VOLUME 35 · NUMBER 9 · MARCH 20, 2017

JOURNAL OF CLINICAL ONCOLOGY

REVIEW ARTICLE

Clinical Implications of Genetic Mutations in Myelodysplastic Syndrome

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Published at jco.org on February 13, 2017.

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0732-183X/17/3509w-968w/$20.00

ABSTRACT

Myelodysplastic syndrome (MDS) is clonal disorder characterized by ineffective hematopoiesis and a tendency to evolve into acute myeloid leukemia (AML). Genetic studies have enabled the identification of a set of recurrently mutated genes central to the pathogenesis of MDS, which can be organized into a limited number of cellular processes, including RNA splicing, epigenetic and traditional transcriptional regulation, and signal transduction. The sequential accumulation of mutations drives disease evolution from asymptomatic clonal hematopoiesis to frank MDS, and, ultimately, to secondary AML. This detailed understanding of the molecular landscape of MDS, coupled with the emergence of cost- and time-effective methodologies for DNA sequencing has led to the introduction of genetic studies into the clinical realm. Here, we review recent advances in our genetic understanding of MDS, with a particular focus on the emerging role for mutational data in clinical management as a potential tool to assist in diagnosis, risk stratification, and therapeutic decision-making.

J Clin Oncol 35:968-974. © 2017 by American Society of Clinical Oncology

INTRODUCTION

Myelodysplastic syndrome (MDS) comprises a heterogeneous group of clonal hematopoietic neoplasms characterized by ineffective and dysplastic hematopoiesis that present clinically as peripheral blood cytopenias, and by a variable propensity to evolve into acute myeloid leukemia (AML).[1] MDS is the most common cause of acquired bone marrow failure in adults, with an incidence in the United States of 75 cases per 100,000 individuals 65 years of age and older.[2] Over the past decade, DNA sequencing has revolutionized our understanding of the pathogenesis of this disease, establishing that MDS arises through the sequential acquisition of somatic mutations in a set of recurrently involved genes. With the advent of cost- and time-effective sequencing technologies, mutational profiling has also entered the clinical realm, with many centers now including these analyses as part of the routine work-up of patients with MDS. In this review, we discuss the molecular pathogenesis of MDS, as well as the emerging role of genetic data in the diagnosis, prognostication, and treatment of patients.

RECURRENTLY MUTATED GENES IN MDS

A detailed understanding of the mutational landscape in MDS has emerged over the past 10 years, first with the advent of high-resolution single nucleotide polymorphism arrays and, subsequently, with methods enabling whole-genome and whole-exome sequencing.[3] Application of these technologies has identified a set of genes recurrently mutated in myeloid malignancies[4-9]; subsequently, several large MDS cohorts have been sequenced using a targeted strategy, focusing on this defined group.[10-12] With this approach, up to 90% of patients have been found to have a somatic mutation in at least one gene.

Though the number of driver genes in MDS is large, these can be organized into a limited number of categories, corresponding to the implicated cellular process: RNA splicing factors, epigenetic regulators, cohesin components, transcription factors, the DNA damage response, and signal transduction molecules (Fig 1). The following sections will provide a brief overview of each group. A detailed discussion of the functional consequences of these mutations is beyond the scope of this review but has been covered elsewhere.[13,14]

Splicing Factors

Components of the spliceosome, most commonly SF3B1, SRSF2, U2AF1, and ZRSR2, are mutated in up to 60% of patients with MDS, with changes occurring as single amino acid

