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The Molecular Anatomy of Myelodysplastic Syndromes: An Update

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic disorders arising from blood stem cells. Their main characteristics are a wide range of cytopenias and ineffective haematopoiesis. The purpose of this review was to summarise the current knowledge on the molecular biology of MDS the impact of gene mutations on the outcome of the disease.
Materials and Methods: A thorough search of PubMed was conducted and a review of the current literature.
Results: The introduction of novel techniques in molecular biology (real-time PCR, next generation sequencing) has led to the identification of a series of mutations associated with prognosis of MDS patients and response to therapy and the development of novel prognostic models classifying MDS patients into risk groups. Those mutations include chromosomal aberrations and point mutations involving genes associated with mRNA splicing, methylation, signal transduction, regulation of transcription and cell cycle and other cellular pathways.
Conclusion: Further studies will be needed in order to define the precise role of those mutations in prognosis and therapy of MDS.

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1. INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal haemopoietic disorders arising from a haematopoietic stem cell. They are characterised by inefficient haematopoiesis, manifesting with morphological dysplasia in one or more haemopoietic cell lineages in bone marrow, blast percentage of less than 20% in the peripheral blood and bone marrow, as well as by the presence of cytogenetic and molecular genetic abnormalities in more than 90% of *de novo* cases, and a variable tendency to develop acute leukaemia in the absence of leukocytosis [1]. The clinical manifestation of MDS varies from indolent disease with mild cytopenia and prolonged life expectancy to aggressive disease with severe cytopenia, increased risk of AML progression and limited life expectancy. MDS are associated either with aging (de novo cases) or with exposure to various compounds such as smoking, benzene, ionising radiation, antineoplastic or immunosuppressive therapy (therapy-related MDS, t-MDS).

The incidence of MDS is 5 cases per 100,000 people, mostly in men (approximately twofold higher) [2-4]; one plausible explanation for this male predominance is the X-linked microRNAs (miRNAs), which target the E3 ubiquitin ligase CBL and seems to play an important role in MDS [5]. In Western countries, the incidence for people over 70 y.o. is 22-45 per 100,000 people and increases with age [6,7].

The main goals of therapy aim at the improvement of life quality of the patients, prolongation of overall survival and delaying progression to AML [8-11].

2. MDS CLASSIFICATION

The last update of *the* MDS classification by *the* World Health Organization (WHO) was in 2016 (Table 1) [12]. In 2001 and 2008, WHO in association with the Society for Haematopathology and the European Association for Haematopathology published a classification of haemopoetic and lymphoid neoplastic disorders, as part of the third edition and fourth edition of *WHO Classification of Tumors* "blue book" monographs. In this revision, various genetic and molecular data were incorporated along with morphological, cytochemical and immunophenotypic characteristics and clinical data in diagnostic algorithms for various myeloid neoplasms. In the WHO classification, 'myeloid' cells include granulocytes (neutrophils, eosinophils and basophiles), monocytes/macrophages, erythroid cells, megakaryocytes and mast cells [13].

According to the WHO criteria, a myeloid neoplasm with 20% or more blasts in the peripheral blood or in the bone marrow are considered acute leukaemia (AML); this could happen either *de novo* or as a progression from a previously diagnosed MDS or a myeloproliferative neoplasm (MPN) [12,13].

The diagnosis of MDS is based on the evaluation of peripheral blood (PB) and bone marrow samples (BM) using standard haematology techniques such as complete blood count (CBC) and optical microscopy in combination with cytochemistry for the detection of iron in BM.

BM biopsy is the gold standard for the estimation of BM cellular content. Additionally, bioptic material is available for immunohistochemical detection of markers, which are useful for the diagnosis and prognosis of the disease such as CD34 [1], TdT [2] and Ki67 [3] [14]. The differential diagnosis of MDS includes other causes of secondary dysplasia such as dyserythropoietic anemia.

Cytopenia is an important marker and a prerequisite for MDS diagnosis. Those criteria have been set in the initial IPSS prognosis index: Hb <10 g/dL, platelet count $<100X10^9$ /L and absolute neutrophil count $<1.8X10^8$/L [12]. Rarely MDS may be present with mild anaemia or thrombocytopenia not correlating with the previous levels. Monocytes in the peripheral blood must be $<1X10^9$/L. However, the WHO classification marks the degree of dysplasia and blast percentages for disease classification and specific cytopenias are less important on MDS classification. Lineages with morphologic dysplasia are not correlated with cytopenias in many MDS cases [15-17]. A diagnosis of MDS can be in some cases with milder levels of

[1] *CD34 is a transmembrane phosphoglycoprotein, first identified on hematopoietic stem and progenitor cells*
[2] *Tdt: Terminal Deoxy Transferase*
[3] *nuclear protein associated with tumour cell proliferation*

cytopenia. Cytopenia must be stable for more than four months, unless it is associated with a specific karyotype or bilineage dysplasia, in which case two months of stable cytopenia are required. Moreover, all other possible underlying causes of cytopenia should be excluded [18,19].

In the examination of PB smears, the occurrence of nucleated red blood cells (RBC) with morphological stigmata or dyserythropoiesis is usual, as well as the detection of two RBC populations. Dysgranulopoiesis of neutrophils is not a standard finding, whereas the nucleus can show abnormal lobulation (such as pseudo-pelgers) [20].

BM cellularity is another important aspect in MDS. In most cases, bone marrow aspirates show hypercellularity of the granulocytic or the erythroid series, whereas in 30-40% of the cases BM show normal cellularity and in 10% hypocellularity [21].

