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<td>Singh, GHH; Leung, AYH; Kwong, YL</td>
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Molecular and Cellular Mechanisms of Myelodysplastic Syndrome: Implications on Targeted Therapy

Harinder Gill, Anskar Y. H. Leung and Yok-Lam Kwong *

Department of Medicine, Queen Mary Hospital, Hong Kong, China; gillhsh@hku.hk (H.G.); ayhleung@hku.hk (A.Y.H.L.)
* Correspondence: ylkwong@hkucc.hku.hk; Tel.: +852-2255-5859; Fax: +852-2974-1165

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Abstract: Myelodysplastic syndrome (MDS) is a group of heterogeneous clonal hematopoietic stem cell disorders characterized by cytopenia, ineffective hematopoiesis, and progression to secondary acute myeloid leukemia in high-risk cases. Conventional prognostication relies on clinicopathological parameters supplemented by cytogenetic information. However, recent studies have shown that genetic aberrations also have critical impacts on treatment outcome. Moreover, these genetic alterations may themselves be a target for treatment. The mutation landscape in MDS is shaped by gene aberrations involved in DNA methylation (TET2, DNMT3A, IDH1/2), histone modification (ASXL1, EZH2), the RNA splicing machinery (SF3B1, SRSF2, ZRSR2, U2AF1/2), transcription (RUNX1, TP53, BCOR, PHF6, NCOA, CEBPA, GATA2), tyrosine kinase receptor signaling (JAK2, MPL, FLT3, GNAS, KIT), RAS pathways (KRAS, NRAS, CBL, NF1, PTPNI1), DNA repair (ATM, BRCC3, DLRE1C, FANCL), and cohesion complexes (STAG2, CTCF, SMC1A, RAD21). A detailed understanding of the pathogenetic mechanisms leading to transformation is critical for designing single-agent or combinatorial approaches in target therapy of MDS.

Keywords: myelodysplastic syndrome; gene mutations; prognostication; target therapy

1. Introduction

Myelodysplastic syndrome (MDS) is a group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis leading to cytopenia, and a significant risk of evolution to acute myeloid leukemia (AML) [1]. Conventional prognostic scoring of MDS is based on the extent of cytopenia, the percentage of bone marrow blast infiltration, and karyotypic abnormalities [2,3]. Risk categories based on prognostic scoring determine the therapeutic approaches. Treatment of high-risk MDS involves the use of hypomethylating agents (HMA) [4–8] and allogeneic hematopoietic stem cell transplantation (HSCT) in younger patients [1]. Clinical studies with HMAs including azacitidine and decitabine have shown a response rate of about 40% in high-risk patients and a median response duration of merely 9 to 15 months. HMA failure is associated with a poor prognosis and a median survival of less than 5 months [9,10]. Allogeneic HSCT is potentially curative for 25%–60% of patients depending on the risk category, but is restricted by age and donor availability. Treatment failure following HMA and allogeneic HSCT in high-risk MDS remains an unmet clinical need.

The pathophysiology of MDS and its progression to AML involve cytogenetic, genetic, and epigenetic aberrations [1] (Figure 1). Genome-wide and targeted analyses from next-generation sequencing have identified novel mutations of prognostic and therapeutic significance [1,11–13]. Recurrent mutations in more than 45 genes are found in over 85% of cases [1,14]. These mutations are found in genes regulating DNA methylation (DNMT3A, TET2, IDH1/2), post-translational chromatin
modification (EZH2, ASXL1), transcription regulation (TP53, RUNX1, GATA2), the RNA spliceosome machinery (SF3B1, U2AF1, SRSF2, ZRSR2), cohesion complexes (STAG2), and signal transduction (JAK2, KRAS, CBL) [14]. Mutations in TP53, EZH2, ETv6, RUNX1, SRSF2 and ASXL1 portend inferior survivals [14]. These mutations may also predict responses to HMA and allogeneic HSCT. Furthermore, specific mutations, such as internal tandem duplication of FLT3 (FLT3-ITD), have been observed during disease progression and are potential therapeutic targets [15,16]. Therefore, a better understanding of the molecular landscape in MDS has important implications on treatment response, prognostication, and novel molecular therapeutic targeting.

![Gene mutations and their roles in disease progression in myelodysplastic syndrome](image)

Figure 1. Gene mutations and their roles in disease progression in myelodysplastic syndrome (DNMT3A: DNA methyltransferase 3A; SF3B1: splicing factor 3b subunit 1; RUNX1: runt related transcription factor 1; IDH1/2: isocitrate dehydrogenase 1/2; ASXL1: additional sex combs like 1; EZH2: enhancer of zeste 2 polycomb repressive complex 2 subunit; SRSF2: serine/arginine-rich splicing factor 2; U2AF1: U2 small nuclear RNA auxiliary factor 1; TP53: tumor protein p53; BCL2: B-cell CLL/lymphoma 2; FLT3-ITD: fms-like tyrosine kinase-3 internal tandem duplication; KIT: KIT proto-oncogene receptor tyrosine kinase; JAK/STAT: Janus kinase/signal transducer and activator of transcription; RAS: rat sarcoma viral oncogene homolog; TET2: ten-eleven translocation methylcytosine dioxygenase 2; N-RAS: neuroblastoma RAS oncogene).

2. Targeting Genes Involved in DNA Methylation

Aberrant DNA methylation represents one of the important mechanisms underlying altered epigenetic regulation in MDS [11]. Frequently mutated genes relevant to this pathway include TET2, DNMT3A, and IDH1/2 [1,11].

