Current Hematologic Malignancy Reports (2019) 14:550–560
https://doi.org/10.1007/s11899-019-00554-4

MYELODYSPLASTIC SYNDROMES (M SAVONA, SECTION EDITOR)

Mutation-Driven Therapy in MDS

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Published online: 23 November 2019

Abstract

Purpose of Review Genetic sequencing in myelodysplastic syndrome (MDS) has provided an improved understanding of the complexity and heterozygosity of the disease. More importantly, our molecular understanding of MDS is leading to rapid advancements and personalized therapy for our patients. Herein, we review the current mutation-driven treatment landscape in MDS, first focusing on individual mutations. We then discuss the effect of specific gene mutations on response and outcomes to standard therapies as well as to cutting edge investigational therapies.

Recent Findings Molecular annotation of MDS can predict response rates and outcomes to our current standard of care therapies including hypomethylating agents, lenalidomide, and allogeneic stem cell transplantation. Clinical trials targeting molecular subsets of MDS are underway with some in very early stages while others advancing to phase III trials. Targeting *TP53* and *IDH1/2* mutations appear to be promising targets with substantial efficacy seen in several trials to date. Furthermore, novel therapeutic strategies such as immuno-oncology agents are of significant interest with future investigation required to understand the molecular predictors of response.

Summary Mutation-driven therapy in MDS is rapidly expanding and has tremendous potential in a disease where limited standard therapy options exist.

Keywords Myelodysplastic syndrome · Genetic mutations · Targeted therapy · Splicing factors · Epigenetic regulators · *TP53*

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by dysplasia, peripheral blood cytopenias, and transformation to acute myeloid leukemia (AML) [1]. Large-scale gene sequencing has uncovered many novel genes and pathways which has led to better understanding of the effect on disease evolution and clinical phenotype of cancer patients [2, 3]. Most commonly, mutated genes in MDS are implicated in RNA splicing (*SF3B1, SRSF2, U2AF1*, and *ZRSR2*), epigenetic regulation (*ASXL1, DNMT3A, EZH2, IDH1/2*, *and TET2*), and transcription regulation (*RUNX1* and *TP53*) [4–6]. MDS has an incredibly diverse clinical course with some patients requiring only observation while others may rapidly progress to AML. Genetic information has furthered our knowledge of the disease complexity of MDS with single genes rather than pathways more closely tied to clinical phenotype.

Additionally, several studies have tried to improve prognostication of MDS patients through inclusion of molecular subsets. Conventional prognostic scoring systems in MDS including the International Prognostic Scoring system (IPSS) and the revised IPSS (IPSS-R) rely on cytogenetics, peripheral blood parameters, and bone marrow blast percentage [7–10]. These systems lack genetic information that can greatly impact the prognosis of this disease. In an early study of 439 MDS patients, *TP53, EZH2, ETV6, RUNX1*, and *ASXL1* were showing to have independent negative prognostic significance. More interestingly, presence of one of these mutations was associated with an overall survival similar to that of the next highest IPSS category [5]. Also, in a large cohort of 3392 MDS patients gathered by International Working Group for prognosis in MDS-molecular committee, *TP53, CBL, EZH2, RUX1, ASXL1*, *and U2AF1* had independent negative prognostic

This article is part of the Topical Collection on *Myelodysplastic Syndromes*

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significance when adjusting for IPSS-R. *SF3B1* was associated with a modest improved survival in the absence of an adverse risk mutation [11]. In another publication, addition of only 3 mutations, *SF3B1, EZH2*, and *TP53,* was able to enhance the predictive ability of the IPSS-R model and notably was dynamic in that the model retained prognostic significant both at diagnosis and subsequently in the patient's treatment course [12]. Finally, to even further personalize predictive modeling using gene mutation, machine learning algorithms have been testing which have outperformed standard IPSS and IPSS-R models; however, they are not available yet for clinical use [13].

