

[REVIEW ARTICLE]

Molecular Pathogenesis and Treatment of Myelodysplastic Syndromes

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Abstract:

Myelodysplastic syndromes (MDS) are clonal hematological disorders arising from hematopoietic stem cells that have accumulated various genetic abnormalities. MDS are heterogeneous in nature but uniformly characterized by chronic and progressive cytopenia from ineffective hematopoiesis, dysplasia in single or multiple lineages, and transformation to acute leukemia in a subset of patients. The genomic landscape revealed by next-generation sequencing has provided a comprehensive picture of the molecular pathways involved in MDS pathogenesis. Recurrent mutational targets in MDS are the genes involved in RNA splicing, DNA methylation, histone modification, transcription, signal transduction, cohesin complex and DNA repair. Sequential acquisition of mutations in these sets of genes serves as a driver for the initiation, clonal evolution and progression of MDS. Based on these findings, novel agents targeting driver mutations of MDS are currently under development and expected to improve the clinical outcome of MDS in the coming decades.

Key words: myelodysplastic syndromes, MDS, molecular pathogenesis, genome, epigenome, treatment

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Introduction

Myelodysplastic syndromes (MDS) are clonal hematological malignancies arising from hematopoietic stem cells (HSCs) that have accumulated various genetic mutations. MDS is heterogeneous in nature but is uniformly characterized by chronic and progressive cytopenia from ineffective hematopoiesis and transformation to acute leukemia in a subset of patients. Single- or multi-lineage dysplasia of blood cells either in the peripheral blood (PB) or bone marrow (BM) and peripheral cytopenia are shared clinical features in MDS. Some patients present with increased blasts in the PB or BM, which is associated with an increased risk of transformation to acute myeloid leukemia (AML). According to a survey by the Ministry of Health and Welfare in Japan, the median age of patients with MDS is 64 years old, and prevalence rate is approximately 3 in 100,000.

The genomic landscape of MDS revealed by next-generation sequencing indicates that mutations of various genes involved in epigenetic, signaling, transcription, apoptosis, DNA repair, RNA splicing and cohesin pathways serve

as drivers for MDS (1). Furthermore, the accumulation of genetic mutations has been shown to be associated with the clonal evolution and development of MDS.

This review will summarize our recent understanding regarding the molecular pathogenesis of MDS and novel approaches to their treatment.

Clinical Features and Classification of MDS

MDS are clonal disorders originating from abnormal HSCs with various genetic mutations. These abnormal HSCs have clonal advantages over wild-type (WT) HSCs and undergo clonal expansion over time in the BM. Hematopoietic cells generated from abnormal HSCs are defective in their proliferation and differentiation capacities, and many of them die from apoptosis during differentiation. This impaired differentiation process, called ineffective hematopoiesis, is a background mechanism for peripheral cytopenia in MDS patients. Half of MDS patients present with pancytopenia, and the others show only anemia or anemia with leukocytopenia or thrombocytopenia.

The World Health Organization (WHO) revised classifica-

Table 1. Classification of MDS (WHO2016).

Name	BM and PB blasts	Ringed Sideroblasts
MDS with single lineage dysplasia (MDS-SLD)	BM<5%, PB<1%, no Auer rods	<15%*
MDS with multilineage dysplasia (MDS-MLD)	BM<5%, PB<1%, no Auer rods	<15%*
MDS with ring sideroblasts (MDS-RS)		
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	BM<5%, PB<1%, no Auer rods	≥15%**
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	BM<5%, PB<1%, no Auer rods	≥15%**
MDS with isolated del(5q)	BM<5%, PB<1%, no Auer rods	None or any
MDS with excess blasts (MDS-EB)		
MDS-EB-1	BM 5%-9% or PB 2%-4%, no Auer rods	None or any
MDS-EB-2	BM 10%-19% or PB 5%-19% or Auer rods	None or any
MDS, unclassifiable (MDS-U)		
with 1% blood blasts	BM<5%, PB=1%, no Auer rods	None or any
with single lineage dysplasia and pancytopenia	BM<5%, PB<1%, no Auer rods	None or any
based on defining cytogenetic abnormality	BM<5%, PB<1%, no Auer rods	<15%
Refractory cytopenia of childhood	BM<5%, PB<2%	None