The percentage of blasts either in BM aspirates or in peripheral blood film preparations is also an essential criterion in MDS classification and a risk factor in the revised international prognostic scoring system (IPSS-R) as shown below [22]. Unclassified MDS is defined by the presence of 1% blasts- in two separate observations- in the PB and <5% in BM aspirates. The upper blast threshold for the diagnosis of MDS is 20% in the PB and/or BM. The nucleus is nucleolated with a finely dispersed chromatin pattern and scarce basophilic cytoplasm [21]. Azurophilic granules may be or may not be present; in case of more granules (granular blasts) the absence of a Golgi area is crucial for the differential diagnosis between blasts and promyelocytes [23]. The presence of Auer rods in blasts either in the PB or BM marks an unfavourable prognosis leading to classification of MDS with excess blasts type 2 (MDS-EB2).

The detection of dysplasia is also usual in MDS in one or in many blood cell lineages (erythroid, neutrophilic and megakaryocytic); dysplastic features should be present at least in 10% of the erythroid precursors and/or in 10% of the granulocytic cells (counting at least 200 cells) and/or in a minimum of 10% of megakaryocytes (counting at least 30) [24]. Alternatively, an elevation of more than 15% of ring sideroblasts is necessary (or more than 5% with the presence of SF3B1 mutation).

BM biopsy is also important in the diagnosis of MDS, especially in 'dry tap' cases. It provides crucial information regarding BM cellularity and architecture, the degree of fibrosis, the anomalous localisation of granulocyte precursors in intertrabecular areas, the presence of micromegakaryocytes or clusters of megakaryocytes [25]. The presence of blasts can be detected using anti-CD34 antibodies, whereas analysis using anti-CD117 [4] is also useful.

The use of karyotype is also important, and the detection of an MDS-related chromosome aberration [del(5q), -7 or complex karyotype] can establish an MDS diagnosis, as discussed elsewhere [19].

The role of immunophenotyping by flow cytometry is important in the characterisation of the blast population in order to evaluate therapeutic results (detection of minimal residual disease), although, according to WHO, the reference method for the diagnosis of MDS is the blast percentage in BM aspirates.

3. PRE-MDS CONDITIONS

A series of other clinical entities have been recognised during the last decade, which should be taken into consideration in the differential diagnosis of MDS. Those include idiopathic cytopenia of unknown significance (ICUS), idiopathic dysplasia of unknown significance (IDUS), clonal haematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of unknown significance (CCUS):

- ICUS: it is characterised by persistent cytopenia of any degree in any of the known blood cell lineages (ICUS-A anemia, ICUS-N neutropenia, ICUS-T thrombopenia or ICUS-PAN bi/pancytopenia) [19], no or mild dysplasia (<10%) and blast cells <5%. The clinical course of this clinical entity is variable and unpredictable. Progression to MDS and AML can be observed in a subset of patients [26-28].
- IDUS: it is characterised by mild or extensive BM dysplasia (≥10%), blast cells <5%, macrocytosis, Pelger-Huet anomaly or hypogranulated neutrophils, with no

[4] *CD117 is a mast/stem cell growth factor receptor , also known as proto-oncogene c-Kit or tyrosine-protein kinase Kit or CD117, and a tyrosine kinase protein receptor that, in humans, is encoded by the KIT gene [19].*

apparent cytopenia and no other MDS criteria [19, 29-31].

- CHIP: it is characterised by the presence of one at least somatic mutation which is also found in MDS (see below), no or mild dysplasia (<10%), blast cells<5%, the absence of persistent cytopenia and the exclusion of MDS or other haematopoietic neoplasms [19,32].
- CCUS: it is characterised by cytopenia and clonal abnormalities, with no or mild dysplasia (<10%), blast cells <5% or other criteria to diagnose MDS or other bone marrow neoplasm [19,32].

4. PROGNOSTIC MODELS IN MDS

An important aspect in the management of patients with MDS is the prognosis and the calculation of risk of progression to AML; several prognostic systems have been developed for that reason [14,33-39]. Those systems include a series of features and variables such as:

(a) Laboratory findings such as haemoglobin concentration, absolute neutrophil count, platelet count, ferritin levels, serum lactate dehydrogenase (LDH) revels, serum albumin levels and peripheral blast percentage
(b) Pathological findings such as WHO classification, bone marrow blast percentage, cytogenetic analysis and flow cytometry results
(c) Biological findings such as molecular data from DNA or RNA sequencing, methylation profile and microRNA profiles

Among them, the most commonly used in clinical practice are the World Health Organization classification-based prognostic scoring system (WPSS) [40], the MD Anderson Global Prognostic Scoring system and lower risk prognostic scoring system (LRPSS) [36], the International Prognostic Scoring System (IPSS) [41], the revised IPSS (IPSS-R) [42] and the MD Anderson Global Prognostic System (MDAPSS) [22].

The WPSS uses pathological, clinical factors associated with patients including WHO subgroups, cytogenetics and the degree of anaemia [40]. This model offers a dynamic risk assessment, although its performance after treatment with hypomethylating agents is limited [18], and it does not apply to patients with secondary or therapy-related MDS.

4.1 International Prognostic Scoring System (IPSS)

The IPSS was developed in 1997 following studies carried out in 816 patients with *de novo* MDS on supportive care [41] . The IPSS model included the following parameters:

1. BM blast percentage
2. Conventional cytogenetics
3. Cytopenias
4. Serum LDH
5. β2-microglobulin
6. Ferritin
7. BM fibrosis
8. Co-morbidity

The first three parameters (1-3) were initially used as the sole criteria for the consideration of MDS patients; the next (4-8) were later added to the criteria and are still in use today [8].

The IPSS model, however, excludes patients with secondary MDS or CMML and can only be used at the time of the initial diagnosis, and before initiation of treatment with hypomethylating agents [11,33,43]. Another disadvantage of the IPSS model is its failure to consider the severity of cytopenias in low-risk individuals.

4.2 World Health Organization (WHO) Classification-Based Prognostic Scoring System (WPSS)

This model has been developed using data from a study of 1165 treatment-naïve patients [44] and includes as prognostic factors WHO subgroups, karyotype and transfusion requirement. According to this model patients are stratified in five risk groups. This system is time-dependent and could be applied as a dynamic model during the course of the disease. Patients with therapy-related MDS and secondary MDS were excluded from the analysis; moreover, the model does not include platelet or white blood counts. The model was revised to include the degree of anaemia (Hb <9 g/dL in men and <8 g/dL in women) [40].