2.1. TET2 Mutations

The TET family comprises the dioxygenase proteins TET1, TET2, and TET3. TET2, its gene mapping to chromosome 4q24, modulates hydroxymethylation by catalyzing the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, which is an intermediate of DNA methylation that blocks the building of silencing proteins to methylated DNA [17]. TET2 mutations are observed in a range of myeloid neoplasms including AML, MDS, and myeloproliferative neoplasm (MPN) [18,19]. In MDS, TET2 mutations are found in 20%–25% of patients, but occur at a higher frequency of 30%–60% in chronic myelomonocytic leukemia (CMML) [20–23]. The prognostic impact of TET2 mutations on survivals in MDS is controversial. Large cohort studies showed that TET2 mutations did not appear to impact on overall survivals (OS) [24,25]. On the other hand, TET2 mutations have also been shown to
confer superior survivals [23]. Furthermore, TET2 mutations may predict a more favorable response to HMAs in high-risk patients [1,11,19].

2.2. DNMT3A Mutations

The DNMT family comprises the enzymes DNMT1, DNMT3A, and DNMT3B, and catalyzes the transfer of methyl group on the 5’-position of cytosines located at CpG dinucleotides [26]. They are involved in physiological processes that include imprinting, X-chromosome inactivation, differentiation, proliferation, and apoptosis [26]. DNMT1 maintains DNA methylation during DNA replication, while DNMT3A and DNMT3B catalyze DNA methylation. DNMT3A is highly expressed in T lymphocytes and neutrophils, while DNMT3B is downregulated in hematopoietic differentiation. Aberrant CpG island promoter methylation in tumor suppressor genes is an important pathogenetic mechanism in malignant tumors, suggesting that DNMTs play important roles in oncogenesis.

DNMT3A mutations occur in 30%–35% of AML with normal karyotype, and about 10% of MDS and 20% of T-lineage acute lymphoblastic leukemia [27–29]. DNMT3A mutations result in loss of function, and are present in pre-leukemic hematopoietic stem cells, remaining stable through disease evolution to MDS and AML [30,31]. MDS patients with DNMT3A mutations have shorter OS and higher risks of leukemic transformation [32,33]. DNMT3A mutations are specific biomarkers of positive response to DNA methyltransferase inhibitors [34]. Azacitidine and decitabine incorporate into DNA, resulting in proteosomal degradation of DNMTs. Guadecitibine (SGI-110) is a second generation HMA and a dinucleotide of decitabine and deoxyguanosine [28], with its use leading to extended decitabine exposure via resistance to deamination. Apart from DNA methylation, histone deacetylation is the other post-translational modification involved in the silencing of genes. Histone deacetylases (HDAC) are enzymes catalyzing histone acetylation, which are over-expressed in various malignancies including AML. However, HDAC inhibitors have limited efficacy as single agents. Phase II trials of HDAC inhibitors in combination with HMAs are ongoing. Examples of HDAC inhibitors that have shown activity in combination with HMAs include pracinostat, vorinostat, and valproic acid [28].

2.3. Isocitrate Dehydrogenases 1 and 2 (IDH1 and IDH2) Mutations

Isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) are a family of NADP-dependent enzymes critically involved in the conversion of isocitrate to α-ketoglutarate (α-KG) [27,28]. IDH1/2 mutations are heterozygous and occur mostly at residues R132 in IDH1, and R140 or R172 in IDH2. IDH1 and IDH2 mutations are mutually exclusive with TET2 mutations in AML, suggesting that they play a similar mechanistic role. Mutant IDH1 and IDH2 proteins have a reduced affinity for isocitrate, but acquire a neomorphic function, not shared by the wild-type enzymes, in converting α-KG to 2-hydroxyglutarate (2-HG) via oxidation of NADP and release of carbon dioxide. As a result, abnormal intracellular accumulation of 2-HG inhibits the dioxygenase enzymes JumonjiC (JmjC) and TET2, prolyl/lysyl hydroxylases, and cytochrome C oxidase (COX). These enzyme inhibitions lead to epigenetic dysregulations [27,28] postulated to be involved in oncogenesis, so that 2-HG is considered an oncometabolite. IDH1/2 mutations are seen in patients with cytogenetically normal AML, MDS, MPN, angioimmunoblastic T-cell lymphoma, glioma, cholangiocarcinoma, and chondrosarcoma [27,35]. In leukemogenesis, additional cooperative genetic mutations (such as in HOXA9 and NPM1) are required. IDH1/2 mutations are seen in 2%–12% of MDS [36,37], being more prevalent in refractory anemia with excess blasts-2 (RAEB-2) than other low-grade MDS [38]. IDH1 mutations, particularly those at codon 132, are associated with inferior OS [36]. IDH2 mutations in MDS are frequently present concurrently with DNMT3A, ASXL1, and SRSF2 mutations and also predict inferior OS, especially those at codon 172 [37]. IDH1/2-mutated myeloid malignancies are potentially targetable by direct blockade of the mutant enzymatic activity or the associated metabolic pathways [28]. The IDH1-R132H inhibitor AGI-5198 is active against glioma cells in vitro [39]. The IDH2-R140Q inhibitor AGI-6780 induces changes in DNA methylation and histone states, resulting in differentiation of AML cell lines and primary AML cells [40,41]. The IDH1
inhibitor HMS-101 blocks colony formation of primary IDH1-mutated AML. In a phase I trial, the oral IDH2 inhibitor AG-221 induced an overall response rate of 62.5% and a complete response rate of 37.5% in patients with relapsed or refractory IDH2-mutated AML and MDS [27]. AG-221 achieved a durable inhibition of plasma 2-HG in more than 90% of patients with IDH2-R140Q mutation and 50% of patients with IDH2-R172K mutation [28]. The selective oral IDH1 inhibitor AG-120 decreased intracellular 2-HG levels and resulted in growth inhibition and differentiation in primary IDH1-mutant AML cells \textit{ex vivo}. A phase I study evaluating AG-120 is ongoing. Intravenous asparaginase \textit{Erwinia chrysanthemi} (Erwinase) is currently being evaluated in IDH1-mutated adult AML [28]. Targeting of the altered metabolic pathways in IDH1/2-mutated AML is another approach, and drugs tested include glutaminidase inhibitors (BPTES, zaprinast and CB-839) and NADPH inhibitors (epigallocatechin-3-gallate) [27].