*<5%, if SF3B1 mutation is present. **≥5%, if SF3B1 mutation is present.

tion of MDS in 2016 did not make drastic changes to the classification in 2008 (Table 1). Notably, a mutation in SF3B1 was incorporated into the diagnosis of MDS with ring sideroblasts (MDS-RS) because of their tight association. MDS with an increased percentage of blasts, which have a high propensity for progressing to AML, were renamed MDS-EB-1, whose blast counts of either BM or PB are 5-9% or 2-4%, respectively, and MDS-EB-2, whose blast counts of either BM or PB are 10-19% or 5-19%, respectively. MDS with single- or multi-lineage dysplasia were termed MDS-SLD or MDS-MLD, respectively.

Chromosomal Abnormalities in MDS

About half of MDS patients have chromosomal abnormalities (2). Most of them are abnormalities affecting genomic copy numbers, such as deletion, monosomy or trisomy, and structural alterations of chromosomes, such as the balanced translocations frequently seen in acute leukemia, are relatively rare. Loss or deletion of chromosome 5 or 7 is particularly common in MDS. Monosomy 7 is known to be associated with a poor prognosis (3). MDS with isolated del(5q), also known as 5q- syndrome, are a unique subtype associated with a favorable outcome and characterized by macrocytic anemia with normal platelet levels (4). The frequency of 5q- syndrome is relatively high in the US and European countries, accounting for approximately 5% of MDS patients, while the frequency is only 1-2% in Japan (5). A commonly deleted chromosomal region of 5q-syndrome is 5q31-5q33, encompassing 1.5Mb (6). Previous studies have identified several pathogenic genes in this region, including RPS14, microRNA (miR)-145 and miR-146a. RPS14 is a ribosomal protein, and its haploinsufficiency is responsible for macrocytic anemia (7). miR-145 and miR-146a are involved in thrombocytosis and neutropenia through interleukin (IL)-6 regulation (8). Lenalidomide is effective for resolving anemia in 5q- syndrome (4).

Genetic Abnormalities in MDS

As stated above, approximately half of MDS patients have normal karyotype, suggesting that genetic mutations play critical roles in the pathogenesis of MDS. Indeed, recent data from next-generation sequencing have revealed a variety of recurrently mutated genes in MDS (Table 2 and Fig. 1). These include genes encoding RNA splicing factors, epigenetic regulators for DNA methylation or histone modification, transcription factors, signaling molecules, cohesin complex and DNA repair. Among these, the mutation frequencies are particularly high in RNA splicing factors and epigenetic regulators (Table 2).

