infection due to neutropenia and granulocyte dysfunction and infection is the principal cause of death in patients with MDS, the use of granulocyte-colony stimulating factors (G-CSFs) [75–77] or granulocyte macrophage colony stimulating factors (GM-CSFs) [78–80] in efficacy trials, including randomized trials, has been disappointing. Antibiotics are indicated for bacterial infections, but no routine prophylaxis is recommended (with the exception of patients with recurrent infections).

Severe bleeding is a rare problem in low-risk MDS patients and platelet counts below 50×10^9 cell/L are generally observed in 30%–50% of patients. Rarely and only in advanced stages MDS patients require repeated platelet transfusions. The current availability of thrombopoietin-receptor agonists romiplostim and eltrombopag approved for therapy of autoimmune thrombocytopenia suggested their use in the setting of MDS patients [81–83]. The development of myeloid malignancies is a concern when administering thrombopoietin receptor agonists. Among 168 MDS subjects treated with romiplostim, progression from MDS to AML was observed in 10 (6%) patients [84]. In another clinical trial a transient increase in bone marrow blasts has been reported in 15% [79]. In conclusion thrombopoietin mimetics should be considered in the setting of MDS patients with bleeding due to low platelet counts who do not respond to transfusions. Further studies to evaluate their safety in the setting of MDS patients with thrombocytopenia are ongoing. Figure 1 summarizes the therapeutic approach to this risk class of patients.

Treatment strategies in intermediate-2 high-risk MDS (IPSS), intermediate high, very high-risk MDS (IPSS-R)

The median overall survival of MDS intermediate-2 or high-risk patients is 1.2 and 0.4 years, respectively; these patients show high risk of developing AML, which means that the aim of the treatment is to modify the natural history of the disease and extend survival [3].

AML-like chemotherapies

AML-like therapy is only recommended for relatively younger patients with favorable karyotype that are candidates for AlloSCT. AML-like protocols in higher-risk MDS patients have generally used classical anthracycline-cytarabin combinations similar to those used in de-novo AML [80]. When used in MDS or AML post-MDS, AML-like therapy results in lower complete remission (CR) rates (40%–60%), shorter CR duration (median duration of 10–12 months) and is associated