3. Targeting Mutation in Histone Modification Genes

3.1. EZH2 Mutations

EZH2, a histone-lysine N-methyltransferase enzyme, catalyzes the addition of methyl groups to histone H3 at lysine 27 (H3K27). It is a key component of Polycomb repressive complexes (PRCs), participating in transcription repression [28]. EZH2 mutations can be detected in lymphomas and myeloid malignancies. In MDS, EZH2 mutations are seen in 6%–12% of patients. EZH2 mutations frequently co-exist with TET2 mutations, and are associated with disease transformation. Consequently, EZH2 mutations are observed in high-grade MDS, and are associated with a higher risk of secondary AML and worse OS. A specific EZH2 inhibitor, GSK-126, has been shown to be active in EZH2-mutated lymphomas [28], and has yet to be tested in myeloid malignancies.

3.2. ASXL1 Mutations

The ASXL1 protein, its gene mapping to chromosome 20q11.21, regulates histone modification by interacting with the PRC2 [11]. PRC2 methylates H3K27 and is a key regulator of hematopoiesis [42,43]. Biologically, ASXL1 loss is associated with hematopoietic transformation and increased self-renewal [28]. ASXL1 mutations are found in 11% of patients with MDS, portending an inferior OS, and 43% of patients with CMML, being associated with a higher risk of secondary AML [44,45].

3.3. UTX Mutations

The \textit{UTX} gene or the lysine (K)-specific demethylase 6A (\textit{KDM6A}) gene is a member of the JumonjiC family and encodes an H3K27 demethylase [46,47]. UTX regulates histone methylation in conjunction with other epigenetic modifiers. \textit{UTX} mutations are seen in 8% of patients with CMML and 1% of patients with MDS [11]. The prognostic impact of \textit{UTX} mutation is unclear. \textit{UTX} (\textit{KDM6A}) can be targeted by small molecule inhibitors that interfere with its activity [28]. GSKJ1 and GSKJ4 are two new agents developed with inhibitory effect against KDM6A activity [28].

4. Targeting Mutations in the RNA Splicing Genes

RNA splicing is a process where mature RNA is formed from pre-messenger RNA (pre-mRNA) through intron removal and exon splicing. Mutation of genes involved in RNA splicing, including \textit{SF3B1}, \textit{SRSF2}, \textit{ZRSR2}, and \textit{U2AF1/2}, occur in approximately 50% of MDS patients [11].

4.1. SF3B1 Mutations

\textit{SF3B1}, encoding the subunit 1 of the splicing factor 3b complex, is an essential component of the U2 snRNP that recognizes the 3' splice site at intron-exon junctions [48]. \textit{SF3B1} mutations are seen in 57%–75% of patients with refractory anemia with ring sideroblasts (RARS), and 6%–18% of patients with other subtypes of MDS [49,50]. Mutant \textit{SF3B1} downregulates genes essential in the mitochondrial pathways. These include the \textit{ACACA} (acetyl-coenzyme A carboxylase alpha) and the \textit{RGL1} (ral
guanine nucleotide dissociation stimulator like-1) genes. SF3B1-mutated RARS have abnormal splicing of the ABCB7 gene in the mitochondria, which leads to deficiency of the ABCB7 protein, resulting in mitochondrial iron overload, reduced heme synthesis, and ineffective erythropoiesis [11,19]. SF3B1-mutated MDS is associated with thrombocytosis, increased ring sideroblasts, fewer cytopenias, and lower blasts percentage [50]. SF3B1 mutations are associated with a favorable prognosis and prolonged survivals [50].

4.2. SRSF2 Mutations

SRSF2, encoding the serine/arginine-rich splicing factor 2, is critically involved in splice site selection, spliceosome assembly, and constitutive and alternative splicing [51]. SRSF2 mutations are stable during disease evolution in MDS, suggesting that they may play a role in disease initiation [51]. SRSF2 mutations are seen in 11%–15% of patients with MDS, frequently co-existing with RUNX1, IDH1, IDH2, and ASXL1 mutations [11], and confer an inferior OS [52,53].

4.3. ZRSR2 Mutations

ZRSR2 encodes a subunit of U2 auxiliary factor. ZRSR2 mutations occur in 3% of patients with MDS. Their prognostic significance is unclear [54].

4.4. U2AF1 Mutations

U2AF1 gene, mapping to chromosome 21q22.2, encodes the U2 auxiliary factor that facilitates the binding of U2 snRNP to the pre-mRNA branch site [54]. Recurrent mutations of the U2AF1 gene occur in 9% of patients with MDS [54]. The prognostic impact of U2AF1 mutations remains unclear [11,54].