Table 1. WHO 2016 classification for Myelodysplastic syndromes

Name	Dysplastic lineages	Cytopenias	Ring sideroblasts as % of marrow erythroid elements	Bone marrow blasts	Peripheral blood blasts	Cytogenetics
MDS with single lineage dysplasia (MDS-SLD)	1	1 or 2	<5*-15%	<5%	<1%	Any unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	2 or 3	1-3	<5*-15%	<5%	<1%	Any unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)						
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	1	1 or 2	≥5*-15%	<5%	<1%	Any unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	2 or 3	1-3	≥5*-15%	<5%	<1%	Any unless fulfills all criteria for MDS with isolated del(5q)
MDS with excess blasts (MDS-EB)						
MDS-EB1	0-3	1-3	None or any	5-9%	2-4%	Any
MDS-EB2	0-3	1-3	None or any	10-19%	5-19%	Any
MDS unclassifiable (MDS-U)						
With 1% blood blasts	1-3	1-3	None or any	<5%	=1%**	Any
With single lineage dysplasia and pancytopenia	1	3	None or any	<5%	<1%	Any
Based on defining cytogenetic abnormality	0	1-3	<15%***	<5%	<1%	MDS-defining abnormality
Refractory cytopenia of childhood (RCC)	1-3	1-3	None	<5%	<2%	Any
MDS with isolated del(5q)	1-3	1-2	None or any	<5%	<1%	del(5q) alone or with 1 additional abnormality except -7 or del(7q)

**The mutation SF3B1 is present*

***1% blasts in peripheral blood should be recorded in 2 separate occasions.*

****In cases with ≥15% ring sideroblasts, there is by definition significant erythroid dysplasia and therefore are classified as MDS-RS-SLD.*

4.3 MD Anderson Global Prognostic Scoring System (MDAPSS) and Lower Risk Prognostic Scoring System

The initial model employed for the classification of MDS was the MDAPSS, which considered both patients who had already received treatment for MDS and those who had not been treated. MDAPSS comprised a multitude of factors, amongst which chromosome 7 abnormalities, platelet and white blood cells count, history of previous transfusions, the percentage of blasts in bone marrow, complex karyotypes, performance status, co-morbidities and age [22]. This model is presently in limited use, due to complexity reasons. Patients with proliferative CMML and MDS related to therapy (secondary) were also taken into consideration [22].

Lower-risk patients, who cannot be accurately assessed with the IPSS model, can be evaluated with another classification model, the MDA Anderson Lower-Risk Prognostic Scoring System (LRPSS) [45]. This system accounts for the risk upgrading of approximately 1/3 of patients with IPSS lower-risk disease and plays a paramount role in therapeutic schemes; it employs age, blast percentage, cytogenetics and the grade of cytopenias (severity of anaemia and thrombocytopenia) as factors for consideration [46-48].

4.4 The Revised International Prognostic Scoring System (IPSS-R)

A revision of the IPSS model occurred in 2013, when the World Health Organization (WHO) included more than 7000 patients with primary MDS and no prior treatment, in a study that led to the development of the IPSS-R model, which further encompasses different blast percentage cutoffs, the severity of cytopenias, and more variable factors in association with cytogenetics [42]. Clinical variables, which were not found to be independent prognostic factors such as serum LDH, ferritin levels and serum B2 microglobulin, were not included in this mfodel (Table 2a, b, c).

Due to the fact that age was not initially viewed as a factor in the IPSS-R model, the following formula can be used to integrate it:

> (Years − 70) X [0.05 − (IPSS-R risk score X 0.005)] [42]

This model stratifies patients into five risk groups and better predicts the disease progression since 18% of patients with high risk MDS according to IPSS were downstaged, whereas 27% were upstaged [42].

The revised IPSS-R classification system can still not be employed with accuracy in the case of patients with secondary/therapy-related MDS [10, 49-52]. However, its validity has been substantiated in individuals who are on first line therapy with HMA, lenalidomide, or have received allogeneic stem cell transplantation [48-54]. This model also included patients with bone marrow blasts of 20-30% which according to WHO are classified as AML. However, even this model is not predictive of the final outcome in patients with secondary MDS [11], and its utility at the time of failure with hypomethylating agents is limited [10].

Table 2a. IPSS-R prognostic score values

Parameter	**Categories and associated scores**				
Cytogenetic risk group	**Very good**	**Good**	**Intermediate**	**Poor**	**Very poor**
	0	*1*	*2*	*3*	*4*
Bone marrow blasts	≤ 2%	2-5%	5-10%	≥10%	-
	0	*1*	*2*	*3*	
Hemoglobin (g/dL)	≥ 10	8-10	<8	-	-
	0	*1*	*1.5*		
Platelet count (x10^3/μL)	≥ 100	50-100	<50	-	-
	0	*0.5*	*1*		
Absolute neutrophile count	≥ 0.8	<0.8	-	-	-
	0	*0.5*			

Table 2b. Cytogenetic prognostic groups within the IPSS-R

Prognostic subgroups	Cytogenetic abnormalities
Very good	−Y, del(11q)
Good	Normal, del(5q), del(12p), del(20q), double abnormalities including del(5q)
Intermediate	del(7q), +8, +19, i(17q), any other single abnormality not listed in other risk groups or double independent clones
Poor	−7, inv(3)/t(3q)/del(3q), double abnormalities including −7/del(7q), Complex with 3 abnormalities
Very poor	>3 abnormalities

Table 2c. Prognostic groups within the IPSS-R system

Risk group	Total score	Proportion of patients in category (%)	Median survival (survival data based on n=7012) (years)	Time until progression (AML data based on n=6485) (years)
Very low	0-1.0	19	8.8	Not reached
Low	1.5-3.0	38	5.3	10.8
Intermediate	3.5-4.5	20	3.0	3.2
High	5.0-6.0	13	1.5	1.4
Very high	>6.0	10	0.8	0.7

4.5 Other Prognostic Systems

Another prognostic system developed by Garcia-Manero et al. [46] stratifies low-risk MDS patients according to IPSS with more aggressive disease; using a multivariate analysis, older patients (≥ 60 years old), anemia (<10 g/dl), low platelets, bone marrow blasts ≥4% and poor risk cytogenetics were independent prognostic factors. Garcia-Manero et al. [46] identified three risk categories: category 1 (21% of patients with median overall survival 80.3 months), category 2 (48% with median overall survival 26.6) and category 3 (31% with median overall survival 14.2 months) [46].

