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Epigenetics in Myelodysplastic Syndromes

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Abstract

Epigenetics links developmental biology, genetics and environment. Dysregulation of epigenetic events can lead to evolution of several diseases including cancer. Various types of epigenetic therapies can potentially treat many diseases such as myelodysplastic syndromes, solid tumors, autoimmune diseases and neurological disorders. The two main classes of epigenetic therapies are inhibitors of the enzymes DNA methyltransferase and histone deacetylase. Despite the progress achieved after introduction of epigenetic therapies, particularly the hypomethylating drugs azacitidine and decitabine, in the management of patients with myelodysplastic syndromes further efforts are needed to improve the outcome of these patients. This is an updated review on epigenetics, epigenetic targets and existing as well as evolving epigenetic therapeutics in myelodysplastic syndromes. However, the hypomethylating agents azacitidine and decitabine as well as histone deacetylase inhibitors will be thoroughly discussed.

Keywords: Epigenetics; Myelodysplastic syndromes; Genetic mutations; Hypomethylating agents; Histone deacetylase inhibitors; Maintenance therapy

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Introduction

Despite having multiple meanings historically, epigenetics is the study of potentially heritable changes in chromatin and DNA or in the pattern of gene expression and function without modification of the underlying DNA sequence [1-5]. In the 1940s, the word epigenetics was coined by Conrad Waddington to link the fields of developmental biology and genetics and to describe the epigenetic landscape [6-11]. Epigenetics has various implications and links with: development and evolution as well as environment and heredity including epigenetic systems of inheritance [8,11,12]. Interactions between DNA and environment through chromatin modifications are responsible for expression of a normal phenotype and development of various pathologies [10].

Griffith and Mahler were the first to suggest that demethylation of DNA might have an important biological role and, in the year 1969, they proposed that demethylation could provide a basis for long-term memory in the brain [6]. The epigenome, which is the bridge between the genome and phenotype, consists of the entire epigenetic code across all the cells in the body [2,10]. Epigenetic mechanisms include: DNA methylation, histone modification, positioning of histone variants, nucleosome remodeling in addition to small and non-coding RNAs [13].

The human epigenome project is expected to:

- Unravel the patterns of DNA methylation in different tissues.
- Determine whether the regulation of gene expression occurs at the level of DNA or chromatin or both, and,
- Provide high-resolution reference epigenetic maps [6,13].

Epigenetic changes such as histone methylation, DNA methylation and histone acetylation alter gene expression at the level of transcription by upregulation, downregulation or complete silencing of genes. Also, dysregulation of epigenetic events can be pathological leading to the development of cardiovascular diseases, neurological and metabolic disorders in addition to cancer. Thus, epigenetics plays a central role in many diseases [14].

The following epigenetic modifiers are genetically altered in patients with cancer: EZH2, IDH1, IDH2 and DNMT3A. These genetic modifiers provide new therapeutic targets for clinical

development [15]. Epigenetic events or modifications are frequently reversible, hence inhibition of epigenetic changes may be a valuable therapeutic potential [14,16,17]. Epigenetic and genetic abnormalities play vital roles in cancer initiation and progression by having frequent mutations [18]. Epigenetic alterations in cancer cells affect virtually all cellular pathways that are associated with tumorigenesis [19]. Epigenetic therapy is intended to reprogram neoplastic cells toward a normal state [18]. Epigenetic drugs can restore defective expression of genes involved in: cell cycle control, apoptosis, cell signaling, tumor cell invasion, metastases, angiogenesis, and immune recognition [19].

Diseases that can be potentially treated with epigenetic therapies include:

- Myelodysplastic syndromes (MDSs);
- Other hematologic malignancies (HMs) such as: multiple myeloma (MM), chronic myelomonocytic leukemia (CMML), acute myeloid leukemia (AML), Hodgkin lymphoma and cutaneous T-cell lymphoma;
- Metabolic and autoimmune disorders such as: diabetes mellitus, rheumatoid arthritis, systemic lupus erythromatosis, multiple sclerosis, systemic sclerosis and Sjögren's syndrome;
- Neurodegenerative and psychological disorders such as: Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis; and
- Miscellaneous disorders such as psoriasis, cardiovascular disorders and idiopathic pulmonary fibrosis [14-16,18,20-26].

There are several classes of epigenetic drugs. The main types of epigenetic therapies and examples of some types are included in **Table 1** [14,15,18,22,27-33].

Epigenetic therapy is a novel therapeutic approach that modulates gene expression by targeting the: DNA methylation machinery, histone covalent modification and micro-RNAs (miRNAs) [20]. A major limitation of epigenetic therapy is the lack of specificity and the consequent global induction of epigenetic changes [20]. Treatment with epigenetic agents can reduce chemotherapy resistance in patients with HMs and solid tumors so epigenetic drugs can be added to cytotoxic chemotherapy or targeted therapy in order to derive chemosentitization benefits [34,35]. Methods that are used in the detection of methylation status of gene promoters and the association between methylation status and clinical parameters in patients with HMs include: methylation-specific polymerase chain reaction (PCR), methylation-specific restriction enzyme digestion, Hpall tiny fragment enrichment by ligation-mediated PCR, bisulphite sequencing and pyrosequencing [15].