5. Targeting Mutations in Transcription Factor Genes

5.1. RUNX1 Mutations

RUNX1 is a key regulator of myeloid differentiation, and was first described in a familial platelet disorder associated with leukemic evolution [55]. RUNX1 mutations are found in 24% of patients with RAEB-1/RAEB-2 and secondary AML [56,57], and independently confer an inferior prognosis [11].

5.2. BCOR/BCORL1 Mutations

BCOR and BCORL1 function as co-repressors. They co-repress BCL6 function and play key roles in embryonic development [58–60]. In addition to its role in transcription regulation, BCOR/BCORL1 are also a component of PRCs [60]. BCOR and BCORL1 mutations are seen in approximately 5% and 1% of patients with MDS and are associated with inferior OS [61].

5.3. ETV6 Mutations

The ETS is a family of transcription factors that play important roles in hematological malignancies. ETV6 (ets-variant 6) co-represses transcription of ETS. ETV6 gene aberrations are commonly secondary to chromosomal translocations. The most common chromosomal translocation involving ETV6 is t(3;12)(q26;p13) [11]. In addition, deletions or somatic mutations of ETV6 have been described in patients with MDS and AML, and are associated with monosomy 7 [11]. ETV6 rearrangements are seen in about 1% of MDS and confer a poor prognosis in MDS [62].

5.4. SETBP1 Mutations

SETBP1 is a nuclear protein that binds to SET, interacting with PP2A, a tumor suppressor gene [11]. It plays an important role in the regulation of cellular proliferation. SETBP1 mutations are seen in 2%–4% of MDS, 6%–15% of CMML, and 24%–32% of atypical chronic myeloid leukemia [11]. Other associations with SETBP1 mutations include leucocytosis, monosomy 7, 7q deletion, isochromosome
17p, and mutations of the other epigenetic regulators ASXL1, EZH2, and SRSF2 [63,64]. SETBP1 mutations are associated with inferior survivals and leukemic transformation [63,64].

5.5. GATA2 Mutations

GATA2 is a transcription factor critical in hematopoietic stem cell proliferation and survival. GATA2 mutations have been described in familial MDS and may play a role in evolution to MDS and AML in patients with chronic neutropenia [11].

6. Targeting DNA Repair/Tumor Suppressor Genes

TP53 Mutations

TP53 is a tumor suppressor gene with key functions in regulating cell cycle and DNA repair [65,66]. TP53 mutations are detected in about 9% of MDS, with a higher frequency in cases of with isolated 5q deletion and complex karyotypes [67]. TP53 mutations are associated with a high risk of leukemic transformation and a poor OS [11]. Therapeutic targeting of TP53 has been explored in MDS patients with del(5q). Canersen is an antisense oligonucleotide complementary to TP53, which is capable of suppressing mutant p53 expression and restoring impaired erythropoiesis in patients with del(5q) not responding to lenalidomide therapy [68].

7. Targeting Mutations in Signal Transduction

7.1. JAK2 Mutations

JAK2 is a member of the family of Janus kinases essential in hematopoiesis. JAK2-V617F, a mutation in the pseudokinase domain, results in constitutional activation of tyrosine kinase activity. Activation of the JAK/STAT pathways is the hallmark of MPN. JAK2-V617F mutation is detectable in patients with MDS/MPN, including RARS with thrombocytosis (RARS-T) and a subgroup of patients with del(5q) [11]. JAK2-V617F-positive MDS has a lower risk of leukemic transformation and better OS [11]. The JAK/STAT pathway is activated in various subtypes of AML. The JAK1/2 inhibitor ruxolitinib is active against secondary AML evolving from MPN [12,69,70]. STAT3 inhibition with tyrosine kinase inhibitors has demonstrated significant anti-proliferative effects in human AML cell lines harboring JAK2-V617F mutation [69].

7.2. FLT3 Mutations

FLT3 is a class III receptor tyrosine kinase consisting of a juxtamembrane domain (JMD), two tyrosine kinase domains (TKD1 and TKD2), and five extracellular immunoglobulin-like domains. It is highly expressed in hematopoietic stem and progenitor cells. Dimerization and auto-phosphorylation follows binding of FLT3 to the FLT3 ligand [71–73]. This leads to downstream activation of the PI3K/AKT and RAS/MAPK pathways. FLT3-ITD occurs in around 30% of AML and is commonly associated with normal karyotype, trisomy 8, t(6;9), and t(15;17) [74,75]. FLT3-ITD occurs as a result of an in-frame duplication of 3 to 400 base pairs at the JMD or TKD1 domains. This results in constitutive activation of FLT3 and aberrant downstream activation of signaling pathways including the STAT5 pathways [76,77]. Clinically, FLT3-ITD positive AML is associated with higher relapse rates and inferior survivals, which are directly related to the FLT3-ITD allele burden [71,78]. Hence, FLT3 inhibition represents an important treatment target. In MDS, FLT3 mutations occur at a much lower frequency, varying from 0.6% to 6% [15,79–85]. Previous studies have examined acquisition of FLT3 mutation in serial samples during disease transformation, showing a higher frequency of FLT3-ITD during leukemia transformation (Table 1) [15,81,84–88]. Intriguingly, among patients with MDS transforming into AML with FLT3-ITD, more than half had prior exposure to HMAs [88]. These observations suggest that FLT3 mutations are involved in treatment failure with HMAs and leukemic transformation.
Various FLT3 inhibitors have been tested in prospective clinical trials in FLT3-ITD-positive AML. They include midostaurin, tandutinib, KW-2449, lestaurtinib, sunitinib, sorafenib, and quizartinib [71,73,89–104]. The second generation dual FLT3 and JAK2 inhibitor pacritinib is currently under prospective evaluation [105]. A response rate of 46%–100% can be achieved with the use of FLT3 inhibitors as monotherapy in FLT3-ITD+ AML [71]. However, responses were generally brief, with a median duration of remission of only 11–13 weeks [71]. Combination therapy with FLT3 inhibitors and chemotherapy resulted in improved rates of complete remission (CR) or complete response with incomplete hematological recovery (CRi) [71], although this did not translate into an improvement in leukemia-free-survival or OS.