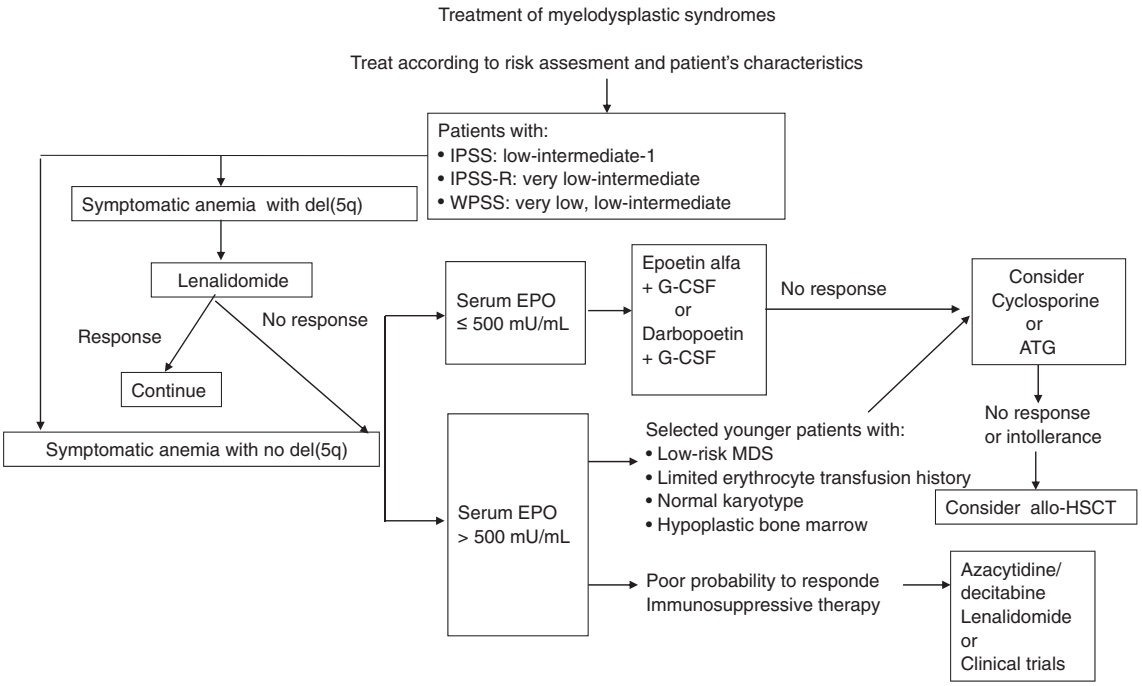

Figure 1: Therapeutic strategies for the treatment of myelodysplastic syndromes.
Treatment strategies for patients with myelodisplastic syndromes according to the International prognostic scoring system (IPSS), WHO Prognostic Scoring System (WPSS) and Revised IPSS (R-IPSS). Anemic very low and low-intermediate myelodisplastic (MDS) patients carrying del 5q should be treated with lenalidomide in order to reduce transfusion request and to obtain cytogenetic response. Lenalidomide should be avoided in patients with a clinically significant decrease in neutophils or platelet count. Although the cost of lenalidomide is higher than that of other treatments, the reduction in the number of transfusions and in transfusion dependence partially offsets the expense. Symptomatic patients with no del(5q) or patients not responding to lenalidomide should be treated according to endogenous levels of eritropoietin (Epo) with erytropoietic stimulating agents (ESAs), eventually associated with granulocyte colony stimulating factors (G-CSFs). Non responding patients should be treated with immusuppressive therapy or with anti-timocytes globulin (ATG) or cyclosporine. Allo hematopoietic stem cell transplantation (allo-HSCT) should be considered in selected fit patients affected by intermediate-1 myelodysplastic syndromes. In selected patients carrying high levels of endogenous Epo who are unlikely to respond to ESAs immunosuppressive therapy should be considered. For patients with poor probability to respond to immunosuppressive therapy alternative therapies with Azacytidine/Decitidine/Lenalidomide should be considered. Red blood cell (leuko-reduced) transfusions are recommended for symptomatic anemia. Platelet transfusions are recommended for thrombocytopenic bleeding. However, they should not be used routinely in patients with thrombocytopenia in the absence of bleeding unless platelet count is $<10{,}000/mm^3$. Irradiated products are suggested for transplant candidates. If >20 to 30 red blood cells transfusions have been received, consider daily chelation with deferoxamine subcutaneously or deferasirox orally to decrease iron overload, particularly for low/intermediate-1 and for potential transplant patients. Patients with low creatinine clearance (<40 mL/min) should not be treated with deferasirox. IPSS, International Prognostic Scoring System; WPSS, World Health Organization Prognostic Scoring System; IPSS-R, Revised IPSS; EPO, erythropoietin; ATG, anti timocytes globulins; allo HSCT, allo hematopoietic stem cell transplantation; MDS, myelodisplastic syndromes.

with more prolonged periods of aplasia. Patients with high risk karyotype have lower CR and shorter durations of remission (DOR) [81]. No chemotherapeutic regimen including fludarabine or topotecan, or gemtuzumab ozogamicin, with cytarabine, with or without G-CSF has shown any survival advantage over classic anthracycline-cytarabine regimens [82–88].

Fifteen percent to 20% of complete or partial remissions are obtained with low-dose cytarabine (20 mg/m^2 daily, 14–21 days every month) in higher-risk MDS patients, but no proven survival advantages [89] have been observed.