MDSs

Introduction to MDSs

MDSs comprise a group of biologically and clinically heterogeneous clonal hematopoietic neoplasms characterized by: peripheral cytopenias, dysplastic changes in at least one hematopoietic lineage, ineffective hematopoiesis due to excessive apoptosis and aberrant myeloid differentiation, genetic instability, clonal evolution and increased risk of transformation into secondary AML [36-44]. MDSs manifest as heterogeneous diseases ranging from indolent conditions with considerable life expectancy to aggressive conditions resembling AML. Therefore, risk-adapted treatment strategy is mandatory for MDSs as these diseases have highly variable clinical courses [45].

Pathogenesis, etiology and associations

Recent studies in humans and in animal models have provided direct evidence that dysplastic hematopoiesis results from the interaction between: bone marrow (BM) microenvironment, hematopoietic stem cells, and stromal mesenchymal stem cells in the BM niche in patients with MDSs [46-54]. Additionally, epigenetic dysregulation plays an important role in the pathogenesis of MDSs [55]. Etiology and associations of MDSs are shown in **Table 2** [40,56-63].

Cytogenetics and molecular genetics

Techniques that are used for the detection of cytogenetic abnormalities in MDSs include:

- Conventional or metaphase cytogenetics to detect visible chromosomal aberrations;
- Fluorescence in situ hybridization (FISH) to detect small and hidden chromosomal aberrations;
- Spectral karyotyping to detect unknown and complex chromosomal abnormalities;
- Single nucleotide polymorphism array (SNP-A) to detect cryptic and complex chromosomal aberrations;
- Microarray-based comparative genome hybridization (CGH) to detect uniparental disomy and copy number variation (CNV);
- Sequencing-based technologies such as next generation sequencing (NGS) to detect CNV and structural variants as well as unknown mutations and aberrations; and
- PCR [64,65]. Conventional cytogenetics and FISH can detect abnormalities in chromosomes: 5, 7 and 8 while array-CGH and PCR can detect the following somatic mutations: ASXL1, EZH2, TP53, TET2, RUNX1, SF3B1 and DNMT3A [65].

Cytogenetic abnormalities, gene mutations and recurrent somatic mutations in MDSs are shown in **Table 3** [42,62,66,67], **Table 4** [45,68,69] and **Table 5** [40,45,62,68,70-73] respectively. MDSs are characterized by mutations in more than 40 genes, a complex structure of gene-gene interactions and extensive subclonal diversification [71,73]. The most frequently mutated genes in MDSs are: TET2, SF3B1, ASXL1, DNMT3A, SRSF2, U2AF1, RUNX1, TP53, EZH2, ZRSR2, STAG2, CBL, NRAS, JAK2, SETBP1, IDH1, IDH2 and ETV6 [73-79]. The following mutated genes are considered epigenetic regulators: TET2, IDH1, IDH2, DNMT3A, ASXL1 and EZH2 [79]. Gene mutations that are independently associated with shorter survival and unfavorable outcome include: ASXL1, U2AF1, TP53, SRSF2, CBL, IDH2, SETBP1, DNMT3A, RUNX1 and EZH2 [75,76,80,81]. However, SF3B1 gene mutation has been associated with longer survival and favorable outcome [80,82]. Identification of somatic mutations in patients with MDSs suggests

Table 1 Types and examples of epigenetic drugs.

Classes or types of epigenetic drugs	Examples
DNA methyltransferase inhibitors [DNMTIs] or demethylating agents	Azacitidine, decitabine, zebularine, S-110 and SGI-1027
Histone deacetylase inhibitors [HDACIs] (5 classes: I, IIa, IIb, III and IV)	Vorinostat, panobinostat, entinostat, givinostat, pracinostat, belinostat, valproic acid, romidespsin, pivanex, CI-994 and ACY-1215
Histone acetylase/acetyltransferase inhibitors	
Histone demethylase inhibitors	
Protein methyltransferase inhibitors	
Sirtuin inhibitors and modulators	
Bromodomain inhibitors	
Nucleosidic DNA methyltransferase inhibitors	6 thioguanine; fazarabine; pseudoisocitidine; 5 fluoro-2-deoxycitidine and 5,6 dihydro-5-azacitidine
Antisense oligonucleotide inhibitors of DNMTs	
Inhibitors of protein binding to acetylated histone	
Inhibitors of protein binding to methylated histone	
Miscellaneous drugs that have epigenetic activities	Procainamide, hydrallazine, methotrexate, thalidomide, statins, neuroleptics, B-blockers, Fluoroquinolones, isotretinoin, cox-2 inhibitors, synthetic estrogens and general anesthetics.

Table 2 Etiology and associations of myelodysplastic syndromes.