**Table 1.** Previous studies evaluating FLT3-ITD mutations in patients with MDS during disease progression.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient No.</th>
<th>Treatment</th>
<th>FLT3 Mutations at Dx</th>
<th>sAML No.</th>
<th>FLT3 Mutations at sAML</th>
<th>HMA Use in Acquired FLT3 Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shih et al. [86]</td>
<td>70 *</td>
<td>Supportive; Ara-C; oral chemotherapy</td>
<td>ITD: 3 (4.3%)</td>
<td>70 *</td>
<td>ITD: 10 (14.2%)</td>
<td>No</td>
</tr>
<tr>
<td>Georgiou et al. [87]</td>
<td>97</td>
<td>Not reported</td>
<td>ITD: 1 (1%)</td>
<td>42</td>
<td>ITD: 5 (42%)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Georgiou et al. [81]</td>
<td>50</td>
<td>Not reported</td>
<td>ITD: 0</td>
<td>2</td>
<td>ITD: 2 #</td>
<td>Not reported</td>
</tr>
<tr>
<td>Dicker et al. [84]</td>
<td>20 **</td>
<td>Supportive care</td>
<td>ITD: 2</td>
<td>74</td>
<td>ITD: 11 (15%)</td>
<td>No</td>
</tr>
<tr>
<td>Takahashi et al. [88]</td>
<td>278</td>
<td>Supportive/HMA/induction chemotherapy/HSCT</td>
<td>ITD: 2</td>
<td>27</td>
<td>ITD: 5 (19%)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Badar et al. [85]</td>
<td>102</td>
<td>Immunomodulators: 7 (7%)</td>
<td>TKD: 0</td>
<td>27</td>
<td>TKD: 6 (16%)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Meggendorfer et al. [15]</td>
<td>38</td>
<td>Not reported</td>
<td>TKD: 0</td>
<td>38</td>
<td>TKD: 3 (8%)</td>
<td>Not reported</td>
</tr>
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</table>

MDS: myelodysplastic syndrome; No.: number; Dx: diagnosis; sAML: secondary acute myeloid leukemia; HMA: hypomethylating agent; ITD: internal tandem duplication; TKD: tyrosine kinase domain mutation; *: 70 patients with sAML were retrospectively evaluated for FLT3 mutation at sAML and MDS stages; #: FLT3-ITD was evaluated serially at 6 and 12 months in this study. FLT3-ITD was detected at 6 months in 2 cases while still at MDS stage. Both progressed to sAML; **: 20 paired samples at MDS and sAML were evaluated; HSCT: hematopoietic stem cell transplantation.

Combination of FLT3 inhibitors with HMAs has also been explored. HMAs have single agent activity in inducing and maintaining remissions in MDS and AML, resulting in the prolongation of OS [106–113]. HMAs increase apoptotic cell death, expression of tumor-necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL), and demethylation of CpG-A elements [114]. HMAs also induce differentiation of the leukemic clones by upregulating differentiation-modulating genes [115,116]. Furthermore, FLT3 ligand is released by the bone marrow microvascular endothelium, T-cells, and myeloid leukemic cells in an autocrine manner [117–119]. A surge of FLT3 ligand may occur because of the use of FLT3 inhibitors, resulting in a decrease in their efficacy. The concomitant use of HMAs is postulated to ameliorate this FLT3 ligand surge. Combination of the FLT3 inhibitor midostaurin with decitabine has shown synergism in a phase I clinical study of elderly AML patients [120]. In a prospective phase II trial combining sorafenib and azacitidine, an overall response rate of 46% (CR = 27%, CRi = 16%, partial remission = 3%) and a median response duration of 2.3 months was achieved [117]. The clinical evidence supports the proposition that reduced FLT3 ligand release and surge with HMA results in improved responses to treatment [121–124]. The addition of azacitidine following sorafenib-induced remission in relapsed or refractory FLT3-ITD positive AML improved the duration of remission, successfully bridging patients to subsequent HSCT [16].
Mutations that disrupt the binding of FLT3 inhibitors to FLT3 lead to resistance (at the activation loop, residues D835V/Y/F/H, D839G, Y842C/H; at the gatekeeper residues F691L/I) [71,73,125]. Second generation tyrosine kinase inhibitors (TKI) have been developed to tackle resistance to first generation FLT3 inhibitors. Ponatinib is a multi kinase inhibitor with proven efficacy against chronic myelogenous leukemia harboring the BCR-ABL1 T315I mutation [51]. It has demonstrable in vitro activities against AML cell lines with FLT3-ITD and FLT3-TKD mutations, especially N767D, F691, and G697R [126–128]. Crenolanib is a novel benzamidine quinolone derivative with highly selective FLT3 inhibitory activity [129] and in vitro efficacy against D835 FLT3 mutants resistant to sorafenib and quizartinib [129]. Significant clinical activity was seen in patients with relapsed or refractory FLT3-ITD+ AML with the FLT3 D835 mutation [130]. Other targetable non-mutational pathways that cause resistance to FLT3 inhibitors include the upregulated pro-survival pathways (MEK/ERK, PI3K/AKT, STAT/PIM), upregulated FLT3 receptor or FLT3 ligand, bone marrow microenvironment/stroma-mediated resistance (CXCL12-CXCR4 pathway), and activated anti-apoptotic proteins [125,131]. With the rapidly developing armamentarium against FLT3-mutated AML, targeting FLT3-mutated MDS and secondary AML is an attractive therapeutic option.

