



Integrating Genomics in Myelodysplastic Syndrome to Predict Outcomes After Allogeneic Hematopoietic Cell Transplantation

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Abstract

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal hematopoietic neoplastic disorders most commonly occurring in the elderly population; MDS has a tendency to progress to acute leukemia. Although epigenetic therapies have improved the outcomes of MDS patients, allogeneic hematopoietic cell transplantation remains the only curative option. Molecular characterization of MDS using next-generation sequencing has expanded not only the knowledge on MDS but also the depth of understanding of evolution and contribution of recurrent somatic mutations in precursor conditions. Rapidly evolving genomic information on MDS may provide clinicians with better risk stratification tools and may also aid in supplying useful information to allow comprehensive therapeutic decision making for MDS patients. In this concise review, we summarize the current knowledge and understanding of recurrent somatic mutations in MDS and discuss salient genomic information predicting response and influencing therapeutic outcomes in the context of allogeneic hematopoietic cell transplantation, as well as the potential application of these findings into future clinical practice.

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Introduction

Myelodysplastic syndrome (MDS) represents a heterogeneous disease with varying prognosis.¹⁻³ Recent advances in the understanding of the pathogenesis and molecular aspects of the disease, coupled with development of various prognostic models to better predict clinical behaviors and outcomes, have significantly improved the treatment of MDS.^{1,3-6} Novel epigenetic therapies have become an integral part of treatment algorithms for this disease.^{7,8} Unfortunately, MDS remains incurable unless patients are offered allogeneic hematopoietic cell transplantation (HCT). Even then, long, durable

remissions occur only in a minority of allografted MDS patients. To date, to our knowledge, there are no published randomized data that compare allogeneic HCT against nontransplant therapies in patients with MDS. Currently, the decision to offer (or not) an allogeneic HCT is based on Markovian decision analysis models that demonstrate a significant benefit in favor of allogeneic HCT earlier in the disease course for patients with higher-risk MDS, whether allografted from human leukocyte antigen (HLA)-histocompatible siblings or unrelated donors.^{9,10}

Allogeneic HCT is capable of yielding durable remissions in approximately half of the patients who undergo the procedure,^{11,12} but significant morbidity and mortality remain a concern despite incorporation of disease-specific comorbidity indices.^{13,14} Graft-versus-malignancy effect is a major immunologic mechanism of allogeneic HCT to potentially cure these patients; however, it has also been long known that relapse of underlying malignancy is a major cause of allogeneic HCT failure.^{11,12,15} Prognostic values of MDS cytogenetic risk scoring and monosomal karyotype in allogeneic HCT outcomes have been demonstrated by single-institution and multicenter registry studies.^{16,17} The presence of mixed T-cell chimerism at day +90 to +120 after myeloablative allogeneic HCT has also been shown to be associated with higher risk of progression in one study.¹⁸ Although traditional prognostic factors are generally helpful, significant limitations exist when

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accurate prediction of relapse/progression risk, disease response, and allogeneic HCT outcomes is needed.

Emerging technologies such as next-generation sequencing (NGS) are revolutionizing the biologic characterization and therapeutic landscape of several hematologic malignancies by identifying somatic mutations with important prognostic impact.¹⁹⁻²¹ This helps physicians to more precisely predict the disease behavior and eventually integrate this information into decision making for better selection of patients more likely to benefit from an allogeneic HCT. Here we summarize the published literature pertaining to the prognostic significance of somatic mutations in patients with MDS in the setting of allogeneic HCT and discuss future application of this growing and exciting genomic information.

Evolving Technologic Advances in MDS Characterization

Disease characterization for MDS has evolved over the last several decades in concert with technological advancement in medicine: microscopic morphologic classification, karyotypic analysis with cytogenetics and fluorescence in-situ hybridization, flow cytometry, and NGS.^{1,19} The recent abundance of genomic analysis in myeloid malignancies could not have been accomplished without the 2 major contributing events in science: the Human Genome Project, which provided a road map of the human genome, and significant technological advancement in DNA sequencing, reducing the time and cost required to sequence the genome.²²⁻²⁴ NGS refers to techniques that allow massively parallel simultaneous sequencing of short (typically 50 to 100 nucleotides) DNA fragments.^{23,24} These DNA sequences are then aligned back to a human reference genome to generate the near-complete characterization of the genome. When studying tumor genome, it is informative to sequence a germ-line control to discern somatic (or acquired) sequence variants in cancer cells and exclude many inherited sequence variants, also termed polymorphisms, which are not part of the somatic mutome. Several different gene sequencing methods exist that have been reviewed extensively by others and are beyond the scope of this review.²⁵⁻²⁷ For the purpose of our concise review, we focus on exome sequencing with target enrichment, which allows evaluation of specific known recurrent mutations in MDS.