That being said, the ultimate goal of gathering molecular information is to provide insight for future treatment strategies. Currently, there are three FDA-approved therapies for MDS that include lenalidomide, azacitidine, and decitabine that are all agnostic to somatic mutations that underlie the disease. With the approval of targeted therapy in AML including midostaurin and gilteritinib for *FLT3* positive and ivosidenib and enasidenib for *IDH1* and *2* mutations, respectively, the search for similar molecular-based therapies in MDS has been of incredible interest. However, in MDS, this could be a challenging endeavor due to the large genetic heterogeneity. In MDS, most genes that are potentially targetable are mutated in < 5% of patients [4–6]. Specifically, some of the most advance mutational targets in AML are seen in relative low frequency in MDS including *FLT3* and *IDH* mutations. Finally, only a minority of mutations are gain of function, thus amenable for targeted inhibition. Despite these challenges, continued endeavors are underway to find affective treatment strategies to target gene mutations in MDS. As our knowledge of these genetic mutations and pathways improve, it not only will influence diagnosis and prognosis but as we enter an era of mutation-driven therapy will greatly impact our treatment decisions [14]. In this review, potential therapeutic targets for individual genetic mutation will be outlined in detail. The main targeted therapeutic trials in MDS are summarized in Table 1. We will then discuss molecular implications on standard and future treatment strategies.

Splicing Factors

SF3B1, SRSF2, U2AF1, ZRSR2

Splicing factor mutations (*SF3B1, SRSF2, U2AF1*, and *ZRSR2*) are frequently seen MDS with approximately 50–60% of MDS patients harboring a mutation. SF*3B1* is the most common of these mutations at 25–35% of patients [4, 6]. *SF3B1* mutations are highly prevalent in MDS patients with elevated ring sideroblasts and define a distinct MDS subtype with indolent course and favorable outcomes [25]. *SRSF2* mutations are seen in approximately 15% of MDS patients [4, 6] and 50% of patients with chronic myelomonocytic leukemia (CMML) [26]. In MDS, *SRSF2* mutations are potentially associated with a poor prognosis [27, 28]. *U2AF1* and *ZRSR2* are seen approximate 6–8% and 5% of MDS patients, respectively. Effect on prognosis of these mutations also remains controversial although both have been associated with a worse prognosis [29, 30]. Due to high frequency of mutations in MDS and CMML, targeting these mutations is an area currently under heavy investigation. Lee et al. initially postulated that because splicing factor mutations are always heterozygous, mutated cells depend on the wild-type alleles for survival. In mouse model and patient-derived xenografts, treatment with the spliceosome inhibitor E107 resulted in significant reductions in leukemic burden and consequent synthetic lethality [31]. H3B-8800 is an orally available small-molecule splicing modulator of the SF3b complex which preferentially kills spliceosome-mutant cancers [15]. Currently, the phase 1 trial of H3B-8800 in MDS, AML and CMML is ongoing. To date, 81 patients have been enrolled with only three dose-limiting toxicities (DLTs) observed. Additionally, PK and PD profiles support dose-dependent spliceosome modulation. Dose exploration is still ongoing [16]. In addition to spliceosome inhibitors, looking for therapies that have improved efficacy in this molecular subset is an area of exploration. In patients with *SF3B1* mutations, erythropoiesis stimulating agents (ESAs) have been shown to have higher response rates compared to wild type (35% vs 16%, $p = 0.032$) [32]. Another prime example is luspatercept in *SF3B1* patients. Luspatercept (ACE-536) is a novel fusion protein that block transforming growth factor beta (TGF-B) superfamily inhibitor of erythropoiesis. In the phase 1/2 multicenter dose finding study (PACE-MDS) of 58 lower risk MDS patients based on IPSS at higher dose of treatment (0.75–1.75 mg/kg subcutaneous every 21 days), 38% of patients achieved transfusion independence and 63% achieved erythroid response. Specifically, patients with *SF3B1* mutation and elevated ring sideroblasts (defined as > 15%) had particular high response rates. Notably, 77% of *SF3B1* mutant patients (all of which were ring sideroblast positive) vs 40% of *SF3B1* wild type patients (3/6 responders had elevated ring sideroblasts) achieved erythroid response [17•]. These positive results led to the phase 3 Medalist Trial in lower risk MDS with ring sideroblasts who require red blood cell transfusions. The trial consistent of 229 patients with 206 (90%) being *SF3B1* mutant. Of the 153 patients receiving therapy 58 (37.9%) achieved the primary endpoint of transfusion independence (TI) for ≥ 8 weeks compared to 13.2% for placebo [18••]. Additionally, erythroid hematologic improvement was significantly increased in the luspatercept group (52.9% versus 11.8%, $p < 0.0001$). Luspatercept is currently undergoing FDA evaluation for approval based on these results.