7.3. KIT Mutations

KIT (CD117) is a type III tyrosine kinase receptor [132]. KIT mutations in exon 8 result in activation of the PI3K/Akt pathways, and those in codon 816 (activation loop) result in STAT3/STAT5 and PI3K/Akt pathway activation. KIT mutations are detected in 20%–30% of patients with core-binding factor (CBF) leukemias that involve t(8;21) and inv(16)/t(16;16). KIT mutations in CBF leukemias are associated with shorter survivals [133]. The combination of the multikinase inhibitor imatinib with chemotherapy was active against AML harboring KIT exon 8 mutations. Phase I/II trials of dasatinib (active against the KIT codon 816 mutation) in combination with induction chemotherapy in CBF AML are ongoing [132]. KIT mutations are found in 1% of MDS, but clinical benefits of KIT inhibition in such patients remain to be defined.

7.4. CBL Mutations

CBL is a tumor suppressor gene that negatively regulates receptor tyrosine kinases [11]. CBL mutations are observed in about 1% of MDS, but with higher frequencies of 19% in CMML and 10% in MDS/MPN. Mutant CBL is unable to attenuate signaling functions that drive oncogenesis. It has been shown that, in juvenile myelomonocytic leukemia, mutant CBL confers resistance to apoptosis via activation of the Src family of kinases and thus the lyn-PI3K/AKT pathway [11]. Lyn-PI3K/AKT is a potentially targetable pathway in CBL-mutated myeloid malignancies [12]. CBL mutations are associated with high-grade MDS [134–136].

7.5. Mutations in the RAS Pathway

Important members of the RAS family include N-RAS, K-RAS and H-RAS. They are GTPases that regulate cell proliferation, differentiation, and survival [11]. N-RAS mutations are seen in 4%–9% of MDS and 12% of CMML [134]. K-RAS mutations are more commonly seen in up to 14% of CMML [134]. The prognostic impact of N-RAS and K-RAS mutations is yet to be determined. Other members of the RAS pathway include NF-1, BRAF, and PTPN-11, but mutations involving these gene are rarely seen in patients with MDS [12].

7.6. Mutations in the Cohesin Gene Family

The cohesin complex is responsible for the cohesion of sister chromatids, DNA repair, and regulation of transcription. Cohesin complex gene mutations or deletions that are seen in myeloid malignancies include STAG2, RAD21, SMC1A, and SMC3 [11]. They are mutually exclusive. STAG2 mutations are seen in approximately 10% of MDS and are associated with RUNX1 mutations commonly seen in high-grade MDS [11]. The impact of cohesin complex gene aberrations on the outcome of MDS is not well defined [11].
8. Other Novel Targeted Approaches

8.1. BCL-2 Inhibition

The intrinsic cellular apoptotic pathway is controlled by the BCL-2 family of proteins comprising pro-apoptotic and anti-apoptotic proteins [137,138]. Important pro-apoptotic proteins include the BAX/BAX-like proteins and the BH3-only proteins. They cause cellular apoptosis via activation of caspase-3 and caspase-7 [138]. The anti-apoptotic or “pro-survival” BCL-2 family members include BCL-2, MCL-1 or BCL-X_L. Aberrant apoptosis is a cardinal feature of MDS owing to deregulated balance between pro-apoptotic and anti-apoptotic BCL-2 proteins [138]. In low-risk MDS, intramedullary apoptosis is increased. In high-risk MDS and secondary AML, there is an acquired resistance to apoptosis associated with a balance shift towards an increased expression of anti-apoptotic BCL-2 proteins [138]. The aberrant apoptotic pathway in MDS is a potential target. It has been shown that inhibition of the anti-apoptotic proteins by ABT-737 or ABT-199 targeted hematopoietic stem and progenitor cells in high-risk MDS and secondary AML samples [137–140]. The anti-apoptotic BCL-2 family of proteins modulates the anti-leukemic effects of azacitidine, and profiling of these proteins might predict response to azacitidine [137,141,142]. Therefore, targeting of the BCL-2 family of proteins may enhance azacitidine effectiveness, so that a combination of BCL-2 inhibitors with HMAs is an attractive option, particularly in cases of failing azacitidine treatment.

8.2. Polo-Like Kinases

Polo-like kinases (Plks) are serine/threonine kinases that regulate cell cycle, including entry into mitosis, DNA replication, and DNA damage responses [143]. Over-expressed in AML, Plk1 is of particular importance, playing a critical role in mitosis, protection against apoptosis, and cancer invasiveness. Volasertib, a dihydropteridinone derivative, is a small molecule competitive kinase inhibitor of Plk1. It is active against relapsed or refractory AML, either as monotherapy or in combination with low-dose cytarabine [143].