Overview of Acquired Somatic Mutations in MDS and Its Precursor State

Comprehensive analysis to characterize recurrent somatic mutations in MDS has been conducted. Many of those mutations are found in genes involved in DNA methylation, chromatin remodeling, and spliceosome. DNA methyltransferases (*DNMT*) transfer a methyl group to the 5' carbon of cytosine (5'-methylcytosine [5-mC]) within CpG dinucleotide-rich regions (ie, DNA methylation). 5-mC can be further modified by α -ketoglutarate-dependent oxygenases *TET1-3* (the ten-eleven-translocation gene), which catalyze the oxidation of 5-mC to 5'-hydroxymethylcytosine (5-hmC).²⁸ α -Ketoglutarate is obtained from isocitrate catalyzed by isocitrate dehydrogenases (*IDH1* in cytoplasm and *IDH2* in mitochondria). *ASXL* and *EZH* are histone modifiers and are members of the polycomb group family, which form protein complexes to maintain the transcriptionally repressive genes such as the clustered

homeobox (*HOX*) genes. *ASXL1* mediates both activation and suppression of *HOX* genes. *EZH2* comprises the catalytic component of the polycomb repressive complex.²⁹ Spliceosomes are complexes of small nuclear RNAs and proteins that splice introns and exons from pre-messenger RNA to assemble mature mRNA.³⁰ There are 8 core spliceosomal genes (*SF3B1*, *SRSF2*, *U2AF35* [*U2AF1*], *ZRSR2*, *SF3A1*, *PRPF40B*, *U2AF65* [*U2AF2*], and *SFI*), and mutations in these genes, with the exception of *ZRSR2*, are considered to be gain of function with RNA splicing defects.³⁰

In a landmark study by Bejar et al,¹⁹ high-throughput mass spectrometry-based genotyping of 953 mutations involving 111 genes and DNA sequencing of selected genes using NGS platform were performed using samples of bone marrow aspirate from 439 MDS patients, with the most frequently mutated genes in MDS being *TET2* (20.5%), *ASXL1* (14.4%), *RUNX1* (8.7%), *TP53* (7.5%), and *EZH2* (6.4%). In a multivariate analysis with the International Prognostic Scoring System (IPSS) risk group as a covariate, mutations in *TP53* (hazard ratio [HR], 2.48; 95% confidence interval [CI], 1.6-3.84), *EZH2* (HR, 2.13; 95% CI, 1.36-3.33), *ETV6* (HR, 2.04; 95% CI, 1.08-3.86), *RUNX1* (HR, 1.47; 95% CI, 1.01-2.15), and *ASXL1* (HR, 1.38; 95% CI, 1.00-1.89) were independent predictors of worse overall survival (OS).¹⁹ In a larger study of 2173 MDS patients with sequencing of 27 genes, several mutated genes were found to have independent prognostic significance when adjusted for revised IPSS risk groups: *TP53* (HR, 2.37; 95% CI, 1.94-2.9), *CBL* (HR, 1.57; 95% CI, 1.22-2.03), *EZH2* (HR, 1.55; 95% CI, 1.22-2.03), *RUNX1* (HR, 1.5; 95% CI, 1.24-1.83), *U2AF1* (HR, 1.29; 95% CI, 1.06-1.58), and *ASXL1* (HR, 1.21; 95% CI, 1.04-1.41). These results suggest that selected somatic mutations in MDS could further refine existing clinical prognostic models and will be likely incorporated in the future molecular risk prognostication of MDS.³¹

Although emerging data on somatic mutations in MDS provide refinement of prognostic characterization of various MDS subtypes, clinical relevance of variant allele frequency (VAF) for individual genes remains largely undetermined. Sallman et al³² profiled *TP53* and other genes in both MDS and secondary acute myeloid leukemia (AML) patients; they demonstrated that MDS patients with *TP53* VAF > 40% had inferior median OS (124 days) compared to VAF < 20% (OS not reached, HR 3.52; $P = .01$), which is also validated in an independent cohort and was shown to be an independent prognostic factor in a multivariate analysis. This study highlights the ongoing need for further characterization of somatic mutations in MDS for better prognostication.