Table 1 Clinical trials targeting somatic mutations in myelodysplastic syndrome

Targeted mutation	Therapy	Mechanism of action	Eligibility	Phase	Reported results	NCT identifier	Reference
Splicing factors	H3B-8800	Inhibition of SF3b complex	MDS (low and high risk) CMML AML	Phase 1	81 enrolled to date 3 DLTs Dose-dependent spliceosome modulation	NCT02841540	[15, 16]
SF3B1	Luspatercept	Inhibition of TGF-B	Lower risk MDS with ringed sideroblast	Phase 3 (completed)	229 enrolled 206 SF3B1 mt TI > 8 weeks = 37.9% HI-E = 52.9%	NCT02631070	[17, 18]
IDH1	Ivosidenib (AG-120)	Inhibition of IDH1	Phase 1: AML/MDS Phase 2: MDS (low and high risk) AML blast < 30%	Phase 1 (completed) Phase 2	Phase 1: 12 patients in MDS cohort ORR = 92% CR = 42% Phase 2: no data reported to date	NCT02074839 NCT03503409	[40]
IDH1	Ivosidenib and venetoclax	IDH1 inhibitor + BCL-2 inhibitor	High risk MDS >10% blasts per PI discretion Treatment naïve not eligible for IC and R/R AML	Phase 1b/2	No data reported to date	NCT03471260	
IDH1	FT-2102	Inhibition of IDH1	Intermediate to high risk MDS AML	Phase 1/2	4 patients in MDS cohort ORR = 25% CR = 25%	NCT02719574 NCT04013880	[19]
IDH1	IDH305	Mutant-selective allosteric inhibition of IDH1	Advance malignancies that harbor IDHR132 (includes MDS)	Phase 1	3 patients in MDS cohort 1 DLT in AML/MDS cohort	NCT02381886	[20]
IDH2	Enasidenib (AG-221)	Inhibition of IDH2	Phase 1: AML/MDS Phase 2: High-risk MDS or MDS post-HMA	Phase 1 (completed) Phase 2	Phase 1:17 patients in MDS cohort ORR = 59% CR = 6% Phase 2: no data reported to date	NCT01915498 NCT03383575	[21]
IDH1/2	Olaparib	Inhibition of PARP which leads to cell death in IDH1/2 mutant cancer cells	MDS-EB and (intermediate to high risk) AML	Phase 2	No data reported to date	NCT03953898	
IDH1/2	AG-881	Inhibition of IDH1/2	Advanced hematologic malignancies (including MDS)	Phase 1	No MDS data reported to date	NCT02492737	
TET2	Vitamin C (IV)	Mimics TET2 function by promoting DNA demethylation	Intermediate or high risk MDS	Phase 1b/2	No data reported to date	NCT03433781	
TET2	Vitamin C (oral)	Mimics TET2 function by promoting DNA demethylation	MDS AML	Phase 2	No data reported to date	NCT03397173	
JAK2	Ruxolitinib	Inhibition of JAK2	Previously treated Low or intermediate-1 risk MDS or CMML	Phase 1 (completed)	18 patients with MDS and CMML ORR = 22% CR = 0%	NCT01895842	[22]
NRAS and KRAS	Trametinib	Inhibition of MEK1/2 which is involved in RAS signaling pathway	High risk MDS CMML R/R AML	Phase 1/2 (completed)	50 enrolled with *RAS* mt (10 with MDS) ORR = 20% CR = 8%	NCT00920140	[23]
TP53	APR-246	Reactivation of mutant TP53	Phase 1b/2: High risk MDS + AML blast < 30% Phase 3: high-risk MDS	Phase1b/2 (completed) Phase 3	Phase1b/2: 11 MDS/AML patient reported ORR = 100% CR = 82% Phase 3: no data to date	NCT03072043 NCT03745716	[24]

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HI-E erythroid hematologic improvement, *mt* mutant, *IC* induction chemotherapy, *R/R* relapse and/or refractory

Epigenetic Regulators

ASXL1

ASXL1 is observed in approximately 15–25% of patients with MDS with 70% being frameshift mutation [4–6, 33]. Due to the relatively high frequency of *ASXL1* mutations and overall poor prognosis in myeloid malignancies, therapies targeting mutant *ASXL1* cells are of great need [5, 33, 34]. Yang et al., using transgenic mouse models engineered with *ASXL1* mutation, showed that truncated *ASXL1*, but not the wild type, binds to BRD4 resulting in a gain of function alteration that promotes myeloid malignancies. Based on this interaction, the authors demonstrated the *ASXL1* mutant cells to be sensitive to BET inhibitors [35]. Currently, there is one ongoing phase 1 trial using a BET inhibitor (FT-1101) in relapsed hematologic malignancies including MDS (NCT02543879). BET inhibition specifically targeting *ASXL1* mutant myeloid malignancies is an area for exploration.