1) RNA splicing factors

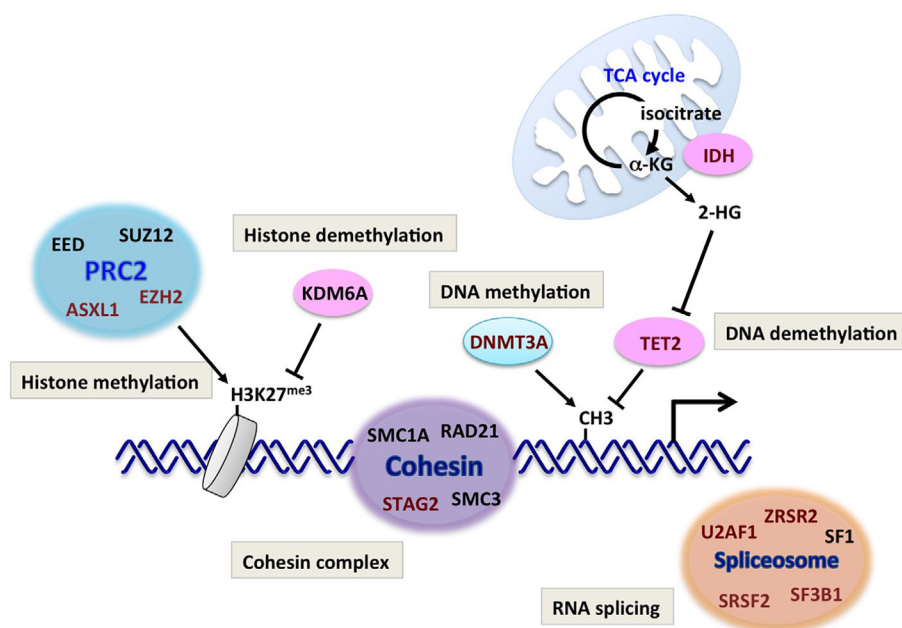
RNA splicing is a complicated process governed by the unique protein complex 'spliceosome,' which comprises more than 50 proteins (9). Among these proteins, recurrent mutations are found in U2AF35, ZRSR2, SRSF2, SF3A1, SF3B1, PRPF40B, U2AF65 and SF1 in MDS patients (10). Interestingly, these mutations are mutually exclusive, suggesting that a single mutation is sufficient to impair the spliceosome function and drive the development of MDS, or mutations in multiple spliceosome genes are detrimental to MDS clones.

The SF3B1 mutation is highly specific to MDS-RS (11). About 70% of MDS-RS patients are positive for the SF3B1 mutation, suggesting its diagnostic significance (12). Mutations in SRSF2, U2AF1 and ZRSF2 are observed in approximately 20%, 10% and 10% of MDS patients, respectively.

The molecular mechanisms underlying how these mutations contribute to MDS pathogenesis are not clear at present. Recent studies have shown that splicing factor mutations can lead to a variety of splicing abnormalities, such as exon skipping and intron retention. By using knock-in mouse models, it was demonstrated that the SRSF2 mutation

Table 2. Recurrently Mutated Genes in MDS.

Gene	Mutation frequency (%)	Function
TET2	33	DNA demethylation
SF3B1	33	RNA splicing
ASXL1	23	Histone modification
SRSF2	18	RNA splicing
DNMT3A	13	DNA methylation
RUNX1	11	Transcription factor
U2AF1	8	RNA splicing
ZRSR2	8	RNA splicing
STAG2	8	Cohesin complex
TP53	6	DNA repair
EZH2	6	Histone modification
CBL	5	Signal transduction (ubiquitin ligase)
JAK2	5	Signal transduction (tyrosine kinase)
BCOR	4	Transcriptional corepressor
IDH2	4	DNA demethylation
NRAS	4	Signal transduction (Ras signaling)
NF1	3	Signal transduction (Ras signaling)

**Figure 1. Genetic abnormalities in MDS. Recurrently mutated genes in MDS (shown in red characters) and their roles in physiological pathways are shown. MDS: myelodysplastic syndromes**

induces alternative recognition of the target sequence, resulting in disordered splicing of major epigenetic regulators, such as EZH2 (13). Mutations in splicing factors are now considered to drive MDS, at least in part, by inducing disordered epigenetic regulation through abnormal RNA splicing.

2) Regulators of DNA methylation

DNA methylation plays a critical role in transcriptional regulation. It is well known that methylation of cytosine residues in the promoter region strongly suppresses the transcription of the downstream gene. The methylation status of DNA is maintained by the fine-tuned, balanced regulation of

methylation and demethylation of DNA. DNA methylation is catalyzed by DNA methyltransferase (DNMT), which comprises DNMT1, DNMT3A and DNMT3B. DNMT1 is critical for the maintenance of the genome-wide methylation pattern upon DNA replication, and DNMT3A and B are required for establishing tissue- or cell type-specific methylation patterns. TET proteins, by contrast, are essential for DNA demethylation. The TET family contains TET1, TET2 and TET3, all of which catalyze the first step of DNA demethylation by converting 5-methyl cytosine (5-mC) to 5-hydroxymethyl cytosine (5-hmC).