Hypomethylating agents

Oligoblastic and secondary myelogenous leukemias have a high prevalence of tumor suppressor gene hypermethylation. 5-Azacytidine is a pyrimidine analog inhibiting DNA methyltransferase, reducing cytosine methylation, and inducing maturation of some leukemic cell lines [90]. Antiproliferative properties have been showed by inhibiting the release of oncostatin-M, IL-6, and IL-11 from mononuclear cells in patients with clonal anemia [91]. 5-Azacytidine at a dose of 75 mg/m^2 once per day given subcutaneously for 7 consecutive days each month provided significantly

more frequent benefit to two thirds of patients than did supportive care [63]. Quality of life was improved and disease progression was delayed. 5-Azacitidine has been studied in higher-risk MDS patients in two major randomized multicenter trials: CALGB 9221 [63] and AZA-001 [81]. In the CALGB 9221 study, 191 patients (median age 68 years) with MDS were randomized to receive 5-azacitidine (75 mg/m^2 per day for 7 consecutive days every 28 days) or best supportive care (BSC). Sixty percent of the patients in the 5-azacitidine group, compared with 5% of control arm patients, responded to treatment ($p<0.0001$). The median time to leukemic transformation or death was 21 months in patients treated with 5-azacitidine vs. 12 months in the BSC arm ($p<0.007$). A survival benefit due to delayed transition to AML was obtained in these studies treating MDS patients with 5-azacytidine treatment (75 mg/m^2 daily subcutaneously, 7 days every 4 weeks). Age, bone marrow blast percentage, and karyotype seem to be independent factors of response. Moreover, these agents have shown a reduction in erythrocyte transfusion requirement. Achievement of any type of hematological improvement, even in the absence of complete or partial remission, was significantly associated with better outcome. The median duration of response to 5-azacitidine was 13.6 months. The median number of cycles was 15 in responders. These data suggest that long-term treatment is needed to obtain a survival benefit.

Moreover, the French group reported that previous therapy with low dose cytarabine, bone marrow blasts >15% and abnormal karyotype were predictors of lower response rate to 5-azacitidine [92]. Poor performance status, intermediate and poor risk cytogenetics, circulating blasts, and more than four units of red blood cells transfused every 8 weeks were associated with worse survival.

Decitabine is another hypomethylating agent that was tested in the EORTC/German MDS trial [92]. During this trial 233 patients with MDS (93% intermediate-2 or high IPSS) were randomly assigned to best supportive care with or without decitabine. Decitabine (15 mg/m^2) was given intravenously over 4 hours three times a day for 3 days in 6-week cycles. At a median follow-up of 2.5 years, median overall survival was 8.5 months for BSC vs. 10.1 months for decitabine and acute myeloid leukemia-free survival was 6.1 months for BSC vs. 8.8 months for decitabine (these differences were both not statistically significant). The statistically significant achieved goals with treatment with decitabine were prolonged progression-free survival (median PFS, 6.6 vs. 3.0 months, respectively) and reduced AML transformation at 1 year (from 33% with BSC to 22% with decitabine). Decitabine treatment was also associated with improvements in patient-reported quality-of-life parameters.

The effectiveness of hypomethylating agents in inducing hematologic improvements and also true remissions with low toxicity justifies the hypothesis of their use in the pre-transplant phase instead of conventional chemotherapy.

Taken together these trials show that the pyrimidine nucleoside analogs of cytidine are the standard of care for patients with higher-risk disease, even in patients eligible for BMT as bridging therapy.

Now there is no therapy approved for patients with higher-risk MDS that do not respond to hypomethylating agents or relapse after AML-like therapy or AlloSCT. The group of patients for whom hypomethylating agents failed has a particularly poor prognosis. For these patients, investigational treatments within carefully designed clinical trials should be considered.

Allogeneic bone marrow transplant (BMT)

Allogeneic bone marrow transplant (BMT) represents the only potentially curative therapy for MDS. Unfortunately, the use of allogeneic BMT for MDS is limited by the older age of most patients and by the fact that only a minority of patients has histocompatible bone marrow donors. However, the use of allogeneic BMT is being extended to older patients, and the use of a national bone marrow donor registry has allowed matched unrelated transplants. Several trials treating MDS with allogeneic BMT have been performed; ~40% of patients in these trials have long-term disease-free survival and may be cured [93]. Patients with MDS show high transplant-related mortality rates (~30% to 35%) due to infections, graft-vs.-host disease and multi organ failure [94]. It is now generally accepted that AlloSCT with myeloablative conditioning is generally indicated only in few patients with MDS while for most other patients, particularly older patients, a reduced-intensity transplant can be still offered from an HLA-identical donor [95]. Different transplant modalities of different intensities and donor sources are now active. Most of them are still investigational. There are several relevant concerns regarding AlloSCT in MDS. These include timing of transplant and choice of the best approach for patients that achieved a complete response to hypomethylating agents prior to AlloSCT. A study from the International Blood and Bone Marrow Transplant Registry (IBMTR) indicated that early transplantation in higher-risk MDS patients was associated with better outcome [96].