DNTM3A

DNTM3A mutations are present in approximately 15% of patients with MDS, most commonly involving the p.R882 codon [4–6, 36]. *DNMT3A* is involved in DNA methylation and appears to act in a dominant-negative manner; however, its exact mechanism is unknown [37]. Studies suggest it may confer a poor prognosis in MDS with more rapid progression to AML [36, 38]. The mutation is thought to be an early event in clonal hematopoiesis, thus making it a challenging molecular target. Notably, the DOT1L methyltransferase is significantly overexpressed in *DNMT3A* mutated hematopoietic stem cells. Pharmalogic inhibition of DOT1L led to inhibition of proliferation and terminal differentiation in *DNMT3A* mutant AML cell lines and patient samples [39]. Pinometostat (EPZ-5676), a small inhibitor of DOT1L, has been trialed in relapse/refractory leukemia with MLL-rearrangement (NCT01684150); however, no study to date has looked at DOT1L inhibition in *DNMT3A* mutant MDS/AML [40].

EZH2

EZH2 is epigenetic regulator serving as a catalytic component of polycomb repressive complex 2 (PRC2) and is often associated with RUNX1 mutations in MDS patients [41]. *EZH2* is seen in approximately 6% of MDS patients [5, 6] and is associated with a poor prognosis [5]. Although EZH2 inhibitor trials are ongoing in non-Hodgkin's lymphoma and multiple solid tumors, the challenge in MDS is that *EZH2* loss is thought to promote development of MDS whereas overexpression or gain of function of *EZH2* is thought to be the driver in carcinomas and lymphomas [41, 42].

IDH1/2

IDH1 and *IDH2* mutations, which are involved in cellular metabolism and epigenetic regulation, occur in approximately 5% of patients with MDS compared to approximately 20% in AML [5, 6, 43, 44]. They are characterized by recurrent pathogenic mutations at the amino acid sites *IDH1*-R132, *IDH2*-R140, and *IDH2*-R172 and occur more frequently in patients with diploid or intermediate cytogenetics and RAEB classification by WHO [43]. The relative prognostic impact of *IDH1/2* mutations in MDS remains unclear [44]. Success of *IDH1/2* inhibitors and venetoclax is changing the paradigm for *IDH* mutant patients with both MDS and AML. Ivosidenib (AG-120) an oral small-molecule inhibitor of *IDH1* is FDA approved for R/R AML based on overall response rate (ORR) of 42% and complete remission (CR) rate of 22% in 125 patients [45••]. In the MDS cohort on phase 1 study, ORR was 92% (11/12) with 5 CRs (42%) [46]. The phase II trial of ivosidenib in IDH1 mutated MDS in underway (NCT03503409). Enasidenib (AG-221), an oral small-molecule inhibitor of *IDH2*, is FDA approved in R/R AML based on ORR of 40% and CR rate of 20% in 176 patients [47••]. In addition, data was reported 17 MDS patients who received enasidenib monotherapy in the phase 1 trial. ORR was 59% (10/17) including 1 CR in a heavily pretreated population [21]. The phase 2 study of enasidenib monotherapy and in combination with azacitidine for high-risk IDH2-mutant MDS in now enrolling patients (NCT03383575). Finally, venetoclax is an oral small molecule BCL-2 inhibitor approved in newly diagnosed AML in combination with azacitidine, decitabine, or low-dose cytarabine [48••, 49••]. Preclinical data suggests that mutation of *IDH* sensitizes leukemic cells to venetoclax [50]. The preclinical findings appear to be supported by venetoclax trials to date suggested by good response rates and prolonged OS in *IDH1/2* patients [8, 9, 11]. Venetoclax in combination with azacitidine is currently has 2 ongoing phase 1b studies in treatment naïve higher risk and relapsed/refractory MDS (NCT02942290 and NCT02966782). Also, a phase 1b/2 study with venetoclax in combination with ivosidenib in AML and MDS pts with > 10% blast is currently underway (NCT03471260). Additional therapies under investigation include FT-2102 and IDH-305 for IDH1 (NCT03471260 and NCT02719574) and AG-881 and olaparib for IDH1/2 (NCT02492737 and NCT03953898). All four studies include patients with MDS [19, 20].