The association of DNA methylation status and clinical outcomes has also been investigated. Shen et al³³ performed quantitative methylation analyses by bisulfite pyrosequencing, the results of which on 10 genes were standardized by the z score method. Although DNA methylation z score at baseline did not correlate with clinical response to decitabine, a greater degree of reduced methylation was observed in patients with complete or partial remission compared to those with only hematologic improvement.³³ Bejar et al³⁴ evaluated 231 MDS patients and correlated the response with either azacitidine or decitabine with respect to 40 sequenced genes. *TET2* mutant patients (> 10% allele burden) without clonal *ASXL1* mutations had the highest response rate (odds ratio, 3.65; $P = .009$), whereas patients with mutations of

TP53 (HR, 2.01; $P = .002$) and *PTPN11* (HR, 3.26; $P = .006$) had shorter OS but had no drug response; *TET2* mutation status was not associated with OS.³⁴ Traina et al³⁵ evaluated 92 patients with predominantly MDS (MDS = 53, MDS/myeloproliferative neoplasm = 28, and secondary AML = 11) who were treated with azacitidine, decitabine, or both and correlated with treatment outcomes and mutational analysis results. Direct sequencing of *TET2*, *DNMT3A*, *IDH1*, *IDH2*, *ASXL1*, *CBL*, *NRAS*, *KRAS*, *SF3B1*, and *TP53* was performed using DNA from bone marrow ($n = 54$) or peripheral blood ($n = 38$). In multivariate analysis, *TET* mutations and/or *DNMT3A* mutations were found to be independent predictors of response to hypomethylating agents (odds ratio, 3.59; 95% CI, 1.14-11.36; $P = .03$). These studies are intriguing such that genome sequencing in MDS may provide not only prognostic but predictive information for better selection of therapeutic interventions.

High-throughput genome sequencing platforms have facilitated the discovery of recurrent somatic mutations that drive the pathogenesis of MDS. Similarly, sequencing studies have also revealed that clonally restricted somatic mutations that are found in MDS may also be found in healthy individuals without apparent MDS or other myeloid disorders.³⁶ These mutations can be seen in individuals with normal blood counts, and the presence of these mutations may suggest potentially increased risk of future development of myeloid disorders.³⁷ Genovese et al³⁸ performed whole-exome sequencing of peripheral blood from 12,380 Swedish individuals (6245 controls, 4970 with schizophrenia, and 1165 with bipolar disorder) with a mean age of 55 years (range, 19-93 years) and identified somatic mutations on the basis of allelic fractions. Clonal hematopoiesis with candidate driver somatic mutations were found in 10% of individuals > 65 years of age but only 1% for those < 50 years of age, with most commonly detected somatic mutations being *DNMT3A*, *ASXL1*, and *TET2*. Those with clonal hematopoiesis were found to have increased risk of subsequent hematologic cancer (HR, 12.9; 95% CI, 5.8-28.7), and 13 (42%) of 31 with subsequent hematologic cancers had clonal hematopoiesis detected in the initial blood samples.³⁸ Jaiswal et al³⁹ performed whole-exome sequencing of peripheral blood from 17,182 subjects (15,801 from 22 cohorts in type 2 diabetes studies and 1381 from a cardiac study) and evaluated 160 recurrently mutated candidate genes in myeloid and lymphoid malignancies. The frequency of detectable somatic mutations increased with age with 9.5% for ages 70 to 79, 11.7% for ages 80 to 89, and 18.4% for ages 90 to 108. The 3 most commonly seen somatic mutations were *DNMT3A*, *TET2*, and *ASXL1*. The presence of a somatic mutation was associated with an increase in the risk of hematologic cancer (HR, 11.1; 95% CI, 3.9-32.6) and all-cause mortality (HR, 1.4; 95% CI, 1.1-1.8).³⁹

The observation that these mutation frequencies increase with aging is considered analogous to other well-described clonal precursor states of hematologic cancers, such as monoclonal gammopathy of undetermined significance and monoclonal B cell lymphocytosis. On the other hand, there are patients with persistent cytopenias without apparent hematologic diagnoses; the condition may be called idiopathic cytopenia of undetermined significance, where bone marrow morphology is unremarkable and no known MDS-related somatic mutations or karyotypic abnormality could be

found.^{40,41} When clonality is demonstrated, these conditions may also be referred as clonal cytopenia of undetermined significance. Steensma et al⁴² have proposed a working definition of clonal hematopoiesis of indeterminate potential (CHIP) where individuals would have cytopenia and no evidence of established hematopoietic neoplasms but have a somatic mutations associated with hematologic cancers at VAF of at least 2% in the peripheral blood.⁴² CHIP would also encompass clonal cytopenia of undetermined significance but excludes traditional idiopathic cytopenia of undetermined significance. CHIP is distinct from MDS on the basis of the proposed definition, although future studies will examine the boundary between health and disease, and further refinement of MDS diagnostic criteria might be on the horizon.