In another model focusing on MDS patients who received chemotherapy or radiation for other cancers [43], multivariate analysis showed that age ≥ 65 years old, poor cytogenetics (-7 and/or complex), Eastern Cooperative Oncology Group performance statuses 2-4, WHO MDS subtype (RARs or RAEB-1/2), anaemia (Hb <11 g/dl), low platelets (<50.000/μl) and transfusion dependency were independent prognostic factors [43]. Using this model they identified three risk categories: good (0-2 risk factors, median OS 34 months), intermediate (3-4 risk factors, median OS 12 months) and poor (5-7 risk factors, median OS5 months) [43].

In older patients with MDS, an important aspect is the high degree of comorbidities, which are present and may affect the therapeutic response [55-58], such as cardiac disease, diabetes, renal, pulmonary and liver problems. In an Italian study of 504 MDS patients, cox regression analysis showed that cardiac disease, severe liver disease, severe pulmonary disease, renal disease and solid tumors were independent prognostic factors for non-leukaemia mortality [59]. The MDS comorbidity index was developed as a result of this analysis and stratified patients in three categories (low, intermediate and high-risk groups) with median OS 43.0, 23.0 and 9.0 months respectively.

Other clinical factors, which seem to affect MDS patients, are bone marrow fibrosis, albumin, ferritin and LDH levels, aberrant expression of certain myeloid markers by flow cytometry and expansion of memory T-cells in patients with lower-risk disease [18,60-64].

5. CYTOGENETIC ABNORMALITIES IN MDS

Certain cytogenetic abnormalities are linked to MDS according to the WHO 2008 classification system (Table 3). Those abnormalities - detected by conventional karyotype- are MDS-defining even in the absence of diagnostic morphologic dysplasia and are located at chromosomes 5, 7, 11, 12, 13, 17 and X.

The del(5q) is the only cytogenetic abnormality which is linked to a separate MDS subtype. It is usually found in women and, due to loss of several genes including *RPS14* (ribosomal protein S14) , *SPARC* (Secreted Protein Acidic and Cystein Rich) and *CSNK1A1* (Caseine Kinase 1 Alpha 1 gene), leads to blockage of erythroid differentiation with hyposegmented or non-segmented megakaryocytes, severe macrocytic anaemia, less than 1% blasts in peripheral blood and 5% in bone marrow, normal or increased platelet count and good prognosis [21,65,66]. del(5q) is also connected with dysregulated expression of certain miRNA mapped in the 5q region such as miRNA-145 and miRNA-146a [67,68].

Inversions and translocations at chromosome 3 [t(3;21) or inv(3)] are found in MDS and AML with increased abnormal megakaryocytes, increased blasts and rapid AML evolution, whereas del(11q) is associated with increased iron deposition. Del (20q) is connected with dyserythropoiesis and dysmoprhic megakaryocytes, whereas -7 is associated with micromegakaryocytes and has a very negative prognostic significance [69]. del(17p)/i(17q) is associated with small neutrophils and Pelger-Huet anomaly with a vacuolated cytoplasm and with poor prognosis, whereas del (20q) as an isolated cytogenetic abnormality is associated with thrombocytopenia [21]. The presence of trisomy 8, Y deletion or del (20q) are not MDS-defining; trisomy 8 is connected with intermediate prognosis in IPSS-R and good response to immunosuppressive therapies, durable reversal and transfusion independence, and Y deletion has a very good prognosis. Loss of Y chromosome (LOY) is observed in 5%-15% of male MDS cases; patients with LOY show a longer overall and AML-free survival, when compared with MDS patients with normal karyotype. A study by Ganster *et al.* [70] showed that CD34 positive myeloid cells have a higher susceptibility for LOY than CD31[5] cells, which may indicate an early step from polyclonality to clonality. In general, monosomal karyotype is associated with worse overall survival independently of other factors [71-73] in cases with 4 or less aberrations, but in cases with 5 or more, monosomy loses its predictive impact [74]. Also, complex karyotype with more than 3 re-arrangements (7-8% of *de novo* MDS cases) is also associated with unfavorable outcome [75, 76].

Although cytogenetic markers are not used to define MDS subtypes, they are directly correlated with prognosis as it is shown in the five cytogenetic prognostic groups in the IPSS-R [42, 78] (see above); therefore a BM karyotype is necessary in each new MDS case.