TET2

TET2 is a member of the TET dioxygenases that catalyzes conversion of 5-methylcytosine to 5-hydroxmethylcytosine which promotes DNA demethylation [51–53]. Somatic *TET2* mutations inhibit the enzymes function leading to hypermethylation which ultimately contributes to increased

stem cell proliferation and leukemogenesis [53]. *TET2* mutations are seen in approximately 20–35% of patients with MDS; however, of the myeloid malignancies, it is seen most frequently in CMML at 50–60% [5, 6, 54]. Effect on prognosis is somewhat unclear; however, in two large meta-analyses, *TET2* mutations did not seem to impact survival in MDS [55, 56]. In all myeloid neoplasms, *TET2* is affected by deletions and loss of function mutations, making it a challenging target [57]. Vitamin C has been recently gaining interest as two studies showed that treatment with vitamin C mimics *TET2* function and is able to restore hematopoiesis in mouse and human cells with *TET2* deficiency [58, 59]. Based on this data, clinical trials in MDS are underway. A phase Ib/II study in being performed evaluating safety and tolerability of Vitamin C in intermediate to high-risk MDS patients with *TET2* mutations (NCT03433781). Also, a phase 2 targeting *TET2* mutations in MDS and AML with azacitidine and ascorbic acid is ongoing (NCT03397173). Furthermore, Cimmino et al. showed that *TET2* mediated DNA oxidation induced by vitamin C treatment in leukemic cells enhanced sensitivity to PARP inhibition. This combination could be another effective strategy to target *TET2*-deficient myeloid malignancies [59]. At this time, there are no active trials testing this combination.

Signal Transduction

JAK2

JAK2 is tyrosine kinase which functions via the STAT signaling pathway. Mutations in *JAK2* most commonly occur at the p.V617F mutational hotspot and are thought to predominantly be gain of function mutations [60]. *JAK2* has relatively low frequency of mutations in MDS at 3–5% in comparison to the high frequency seen in PV and ET at 90 and 50%, respectively [5, 6, 60]. Additionally, higher prevalence is seen in MDS/MPN-RS-T, an overlap syndrome characterized by ring sideroblasts and thrombocytosis with approximately 50% of patients harboring the mutation [22]. In MDS, *JAK2* is thought to identify a subgroup of patients with isolated 5q and proliferative bone marrow [61]. In recent years, targeting *JAK2* has been an area of strong interest. Ruxolitinib is a JAK1/2 inhibitor that is FDA approved for polycythemia vera, primary myelofibrosis, and steroid refractory graft versus host disease (GVHD). A phase 1 trial using ruxolitinib in patients with low or intermediate-1 risk MDS has recently been completed. In the 14 patients presented, ORR was 21% with 2 patients achieving hematological improvement in platelets and 1 achieving partial cytogenetic response [62]. One other potential therapeutic option is chidamide, a novel histone deacetylase inhibitor, which inhibits viability of MDS/AML cell lines through suppression of JAK2/STAT3 signaling [63]. Finally, NS-018, a selective JAK2 inhibitor, was shown to suppress colony forming ability of bone marrow mononuclear cells in higher risk MDS patients [64]. Novel therapies targeting the JAK/STAT pathway are rapidly expanding with many ongoing clinical trials in hematologic malignancies; however, trials in MDS remain limited.