Somatic Mutations Present in MDS Patients Undergoing Allogeneic HCT

A single-institution retrospective study of 87 patients (median age, 58 years) who received an allogeneic HCT for various subtypes of MDS identified somatic mutations in 92% of cases.⁴³ The largest proportion of patients had refractory anemia with excess blasts (48%), a complex karyotype was documented in 32%, and 92% were allografted using filgrastim-mobilized peripheral blood stem cells.⁴³ A reduced-intensity conditioning regimen was used in 71% of cases.⁴³ After the authors analyzed the coding sequence of 40 genes known to be recurrently mutated in MDS and related myeloid malignancies, *ASXL1* was reported as the most frequently identified mutation in 29%, followed by *TP53* in 21% and *DNMT3A* in 18%.⁴³ All patients with *TP53* mutations died before 5 years from allogeneic HCT, with 83% having evidence of MDS at the time of death.⁴³ The median survival of patients harboring *TP53* mutations was dismal, at 4.6 months. Mutations in *TET2* and *DNMT3A* were also reported to be associated with inferior OS.⁴³ These results suggest that incorporating genomic information would help to better predict posttransplantation outcomes in patients with MDS and develop therapeutic strategies to help mitigate the risk of relapse or progression in patients with poor-risk somatic mutations.

Another single-institution study by Kharfan-Dabaja et al⁴⁴ evaluated the incidence and prognostic significance of somatic mutations in 101 patients (median age, 58 years) who received an allogeneic HCT for MDS. Different from the study of Bejar et al,⁴³ somatic mutations were evaluated using a panel of the 26 most frequently mutated genes in MDS, and DNA was extracted from paraffin-embedded bone marrow samples that were obtained as part of the pretransplantation disease staging assessment.⁴⁴ Mutations were identified in 39% of cases, with the cutoff for VAF set at 20%; the most common ones were *ASXL1* (11%), *DNMT3A* (6%), *IDH2* (5%), *TP53* (4%), *KRAS* (4%), and *RUNX1* (4%).⁴⁴ Consistent with previously published findings, the presence of *TP53* mutations was associated with a significantly inferior OS (6 months, 95% CI, 0-17; vs. 34 months, 95% CI, 14-53; $P = .02$).⁴⁴ Additionally, this study identified the presence of *IDH2* mutations as a significant predictor of poor survival in allografted patients.⁴⁴ Contrary to the study by Bejar et al,⁴³ the presence of *DNMT3A* or *TET2* mutations did not appear to affect OS⁴⁴; but we caution that the small sample size and the low frequency of those

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mutations might explain in part this discrepancy.⁴⁴ Similar to study of Bejar et al,⁴³ the presence of *ASXL1* mutations did not predict worse OS in MDS allografted patients.⁴⁴ This is in contrast to published data of MDS patients treated outside the transplant setting,^{19,45} possibly suggesting MDS with *ASXL1* mutation is perhaps susceptible to the graft-versus-malignancy effect mediated by alloreactive donor T cells.

A multicenter Japanese study of 719 patients (median age, 53 years) with MDS who underwent an allogeneic HCT from unrelated bone marrow donors that utilized targeted deep sequencing of peripheral blood DNA samples demonstrated gene mutations in 75% of cases, with *TP53* being the most common (14%), followed by *U2AF1* (13%), *RUNX1* (12%), *ASXL1* (11%), and *DNMT3A* (9.3%).⁴⁶ Presence of mutations in *TP53* (HR, 2.31; $P = .015$) and *ETV6* (HR, 2.57; $P = .015$) adversely impacted OS. This study also identified mutations in *ETV6* as independently predictive of inferior OS.⁴⁶

A multicenter German study reported outcomes of 308 patients (median age, 58 years) with MDS (47%) or secondary AML (53%) who received an allogeneic HCT and for whom genomic DNA was available at a time with active disease before transplantation.⁴⁷ In this study, somatic mutations were detected using a 54-gene panel by Illumina high-throughput sequencing.⁴⁷ The authors identified mutations in *PTPN11*, *IDH2*, *PHF6*, and *NRAS* as significant predictors of OS in multivariate analysis. However, contrary to the aforementioned studies, the presence of *TP53* mutations lost their unfavorable prognostic impact when complex karyotype was included in the multivariate model.⁴⁷ When interpreting the result of this study, it is important to keep in mind that secondary AML comprised the large majority of cases (53%).