Due to the observation that blast percentage before transplantation (especially if >10%) is clinically associated with higher risk of relapse [97], a cytoreductive

regimen (chemotherapy or hypomethylating agents) are generally administered to patients with excess of blasts. Prospective studies on the topic are lacking to drive definitive conclusions.

Regarding the timing of BMT, both in younger higher-risk patients treated with myeloablative conditioning transplantation and in older patients receiving non-myeloablative regimens, early stem-cell transplantation was associated with a survival advantage compared with other therapeutic options [97, 98]. By contrast, early stem-cell transplantation had an adverse effect on survival in lower-risk patients [96, 97].

The evolution of transplantation techniques, today characterized by a better control of graft-vs.-host disease (GVHD) and infectious complications, the consequent increased availability of unrelated donors with the use of peripheral blood stem cells and the overall lower toxicity of the transplant have extended the availability of this procedure to patients older than 60 years if clinically fit [99].

There is no consensus regarding the optimal treatment of patients with intermediate-1 IPSS and intermediate IPSS-R risk and this remains a burning issue in the treatment of MDS patients. Patients who want to focus on quality rather than quantity of remaining years may favor supportive care and hypomethylating agents. In contrast, more intensive chemotherapy with or without transplantation may be chosen by younger, fit patients who prefer to deal with the higher risk of treatment-related mortality and morbidity in order to achieve an increase in survival. The possible role of hypomethylating agents as a bridge therapy to bone marrow transplantation is under consideration. Treatment with hypomethylating agents may delay progression to AML before HSCT. Such a strategy is especially relevant for MDS patients with intermediate-2 or high-risk IPSS disease, for whom the average time to AML progression may be short [100].

Figure 2 summarizes the approach to treatment in these subgroups of patients.