6. GENE MUTATIONS

Except from cytogenetic abnormalities, a series of mutations have also been identified in the majority of MDS patients [37, 38]. Some of them are frequently detected and are associated with unfavorable or favorable prognosis in MDS cases as depicted in Table 4 These include:

(a) Mutations in the spliceosome machinery which include the following genes: Mainly *SF3B1* (Splicing Factor 3b subunit 1), *SRSF2* (arginine-rich splicing factor 2), *U2AF1* (U2 auxilliary factor 1), *ZRSR2* (Zinc finger CCCH-type, RNA Binding Motif and Serine/Arginine Rich 2) and less frequently (1-2% of the cases) *PRPF8* (pre-mRNA processing factor 8 homolog), *SF1* (Splicing Factor 1), *SF3A1* (Splicing Factor 3A subunit 1) and *U2AF2* (U2 Small Nuclear RNA Auxiliary Factor 2) . They are the most commonly mutated gene class in MDS cases (45-85%) [79, 80]. *SF3B1* mutations seem to be associated with a lesser degree of cytopenias, improved overall survival and improved leukaemia-free survival [35, 74, 79-81]; they are detected frequently in refractory anemia with ring sideroblasts (MDS-RS) and MDS with multilineage dysplasia (MDS-MLD) with ring sideroblasts [82-86]. *U2AF1* mutations are associated with reduced cellular proliferation and inferior overall survival [76,79,87,88] *SRSF2* mutations are associated with neutropenia and thrombocytopenia, and, therefore, have a poor prognosis and increased incidence of transformation to AML [89]. Similarly *ZRSR2* mutations are connected with isolated neutropenia in MDS cases, but no detectable effect on clinical outcomes [87,90], whereas *PRPF8* mutations with ring sideroblast phenotype [91].

(b) Epigenetic mechanisms which include the following genes: *TET2* (TET methylcytosine dioxygenase 2), *IDH1*, *IDH2* (isocitrate dehydrogenase 1 and 2),

[5] *Platelet endothelial cell adhesion molecule (PECAM-1) also known as cluster of differentiation 31 (CD31) is a protein that in humans is encoded by the PECAM1*

DNMT3A (DNA methyltransferase 3A), *ASXL1* (additional sex combs-like 1) and *EZH2* (enhancer of zeste homologue 2). The role of hypermethylation in MDS has been proven to be crucial, and, therefore, hypomethylating agents are one of the most effective treatments [92]. *TET2* mutations are associated with aging of hematopoietic cells and are considered to be initial mutations during the course of malignant transformation [82,93]. Although they seem to have no prognostic significance in MDS, their presence is associated with better response to hypomethylating agents such as azacitidine and decitabine [18,94,95]. *IDH1* and *IDH2* are connected with an unfavourable and controversial outcome respectively since they are detected in 4-12% of MDS cases and in 10-15% of AML cases [69]. *DNMT3A* are rare in MDS and are connected with unfavorable prognosis and faster AML transformation [79, 82, 96-98]. *EZH2* mutations have been reported in 6-12% of MDS cases with unfavorable prognosis [98]. *ASXL1* is also common mutation in MDS cases (14-21%) and it is also associated with worse prognosis and AML transformation [89,99].

(c) Signal transduction kinases which include the following genes: *FLT3-ITD* (FMS-like tyrosine kinase-3, Internal Tandem Duplication), *MPL* (Myeloproliferative Leukaemia Protein), *KIT*, members of the *RAS/RAF/MEK* pathway (*KRAS, NRAS, CBL, NF1, PTPN11)*, *GNAS* and *JAK2*. All of them are rather rare mutations and are mostly connected with AML transformation, except for *JAK2,* which is described in 5% of MDS cases with megakaryocytic proliferation and in 50% of MDS/MPN overlapping refractory anemia with ring sideroblasts and thrombocytosis [71,100,101].

(d) Transcription factors, tumor suppressors and cell cycle regulators including the following genes: *RUNX1* (RUNt related transcription factor 1), *ETV6* (ETS varian gene 6), *TP53*, *NPM1* (nucleophosmin 1), *CEBPA* (CCAAT enhancer binding protein alpha), *WT1* (Wilms tumor 1), *GATA1/2* (GATA binding protein 1 and 2), *SPI1* (Spi-1 proto-oncogene) [82,102]. *RUNX1* mutants are common in MDS cases (10-20%) and are associated with severe thrombocytopenia and adverse outcome. *ETV6* is rather rare (2-5%) and their role is rather unfavorable [89]. *ETV6-RUNX1* translocations have been detected frequently in B-ALL [103]. *TP53* is always associated with poor prognosis, but in most of the MDS cases *TP53* mutations are associated with complex karyotypes [104]. The other genes (*NPM1/CEBPA/GATA2/GATA1/ SPI1/WT1*) are more frequently mutated in AML and in less than 5% of MDS cases [105,106].

(e) Cohesin complex genes which include: *STAG2* (Stromal Antigen 2), *RAD21*, SMC1A (Structural Maintenance of Chromosomes 1A) and *SMC3* (Structural Maintenance of Chromosomes 3) [107]. Mutations in those genes are associated with poor prognosis, especially at STAG2.

(f) Other genes: A series of other mutations have also been described in MDS patients, but their prognostic role is not clear; these include mutations in chromatin modifiers (Histone-lysine N-methyltransferase 2D-*MLL2/KMT2D*, Lysine Demethylase 6A-*KDM6A*, Alpha Thalassemia/Mental Retardation Syndrome X-linked *ATRX*), other transcription factors (cut-like homeobox 1-*CUX1*, E1A associated protein P300 -*EP300*, Interferon Regulatory Factor 1- *IRF1*) and signaling factors (Cycline dependent kinase inhibitor 2A-*CDKN2A*). The role of miRNAs in the development of MDS is also noted in mouse models as well as the association of MIR145 with del(5q) MDS [108, 109]. A series of other studies indicate the role of mitochondrial DNA in MDS pathogenesis; mutations of *ABCB7* (ATP Binding Cassette Subfamily B Member 7) are associated with ring sideroblast formation [110, 111]. Mutations in *SETBP1* (Set binding protein 1) are associated with leukaemic transformation and poor prognosis. A recent study by Visani et al. [112] showed that mutations at *MTHFR1* (methylenetetrahydrofolate reductase 1), *TS* (thimidylate synthase) and *XRCC1* (X-ray repair cross-complementing protein 1) may be connected with worse prognosis in MDS patients. Gene expression studies have shown that decreased expression of *LEF1* (Lymphoid Enhancer Binding Factor 1), *CHD1* (Chromosome helicase DNA binding protein 1) and increased expression of *WT1*, *MN1* (Meningioma 1) and *PTH2R* (Parathyroid hormone 2 receptor) are associated with inferior overall survival [113]. The role of CD95[6] (Fas, APO-1, TNFRSF6, APT1), a

[6] *Fas or FasR, also known as **apoptosis antigen 1** (**APO-1** or **APT**), cluster of differentiation 95 (CD95) or tumour*

member of the death receptor family was explored by Raimbault et al. [114]; CD95 was found to be overexpressed on CD34+ progenitor and erythroblasts in two thirds of patients with lower-risk MDS. Moreover, the genetic polymorphism 1377G>A was shown to be associated with risk of developing AML [114].