RAS

RAS regulates cell growth and differentiation though RAS signaling pathways which involve MEK and PI3K [23]. *NRAS and KRAS* mutations are present in approximately 4 and 1–2% of patients with MDS [6, 23]; however, higher frequencies are seen in CMML and AML. Patients with *RAS* mutations in MDS/CMML are thought to have worse prognosis and higher risk of transformation to AML [65]. However, when focusing on MDS alone, this impact is less clear [23]. Targeting *RAS* signaling has been of great interest in myeloid malignancies. Given MEK's involvement in the RAS signaling pathway, MEK inhibitors have been studies in *RAS* mutant refractory myeloid malignancies. In the phase 1/2 of trametinib monotherapy, for RAS mutant AML/high-risk MDS in which 10/50 patients had MDS, the ORR was 20% with 4 CRs and 1 complete remission with incomplete count recovery (Cri). This was much higher than the ORR for RAS wild type (wt) at 3%; however, it did not provide a survival benefit [66]. Tipifarnib is a potent and selective inhibitor of farnesyltransferase (FT). RAS isoforms are FT substrates; however, only *HRAS* is exclusively dependent on farnesylation for signal activation [67]. Tipifarnib has been studied in MDS, AML, and CMML; however, to date, no increased responses have been seen in *RAS* mutant patients [67–69]. Finally, rigosertib is an oral Ras-mimetic that inhibits PI3K and PLK cellular signaling pathways. Results from phase 1/2 study in combination with HMA, in 15 evaluable patients with MDS at recommended phase II dose, showed ORR of 67% with 1 CR and 7 marrow CR. *RAS* mutation status in this cohort was not reported [70]. There are no ongoing clinical trials specifically targeting *RAS* mutations in MDS.

Transcription Factors

RUNX1

RUNX1 is an important regulator of myeloid differentiation and effective hematopoiesis [71]. It was first described in an inherited disordered causing familial thrombocytopenia with propensity to develop acute myeloid leukemia [72]. *RUNX1* is present in approximately 10% of patients with MDS and independently confers an inferior prognosis [5, 6]. Currently, there are no clinical trials targeting *RUNX1* in MDS/AML. However, novel therapeutic agents targeting *RUNX1* may exist. In mice engrafted with AML expressing mutant *RUNX1*, BET inhibition or degradation has been shown to repress

RUNX1 and its targets, inducing apoptosis and improving survival of mice. [73]. Whether this finding will apply clinically in the future is uncertain.

TP53

TP53 mutant MDS accounts for approximately 5–10% of patients with de novo MDS and 25–30% of therapy-related MDS and is associated with complex karyotype. This mutant cohort represents the worst outcomes in MDS with median overall survival of 6–12 months, and importantly, the clonal burden of TP53 is strongly concordant with inferior overall survival [5, 6, 74]. Historically, patients have significant inferior OS with hypomethylating agents compared to wild type, and no effective disease-modifying therapy exists which supports the strong need for novel therapies targeting this molecular subgroup [24, 75]. APR-246 is novel small molecule currently in trial for treatment of *TP53* mutant patients. APR-246 has been shown to selectively induce apoptosis in *TP53* mutant cells by reactivating mutant *TP53* and restoring it to the wild-type conformation [76]. In the phase 1b/2 combination study of APR-246 and azacitidine in TP53 mutant MDS/AML blast< 30%, preliminary data showed an ORR of 100% with CR rate of 82% in 11 patients [77••]. Currently, the phase III multicenter trial using APR-246 in *TP53* mutant high-risk MDS is underway (NCT03745716). Additionally, phase 1b data from pevonedistat, a first-in-class NEDD8-activating enzyme inhibitor, was presented with an ORR of 50% in treatment naïve AML patients. Interestingly, in patients with TP53 mutations, the composite CR/partial response (PR) rate was 80% (4/5) [78]. Whether this combination will yield similar response rates in a larger cohort and if it could have implications in *TP53* mutant MDS remain unclear. The phase III trial of frontline therapy with pevonedistat plus azacitidine for higher risk MDS, CMML, and low-blast AML (NCT03268954) is underway. In addition to targeting mutant *TP53,* there has also been interest in activating wild type p53 through inhibition of critical regulators (MDM2 and its homolog MDMX (MDM4)) [79]. RG7112, a small-molecule MDM2 antagonist, demonstrated in a proof of concept phase 1 trial that MDM2 inhibition resulted in p53 stabilization and transcription activation of p53 target gene [80]. To add to this, ALRN-6924, an antagonist of both MDM2 and MDMX, is currently under investigation as monotherapy and in combination with cytarabine in patients with MDS and AML. In the preliminary data from the phase 1/1b trial, marrow CR was reported in 2/4 (50%) patients treated with 4.4 mg/kg ALRN-6924 and 200 mg/M2 cytarabine, one of whom went to transplant [81]. Notably, MDM2 and/or MDMX inhibition would be focused in *TP53* wild-type patients given loss of function p53. Effective treatment strategies in *TP53* mutant MDS are of dire need to improve the otherwise dismal survival in this challenging group of patients.