Dysregulated DNA methylation is critical for MDS patho-

genesis, since the mutation rates of TET2 and DNMT3A are high in MDS (10-30% and 10-20%, respectively) (14-16). Notably, mutations in DNMT3A or TET2 are also frequent in AML, myeloproliferative neoplasm (MPN) and malignant lymphoma, suggesting that disordered DNA methylation is fundamental for various hematological malignancies.

TET2 mutations are considered to be loss-of-function, since nonsense mutations are clustered in the N-terminal region and missense mutations are mostly localized in C-terminal catalytic domain (15). In fact, TET2-deficient HSCs have enhanced self-renewal capacity and show clonal advantage *in vivo* (17, 18). Furthermore, TET2-deficient mice develop hematological malignancies, such as myeloid neoplasm or malignant lymphoma, after a long latency (18).

For DNMT3A, arginine 882 (R882), located in the catalytic domain of methyltransferase, is a hotspot for mutations, and its mutation profoundly affects its catalytic activity (19). Similarly to TET2, DNMT3A plays a crucial role in the HSC function, and DNMT3A mutations confer HSCs with an enhanced stem cell capacity and clonal advantage (20, 21).

Recent findings from mouse models and clonal hematopoiesis in human settings indicate that abnormal DNA methylation alone is not sufficient to cause MDS, and additional mutations are required for the development of hematological malignancies (22-26).

3) Histone modifiers

Histones are major components for chromatin that are required for maintaining its integrity and structure. Histone octamer, consisting of histone H2A, H2B, H3 and H4, serves as the core of the nucleosome, the smallest unit of chromatin structure. Histones are targets of various post-translational modifications, such as methylation, acetylation and phosphorylation. Histone modifications induce dynamic changes in the local chromatin structure, leading to the transcriptional activation or suppression of the genes in the surrounding region.

Polycomb group complex (PRC) 2 is a well-known histone modifying complex frequently targeted in MDS. PRC2 induces tri-methylation of lysine 27 of histone H3 (H3K27), which suppresses transcription of the downstream gene. The loss of function mutation of EZH2, a major component of PRC2 with histone methyltransferase activity, is reported in 5-10% of MDS patients and has been shown to be associated with a poor prognosis (27, 28). Interestingly, the *EZH2* gene is localized on chromosome 7q, suggesting that haploinsufficiency of *EZH2* might be associated with the poor prognosis of the -7/-7q karyotype (29). *ASXL1*, another frequent target in MDS, is considered to be critical for the PRC function and is mutated in 20-25% of MDS patients (30).

4) Cohesin complex

Cohesin complex, consisting of four proteins (STAG2, SMC3, SMC1A and RAD21), is known to play critical roles

in sister chromatid segregation during cell division (31). Recurrent mutations of cohesin complex have been reported in various myeloid malignancies, including MDS (32). Mutations in four components of cohesin complex are mutually exclusive, indicating that functional disruption of the whole complex is important for the pathogenesis of MDS. Recent studies have suggested that cohesin complex plays a role in maintaining the higher-order DNA structure, such as facilitating chromatin looping by combining DNA at distant sites, and is thereby deeply involved in transcriptional regulation. Cohesin mutations have been shown to induce abnormal myeloid differentiation and enhance the HSC function in mouse models (33, 34). Therefore, it is now postulated that cohesin mutations contribute to the development of MDS through the structural alteration of chromatin and resultant transcriptional dysregulation of the genes critical for HSCs and hematopoietic differentiation (35).