Similar mutations have also been identified in haematopoietic cells of healthy elderly patients without MDS, a condition called "clonal haematopoiesis of indeterminate potential" or "age-related clonal haematopoiesis (CHIP or ARCH) and which is not yet fully understood [32, 115, 116]. This condition has been recognised since the early 1990s in 30-40% of elderly women and it is connected mostly with mutations in *DNMT3A*, *TET2* and *ASXL1* [3,115-121], but also in *SF3B1* and *SRSF2* in older individuals [117,122,123]. Somatic *TET2* mutations are present in elderly individuals [93]. Other genetic alterations include detection of *BCL-2* and *BCR-ABL* re-arrangements, copy number variations at 5q, 11q, 17p and 20q, altered protein function of more than 40 somatic point-mutations [93, 120, 124-126]. It is quite possible that the accumulation of mutations in healthy asymptomatic individuals initiates clonal expansion and precedes the development of cancer for many years [93,115,116,120,122, 127].

Several other mutations have been detected in cases of juvenile/familial MDS/AML in other cancer genes such as *CEBPA, GATA2, BRCA1* (breast cancer 1)*, DDX41 (Dead Box Helicase 1), SAMD9* (sterile alpha motif domain-containing 9) and *SAMD9L* (sterile alpha motif domain-containing 9 ligand) [128-131]. 10-20% of childhood MDS harbor germline mutations and belong to the newly established WHO category of myeloid neoplasms with germline predisposition. Most of them are connected with mutations in the *RAS* pathway such as in *NF1* (neurofibromatosis type 1)*, PTPN11* (protein tyrosine phosphatase non-receptor type 11) and *CBL* (casitas b-lymphoma) [4,132-134].

Next generation sequencing (NGS) technologies such as whole genome and whole exome sequencing, gene expression profiling (GEP) and single nucleotide polymorphism arrays (SNPs) have been employed in the search of novel mutations associated with MDS [34,35,80,82, 102]. Although technology has revolutionised the research of mutations in MDS patients, it is still unclear which of them have a prognostic value and seem to affect various biological pathways such as DNA methylation, chromatin modification and RNA splicing; a few of them are present in more than 10% of MDS patients, whereas most of them are in less than 1-2% [82,102].

The role of those mutations in MDS pathogenesis has been described. Spliceosome mutations seem to contribute to dysplasia in MDS. *SF3B1* and PRP *are* associated with ring sideroblasts, *define* MDS-RS and *are* connected with indolent clinical course and better prognosis [82,84,102,135]; *SF3B1* mutations are present in ~25% of all MDS cases and in more than 85% of cases with refractory anaemia with ring sideroblasts. The mutation K700E seems to be sufficient to cause the characteristic features of MDS including macrocytic anemia, erythroid dysplasia and expansion of LT-HSCs in the bone marrow [136]. On the other hand, *SF3B1* mutations are associated with adverse outcome in chronic lymphocytic leukaemia [84, 137, 138]. DNA methylation genes are also associated with MDS; *TET2* is detected in patients with normal karyotype and is associated with CMML in combination with *SRSF2* or *ZRSR2* [82,102]. *DNMT3A* and *TET2* mutations are associated with overexpression of arginase 1, a biomarker of immune deregulation in MDS and CMML [139]. Mutations in the *PRC2* (polycomb repressive complex 2) are also present in MDS; *PRC2* functions as a histone methyltransferase that trimethylates histone H3 on lysine 27, a mark of transcriptionally silent chromatin. *EZH2* encodes a catalytic subunit of PRC2 and it is also frequently mutated in MDS cases. *EZH2* mutations lead to loss of function. ASXL1 is a tumor suppressor protein, which is also mutated in MDS, stabilises PRC2 [33,140,141]. *ASXL1* also seems to interact with *BRCA-1* associated protein (BAP1) [5]. Loss of *ASXL1* leads to reduced erythroid differentiation and progenitor development due to increase apoptosis and increased accumulation of cells in the G0/G1 phase, as shown by knockdown of *ASXL1* experiments [142]. Mutations in genes that encode transcriptional regulators are also common in MDS cases. *RUNX1* mutations are usually associated with thrombocytopenia and adverse outcome [140,143]. *CEBPA* (CCAAT/enhancer binding protein-α) and *NPM1* (nucleophosmin) are also mutated in MDS cases

necrosis factor receptor superfamily member 6 (TNFRSF6) is a protein that in humans is encoded by the FAS gene

with undefined and favorable role respectively [144-146]. The role of other transcriptional factors that are associated with MDS patients such as *CUX1, PHF6* (PHD finger protein 6) and *BCOR* (transcriptional co-repressor BCL6) remains to be clarified [5].

In most MDS cases, the founder mutations are located in genes involved in DNA methylation (*TET2, DNMT3A*), in chromatin remodeling (*ASXL1, EZH2*) and in RNA splicing (*SF3B1, SRSF2, U2AF1, ZRSR2*) [79,147-149]. Mutations affecting cell differentiation or proliferation (*RUNX1, GATA2, BCOR, N/KRAS, CBL*) or cohesins (*STAG2, RAD21*) are observed during the progression from MDS to AML [150,151].