In a single-center retrospective study, Christopheit et al⁴⁸ evaluated the impact of somatic mutations in 62 MDS patients with a median age of 61 who were treated with fludarabine, amsacrine, and cytarabine chemotherapy followed by a busulfan-based conditioning regimen (91.9% reduced-intensity conditioning regimen) and allogeneic HCT from various donor types (16.1% related matched donors, 46.8% matched unrelated, and 35.5% mismatch unrelated). A panel of 19 genes (but not including *EZH2* and spliceosomal genes) was analyzed by amplicon-based NGS using peripheral blood ($n = 58$) and bone marrow ($n = 4$) samples. Mutations were found in 95% of the cases, with *RUNX1*, *GATA2*, *TET2*, and *CEBPA* being the most commonly mutated genes. In their analysis, no statistically significant difference in OS or disease-free survival was seen for any of the mutated genes. When patients with mutated *TP53* were compared to those with wild-type *TP53*, OS and disease-free survival did not differ.⁴⁸ These studies are summarized in Table 1.

Discussion

With the advent of NGS and other technologies, it became clear that MDS is more complex and widely heterogeneous than previously anticipated, with unique profiles and molecular signatures. While emerging information, including genomics, represents a step in the right direction of personalizing care of MDS patients, the fast pace of emergence of new knowledge poses a serious ongoing challenge to incorporate this information into more personalized treatment algorithms including allogeneic HCT.

The incidence of somatic mutations (at least 1) in patients with MDS who underwent an allogeneic HCT ranged from 39% to 95% and partly depends on the targeted mutation panel used for the analysis and underlying pathology.^{43,44,46-48} It is of note that in the study by Heuser et al,⁴⁷ 53% of cases comprised secondary AML that progressed from MDS. Moreover, the difference in incidence of mutations among studies with predominantly MDS cases could be explained by several reasons. First, in the study by Bejar et al,⁴³ a panel of 40 genes was used compared to 26 genes by Kharfan-Dabaja et al⁴⁴ and 68 genes by Yoshizato et al.⁴⁶ Moreover, the source of DNA varied among those studies: the study by Kharfan-Dabaja et al used DNA from formalin-fixed, paraffin-embedded bone marrow samples, 2 others used solely peripheral blood for the targeted NGS analysis, a German single-center study used both peripheral blood and bone marrow, and one study did not include information on the DNA source.^{43,44,46-48} Additionally, the timing of sample acquisition relative to allogeneic HCT may also influence the prognostic significance of somatic mutations, as earlier time points may potentially underestimate the mutational burden.

At least 3 of these studies identified *TP53* mutations as independent predictors of inferior OS after transplantation.^{43,44,46} In 2 studies, the median OS of patients harboring *TP53* mutations ranged from 4.6 to 6.0 months,^{43,44} raising questions regarding the efficacy of allogeneic HCT with current platforms whenever *TP53* mutation is present. In the studies by Heuser et al⁴⁷ and Christopheit et al,⁴⁸ mutations in *TP53* did not confer an adverse prognosis on OS, but one must be cautious when interpreting this study, as 53% of cases in the former study comprised secondary AML. In 2 studies, the presence of *IDH2* mutations also conferred an adverse impact on posttransplantation OS, with a reported median OS of 11 months in one of the studies.^{44,47} Also, in the Japanese multicenter study, *ETV6* was identified as an independent predictor of poor OS.⁴⁶

Identifying these extremely high-risk populations—for instance, mutations in *TP53*, *IDH2*, *ETV6*, and others—would certainly be useful, as standard allogeneic HCT may be less effective in their presence, with an anticipated median OS of less than 12 months (for *TP53* and *IDH2*), although the observation is not consistently supported by other studies. It is possible that the timing of sample acquisition, sample type (peripheral blood vs. bone marrow), age and type of sample (fresh blood or bone marrow aspirate vs. archived materials), somatic mutation gene panel, frequency of mutations, MDS subtype, age of patients, transplant conditioning intensity, donor type, HLA disparity, and other donor–recipient differences (such as killer immunoglobulin-like receptor mismatch)^{49,50} may all contribute to the outcomes of allogeneic HCT in MDS. Emerging information on somatic mutations in MDS clearly opens the door for reshaping the transplant indications in MDS and elucidates the need for further research to improve current allogeneic HCT platforms for MDS.

Somatic mutations in MDS may provide predictive information that may aid therapeutic decision making. At least 2 studies have shown an increased likelihood of response to hypomethylating agents when *TET2* mutations are present,^{34,35} and one study observed an even stronger predictive value when *ASXL1* is wild type.³⁴ This finding has potential implications in the allogeneic HCT strategy. If *TET2* mutations are associated with better and

Table 1 Summary of Studies Evaluating Somatic Mutations in MDS Patients Undergoing Allogeneic Hematopoietic Cell Transplantation