1. Unknown etiology.
2. Old age; more than 50 years.
3. Male gender.
4. Obesity.
5. Tobacco use.
6. Alcohol intake.
7. Sweet syndrome; neutrophilic dermatosis.
8. Vitamin deficiencies: - Folic acid
- Vitamin-B12.
9. Infections: - Human immunodeficiency virus.
- Tuberculosis.
- Brucellosis.
10. Occupational and environmental exposure: solvents, benzene, lead, arsenic, pesticides, herbicides, hair dyes, and agricultural chemicals.
11. Autoimmune disorders: - Systemic lupus erythromatosis.
- Fibrosing alveolitis.
- Behcet syndrome.
- Other vasculitis disorders and seronegative polyarthropathies
12. Therapy-related myelodysplastic syndromes: - Alkylating agents.
- Topoisomerase II inhibitors.
- Radiotherapy.
13. Bone marrow failure syndromes: - Aplastic anemia. - Diamond Blackfan syndrome.
- Fanconi anemia. - Paroxysmal nocturnal hemoglobinuria.
- Dyskeratosis congenita. - Congenital neutropenias.
14. Genetic, familial and hereditary disorders: - Ataxia telangiectasia - Down's syndrome
- Xeroderma pigmentosa - Trisomy 8 mosaicism
- Bloom's syndrome - Neurofibromatosis

new targets for therapeutic interventions [68]. For example TP53 mutations, which are less likely to respond to single agent lenalidomide, have been reported to occur in approximately 20% of patients with del 5q, low and intermediate I MDSs [45].

Diagnosis of MDSs using peripheral blood

Recently, several studies have shown that peripheral blood cell-free DNA (PB-CF-DNA) is safer, easier and even more sensitive for genetic and epigenetic analyses than whole BM samples [70,83-87]. After obtaining PB-CF-DNA from plasma or serum, high resolution SNP-A is used for karyotyping then mutation analysis of genes is performed using PCR or sequencing (Sanger, parallel or targeted NGS) [83,85,87]. Studies have shown high concordance rates reaching 100% in cytogenetic or mutational profiles between PB and BM in patients with MDSs [83,86]. Mutations in the following genes can be determined by PB-CF-DNA: SF3B1, DNMT3A, ASXL1, SRSF2, IDH1, IDH2, TET2, U2AF1, ZRSR2, RUNX1, ETV6, NRAS, KRAS, TP53, CBL, JAK2, MPL, CEBPα,

Table 3 Cytogenetic abnormalities in myelodysplastic syndromes.

Risk Category	Examples
Very Good	del 11q; -Y
Good	Normal cytogenetics; del 20q; del 5q; single or double; del 12p
Intermediate	+8; del 7q; i17q; +19; +21; any other single or double abnormality; independent clones
Poor	-7; inv 3; del3q/t3q; 2 abnormalities including -7/del 7q; complex cytogenetics (3 abnormalities)
Very Poor	Complex cytogenetics: >3 abnormalities

Table 4 Genetic mutations in myelodysplastic syndromes (MDSs).

Mutated Genes	Frequency (%)	Prognosis
SF3B1	15-30 (up to 80% in MDS-RARS)	Good, favorable outcome with longer event free survival
SRSF2	2-12	Poor with short overall survival
U2AF1/U2AF35	5-12	Poor with rapid transformation into acute myeloid leukemia
ZRSR2	5	Neutral
DNMT3A TET2	5-22 15-26	Poor Neutral with no impact on survival
IDH1/IDH2	4-11	Mixed evidence
ASXL1 EZH2	10-20 3-7	Poor Poor
RUNX1 TP53 BCOR ETV6	5-10 5-10 5-6 3	Poor Poor Poor Poor
NRAS/KRAS	5-10	Poor

- RARS: Refractory anaemia ring sideroblasts

Table 5 Recurrent somatic mutations in myelodysplastic syndromes.

Pathway	Examples of genetic mutations
DNA methylation (epigenetic regulatory genes)	- DNMT3A - IDH1 - TET 2 - IDH2
DNA repair	- ATM - DLRE1C - FANCL - BRCC3 - TP53
Chromatin modification	- ASXL1 - EZH2
Signal transduction (Kinases/RAS pathway)	- NRAS / KRAS - CBL/NF1 - JAK2 - PTPN11 - FLT3
Cohesion complex	- STAG2 - RAD21 - SMC1A/SMC3 - CTCF
RNA splicing (splicing factor genes)	- SF3B1 - U2AF1 - SRSF2 - ZRSR2
Transcription factors and transcriptional regulation	CEBPA - RUNX1 - BCOR1/ BCORL1 - GATA2 - ETV6/EVI1

SETBP1, FLT3, BRAF and NPM1 [70,85-87]. PB-CF-DNA analysis has the following advantages:

- Detection of cytogenetic abnormalities and genetic mutations that predict evolution of new clones and disease progression,

Table 6 The revised international prognostic scoring system (R-IPSS) for myelodysplastic syndromes.

Prognostic variable	Points						
	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	-	Good	-	Intermediate	Poor	Very poor
Bone marrow blasts %	≤ 2	-	>2 - 5%	-	5% - 10%	> 10 %	-
Hemoglobin (g/dL)	≥ 10	-	8 - <10	< 8	-	-	-
Platelet count × 10^9/L	≥ 100	50 - 100	<50	-	-	-	-
Absolute neutrophil count × 10^9/L	≥ 0.8	< 0.8	-	-	-	-	-

Table 7 Current clinical picture of personalized medicine in MDSs.