In order to clarify the role of mutations with a low frequency, a large dataset of MDS patients will be necessary to be analysed. Moreover, many of those mutations are also present in other haematological conditions such as myeloproliferative neoplasms (MPNs) and chronic myelomonocytic leukaemia (CMML). In many studies, the impact of those mutations depend on other characteristics of the MDS patients; *ASXL1, NRAS* and *RUNX1* are associated with poor prognosis in univariate analysis, but in multivariate analysis controlling for age and the IPSS-R clinical variables have no effect, whereas SF3B1 are independently associated with improved OS [98,135]. In a study by Papaemmanouil et al. [82] the number of mutations were associated with overall survival: patients with one mutation have a better leukaemia-free survival compared with patients with two, three, four or five and less than six mutations (49, 42, 27, 18 and 4 months respectively). The role of the mutations is more complex since their impact on the outcome of the disease depends on the location and type of mutations, the presence of other mutant alleles in different genetic loci and the variant allele frequency (VAF). In a study by Al-Issa et al. [152] of 610 treated MDS patients, TP53 mutations were connected with poorer OS, but patients with VAF less than 25% had better OS compared with patients with VAF >50% (12.4 vs 3.4 months respectively).

The addition of mutations in the pre-existing prognostic models can improve predictability. In a study of 439 MDS patients by Bejar *et al.* in 2011 [35], in multivariate analysis including age, sex and IPSS score *ASXL1, RUNX1, TP53, EZH2* and *ETV6* were shown to be independent prognostic markers; more specifically the addition of one of those mutations can upstage patients to a higher IPSS risk group. This study, *though* important, had several drawbacks; only 51% of the patients showed detectable mutations [82, 153] and it preceded the 2012 publication of the IPSS-R prognostic score which stratified MDS *patients better*. The same group in a study with IPSS low or intermediate-1 risk MDS cases showed that ASXL1, TP53, RUNX1 and EZH2 conferred adverse survival impact independent of *the* LR-PSS score, in univariate analysis; in multivariate analysis only *EZH2* mutations retained their significance [48]. In another study by Halerfach et al. [102] in 944 MDS patients, 25 genes were negatively associated with OS including PTPN11, *NPM1, TP53, PRPF8, EZH2, LUC7L2, NRAS, KRAS, FLT3, RUNX1, NF1, LAMB4, GATA2, ASXL1, SMC1A* and *STAG2*, whereas *SF3B1* had a positive impact. After adjusting for age, sex and IPSS-R variables only *ASXL1, KRAS, PRPF8, SF3B1* and *RUNX1* remained significant; this study proposed a prognostic model classifying patients in four risk groups (low, intermediate, high and very high risk) with 3-year OS 95.2, 69.3, 32.8 and 5.3 months respectively. Bejar et al. in a large meta-analysis of 3562 MDS samples showed that SF3B1 mutations were associated with favorable prognosis in patients with less than 5% bone marrow blasts, but this association was lost in patients with higher blast percentages [154]. SF3B1 was frequently mutated in patients with ring sideroblasts [79,84]. In a similar manner, *ASXL1, U2AF1* and *SRSF2* had a negative impact on OS in patients with less than 5% BM blasts, but this association was not significant in patients with higher blast percentage. Another set of 12 genes were independently associated with OS: *TP53, RUNX1, EZH2, NRAS, SF3B1, CBL, ASXL1, TET2, IDH2*, *KRAS* and *NPM1*. In multivariate analysis, mutations at *TP53, RUNX1, EZH2, NRAS* and *SF3B1* remained independent prognostic indicators after adjusting for IPSS-R risk categories. In a large meta-analysis Bejar et al. [154] showed in multivariate analysis that *TP53, RUNX1, EZH2, NRAS* and *SF3B1* were independent predictors after adjustment for IPSS-R risk categories. *TP53, EZH2, ETV6, RUNX1* or *ASXL1* could upstage patients with low IPSS, intermediate-1 or intermediate-2 risk to one risk category [155]. In another study of 508 MDS patients [135] treated at the Cleveland Clinic between 2000-2012, age, IPSS-R score*, EZH2, SF3B1* and *TP53* were included in a score to stratify patients in four risk groups (low, intermediate-1,

intermediate-2 and high) (Table 5) [135]. Based on those coefficients, a linear score was developed:

Age X 0.04+IPSS-R score X 0.3+EZH2 X 0.7+SF3B1 X 0.5+TP53 X 1

A study by Tefferi et al. in 179 MDS patients showed that mutations *ASXL1, SETBP1, TP53, SRSF2, IDH2* and *CSF3R* were age and IPSS-R independent risk factors for overall and leukaemia-free survival [156]. The prognostic impact of adverse mutations was more pronounced in IPSS-R lower risk disease and, therefore, might constitute relevant information for treatment decision making [156]. In another study by the same group in 685 MDS patients by Tefferi et al. [157] from the Mayo Clinic, monosomal karyotype, non-MK abnormalities other than single/double del (5q), *RUNX1*, *ASXL1* mutations, absence of *SF3B1* mutations, age greater than 70 years, Hb <8 g/dL in women and <9 g/dL in men, platelet count less than $75X10^9$/L and 10% or more bone marrow blasts were associated with worse prognosis and inferior overall survival. Patients were stratified according to this model in four groups (low, intermediate-1, intermediate-2 and high) with respective median 5-year OS rates of 73%, 34%, 7% and 0%. Gangat et al. [158] in a study of 300 MDS patients showed that age, the Mayo cytogenetics risk model and the number of adverse mutations (*RUNX1, ASXL1* and *SF3B1*) could serve as a prognostic model; their analysis resulted in HR 5.3 for three adverse mutations, 2.4 for two adverse mutations and 1.5 for one, 5.6 for high-risk karyotype, 1.5 for intermediate risk karyotype and 2.4 for age >70 years. HR-weighted risk point assignment generated a three-tiered genetic risk model (high, 5-year survival 2%), intermediate (5-year survival 18%) and low (5-year survival 56%).

Therapy related MDS (t-MDS) seem to have a different mutational context compared to de novo MDS. T-MDS patients have a significantly worse overall survival compared with patients with de novo MDS [121]. In a recent study by Linsley et al. [121], *TP53* and *PPM1D* were the only genes significantly enriched in t-MDS.