Study	Year	Study Type	No. of Patients	Three Most Common MDS Subtypes	No. of Genes in Panel	Source of DNA	Incidence of Mutations (At least 1)	Frequency of Mutations	Intensity of Preparative Regimen	OS Based on Specific Gene Mutations ^a
Bejar ⁴³	2014	Retrospective, single-center	87	RAEB = 48%; RA = 28%; RARS = 8%; other/unknown = 8%	40	PB	92%	<i>ASXL1</i> , 29%; <i>TP53</i> , 21%; <i>DNMT3A</i> , 18%; <i>RUNX1</i> , 16%	RIC/NMA = 71% MAC = 29%	Median OS for <i>TP53</i> mutations = 4.6 months (HR = 4.22); <i>TET2</i> (HR = 2.29); <i>DNMT3A</i> ^b (HR = 2.62)
Kharfan-Dabaja ⁴⁴	2015	Retrospective, single-center	101	RAEB-1 = 31%; RAEB-2 = 26%; RCMD = 16%; CMML = 8%; MDS unclassified = 5%; MDS-RS = 5%, AML = 4%; RARS = 3%; RA = 2%; MDS/MPN = 1%	26	FFPE-BM	39% ^c	<i>ASXL1</i> , 11% <i>DNMT3A</i> , 6% <i>IDH2</i> , 5% <i>KRAS</i> , 4% <i>RUNX1</i> , 4% <i>TP53</i> , 4%	RIC/NMA = 8% MAC = 92%	Median OS for <i>TP53</i> mutations = 6 months; Median OS for <i>IDH2</i> mutation = 11 months
Yoshizato ⁴⁶	2015	Retrospective, multicenter	719	NR	68	PB	75%	<i>TP53</i> , 14%; <i>U2AF1</i> , 13%; <i>RUNX1</i> , 12%; <i>ASXL1</i> , 11%	NR	<i>TP53</i> (HR = 2.31); <i>ETV6</i> (HR = 2.57)
Heuser ⁴⁷	2015	Retrospective, multicenter	308 (MDS = 47%)	NR	54	NR	82%	<i>ASXL1</i> , 24%; <i>DNMT3A</i> , 23%; <i>RUNX1</i> , 17%; <i>TET2</i> , 17%; <i>STAG2</i> , 12%; <i>TP53</i> , 12%	NR	<i>PTPN11</i> (HR = 3.1); <i>IDH2</i> (HR = 2.6); <i>PHF6</i> (HR = 2.2); <i>NRAS</i> (HR = 1.8); <i>TP53</i> ; (not significant in multivariable analysis)
Christopeit ⁴⁸	2015	Retrospective, single-center	62	RAEB-2 = 40.3%; RAEB = 24.2%; RAEB-1 = 19.4%; unknown = 8.1%	19	PB (94%)/ BM (6%)	95%	<i>RUNX1</i> , 31%; <i>GATA2</i> , 29%; <i>TET2</i> , 27%; <i>CEBPA</i> , 27%; <i>DNMT3A</i> , 23%; <i>ASXL1</i> , 15%; <i>TP53</i> , 13%	RIC = 8.1% MAC = 91.9%	Median OS = 43 months; Median DFS = 34 months; (none of the mutations affected OS or DFS)

Abbreviations: AML = acute myeloid leukemia; CMML = chronic myelomonocytic leukemia; DFS = disease-free survival; FFPE-BM = formalin-fixed, paraffin-embedded bone marrow; HR = hazard ratio; MAC = myeloablative conditioning; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasms; NMA = nonmyeloablative conditioning; NR = not reported; OS = overall survival; PB = peripheral blood; RA = refractory anemia; RAEB = refractory anemia with excess blasts; RARS = refractory anemia with ringed sideroblasts; RCMD = refractory cytopenia with multilineage dysplasia; RIC = reduced intensity conditioning.

^aWith statistical significance in multivariable analysis.

^bOn day 100 landmark analysis.

^cCutoff for variant allele frequency was set at 20%.

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longer duration of response, one could consider developing new treatment algorithms that evaluate the presence of *TET2* in the context of other known clinical prognostic factors. Another point of consideration would be to further evaluate the impact of allele burden (ie, VAF) for individual somatic mutations on allogeneic HCT outcomes. It is plausible that reduction of allele burden with therapy such as hypomethylating agents or novel anti-MDS therapy before allogeneic HCT may improve outcomes after allografting; future clinical trials are needed to analyze these hypotheses. Additionally, hypomethylating agents can be considered for post-allografting maintenance, especially in those MDS patients on the basis of the available data on *TET2* mutations predicting better response outside of the allogeneic HCT setting.^{34,35} Last, either mutation-specific or mutation-targeted agents such as IDH inhibitors AG-120 and AG-221 (NCT02632708, NCT02677922, NCT02577406), as well as a mutant p53 reactivating compound, APR-246 (NCT00900614, NCT02098343), have been investigated in various malignancies.⁵¹⁻⁵³ Application of these new mutation-specific compounds may pave the way for revolutionary therapeutic strategies in the context of allogeneic HCT.