Variable	Grading	Potential clinical consequences
Performance status	Good Poor	Standard treatment including allogeneic HSCT Supportive care only
Erythropoietin level	Low High	Treatment with erythropoietin stimulating agents No treatment with erythropoietin stimulating agent in case of anemia
Ferritin level	High	Treatment with iron chelation
Prognostic scoring index (IPSS/R-IPSS)	Good risk Poor risk	Supportive care only Hypomethylating agents and allogeneic HSCT
Cytogenetics	Del 5q	Treatment with lenalidomide
Genetic mutations	Good risk Poor risk	Supportive care only Standard treatment including allogeneic HSCT Intensified surveillance or early pre-emptive therapy in otherwise good-risk MDSs (e.g., by R-IPSS)

● MDSs: myelodysplastic syndromes ● HSCT: hematopoietic stem cell transplantation ● IPSS: international prognostic scoring index ● R-IPSS: revised international prognostic scoring index

- Establishing the diagnosis of MDSs in patients with cytopenias,
- Obviating the need for repeated BM examinations particularly in elderly patients and those with hypocellular or fibrotic BMs, and
- Monitoring response to cytotoxic chemotherapy and targeted agents including epigenetic therapies [83,86,87].

New techniques in the diagnosis of MDSs

The following are new techniques that are helpful in establishing the diagnosis of MDSs:

- Immunophenotyping by flowcytometry of PB neutrophils and monocytes particularly in low-risk MDSs,
- Proliferation index of specific compartments of BM cells that reflects the rate of production of hematopoietic cells in MDSs, and
- Measurement of telomere length in PB leukocytes as shorter telomeres have been found to be associated with occupational exposure to paints and pesticides [88-90].

Therapeutic options in MDSs

Treatment of MDSs is selected based on: risk stratification by the international prognostic scoring index (IPSS) and the revised IPSS, transfusion needs, percentage of BM blasts and cytogenetic as well as mutational profiles [42]. The revised IPSS in patients with MDSs is shown in **Table 6** [38-40]. Therapeutic options for low-risk MDSs with <10% blasts include:

- Growth factors such as erythropoietin and granulocyte-colony stimulating factor (G-CSF),
- Immune therapies including: corticosteroids, cyclosporine-A and antithymocyte globulin,
- Lenalidomide for 5q31,
- Decitabine and azacitidine,
- Iron chelation and blood transfusion,
- Imatinib for t5,12 and 5q33 variant with platelet-derived growth factor receptor (PDGFR)-β; and
- Investigational therapies such as clofarabine and homoharringtonine.
- For higher-risk (HR) MDSs with ≥ 10% blasts and chromosome 7 abnormalities or complex cytogenetics, therapeutic options include:
- Decitabine and azacytidine,
- Intensive chemotherapy for younger patients and those with diploid karyotype,
- Allogeneic hematopoietic stem cell transplantation (HSCT),
- Imatinib for t5,12 and 5q33 variant with PDGFR-β,
- Iron chelation and blood transfusion; and
- Investigational therapies [37,39,40,42,56,91].

Epigenetic modifying agents that are used in patients with MDSs include:

Table 8 Drugs in clinical trials and future therapies for myelodysplastic syndromes.

Genetic mutations, pathway or target	Drugs in clinical trials
IDH1/IDH2/R132	FT-2102, AG-881, ivosidenib, venetoclax and enasidenib
SF3B1, SRSF2, U2AF1, ZRSR2	H3B-8800 (oral)
TET 2	Ascorbic acid (oral and intravenous) and hypomethylating agents
TP53 including 5q- syndrome	APR-246 (intravenous) and decitabine
Epidermal growth factor receptor	Erlotinib
Dual inhibitor [phosphoinositide-3 kinase and polo-like kinase]	Rigosertib
Programmed cell death 1 protein (PD-1) and PD-1 ligand-1(PD-1L-1)	PD-1 and PD-1L1 inhibitors
Bone marrow megakaryocytes	Eltrombopag and romiplostim

- Demethylating agents such as: azacitidine licensed for Demethylating agents such as: azacitidine licensed for MDSs and AML, decitabine licensed for HR-MDSs and AML, and zebularine which is still investigational, and
- HDACIs which are still investigational and they include: panobinostat, vorinostat, entinostat, belinostat and romidepsin and valpoic acid [69].

Prognosis in MDSs

In patients with MDSs, prognosis is determined by:

- IPSS and R-IPSS,
- Age,
- Performance status,
- Comorbid medical conditions,
- Transfusion dependence, and
- Molecular biomarkers such as somatic mutations that can be detected by several methods including DNA sequencing [92].

Epigenetic Therapies in MDSs

Hypomethylating Agents (HMAs)

Epigenetic mechanisms such as abnormal DNA methylation are considered the first markers of tumorigenesis [93]. Methylation of the tumor suppressor gene CDKN2B is frequent in patients with MDSs and is usually acquired during disease progression [94]. DNA hypermethylation is well documented in the pathogenesis of MDSs [95,96]. Reversal of unfavorable methylation status in malignant cells has been a subject for epigenetic therapy of cancer using HMAs [93]. Reactivation by demethylation may halt disease progression [94]. Restoration of transcriptionally silenced genes by means of DNA methyltransferase inhibitors (DNMTIs) plays an important role in the current management of MDSs [93]. Thus, methylation status may serve a marker to monitor response to epigenetic therapies [96].