8.3. Mitogen-Activated Protein Kinase (MAPK) and Mammalian Target of Rapamycin (mTOR)

The mitogen-activated protein kinase (MAPK) and the mammalian target of rapamycin (mTOR) pathways are involved in the expansion and survival of leukemic cells [69]. The downstream effectors of MAPK, namely Mnk kinases, are potential targets for treatment in AML. Inhibition of mTOR complex 1 and mTOR complex 2 has shown potent anti-leukemic effect [69].

8.4. Arsenic Trioxide

Arsenic trioxide (As_{2}O_{3}) has been shown to be active against MDS via its pro-apoptotic, anti-proliferative, and anti-angiogenic properties [144]. As_{2}O_{3} promote apoptosis via downregulation of the BCL-2 family of proteins, inhibition of glutathione peroxidase activity, caspase activation, and NF-κB inhibition [144–146]. As_{2}O_{3} as a single agent or in combination results in a response in about 25% of cases [144,147,148]. Combined use with thalidomide, gemtuzumab ozogamicin (GO), and azacitidine has been reported [149–151]. The combination of arsenic trioxide with thalidomide has shown activity against MDS with inv(3)(3q21;3q26), targeting the EVI1 pathway [144].

8.5. Monoclonal Antibodies and Novel Immunotherapy

Gemtuzumab ozogamicin (GO) is an immuno-conjugate composed of the anti-CD33 monoclonal IgG4 antibody conjugated to the cytotoxin calicheamicin [27,69,152]. There is clinical benefit, which has been shown recently, in the management in older AML patients [69]. SGN-CD33a is an anti-CD33 monoclonal antibody conjugated to pyrrolo-benzodiazepine dimer [69]. It cross-links DNA leading to cell death and is as least three times as potent as GO in preliminary studies. CD123 (IL-3R) is a leukemia stem cell marker targetable using immuno-conjugates, bi-specific antibodies (combined with diphtheria toxin and an antibody against CD95), and engineered T-cells expressing chimeric
antigen receptors (CAR-T) [27,69]. Deregulation of the programmed cell death (PD-1/PD-L1 axis has also been shown to contribute to the pathogenesis of myeloid malignancies including AML and MDS [153]. PD-L1 is over-expressed in various malignancies. Blockade of the PD-1 pathway in solid tumors results in CD8+ T-cell infiltration, leading to clinical response [69]. Phase I studies of immune checkpoint inhibitors in acute myeloid leukemia and myelodysplastic syndromes are now ongoing.

9. Conclusions and Future Directions

Data arising from whole-genome sequencing (WGS) have shown that the clonal evolution of MDS to AML is complex [154]. More tantalizing is the observation that, in individuals who are either healthy or have minor cytopenias without definite evidence of MDS, next-generation sequencing has shown the presence of MDS-related mutations in genes including ASXL1, TP53, BCORL1, GNAS, SF3B1, DNMT3A, TET2, and JAK2. The frequency of detecting these mutations increases with age, being very rare in people <40 years old, but present in about 10% of people aged 70 to 79 years, and reaching 18% in people >90 years old. The risk of development of MDS or leukemia in these people has been estimated to be 0.5%–1% per year. This condition, referred to as clonal hematopoiesis of indeterminate potential (CHIP), highlights the dynamic nature of clonal evolution in myeloid cells [155]. When MDS has arisen, the selection of clones during transformation is shaped by acquisition of genetic alterations during clonal expansion, as well as exposure to genotoxic chemotherapy [156].

Better understanding of the molecular landscape of MDS has important clinical implications. Firstly, prognosticating MDS based on molecular aberrations will supplement current models in stratifying patients for treatment including allogeneic HSCT. Somatic mutations of TP53, TET2, and DNMT3A have been shown to identify patients with MDS with shorter OS after allogeneic HSCT [157], suggesting that newer treatment strategies other than transplantation are needed for these patients. Secondly, molecular markers may better predict response and resistance to treatment with HMAs. Thirdly, detection of targetable molecular markers during treatment resistance or leukemic transformation may provide an opportunity for specific therapy, as exemplified by the use of FLT3 inhibitors in FLT3-ITD positive secondary AML. Table 2 summarizes the molecular aberrations in MDS with respect to their prognostic impact and their targetability. Finally, understanding the molecular dynamism of myeloid cell mutation has important implications on treatment, particularly in detecting CHIP that precedes the development of clinical MDS [155,156]. Currently, there is as yet no specific treatment for these mutations. However, rapid advances in molecular biology and drug development lead to the optimism that these mutations may soon be targetable, with a view to early treatment perhaps even at the stage of CHIP so as to avert the development of myeloid malignancies. Hence, future treatment strategies for MDS may involve exploitation of genetic information in designing more effective therapy encompassing single agents or combinatorial approaches.