DOI: 10.1200/JCO.2016.71.0806

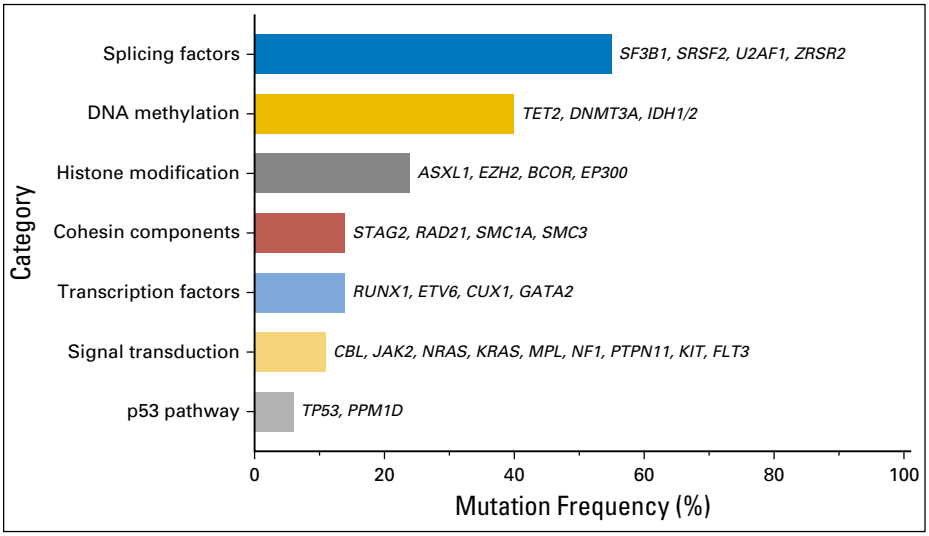

Fig 1. The recurrently mutated genes in myelodysplastic syndrome (MDS) can be organized into a limited number of biologic categories. Estimated mutation frequencies within an unselected population of patients with MDS are displayed, with examples of the most commonly implicated genes in each category listed to the right of each bar. Data are from Papaemmanuil et al,[11] Haferlach et al,[12] and R.C. Lindsley (personal communication, October 2016).

substitutions at defined hotspots.[8,11,12] *SF3B1* was the first spliceosome family member to be implicated, and is mutated in up to 80% of MDS cases with ringed sideroblasts.[6,15] Splicing factor mutations are heterozygous and generally mutually exclusive of one another, suggesting that cells cannot tolerate two mutations or, alternatively, that these changes have a redundant role in disease pathogenesis. The spliceosome functions to mediate intron excision and exon ligation in the generation of mature messenger RNA molecules. Mutant splicing factors result in altered patterns of splicing, and investigation of the role of these alternate transcripts in MDS pathogenesis is ongoing.[16-18]

Epigenetic Regulators

Genes involved in DNA methylation and histone modification make up a second common class of mutations in MDS. Recurrent missense, nonsense, splice site, and frameshift mutations have been identified in *DNMT3a*, a de novo DNA methyltransferase, and *TET2*, an enzyme that hydroxylates methylated cytosines to initiate the process of DNA demethylation.[9,19] TET2 activity is also affected by mutations in isocitrate dehydrogenase 1 (*IDH1*) and *IDH2*. Heterozygous hotspot changes alter isocitrate dehydrogenase enzymatic activity, resulting in the generation of 2-hydroxyglutarate, an oncometabolite that inhibits the activity of numerous targets, including TET2.[20,21] Components of histone modification complexes are also recurrently mutated in MDS, most commonly *ASXL1* and *EZH2*, which are affected by loss-of-function mutations in approximately 20% and 5% of cases, respectively.[12]

Cohesins

Cohesin is a closed-loop multiprotein complex composed of SMC1A, SMC3, RAD21, STAG1, and STAG2. Mutually exclusive loss of function nonsense and frameshift mutations in the cohesin components are found in 11% and 17% of low- and high-risk MDS, respectively.[22,23] Cohesin normally functions to align sister chromatids during mitosis; however, mutations do not result in gross aneuploidy.[7,24] Instead, recent studies have shown that cohesin mutations can promote transformation by driving aberrant transcriptional programs, potentially by disrupting this complex's role in stabilizing DNA loops, such as those involved in enhancer-promoter interactions.[25]

Transcription Factors

Hematopoietic differentiation involves the activation of lineage- specific gene-expression programs by core transcription factors, such as GATA2 and RUNX1. Recurrent loss-of-function mutations in these molecules occur somatically in MDS and can also be inherited in the germline, where they cause familial bone marrow failure syndromes with a propensity to evolve into myeloid malignancies.[11,12,26]

TP53

The tumor suppressor TP53 plays a key role in coordinating responses to cellular stresses such as DNA damage. Missense *TP53* mutations are particularly prevalent among patients with MDS who have undergone chemotherapy, in whom their frequency approaches 40%.[27] These changes often occur alongside loss of the second *TP53* allele via deletion of the short arm of chromosome 17 and are associated with thrombocytopenia, complex karyotype, and a particularly poor prognosis.[10,28]

THE GENETIC TRAJECTORY OF MDS: FROM CLONAL HEMATOPOIESIS TO SECONDARY AML

The sequential acquisition of mutations during MDS pathogenesis implies a series of genetic states that correspond to distinct clinical phenotypes (Fig 2).[29] According to this model, during disease initiation, founder mutations drive asymptomatic clonal expansion within the hematopoietic compartment. During progression, further mutations are acquired within this clone, which impair normal hematopoiesis and alter blood counts, ultimately resulting in overt MDS and in some instances, eventual secondary AML (sAML).