Azacitidine: Azacitidine is a pyrimidine nucleoside analog that was chemically synthesized and characterized by Frantisek Sorm et al in Czechoslovakia in the 1960s. It differs from cytosine primarily by the presence of nitrogen at position 5 [97]. Azacitidine is a DNMTI that leads to reduction of DNA methylation in patients with MDSs [93]. Azacitidine was the first HMA to be approved by the food and drug authority in the United States of America (USA-FDA) for the treatment of all subtypes of MDSs in the year 2004, then 5 years later it was granted extended approval for use in HR-MDSs [94,97-99]. Studies have shown that the following groups of patients with MDSs appear to have particular benefit:

- Chromosome 7 abnormalities including monosomy 7,
- Trisomy 8,
- Diploid karyotype, and
- HR-del 5q harboring TP53 mutations [94,100]. Azacitidine is indicated not only in MDSs but also in AML and CMML [94,97-99,101-107].

Azacitidine is a disease modifying agent that has changed the history of MDSs and it has been shown to impact positively all the 3 cell lines [94,97]. At high doses, azacitidine is cytotoxic and its cytotoxicity results from incorporation into DNA and RNA, while at lower doses the drug has hypomethylating effects as it induces differentiation and demethylation resulting in restoration of normal growth control and differentiation into hematopoietic cells [94,97,99]. The effectiveness of azacitidine was first demonstrated in the following 3 studies:

- A single randomized controlled trial comparing azacitidine administered subcutaneously (SC) with best supportive care (observational group) which showed 16% response rate in the study group and 0.0% response rate in the observational group, and
- Two single arm studies, in one azacitidine was administered intravenously (IV) and in the other it was given SC and these 2 studies showed response rates of 13% and 19% respectively and these responses were sustained for 11 and 17 months respectively [97,99,102].

Several studies have shown that azacitidine can:

- Prolong survival,
- Prolong time to leukemic transformation from 12 to 21 months,
- Reduce transfusion requirements of blood products, and
- Improve quality of life, while maintaining a relatively safe toxicity profile even in elderly individuals [95,97,101,103-105,108-112].

Complete remissions (CRs) can be encountered in up to 25% of patients, but unfortunately some patients do not respond to azacitidine possibly due to having inadequate plasma levels of the drug [94]. In order to improve response rates further, azacitidine

can be combined with lenalidomide, histone deacetylase inhibitors (HDACIs) and growth factors [97,100]. Although prolonged use of the drug is generally practiced, patients may benefit from a limited number of cycles of azacitidine [113]. The drug can induce complete and partial responses in approximately 50% of patients, these responses are usually not durable or sustainable as most responding patients lose their responses within 2 years [106,113,114].

Azacitidine can be given IV or SC. The standard and approved dose of 75 mg/m^2/day for 7 days every 28 days has been proven to show objective response rates, while the other dose schedule of 100 mg/m^2/day for 5 days has not been approved although this schedule is given taking into consideration convenience and logistic factors [97-99,102,109,110]. Azacitidine is rapidly absorbed after SC administration and maximum plasma concentration is reached within 30 minutes of SC administration and 10 minutes of IV administration [97,99]. The drug is widely distributed in tissues. Its bioavailability after SC administration is 89% of that after IV administration and plasma half-life is approximately 41 minutes after SC administration and about 22 minutes after IV administration [97,99].

The adverse effects of azacitidine include:

- Gastrointestinal tract (GIT) manifestations such as: nausea, vomiting, diarrhea, and constipation;
- Myelosuppression: neutropenia causing febrile neutropenia and infections in addition to thrombocytopenia causing petechiae, ecchymoses and other bleeding complications;
- Injection site reactions;
- Fever and rigors;
- Headache, dizziness and arthralgia;
- Liver dysfunction;
- Renal failure particularly in patients with hypotension and sepsis; and
- Treatment-related mortality (TRM) [94,98,99,101,113-115].

Oral azacitidine: In early phase clinical trials, oral azacitidine (CC-486) has been shown to be biologically and clinically active in patients with MDSs. Hence, it is currently evaluated in ongoing phase III clinical trials [108,115]. Oral azacitidine improves convenience and eliminates injection-site reactions and it enables testing of novel longer term low-dose schedules that enhance therapeutic activity of the drug by increasing exposure to circulating malignant cells [108].

Oral azacitidine can be given at doses ranging from 300 mg to 400 mg per day for 14-21 days each cycle [108,115,116]. In patients with MDSs (including lower-risk groups and patients with pre-treatment thrombocytopenia), AML and CMML, oral azacitidine in extended dosing regimens has been shown to be associated with significant DNA hypomethylation effect and overall response rates (ORRs) ranging from 35% to 73% [108,115-118]. The adverse effects of oral azacitidine include: GIT disturbances, myelosuppression, bleeding and TRM [108,115,116].