### Table 2. Recurrent mutations in myelodysplastic syndrome with prognostic and treatment implications.

<table>
<thead>
<tr>
<th>Mutated Genes</th>
<th>Function</th>
<th>Frequency</th>
<th>Prognostic Impact</th>
<th>Potential Targetted Therapy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA methylation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TET2</td>
<td>Conversion of 5mC to 5-hmC</td>
<td>20%–30% in MDS 50%–60% in CMML</td>
<td>None</td>
<td>DNA methyltransferase inhibitor (DNMTI)</td>
<td>[1,11,17–25]</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>DNA methyltransferase, histone methylation and transcription repression</td>
<td>10% in MDS</td>
<td>Unfavorable</td>
<td>DNMT1</td>
<td>[26–29,32–34]</td>
</tr>
<tr>
<td>IDH1/IDH2</td>
<td>Convert isocitrate to α-KG, regulates TET2</td>
<td>5%</td>
<td>Unfavorable</td>
<td>DNMT1, IDH1/2 inhibitors</td>
<td>[27,28,35–41]</td>
</tr>
<tr>
<td>Histone modification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EZH2</td>
<td>Histone methyltransferase, gene repression</td>
<td>5%</td>
<td>Unfavorable</td>
<td>HDAC inhibitors, EZH2 inhibitors</td>
<td>[28]</td>
</tr>
<tr>
<td>ASXL1</td>
<td>H3 methylation</td>
<td>15%–20%</td>
<td>Unfavorable</td>
<td>HDAC inhibitors</td>
<td>[11,28,42–45]</td>
</tr>
<tr>
<td>UTX</td>
<td>H3K27 demethylase</td>
<td>1%</td>
<td>None</td>
<td>HDAC inhibitors</td>
<td>[11,28,46,47]</td>
</tr>
</tbody>
</table>
Table 2. Cont.

<table>
<thead>
<tr>
<th>Mutated Genes</th>
<th>Function</th>
<th>Frequency</th>
<th>Prognostic Impact</th>
<th>Potential Targetted Therapy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RNA splicing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF3B1</td>
<td>Pre-mRNA splicing</td>
<td>15%–30%</td>
<td>Favorable</td>
<td>None</td>
<td>[11,19,49,50]</td>
</tr>
<tr>
<td>SRSF2</td>
<td>Spliceosome assembly</td>
<td>10%–20%</td>
<td>Unfavorable</td>
<td>None</td>
<td>[11,51–53]</td>
</tr>
<tr>
<td>ZRSR2</td>
<td>Spliceosome assembly</td>
<td>&lt;5%</td>
<td>None</td>
<td>None</td>
<td>[54]</td>
</tr>
<tr>
<td>U2AF1</td>
<td>Spliceosome assembly</td>
<td>5%–10%</td>
<td>None</td>
<td>None</td>
<td>[11,54]</td>
</tr>
<tr>
<td><strong>Transcription</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUNX1</td>
<td>Regulates myeloid differentiation</td>
<td>10%</td>
<td>Unfavorable</td>
<td>None</td>
<td>[11,55–57]</td>
</tr>
<tr>
<td>BCOR/BCORL1</td>
<td>BCL6 co-repressor</td>
<td>5%</td>
<td>Unfavorable</td>
<td>HDAC inhibitors</td>
<td>[58–61]</td>
</tr>
<tr>
<td>ETV6</td>
<td>ETS transcription factor</td>
<td>&lt;5%</td>
<td>Unfavorable</td>
<td>None</td>
<td>[11,62]</td>
</tr>
<tr>
<td>SETBP1</td>
<td>Cell proliferation</td>
<td>2%–5%</td>
<td>Unfavorable</td>
<td>None</td>
<td>[11,63,64]</td>
</tr>
<tr>
<td>GATA2</td>
<td>Transcriptional activator</td>
<td>Rare</td>
<td>Unfavorable</td>
<td>None</td>
<td>[11]</td>
</tr>
<tr>
<td><strong>DNA repair</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>Cell cycle regulation, tumor suppressor gene</td>
<td>10%</td>
<td>Unfavorable</td>
<td>Antisense TP53 oligonucleotide</td>
<td>[11,65–68]</td>
</tr>
<tr>
<td><strong>Signal transduction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK2</td>
<td>Tyrosine kinase activation</td>
<td>5%</td>
<td>None</td>
<td>JAK1/2 inhibitors</td>
<td>[11,69,70]</td>
</tr>
<tr>
<td>FLT3</td>
<td>Tyrosine kinase activation</td>
<td>&lt;5%</td>
<td>Unfavorable</td>
<td>FLT3 inhibitors</td>
<td>[15,16,71,81–105]</td>
</tr>
<tr>
<td>KIT</td>
<td>Tyrosine kinase activation</td>
<td>Rare</td>
<td>None</td>
<td>TKI (imatinib, dasatinib)</td>
<td>[132,133]</td>
</tr>
<tr>
<td>CBL</td>
<td>Proto-oncogene</td>
<td>5%</td>
<td>Unfavorable</td>
<td>None</td>
<td>[11,12,134–136]</td>
</tr>
<tr>
<td><strong>RAS pathway</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS222</td>
<td>GTPase signal transduction</td>
<td>10%</td>
<td>Unfavorable</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td><strong>Cohesin complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAG2</td>
<td>Control of cell division</td>
<td>5%–10%</td>
<td>Unfavorable</td>
<td>None</td>
<td>[11]</td>
</tr>
<tr>
<td>RAD21</td>
<td>Component of cohesin complex</td>
<td>&lt;3%</td>
<td>None</td>
<td>None</td>
<td>[11]</td>
</tr>
<tr>
<td>SMC3</td>
<td>Structure and function role in cohesin complex</td>
<td>&lt;3%</td>
<td>None</td>
<td>None</td>
<td>[11]</td>
</tr>
</tbody>
</table>

MDS: myelodysplastic syndrome; CMML: chronic myelomonocytic leukemia; α-KG: α-ketoglutarate; HDAC: histone deacetylase; TKI: tyrosine kinase inhibitor.

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