Available data on somatic mutations in MDS from allografted patients highlight a large unmet need to improve allogeneic HCT outcomes with future clinical trials investigating novel conditioning regimen, better donor selection to optimize antitumor alloreactivity, and integration of novel anti-MDS agents, epigenetic therapy, and/or mutation-specific newer compounds as pre-HCT therapy, early posttransplantation consolidation, or maintenance.

Disclosure

The authors have stated that they have no conflict of interest.

References

- Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood* 2012; 120:2454-65.
- Schanz J, Tuechler H, Sole F, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. *J Clin Oncol* 2012; 30:820-9.
- Mufti GJ, Bennett JM, Goasguen J, et al. Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of Myelodysplastic Syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. *Haematologica* 2008; 93:1712-7.
- Mughal TI, Cross NC, Padron E, et al. An International MDS/MPN Working Group's perspective and recommendations on molecular pathogenesis, diagnosis and clinical characterization of myelodysplastic/myeloproliferative neoplasms. *Haematologica* 2015; 100:1117-30.
- Della Porta MG, Tuechler H, Malcovati L, et al. Validation of WHO classification-based Prognostic Scoring System (WPSS) for myelodysplastic syndromes and comparison with the revised International Prognostic Scoring System (IPSS-R). A study of the International Working Group for Prognosis in Myelodysplasia (IWG-PM). *Leukemia* 2015; 29:1502-13.
- Porwit A, van de Loosdrecht AA, Bettelheim P, et al. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes—proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. *Leukemia* 2014; 28:1793-8.
- Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol* 2009; 10:223-32.
- Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol* 2012; 30:2670-7.
- Cutler CS, Lee SJ, Greenberg P, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. *Blood* 2004; 104:579-85.

- Koreth J, Pidalá J, Pérez WS, et al. Role of reduced-intensity conditioning allogeneic hematopoietic stem-cell transplantation in older patients with de novo myelodysplastic syndromes: an international collaborative decision analysis. *J Clin Oncol* 2013; 31:2662-70.
- Deeg HJ. Hematopoietic cell transplantation for myelodysplastic syndrome. *Am Soc Clin Oncol Educ Book* 2015:e375-80.
- Cutler C. Timing of allogeneic stem cell transplantation for myelodysplastic syndromes and aplastic anemia. *Hematol Am Soc Hematol Educ Program* 2014; 2014:77-81.
- Sorror ML, Sandmaier BM, Storer BE, et al. Comorbidity and disease status based risk stratification of outcomes among patients with acute myeloid leukemia or myelodysplasia receiving allogeneic hematopoietic cell transplantation. *J Clin Oncol* 2007; 25:4246-54.
- Armand P, Gibson CJ, Cutler C, et al. A disease risk index for patients undergoing allogeneic stem cell transplantation. *Blood* 2012; 120:905-13.
- Field T, Perkins J, Huang Y, et al. 5-Azacitidine for myelodysplasia before allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant* 2010; 45:255-60.
- Shaffer BC, Ahn KW, Hu ZH, et al. Scoring system prognostic of outcome in patients undergoing allogeneic hematopoietic cell transplantation for myelodysplastic syndrome. *J Clin Oncol* 2016; 34:1864-71.
- Deeg HJ, Scott BL, Fang M, et al. Five-group cytogenetic risk classification, monosomal karyotype, and outcome after hematopoietic cell transplantation for MDS or acute leukemia evolving from MDS. *Blood* 2012; 120:1398-408.
- Lee HC, Saliba RM, Rondon G, et al. Mixed T lymphocyte chimerism after allogeneic hematopoietic transplantation is predictive for relapse of acute myeloid leukemia and myelodysplastic syndromes. *Biol Blood Marrow Transplant* 2015; 21:1948-54.
- Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med* 2011; 364:2496-506.
- Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med* 2012; 366:1079-89.
- Suela J, Alvarez S, Cigudosa JC. DNA profiling by arrayCGH in acute myeloid leukemia and myelodysplastic syndromes. *Cytogenet Genome Res* 2007; 118:304-9.
- Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature* 2001; 409:860-921.
- Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. *Nat Rev Genet* 2010; 11:685-96.
- Metzker ML. Sequencing technologies—the next generation. *Nat Rev Genet* 2010; 11:31-46.
- Lister R, Pelizzola M, Dowen RH, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 2009; 462:315-22.
- Neff T, Armstrong SA. Chromatin maps, histone modifications and leukemia. *Leukemia* 2009; 23:1243-51.
- Blencowe BJ, Ahmad S, Lee LJ. Current-generation high-throughput sequencing: deepening insights into mammalian transcriptomes. *Genes Dev* 2009; 23:1379-86.
- Figueroa ME, Abdel-Wahab O, Lu C, et al. Leukemic *IDH1* and *IDH2* mutations result in a hypermethylation phenotype, disrupt *TET2* function, and impair hematopoietic differentiation. *Cancer Cell* 2010; 18:553-67.
- Larsson CA, Cote G, Quintás-Cardama A. The changing mutational landscape of acute myeloid leukemia and myelodysplastic syndrome. *Mol Cancer Res* 2013; 11:815-27.
- Singh RK, Cooper TA. Pre-mRNA splicing in disease and therapeutics. *Trends Mol Med* 2012; 18:472-82.
- Bejar R, Papaemmanuil E, Haferlach T, et al. Somatic mutations in MDS patients are associated with clinical features and predict prognosis independent of the IPSS-R: analysis of combined datasets from the International Working Group for Prognosis in MDS—Molecular Committee. *Blood* 2015; 126:907.
- Sallman DA, Komrokji R, Vaupel C, et al. Impact of *TP53* mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes. *Leukemia* 2016; 30:666-73.
- Shen L, Kantarjian H, Guo Y, et al. DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. *J Clin Oncol* 2010; 28:605-13.
- Bejar R, Lord A, Stevenson K, et al. *TET2* mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood* 2014; 124:2705-12.
- Traina F, Visconte V, Elson P, et al. Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms. *Leukemia* 2014; 28:78-87.
- Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic *TET2* mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet* 2012; 44:1179-81.
- Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 2014; 20:1472-8.
- Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014; 371:2477-87.
- Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014; 371:2488-98.
- Valent P, Bain BJ, Bennett JM, et al. Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS. *Leuk Res* 2012; 36:1-5.