Standard Therapies and Future Treatment Strategies

Lenalidomide

Lenalidomide was the first therapy in MDS to specifically target a chromosomal or genetic change. Lenalidomide induces ubiquitination and degradation of CK1a. CK1a is encoded by a gene within the deleted region of del5q and haploinsufficient expression sensitizes cells to lenalidomide therapy [82]. When specifically targeting lower risk MDS patient with del5q, rate of transfusion independence was 66% with a median duration of 2.2 years. Partial or complete cytogenetic responses were seen in 72% of patients [83]. In Non-del5q, the rate of transfusion independence is much lower at 27% [84]. Despite great responses in 5q patients, the genetic makeup of the disease impacts response to therapy, primarily through *TP53* mutations. In one study, *TP53* mutations were present in approximately 20% of patients with del5q lower risk MDS. *TP53* mutations appeared to confer resistance to lenalidomide based on significantly lower complete cytogenetic response in mutated patients (0/7 vs 12/24, $p = 0.024$) [85]. Consistent with these findings, another study looked at 52 patients carrying the 5q deletion and again no complete cytogenetic responses were observed [86].

Hypomethylating Agents

Hypomethylating agents (HMAs) have become a staple of MDS therapy especially in higher risk MDS patients. These agents work by incorporating in DNA (additionally in RNA with azacitidine) and inhibit DNA methyltransferases leading to DNA hypomethylation resulting in DNA damage [87]. Based on the updated International Working Group (IWG) criteria, azacitidine monotherapy has a 14% CR + PR rate with a 30% rate of hematologic improvement (HI) whereas decitabine has been shown to have a CR + PR rate of 17% with a 13% rate of HI in high-risk MDS patients [88, 89]. As we learn more about molecular subsets of MDS, understanding the effect of gene mutations on response to HMA has been explored. In 213 MDS patients with sequencing performed prior to azacitidine or decitabine therapy, clonal TET2 mutations predicted response (when subclones with VAF < 10% were excluded). In particular, patients with *TET2* mutant/*ASXL1* wild type had highest response rates to HMA therapy (ORR 65% vs 44% OR 2.37, $p = 0.049$). Despite the improved response rates, *TET2* mutation was not associated with improved overall survival. Mutations in *TP53* and the rarer *PTPN11* were associated with decreased overall survival. Interestingly, RUNX1, ASXL1, EZH2, and ETV6 which are historically thought to be associated with a poor prognosis did not predict shorter survival in these patients on therapy [90]. In another study evaluating 134 patients with higher risk MDS treated with azacitidine, *ASXL1* and *EZH2*

mutations were associated with prolonged survival; however, increased rates of response did not reach statistical significance [91].

Effect of *TP53* mutation specifically on response rates to HMAs has been an area of heavy intrigue. Preclinical data suggest that hypomethylating agents preferentially kill human and mouse cells that contain *TP53* mutations or deficiencies [92]. In the study by Bejar et al. above, although *TP53* had a negative effect on overall survival, response rates between mutant and wild type were similar [91]. This finding has been since validated in the study of 100 patients with myeloid malignancies, 53 of which were high-risk MDS patients [93]. Additionally, the optimal HMA regimen for *TP53* mutated MDS has been evaluated. Two studies suggest the use of decitabine in *TP53* mutant patients. In 116 patients (26 with MDS), treatment with 10-day decitabine resulted in higher rate of blast clearance in *TP53* mutant vs wild-type patients (21 of 21 [100%] vs. 32 of 78 [41%], $p < 0.001$); however, similar rates of CRs were seen (19% vs 14%, $p = 0.73$) [94]. In 109 MDS patients receiving 5-day decitabine, 10 of 15 patients with *TP53* mutation (66.7%) had achieved a CR [95]. Despite these finding, the true advantage of decitabine over azacitidine in *TP53* mutant patients remain in question. In a recent large retrospective analysis, TP53 mutant MDS/AML had similar outcomes with frontline azacitidine and decitabine. Additionally, increased overall survival was seen in patient with *TP53* clonal clearance (defined as VAF < 5%) on therapy [96]. Optimal use of HMA therapy remains critically important as it remains the backbone of many future targeted therapies.