5) Isocitrate dehydrogenase (IDH)

IDH is an enzyme of the tricarboxylic acid (TCA) cycle that converts isocitrate to α -ketoglutarate (α -KG). α -KG is an essential cofactor for TET and histone demethylases, such as KDM6A, and is therefore critical for epigenetic regulation, such as DNA and histone methylation. IDH has two isoforms (IDH1 and IDH2) localized in the cytoplasm and mitochondria, respectively. Heterozygous missense mutations of IDH1 and IDH2 (IDH 1/2), mostly at the critical arginine residue in the catalytic domain, have been reported in about 5-12% of MDS patients (36, 37). Interestingly, this mutation confers the enzyme with novel activity to convert α -KG to the oncometabolite 2-hydroxy glutarate (2-HG) (38). 2-HG was shown to inhibit the function of α -KG-dependent enzymes, such as TET and KDM6A, by competing with α -KG (39). Therefore, IDH mutations are considered to affect the genome-wide methylation status of DNA and histones, serving as a background for MDS pathogenesis. Supporting this notion, mutations in IDH1, IDH2 and TET2 are mutually exclusive. IDH mutations in MDS are reported to be associated with mutations in DNMT3A, ASXL1 and SRSF2 (36, 37).

6) Transcription factors

Transcription factors involved in hematopoietic differentiation and HSC regulation, such as RUNX1, ETV6 or GATA2, are recurrently mutated in MDS.

RUNX1 is a core binding factor (CBF) family transcription factor that plays critical roles in the emergence of HSCs during ontogeny, the function of hematopoietic stem and progenitor cells and the differentiation of megakaryocytes and lymphocytes. RUNX1 is mutated in about 10% of MDS patients, placing itself as the most frequently targeted transcription factor in MDS (40). The mutation frequency is reported to be substantially higher in radiation-associated and therapy-related MDS (41, 42). Most mutations are loss-of-function, while some mutants show a dominant negative effect on the residual WT allele. RUNX1 mutations are known

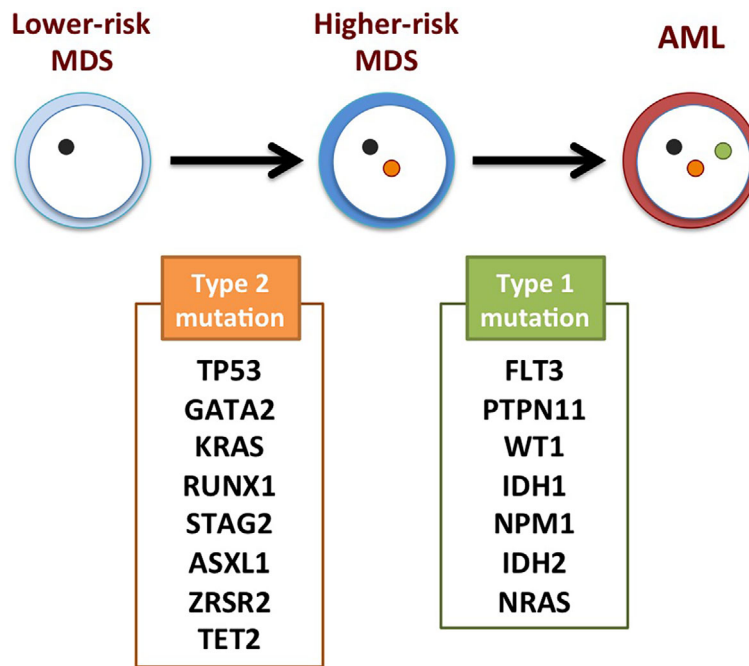


Figure 2. Molecular mechanism underlying the clonal evolution from lower-risk MDS to AML. Type 2 mutations are commonly seen during the evolution from lower-risk MDS to higher-risk MDS. Type 1 mutations are mainly involved in the transformation from higher-risk MDS to AML. MDS: myelodysplastic syndromes, AML: acute myeloid leukemia

to be associated with a poor prognosis.

Germline mutations in transcription factors, such as RUNX1, ETV6, GATA2 or CEBPA, are known to cause familial MDS/AML syndromes (43).