Response to azacitidine: In patients with MDSs treated with azacitidine, several studies have shown that the following factors predict response, longer overall survival (OS) and longer duration of response: red blood cell transfusion requirements, performance status, circulating blast cells, doubling of platelet count after first cycle of therapy, type of induction therapy given prior to HSCT, karyotype particularly HR cytogenetics, preceding 5q- syndrome, therapy-related MDSs, and mutational profile particularly TET2, SRSF2, TP53 and KDM6A mutations [45,114,119,120]. However, negative outcome, shorter OS and shorter duration of response to azacitidine have been reported with CDKN2A mutations [121-125]. Despite the presence of several publications indicating the presence of predictive biomarkers for response to azacitidine, a study that included 128 patients with MDSs and AML treated with azacitidine has shown no clear biomarkers for response to azacitidine and survival that could be identified [121-123].

In patients with MDSs treated with azacitidine, P53 expression which is a surrogate for the presence of TP53 mutation does not have negative impact on treatment response indicating that response to azacitidine is independent of P53 expression in patients with HR-MDSs [126]. Thus, the combination of azacitidine and lenalidomide may be beneficial in patients with del 5q harboring TP53 mutations [126].

Decitabine

Decitabine, 2-deoxy-5-azacitidine, is similar to azacitidine in structure and inhibition of the enzyme DNMT, but has different mechanisms of action [73,127-129]. Decitabine, a cytosine analog, is cytotoxic at high doses and has DNA demethylating activity at low doses [130]. In the year 2006, decitabine was approved by the USA-FDA for the treatment of de novo, secondary and therapy-related MDSs [127]. Although the antitumor activity of decitabine is not fully understood, it may involve one or more of the following:

- Reversal of cancer-associated hypermethylation events,
- Reactivation of genes that are responsible for cellular differentiation,
- Stimulation or induction of immune responses,
- Induction of DNA-damage response pathways,
- Augmentation of stem cell renewal, and
- Changes in the rate of apoptosis or apoptotic response pathways [127].

Decitabine has been used in the treatment of HR-MDSs, CMML, and AML particularly in elderly individuals [127,128,131-134]. It can be given: in upfront setting in the treatment of MDSs patients, in the maintenance therapy after allogeneic HSCT, and with fludarabine and total body irradiation (TBI) conditioning therapy prior to HSCT [127-129,132,133,135]. In patients with MDSs, decitabine has been used in combination with the following medications:

- Traditional Chinese medicine,
- Fludarabine and TBI conditioning therapy,
- Aclarubicin hydrochloride, cytosine arabinoside and G-CSF,
- Low dose chemotherapy particularly cytosine arabinoside,
- Tosedostat, and
- Valproic acid.

However, in patients with MDSs and AML, the combinations of decitabine with these medications have yielded variable responses [55,73,129,131,132,134,136-139].

The side effects of decitabine include: hematologic toxicity with neutropenia causing febrile neutropenia and infections such as pneumonia and thrombocytopenia causing bleeding from various sites; GIT toxicity such as nausea, vomiting, diarrhea and mucositis; hyperbilirubinemia; cardiovascular toxicity; renal failure; fatigue; and hair loss [127,128,133-135].

Several studies and 2 meta-analyses have shown superiority of azacitidine to decitabine in the treatment of patients with MDSs [127]. Hence, the use of decitabine in the treatment of HR-MDSs is not recommended after failure of azacitidine due to short duration of response and poor OS [136,137]. Also, the addition of valproic acid to decitabine has not been associated with improved outcome in patients with MDSs [131].

Disappearance of TP53 mutation has been shown to be an indication of response to decitabine in patients with MDSs and AML [128]. Recovery of platelet count by the second cycle of decitabine therapy can be used as an early predictor marker of improved survival and an increased response rate [138]. Several studies have sought to identify biomarkers that may predict response to decitabine such as: DNA methylation changes, expression of miR-29b, and specific genetic mutations such as: DNMT3A, IDH1, IDH2 and TET2 [128]. However, controversy still exists regarding the predictive value of these mutations. Additionally, none of the above suggested biomarkers is currently used to guide decitabine treatment for individual patients [128].

HDACIs in MDSs

In the nucleus, DNA is wound around 4 core histone proteins to form nucleosomes that, when compacted, form the condensed structure of chromatin [140]. Histones can be modified by several processes that include: acetylation, methylation, phosphorylation, sumoylation, ubiquitination, and citrullination [140]. Modifications of DNA or histones via methylation or acetylation lead to gene silencing and altered physiology relevant to MDSs [141]. Acetylation, which is one of the main histone modifications associated with gene expression, is controlled by 2 groups of enzymes: histone acetyltransferases and histone deacetylases (HDACs) [140,142].

Classes, mechanisms of action and resistance to HDACIs

HDACIs are epigenetic agents that act by modifying gene expression to restore the normal differentiation or death programs of transformed cells [143]. They regulate the acetylation of histones as well as non-histone protein targets [142]. There are 5 classes of HDACIs: class I, class IIA, class IIB, class III and class IV [140]. HDACIs have various mechanisms of action that include:

- Chromatin remodeling thus permitting re-expression of tumor suppressor genes that are abnormally suppressed or silenced in cancer cells,
- Relaxation of DNA, induction of DNA damage and inhibition of DNA repair,
- Interference with or inhibition of chaperone protein functions,
- Upregulation of endogenous inhibitors of cell cycle progression such as p21 and disruption of cell cycle checkpoints thus causing cell cycle arrest,
- Generation of free radicals and induction of autophagy,
- Promotion of apoptosis by inhibition of anti-apoptotic proteins, and
- Inhibition of angiogenesis and proteasome function [142,143].