41. Valent P, Horny HP. Minimal diagnostic criteria for myelodysplastic syndromes and separation from ICUS and IDUS: update and open questions. *Eur J Clin Invest* 2009; 39:548-53.
42. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* 2015; 126: 9-16.
43. Bejar R, Stevenson KE, Caughey B, et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. *J Clin Oncol* 2014; 32:2691-8.
44. Kharfan-Dabaja MA, Komrokji RS, Zhang Q, et al. *TP53* and *IDH2* somatic mutations are associated with poor outcomes following allogeneic hematopoietic cell transplantation for myelodysplastic syndrome. *Blood* 2015; 126: 4382.
45. Thol F, Friesen I, Damm F, et al. Prognostic significance of *ASXL1* mutations in patients with myelodysplastic syndromes. *J Clin Oncol* 2011; 29:2499-506.
46. Yoshizato T, Shiozawa Y, Yoshida K, et al. Impact of somatic mutations on outcome in patients with MDS after stem-cell transplantation. *Blood* 2015; 126: 711.
47. Heuser M, Koenecke C, Gabdoulline R, et al. Molecular predictors of outcome in patients with MDS and AML following MDS after allogeneic hematopoietic stem cell transplantation. *Blood* 2015; 126:912.
48. Christopeit M, Badbaran A, Alawi M, et al. Correlation of somatic mutations with outcome after FLAMSA-busulfan sequential conditioning and allogeneic stem cell transplantation in patients with MDS. *Eur J Haematol* 2016; 97:288-96.
49. Sobucks RM, Wang T, Askar M, et al. Impact of KIR and HLA genotypes on outcomes after reduced-intensity conditioning hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2015; 21:1589-96.
50. Bao X, Wang M, Zhou H, et al. Donor killer immunoglobulin-like receptor profile Bx1 imparts a negative effect and centromeric b-specific gene motifs render a positive effect on standard-risk acute myeloid leukemia/myelodysplastic syndrome patient survival after unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2016; 22:232-9.
51. Deneberg S, Cherif H, Lazarevic V, et al. An open-label phase I dose-finding study of APR-246 in hematological malignancies. *Blood Cancer J* 2016; 6:e447.
52. Birendra KC, DiNardo CD. Evidence for clinical differentiation and differentiation syndrome in patients with acute myeloid leukemia and *IDH1* mutations treated with the targeted mutant *IDH1* inhibitor, AG-120. *Clin Lymphoma Myeloma Leuk* 2016; 16:460-5.
53. Stein EM, Altman JK, Collins R, et al. AG-221, an oral, selective, first-in-class, potent inhibitor of the *IDH2* mutant metabolic enzyme, induces durable remissions in a phase I study in patients with *IDH2* mutation positive advanced hematologic malignancies. *Blood* 2014; 124:115.