7) TP53

TP53 is a well-known tumor suppressor gene mutated in a wide variety of tumors. TP53 encodes a transcription factor that induces apoptosis, cell cycle arrest and senescence in response to DNA damage or oncogenic stress (44). Mutations of TP53 are found in 5-10% of MDS patients and are particularly associated with secondary MDS and patients with del(5q) cytogenetics. TP53 mutations are well known to be associated with a poor prognosis (45).

8) Signaling molecules

Activating mutations of tyrosine kinase or serine/threonine kinase, leading to constitutive activation of the JAK-STAT or RAS-MAPK pathways, are commonly seen in various myeloid malignancies. In MDS, mutations in JAK2, CBL, NRAS and NF1 have been reported, each in about 5% of MDS patients. Active signaling evoked by these mutations simulates cell proliferation and suppresses apoptosis, contributing to the expansion of abnormal clones that have accumulated various mutations.

Clonal Evolution of MDS and AML Transformation

Understanding the clonal dynamics and the molecular ba-

sis behind the initiation, progression and transformation of MDS are crucial for developing novel therapies, better clinical care and prevention of disease progression in the future practice. It has been considered that MDS clones sequentially acquire mutations as they evolve and selected clones would expand and dominate during disease progression. However, recent studies using deep sequencing and single-cell analyses have revealed the complex and heterogeneous nature of clonal evolution in MDS.

Makishima et al. have revealed the genetic basis for the clonal evolution of MDS by sequencing DNA samples collected from 2,250 patients who had progressed from lower-risk MDS to secondary AML (46). They reported that the number of genetic mutations found in the patient BM cells increased with disease progression. Furthermore, mutations were able to be classified as type 1 or type 2, which are involved in the transformation from MDS to AML or the progression from lower-risk to higher-risk MDS, respectively (Fig. 2). Type 1 mutations include FLT3, PTPN11, WT1, IDH1, NPM1, IDH2 and NRAS; and type 2 mutations include TP53, GATA2, KRAS, RUNX1, STAG2, ASXL1, ZRSR2 and TET2. It was also shown that patients with SF3B1 mutations have a lower risk of AML transformation, whereas those with type 1 mutations have a worse outcome. These data clearly demonstrate that each mutation has a unique role in the pathogenesis and progression of MDS, and the order as well as the type of mutations critically shapes the clinical features of MDS. In addition, they also analyzed the dynamics of the clonal architecture during progression from MDS to AML. They observed two distinct

patterns of clonal evolution: 'linear evolution' and 'clone sweeping'. In the 'linear evolution' pattern, subclones that have acquired new mutations emerge recursively within the dominant population and take over the latter population. In contrast, in the 'clone sweeping' pattern, a new or pre-existing small subclone sweeps out the other clones and dominates during progression.

Recently, a detailed picture of clonal evolution from MDS to AML was revealed by single-cell analyses of highly fractionated disease stem cells from longitudinally collected samples of the same patient (47). The study examined genetic mutations in pre-stem cell, stem cell and blast fractions in MDS and AML samples at the single-cell level and found that the MDS blasts and AML blasts were clonally distinct. In turn, it was revealed that a small subclone pre-existing in the MDS stem cell or pre-MDS stem cell fraction expanded during transformation and became dominant in AML. These findings are not compatible with the linear progression model, which predicts that MDS blasts give rise to AML blasts by acquiring additional mutations, but clearly supports the non-linear, parallel-type clonal evolution at the disease stem cell level. However, that study was conducted with only a small number of patients, and the proposed model must be confirmed in a larger-scale study.

Novel Treatments for MDS

Accumulating knowledge on the molecular pathogenesis of MDS has enabled us to develop novel agents for MDS. Drug development at present is mainly focused on epigenetics (DNA methylation and histone modification), RNA splicing and signal transduction because of the major role of these factors in MDS pathogenesis.