Unfortunately, resistance to HDACIs frequently evolves and the following mechanisms of resistance have been described:

- Increased expression of multidrug resistance-associated proteins;
- Enhanced expression of p21 cell cycle protein;
- Increased expression of thioredoxin;
- Enhanced expression of anti-apoptotic proteins and inability to upregulate pro-apoptotic proteins;
- Alterations of HDAC protein levels;
- Increased protein signaling via the following pathways: mitogen-activated protein kinase, phosphoinositide 3-kinase, as well as signal transducer and activator of transcription; and
- Activation of nuclear factor kappa light chain enhancer signaling pathway and acetylation of p65 [140].

Clinical activity of HDACIs

In general, when used as single agents, HDACIs have shown only modest clinical activity in the treatment of patients with MDSs. However, marked responses have been observed in selected subsets of patients and once HDACIs are used in combination with other agents particularly HMAs [142,144]. Nevertheless, a recently published systematic review and a meta-analysis that included 7 clinical studies comprising 922 patients; 458 patients treated with HMAs alone and 464 patients treated with combination of HMAs and HDACIs; showed no significant differences in CR rates, hematologic improvement, ORRs, OS and toxicities between patients treated with HMAs alone or combined therapy [138]. Additionally, while significant results have been achieved with the use of HDACIs in the treatment of lymphomas and MM, efficacy in patients with myeloid malignancies has remained limited [145].

Obviously, many issues related to HDACIs remain incompletely understood and pose clinical and translational challenges [141]. Hopefully, the recent advances in disease biology and the design of more specific third generation HDACIs may drive the future clinical development of HDACIs in patients with myeloid malignancies in particular [146].

Specific HDACIs

Vorinostat: Vorinostat, suberoyalanilide hydroxamic acid, is a HDACI that was approved by the US-FDA in December 2006 for the treatment of relapsed or refractory cutaneous T-cell lymphoma [147-149]. It promotes protein acetylation; modulates gene expression; and induces differentiation, growth arrest and apoptosis of tumor cells [148,149]. It has shown promising

clinical activity against certain hematologic malignancies and solid tumors such as: MDSs, AML, CMML, MM, cutaneous T-cell lymphoma, diffuse large B-cell lymphoma, Hodgkin's lymphoma, in additions to carcinomas of the: breast, prostate, colon and lung [147-154].

In patients with MDSs, CMML and AML, the efficacy of vorinostat as a single agent is limited but several phase I and II clinical trials using combinations of vorinostat with conventional chemotherapeutic agents such as idarubicin and cytarabine or investigational drugs such as HMAs or lenalidomide have shown more promising results [147,148,150,154]. However, in patients with MDSs and AML, the use of vorinostat in combination with bortezomib or alvocidib has not shown any objective clinical responses [151,153].

As a single agent or in various drug combinations, vorinostat has acceptable toxicity profile with mainly gastrointestinal and constitutional side effects [148,154]. The main adverse effects of the drug include: nausea, vomiting, diarrhea, dehydration, anorexia, fatigue, cytopenias including thrombocytopenia, prolongation of QT interval on electrocardiogram, abnormal liver profile and metabolic disturbances including hypokalemia, hyperglycemia and hypophosphatemia [147,148,150-154].

Panobinostat: Panibinostat is a potent oral pan-deacetylase inhibitor of HDAC enzymes implicated in cancer development and progression that has been approved for the treatment of MM in the USA, Japan and Europe [155,156]. It modulates the acetylation of histone proteins and protein chaperones in malignant cells and its epigenetic regulation is primarily modulated through inhibition of class I histone deacetylase enzymes leading to: increased histone acetylation, relaxation of chromatin and alteration of expression of certain genes including tumor suppressor genes [155].

Phase I and II clinical trials on the use of panobinostat in low or intermediate risk MDSs have shown either limited or no meaningful clinical activity [157,158]. However, the use of the drug in combination with azacitidine in the treatment of MDSs, CMML and AML has shown variable clinical activity [159-161]. Additionally, the use of panobinostat in the maintenance therapy after allogeneic HSCT in patients with HR-MDSs and AML has been shown to prolong OS and to reduce rate of relapse of the primary disease [162]. The following adverse effects have been reported with the use of panobinostat: constitutional symptoms, GIT upset, BM suppression, infections, neuropathy and metabolic disturbances [155,159-162].

Romidepsin: Romidepsin (depsipeptide) is a bicyclic peptide that showed class I selective HDACI activity in the year 1998. Subsequently, it was approved by the USA-FDA for the treatment of cutaneous T-cell lymphoma [163,164]. Promising results have emerged from early clinical trials supporting the use of romidepsin in conjunction with other drugs for the treatment of other types of lymphoma, MM as well as certain solid tumors [163]. As monotherapy in unselected patients with MDSs and AML, romidepsin has shown limited clinical activity [164]. However, its use in patients with core binding factor AML has shown differential anti-leukemic and molecular activity [165]. The adverse effects of romidepsin include: GIT toxicity, myelosuppression with subsequent bleeding and infectious complications, constitutional symptoms, and metabolic as well as electrolytic disturbances [164].