Consistent with this model, recurrent somatic mutations in MDS-associated genes, such as *DNMT3a, TET2*, and *ASXL1,* have been identified in the peripheral blood of healthy individuals with

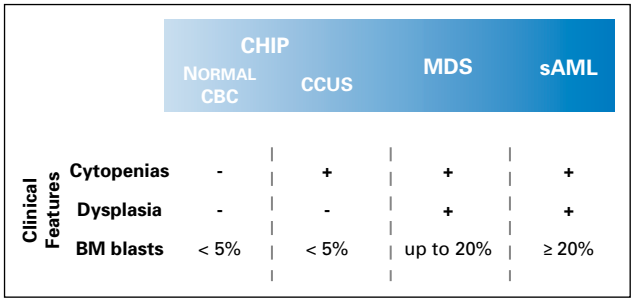

Fig 2. The spectrum of clonal myeloid disorders and their defining clinical features. The sequential acquisition of somatic mutations drives the evolution of asymptomatic clonal hematopoiesis through CCUS to frank MDS and sAML. CHIP, originally defined by Steensma et al,[29] encompasses individuals who possess a somatic mutation in a gene associated with myeloid malignancy but do not meet diagnostic criteria for another hematologic neoplasm. Of note, only a minority of patients at each stage along this spectrum proceed to the next; for example, CHIP evolves into frank MDS at a rate of 0.5% to 1% per year.[29] CBC, complete blood cell count; CCUS, clonal cytopenias of undetermined significance; CHIP, clonal hematopoiesis of indeterminate potential; MDS, myelodysplastic syndrome; sAML, secondary acute myeloid leukemia.

normal blood counts and are a strong independent predictor for the future development of hematologic malignancies.[30-33] However, the absolute risk of malignant transformation is low, approximately 0.5% to 1% per year, leading to this entity being termed "clonal hematopoiesis of indeterminate potential" (CHIP).[29] The acquisition of further mutations drives the progression of CHIP to overt malignancy, as demonstrated by the common clonal origin of paired CHIP and AML samples.[31] Moreover, analysis of mutational hierarchies in patients with MDS has identified that mutations in epigenetic regulators, the genes most commonly implicated in clonal hematopoiesis, are founder events, providing additional support that CHIP represents the earliest genetic step in MDS pathogenesis.[34]

Given the low rate of malignant transformation in CHIP, identification of factors that influence its natural history (ie, the development of clonal dominance and/or the risk of acquiring cooperating mutations) is the focus of much ongoing investigation. Potential contributors include not only the identity of the CHIP mutation itself but also germline polymorphisms and cell extrinsic factors.[35] In one example of the latter, treatment with chemotherapy has been shown to enable the preferential expansion of clones carrying mutations in *TP53*.[36]

Whereas CHIP may precede MDS, evolution to sAML can be considered the final stage of disease progression. This transition involves the acquisition of characteristic AML-associated genetic changes, such as activating mutations in signaling molecules such as *FLT3* and *N-RAS*, as well as inactivating mutations in *CEBPA*.[37] At the time of leukemic transformation, the antecedent MDS clone persists but is outcompeted by aggressive subclones that drive the development of AML.[38] Interestingly, compared with de novo AML, sAML has a distinct genetic signature, characterized by the presence of mutations in *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, and *STAG2*, all highly specific for prior MDS.[39] Of note, mutations in these genes can also identify a subset of patients with AML who have no history of preceding MDS and who share the aggressive clinical behavior of sAML.[39]

THE ROLE OF GENOMICS IN MDS DIAGNOSIS

At present, the diagnostic evaluation of MDS relies on morphologic assessment of the peripheral blood and bone marrow, conventional cytogenetics, and exclusion of secondary causes of dysplasia. The WHO has defined various disease subtypes on the basis of the number of dysplastic and cytopenic lineages, the prevalence of blasts, the percentage of ring sideroblasts (RS), and the presence of specific cytogenetic abnormalities.[40] However, given this heavy reliance on morphologic assessment, MDS can be challenging to diagnose, with well-documented interobserver variability.[41,42]

In light of recent insights into MDS genetics, consideration has been given to the incorporation of mutational data into its diagnostic criteria, similar to the use of *JAK2* mutations in myeloproliferative neoplasms (MPNs).[40] However, in the 2016 WHO classification scheme, the role of genetics has appropriately remained limited. The recurrent somatic mutations observed in MDS are not specific for this disease entity; for example, *TET2* mutations are prevalent in conditions on the differential diagnosis of MDS, namely AML, MPNs, and MPN/MDS overlap syndromes.[9] Moreover, somatic mutations in MDS-associated genes have been identified in healthy individuals with CHIP.[30-33] Similarly, patients with idiopathic cytopenias of undetermined significance, who have low blood cell counts, a normal karyotype, and lack significant dysplasia, have somatic mutations in MDS-related genes greater than one-third of the time.[43] Together, these studies highlight that the mere presence of MDS-associated somatic mutations should not be considered as definitive evidence of this diagnosis.