1) Hypomethylating agents (HMAs)

Recurrent mutations in DNA methylation pathways in MDS suggest that aberrant DNA methylation serves as a critical basis for MDS development. Indeed, genome-wide hypermethylation of DNA has been reported in MDS patients (48). Azacitidine (AZA) and Decitabine are HMAs currently approved for the treatment of MDS (only AZA is approved in Japan) and are the first choice for transplant-ineligible higher risk MDS (49-51). HMA has been shown to improve the overall survival of higher-risk MDS by delaying the transformation to AML (52). It is also effective for inducing hematopoietic recovery in less than 50% of patients with lower-risk MDS, although it does not improve the survival (53-55). The response rate of HMA in higher-risk MDS is 40-50%, and it normally takes 3-6 cycles to obtain a substantial hematological response. Factors predicting the response to HMA are reported to be mutations of TET2 or DNMT3A, DNA hypomethylation and the absence of an ASXL1 mutation.

Guadecitabine (GDAC) is a next-generation HMA with a prolonged half-life. It is a novel dinucleotide of decitabine and deoxyguanosine, which makes decitabine resistant to

degradation by cytidine deaminase. It is currently under clinical trials for MDS and AML (56-58). It was shown to be effective for higher-risk MDS, CMML and low-blast-count AML cases with AZA failure in phase 2 trials (56, 58).

2) Erythropoiesis stimulating agents (ESAs)

Anemia is commonly observed in MDS, and a majority of patients require frequent red blood cell (RBC) transfusions. This results in iron overload, which eventually leads to organ dysfunction due to hemosiderosis and significantly impairs the quality of life as well as the survival of the affected patients. Treatment of anemia with ESAs is a standard of care, particularly for transfusion-dependent lower-risk MDS patients. Darbepoietin, a modified recombinant human erythropoietin with a long serum half-life, is widely used for treating anemia in lower-risk MDS. In a phase 3 trial, Darbepoietin 500 µg every 3 weeks induced a significant hematological response and reduction in the transfusion frequency (59). A phase 2 trial conducted in Japan and Korea showed that the weekly administration of Darbepoietin 240 µg led to a 50% reduction in the transfusion burden in 67% of patients. Predictive factors for a response to ESA included a low serum concentration of Epo and low transfusion burden. Based on these findings, it is recommended that ESA be used in patients with a serum Epo value of < 500 IU/mL (60). Response to ESA usually occurs within 3 months of treatment, and the median duration of response is 15 to 18 months (60). The combination of granulocyte-colony stimulating factor (G-CSF) with ESA was shown to enhance its efficacy in a subset of patients refractory to ESA.

3) Erythropoiesis-maturing agents (EMAs)

Luspatercept and Sotatercept are novel agents stimulating the late stage of erythropoiesis (61-64). They are fusion proteins of the human immunoglobulin G₁ (IgG₁) Fc domain and extracellular domain of activin receptor type IIB (Luspatercept) or IIA (Sotatercept). They act as ligand traps to neutralize TGFβ superfamily such as GDF11, which is a negative regulator for the late stage of erythropoiesis. Since Luspatercept and Sotatercept stimulate erythropoiesis through a different mechanism from Epo, which works at the early stage of erythropoiesis, they are expected to be effective for MDS patients refractory to ESA.

Luspatercept was well-tolerated and showed promising activity for increasing hemoglobin levels in lower-risk MDS patients in a phase 2 clinical trial (61). In that trial, an erythroid response and transfusion independence were observed in about 60% and 40% of the patients, respectively. Interestingly, patients with ring sideroblasts or an SF3B1 mutation showed a better response to Luspatercept than patients without them. A randomized, placebo-controlled phase 3 trial (Medalist Trial) was recently conducted by enrolling 229 transfusion-dependent, lower-risk MDS-RS patients refractory to ESA or with high serum Epo levels. The rates

for an erythroid response and transfusion independence of Luspatercept vs. placebo were 53% vs. 12% and 38% vs. 13%, respectively (63). The median duration for the response to Luspatercept was 30.6 weeks.