Valproic acid (phenylbutyrate): Valproic acid (VPA), which has been known as an epileptic agent for many years, is a short-chain fatty acid and a HDACI that can reduce proliferation and induce differentiation of myeloid blast cells in patients with MDSs and AML particularly when given in combination with all-trans retinoic acid (ATRA) [166-169]. In patients with MDSs and AML, VPA as a single agent has shown limited clinical activity, but once used in combination with other drugs; such as: azacitidine, ATRA, bortezomib, hydralazine, decitabine or cytotoxic chemotherapeutic agents; it has been reported to be clinically active due to synergistic anti-leukemic activity and positive effects on blood indices with a significant increase in platelet count in particular [131,166-175]. Although VPA is generally well tolerated with modest side effects, moderate to severe hematologic toxicity including myelosuppression and evolution of myelodysplasia has been reported with the prolonged use of the drug [166,176-182]. The adverse effects are more pronounced once the drug is given in combination with other agents and these include various degrees of myelosuppression, evolution of MDSs and neurotoxicity [131,168,169].

Other HDACIs: Unfortunately, clinical trials combining entinostat or pracinostat with azacitidine in the treatment of patients with MDSs and AML have not shown any additional beneficial effects [183,184]. LBH589 is a novel HDACI that inhibits proliferation and induces apoptosis in tumor cell lines [185]. A phase I study on the IV use of LBH589 in a limited number of patients with HMs including AML and MDSs has shown consistent but transient anti-leukemic and biological effects [185]. A phase II clinical trial on the use of belinostat in the treatment of 21 patients with MDSs showed only 5% ORR with significant grade 3-4 toxicities so the study was ultimately terminated [186].

Challenges in the Current Management of MDSs

Thrombocytopenia and its future therapies in MDSs

Thrombocytopenia, which is commonly encountered in patients with MDSs, has multifactorial etiology and its associated bleeding complications represent a major cause of morbidity and mortality [187-190]. The thrombopoietin agonists, eltrombopag and romiplostim, have shown clinical activity in trials performed in patients with MDSs and thus they represent a potential alternative therapeutic option to platelet transfusions [187,191]. Several phase I and phase II clinical trials have shown not only safety but also efficacy of eltrombopag in the treatment of thrombocytopenia in patients with advanced MDSs [190,192-194]. Eltrombopag has been shown to increase megakaryocytic differentiation thus leading to the formation of normal megakaryocytic clones [195]. A single phase II clinical trial on the use of romiplostim in patients with low and intermediate-risk MDSs receiving azacitidine therapy has shown clinical benefit [189]. More prospective and ramdomized clinical trials on the

use of thrombopoietin agonists in different subtypes of MDSs are needed to: determine their future role as adjunctive therapies in patients with MDSs receiving novel agents including epigenetic therapies and prove or disprove the concern that these agents may increase the risks of clonal evolution and transformation into AML [196,197].

Personalized medicine in MDSs and its challenges

The main problems encountered in the treatment of patients with MDSs are:

- Unremarkable effects of conventional therapies,
- Only a minority of patients with MDSs are eligible for allogeneic HSCT which is still the only proven curative therapeutic modality, and
- Despite the superiority of HMAs when compared to HDACIs, treatment with both HMAs and HDACIs has shown limited efficacy [198].

Additional challenges include:

- despite the molecular advances in MDSs, response rates and their durations are suboptimal as CR rates are less than 20% and they rarely exceed 2 years;
- over the last 12 years, only 3 drugs (azacitidine, decitabine and lenalidomide) have been approved for the treatment of MDSs; and
- the progress in the therapeutics of MDSs is lagging behind those of MM, lymphomas and acute lymphoblastic leukemia [199-201]. The current clinical picture of personalized medicine in MDSs is illustrated in **Table 7** [39,45,200-206]. Examples of the investigational drugs; mainly in phase I/II clinical trials; and the potential future therapies for MDSs are included in **Table 8** [187,190,196,206-223].

Conclusions and Future Directions

MDSs comprise a group of clonal disorders that are clinically and biologically heterogeneous. Dysplastic hematopoiesis and epigenetic dysregulation are major players in the complex pathogenesis of MDSs. Despite the progress achieved in the molecular biology and epigenetics of MDSs, the response rates to the currently available epigenetic therapies are still suboptimal. Additionally, no new novel therapies have been approved for the treatment of MDSs over the last 12 years.

As single agents, the HMAs azacitidine and decitabine have already shown remarkable clinical activity, but complete responses are encountered in about 20% of patients and these responses hardly last longer than 2 years. However, once these epigenetic therapies are used in combination with cytotoxic chemotherapeutic agents or other novel therapies, response rates can improve further.

Despite having several classes of HDACIs with various mechanisms of action, these agents have shown only modest activity in the treatment of patients with MDSs, possibly due to the frequently evolving drug resistance. Hopefully, the ongoing clinical trials on several novel agents targeting various pathological pathways may ultimately translate into real progress in the clinical arena so that patients with various types of MDSs can enjoy cure or at least more durable responses.

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