Allogeneic Stem Cell Transplant

Transplant remains the only curative therapy for patients with MDS. As we began to expand our knowledge of recurrent mutated genes, their impact on transplant outcomes was explored. In an early study of 87 patients with MDS before allogeneic hematopoietic stem-cell transplant (HSCT), mutations in *TP53, DNMT3A*, and *TET2* were associated with shorted overall survival after HSCT. Mutations in these genes were present in nearly half of the patients in this cohort [97]. In addition to effect on survival, gene mutations have also been shown to play a role in relapse. Initially, a study of 401 patients with MDS/AML showed that *ASXL1, RUNX1*, and *TP53* were independent predictors of relapse and overall survival after HSCT [98]. Later, in a large study of 1514 patients enrolled in the Center for International Blood and Marrow Transplant Research Repository, *TP53 and RAS* pathways mutations had higher risk of relapse which leads to shorter overall survivals. However, the effect of RAS pathway mutations was only seen in patients who had received reduced intensity conditioning regimen, leading author to propose that the effect of the mutation can be overcome by myeloablative conditions [99•]. Two additional studies in both Japanese and German cohorts again showed unfavorable outcomes in patient with *TP53* and *RAS* mutations [100, 101].

As noted above, *TP53* is the only mutation that has been uniformly shown to have increase rate of relapses and worse overall survival. This has led to many centers recommending against transplant in *TP53* mutated patients. However, this had remained a topic of heavy debate as 20% long-term survival was observed in data from CIBMTR registry. Researchers have proposed that clearance of the mutational clone could improve outcomes in these patients. A recent study showed patients who achieve clonal suppression of *TP53* (as defined by VAF < 5%) have improved outcomes with HSCT [96]. As further therapies targeting *TP53* mutant clones emerge, the overall outcome of these patients post-transplant could drastically change.

Immune-Oncology

Immunotherapy has been an area of tremendous success in oncology. These therapies unleash patients own immune system to detect and eliminate cancer cells. Trials exploring the use of immunotherapy in MDS/AML have had variable success. Whether specific genetic subsets would preferentially benefit from these therapies is an area in early exploration. Checkpoint inhibitors block important inhibitory coreceptors on T-cells (for example PD-1 and CTLA4) which help cancer cells invade the immune system. In the phase II study of nivolumab or ipilimumab monotherapy or in combination with azacitidine in higher risk MDS, 76 patients were enrolled. ORR was 15/20 (75%), 15/21(71%), 2/15 (13%) and 7/20 (35%) in azacitidine and nivolumab, azacitidine and ipilimumab, nivolumab monotherapy and ipilimumab monotherapy respectively. Next generation sequencing was performed on all patients. Clearance of detectable mutations was seen in 3(15%) patients treated with ipilimumab monotherapy and 3(14%) and 4 (20%) patients on ipilimumab and nivolumab and azacitidine combinations [102•]. Another potential therapy in MDS is Hu5F9-G4 (5F9) a targeted antibody against CD47. CD47 is an immune checkpoint on both normal and cancer cells that acts as a "don't eat me" signal to macrophages. It is known to be upregulated in leukemic stem cells in higher risk MDS [103]. CD47 blockage leads to tumor phagocytosis and elimination of LSC in AML models [104]. Early results of phase 1b of 5F9 in combination with azacitidine in higher risk MDS and AML showed CR/CRi rate of 50% in AML and 60% (3/5) in MDS patients with 100% of MDS patients having an objective response [105]. Molecular subsets were not reported in this study.

Finally, with the rapid expansion of bispecific antibodies (Bi-specific T cell Engager, BiTE or Dual-affinity Re-Targeting, DART) and cellular therapies including chimeric antigen receptor (CAR) T cell therapies in hematologic

malignancies including MDS/AML, further investigation is needed to determine what role molecular subsets will play. Specifically, whether or not certain genetic mutations alter the immune microenvironments to allow for improved responses to these therapies is an area of future exploration.

Conclusion

The genetic heterogeneity of MDS provides a significant challenge in the development of targeted therapy. However, it is obvious that successful therapies are on the horizon, with the furthest along likely involving *TP53*, *IDH1/2*, and splicing factor mutations. As our understanding continues to expand, both novel genetic targets and optimization of known targets will arise. Continued research on the effects of genetic mutations on hypomethylating and allogeneic transplant is needed as they will likely remain the backbone of future treatment. In the rapidly expanding field of immune-oncology in MDS patients, whether somatic mutations will factor into our approach of immune-based therapies is unknown. Despite the uncertainty, one thing is apparent, genetic information is now an integral part of the clinical phenotype, prognosis, and treatment of this complex disease.

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