Except for their prognostic value, mutations may be helpful to predict response to specific therapies. For example, MDS patients with *TET2* mutations are more likely to respond to DNA hypomethylating agents azacitidine and decitabine [94,159,160], whereas *ASXL1* mutations predict a less favorable response [94, 161,162]. *TP53* is associated with patients who are likely to proceed to higher-risk MDS or AML when treated with lenalidomide [163]. Patients who underwent allogeneic stem cell transplantation and harbored complex karyotype or mutations in *TP53, RAS* or *JAK2* genes were at higher risk of negative outcome [164]; patients with both TP53 mutations and complex karyotype had the worst outcome. In another study with 1514 MDS patients, *TP53* mutations were connected with shorter OS and shorter time to relapse after receiving stem cell transplantation [121]. In the same study, patients older than 40 years old with wild type *TP53*, mutations in the RAS pathway were associated with inferior outcome or higher risk for relapse, whereas mutations in the JAK2 pathway were associated with higher risk of death without relapse and shorter OS. In another study of 797 MDS patients who received allogeneic SCT, complex karyotype or mutations in *TP53* or RAS-pathway genes were associated with inferior outcome post-transplantation [164].

The discovery of novel targets such as IDH1 or IDH2 mutants may suggest the possibility of *using* enasidenib for MDS patients with IDH2 mutations [165], although *these* mutations are uncommon.

The role of gene mutations in the diagnostic evaluation of patients with cytopenias is also a challenging field. Patients with aplastic anaemia harbor mutations at *PIGA*, *BCOR* and *BCORL1* whereas mutations in splicing factors and in *ASXL1* are associated with poor outcome and clonal evolution towards MDS [166]. In patients with ICUS (idiopathic cytopenias of unknown significance), the detection of a clonal mutation may be helpful since it is associated with poorer outcome or progression to MDS or a clonal myeloid neoplasm [28,30]. However, the presence of such mutations in elderly patients in a state known as clonal hematopoiesis of indeterminant potential (CHIP) shows that mutations are not enough to diagnose MDS [32, 115,116]. Moreover, in some patients with a state known as clonal cytopenia of undetermined significance (CCUS), which is associated with a higher risk, there are no mutations for progression associated with MDS or AML [118, 153,167].

Table 3. Recurring chromosomal abnormalities deemed as presumptive evidence of MDS in the setting of persistent cytopenia of undetermined origin, but in the absence of morphologic features of MDS (obtained by [77])

Unbalanced abnormalities	Balanced abnormalities
del(3q)	t(11;16)(q23.3;p13.3)
-5 or del (5q) or t(5q)	t(3;21)(q26.2;q22.1)
-7/del (7q)	t(2;1)(p21;q23.3)
+8	inv(3)(q21q26.2)
del (11q)/t(11q)	t(6;9)(p23;q34)
del (12p)/t(12p)	t(1;3)(p36.3;q21.2)
-13/del (13q)	t(5;12)(q32;p13.2)
Idic(X)(q13)	t(5;7)(q32;q11.2)
-17/del(17p)/i(17q) and t(17p)	t(5;17)(q32;p13.2)
+19/t(19)	t(5;10)(q32;q21.2)
del(20q)	t(3;5)(q25.3;q35.1)
-Y	

Table 4. Impact of mutations on overall survival in patients with MDS

Gene	Position	Frequency, %	Impact on Overall survival
RNA splicing			
SF3B1	2q33.1	20-25	Favorable
SRSF2	17q25.1	10-20	Unfavorable
U2AF1	21q22.3	5-10	Unfavorable
ZRSR2	Xp22.1	5-10	Not defined
PRPF8	17p13.3	<5	Not defined
Epigenetic modifiers			
TET2	4q24	20-30	Possibly favorable
DNMT3A	2p23	10-15	Unfavorable
IDH1	2q34	<10	Unfavorable
IDH2	15q26	<10	Controversial
ASXL1	20q11	15-20	Unfavorable
EZH2	7q35-36	5	Unfavorable
Transcription factors			
RUNX1	21q22.3	10	Unfavorable
ETV6	12p13	<5	Unfavorable
CEBPA	19q13.1	<5	Not defined
NPM1	5q35.1	<5	Favorable
BCOR	Xp11.4	<5	Unfavorable
GATA2	3q21.3	<5	Unfavorable
Signal transduction proteins			
CBL	11q23.3	<5	Favorable
NRAS	1p13.2	<5	Unfavorable
BCOR		<5	Unfavorable
FLT3-ITD	13q12	<5	Not defined
JAK2	9p24	<5	Unfavorable
KIT	4q12	<5	Not defined
Tumor suppressors			
TP53	17p13.1	5-10	Very unfavorable
Components of the cohesion complex			
STAG2	Xq25	<10	Unfavorable
Other genes			
SETBP1	18q21.1	<5	Unfavorable

Table 5. Risk category and cutoff score in MDS patients according to the Cleveland Clinic study [135]

Risk category	Score cutoff	Median overall survival (months)
Low	≤3	37.4
Intermediate-1	3.1-3.6	23.2
Intermediate-2	3.7-4.6	19.9
High	≥4.7	12.2

It is important to note that genetic testing should be incorporated properly, so as to be of clinical importance. Mutations may have a different effect when they are solely detected or in combination with other mutations. The same gene may have different alleles, which may have a different effect and prognostic significance. Many alleles are present as germline polymorphisms whereas others are not present in myeloid cells; *KRAS* mutants are present in MDS and AML as well as lymphoproliferative disease and other non-myeloid neoplasms [168, 169]. The incorporation of more sensitive techniques such as whole genome and whole exome sequencing will bring more data to be incorporated in the growing panel of genes associated with MDS.

7. CONCLUSION

Further studies will be needed in order to define the precise role of those mutations in prognosis and therapy of MDS

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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