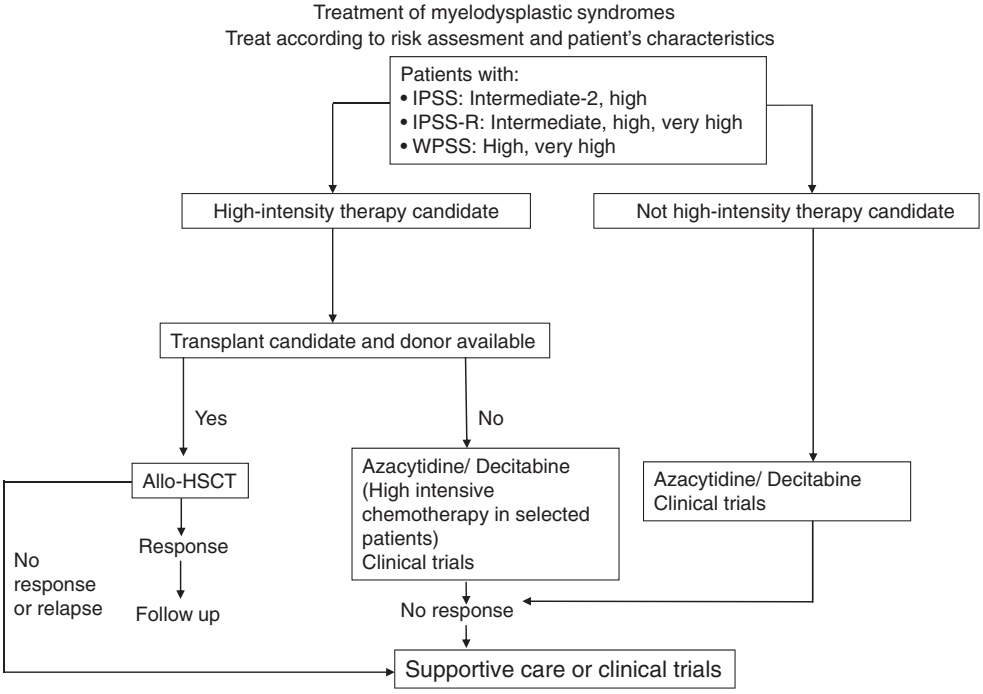

Figure 2: Treatment strategies for patients with myelodysplastic syndromes according to the International Prognostic Scoring System (IPSS), WHO Prognostic Scoring System (WPSS) and Revised IPSS (R-IPSS).
Intermediate-2, high or very high risk myelodysplastic patients have different treatment strategies based on patients' characteristics and their elegibility for high intensity therapy. If they are candidate to bone marrow transplant and a donor is available, patients should be treated with allo hematopoietic stem cell transplantation. A bridging therapy should be considered in order to decrease marrow blasts to an acceptable level prior to transplant. High intensity chemotherapy (only in selected fit patients) or azacytdine/decitabine treatment should be considered for fit patients eligible for high intensity chemotherapy. Not high intensity therapy candidates should be treated with azacytdine/decitabine. Supportive care or clinical trials should be considered for non-responding or relapsing patients. IPSS, International Prognostic Scoring System; WPSS, World Health Organization Prognostic Scoring System; IPSS-R, Revised IPSS; allo HSCT, allo hematopoietic stem cell transplantation.

Conclusions and future directions

The central problem with MDS is their heterogeneity. MDS are a heterogeneous group of hematological diseases, with different clinical and prognostic features. The MDS are challenging for clinicians and pathologists due to the clinicopathologic heterogeneity of the disease and overlapping features with other benign and malignant disorders. Currently, the initial evaluation of a patient with suspected MDS focuses on a detailed medical history, review of the peripheral blood and bone marrow by an expert hematopathologist and risk stratification using laboratory results, morphology and cytogenetics. More sophisticated technologies, including multi-color flow cytometry, FISH, next-generation sequencing, and others are emerging and promise to offer significant refinements in diagnostic, prognostic and, hopefully, therapeutic information.

Since MDS range from indolent conditions with a long natural history to subtypes analogous to AML, clinical decision-making concerning treatment modalities and timing of interventions is challenging. Currently the prognosis of patients with MDS can be predicted using a number of scoring systems. In general, all these scoring systems include analysis of peripheral cytopenias, percentage of blasts in the bone marrow and cytogenetic characteristics. The most common used system was IPSS and tends to shift to IPSS-R. Although parameters such as hemoglobin level, blast count, and high-risk cytogenetic abnormalities will continue to retain strong independent prognostic value, the current era of genomics will provide us with additional parameters including new molecular markers, which may significantly contribute to a refined risk assessment of MDS and allow us to move towards a more patient-tailored therapeutic approach. Newer technologies with next-generation targeted deep sequencing and whole-genome and -exone sequencing have identified several recurrent mutations that play a pivotal role in the pathophysiology of MDS and the impact of these genetic changes on disease phenotype.

In recent years, several gene mutations have been identified among patients with MDS that may, at least partly, explain the clinical heterogeneity of the disease course and may influence prognosis. A large variety of gene mutations will be present in most patients with newly diagnosed MDS, including most patients with normal cytogenetics. Several studies examining large numbers of MDS bone marrow or peripheral blood samples have identified more than 40 recurrently mutated genes, with more than 80% of patients harboring at least one mutation.

Thus, the combined analysis of these gene mutations and the IPSS or IPSS-R may improve the risk stratification provided by these prognostic models alone.

Future molecular analysis could predict not only the risk of disease, but also the response to therapy allowing a molecular based tailored therapy. Other important goals include the determination of the clinical impact of all these mutations on response to therapy and MDS patients' survival in large cohorts of patients. With the introduction of more sophisticated molecular techniques like gene expression profiling, it might become possible not only to predict the natural course of the disease, but also to identify patient populations that are prone to respond to specific drugs especially designed for specific genetic lesions. Sequencing-based studies suggest that multiple mutations may play a role in the progression of MDS to AML. Further work is necessary to understand the molecular basis of leukemic transformation in MDS syndromes.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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