Regarding Sotatercept, a dose-escalating phase 2 clinical trial for lower-risk MDS refractory to ESA showed favorable erythroid responses in approximately 50% of the patients both with high and low transfusion dependency (62). Similar to Luspatercept, the response rates were higher among patients with MDS-RS (58.8%) than those with other lower-risk MDS without ring sideroblasts (22.2%).

In summary, these results show that both Luspatercept and Sotatercept are promising agents for treating ESA-refractory anemia in MDS-RS patients.

4) Rigosertib

Rigosertib is a multi-kinase inhibitor that selectively induces apoptosis in tumor cells by inhibiting Ras-driven signals. It is a Ras-mimetic small molecule that binds to a variety of Ras effectors, such as Raf and phosphoinositide-3 kinase, and blocks their activation. Randomized phase 3 clinical trial of Rigosertib for HMA-refractory higher-risk MDS failed to show a survival benefit. However, a subgroup analysis suggested a potential benefit for patients with early failure of HMAs (within the first nine months) (65), and a study to confirm this finding is currently ongoing.

5) IDH inhibitors

IDH mutations are gain-of-function mutations that are capable of generating the oncometabolite 2-HG from α -KG. Since IDH mutations lead to disordered methylation of DNA and histones that are fundamental for MDS development, it is reasonable to target this mutation when developing drugs for MDS. Enasidenib (AG-221) is a covalent inhibitor of mutant IDH2 and is approved in the US for relapsed or refractory AML with IDH2 mutations. In a phase 1 study, Enasidenib was effective for a subset of MDS patients with an overall response rate of 53% (66). Based on this promising result, a phase 2 trial of Enasidenib alone or in combination with AZA is currently ongoing. The IDH1 inhibitor AG-120 (Ivosidenib) has proven its efficacy in relapsed or refractory AML (67), and other IDH1 inhibitors, such as IDH305 or FT-2102, are under clinical trials for MDS.

6) Spliceosome inhibitors

Recurrent and frequent mutations of spliceosome components suggest that alterations of RNA splicing play critical roles in MDS pathogenesis. Based on the hypothesis that inhibiting the spliceosome function may be detrimental to cells with aberrant RNA splicing, spliceosome inhibitors have been tested for their anti-tumor activity in preclinical models and clinical trials. E7107, a novel spliceosome inhibitor, showed anti-leukemic activity in mice with heterozygous SRSF2 P95H mutation (13, 68). H3B-8800 is an orally available small molecule that selectively modulates SF3B complex and has been shown to selectively kill leukemia

cells with an SF3B1 or SRSF2 mutation in PDX mouse models *in vivo* (69). H3B-8800 is currently under phase 1/2 multicenter clinical trials for patients with MDS, CMML and AML.

7) Pevonedistat

Pevonedistat is an inhibitor of the neural cell developmentally downregulated 8 (NEDD8)-activating enzyme (NAE). NAE processes NEDD8 to activate cullin-RING E3 ubiquitin ligases (70-72), which then induce proteosomal degradation of their substrates, such as p27, CDT1 and Nrf-2, through ubiquitination. Pevonedistat is thus considered to suppress the proliferation of leukemic cells by inducing the accumulation of specific substrates of cullin-RING E3 ubiquitin ligases (73). Pevonedistat in combination with AZA was shown to be safe and effective for AML in a phase 1b study (74). A randomized, controlled phase 3 study comparing AZA alone and AZA+pevonedistat for higher-risk MDS and oligoblastic AML is currently underway.

Conclusion

Next-generation sequencing has revealed the recurrent genetic mutations involved in MDS and their correlation with the clinical features, such as diagnostic classification, response to therapy and prognosis. Furthermore, the analysis of serial samples taken from the same patient has provided molecular insights into clonal evolution. Novel agents targeting the molecular pathways involved in MDS pathogenesis are currently under development and are expected to be available for improving the clinical outcomes of MDS in the coming decades.

Author's disclosure of potential Conflicts of Interest (COI).

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