However, in certain contexts, genetic data may provide diagnostic utility in MDS. Most notably, mutations in the spliceosome gene *SF3B1* define a subgroup of patients with RSs and favorable prognosis.[6,15] The marked specificity of this association is reflected in the updated WHO criteria, where, in the presence of cytopenias and dysplasia, detection of an *SF3B1* mutation can establish a diagnosis of MDS-RS when RSs make up as few as 5% of all nucleated erythroid cells, compared with the traditional cutoff of 15%.[40] Though currently limited to *SF3B1*, the role of genetics in defining MDS subclasses may continue to expand. For example, analysis of genotype–phenotype relationships has demonstrated that mutations of genes involved in DNA methylation, cohesin, the RAS pathway, and splicing factors (other than *SF3B1*) are associated with multilineage dysplasia.[44] Moving forward, although the diagnostic value of single mutations is likely to remain limited, it is possible that certain combinations of changes may hold high specificity for MDS, a hypothesis that will be informed by ongoing large-scale sequencing studies.

THE UTILITY OF GENETICS IN INFORMING PROGNOSIS

Given the variability in risk of leukemic transformation and survival among patients with MDS, a number of prognostic risk stratification systems have been developed to facilitate clinical decision-making. The most widely used tools are the International Prognostic Scoring System (IPSS),[45] and a revised version of the IPSS (IPSS-R),[46] which use a combination of bone marrow

morphology, conventional cytogenetic findings, and the degree of cytopenias to risk stratify patients. However, patient outcomes remain highly variable within the subsets defined by these and other prognostic systems.[47]

To date, there have been three large studies that have assessed the prognostic impact of MDS-associated gene mutations across a broad cross-section of patients.[10-12] Although the number of interrogated genes, patient populations, and statistical methods differ among these, several common themes have emerged. First, as the number of oncogenic mutations increases, patient outcomes progressively worsen.[10,11] Second, in univariate analyses, somatic mutations in certain genes reproducibly predict patient outcomes. Across studies, *TP53, EZH2, ETV6, RUNX1*, *ASXL1*, and *SRSF2* mutations predict poor overall survival, whereas *SF3B1* mutations are associated with better clinical outcomes. Interestingly, the prognostic significance of these mutations seems to be maintained regardless of whether these are early or late events in disease progression.[11] However, generalization of this finding to all somatic mutations may not be warranted, because the acquisition of subclonal mutations in *FLT3* and *N-RAS* in cases of low-risk MDS has been associated with impending leukemic transformation.[37]

Notably, somatic mutations can predict overall survival independent of clinical prognostic scoring systems, including the IPSS-R (Fig 3).[10,48] However, given that cytopenias, blast count, and morphology are likely closely linked to the genetic makeup of the MDS clone, it follows that prognostic models that include a detailed set of clinical and cytogenetic variables are only modestly improved by the inclusion of mutational data.[11,12] This interdependency was also demonstrated in a recent study that combined genetic, cytogenetic, transcriptomic, and hematologic data to predict leukemia-free survival in patients with MDS; in the resulting model, genetics made only a minor contribution to risk estimates.[49] Thus, traditional morphologic and clinical criteria will continue to play a central role in evaluating MDS prognosis. These variables reflect not only the genetics of the neoplastic clone, but also other potential contributors to MDS biology, such as the microenvironment, which has been shown to contribute to disease pathogenesis in animal models.[50]

In light of this, before the routine use of mutational data for MDS prognostication, further investigation is required. Large multicenter studies, complimented by detailed clinical annotation, are necessary to precisely integrate genetic data into existing schemes. To this end, a collaboration organized by the International Working Group for Prognosis in MDS is ongoing. Moving forward, it is possible that genetic data may hold greatest prognostic value among specific patient subsets. Consistent with this notion, in low-risk MDS, *EZH2* mutations can identify patients with shorter than expected survival.[48] Another example is individuals with complex karyotypes, traditionally considered to be an indicator of high-risk disease; within this group, the absence of *TP53* mutations is associated with significantly improved outcomes, comparable to patients with noncomplex karyotypes (Fig 4).[10] These findings highlight the potential utility of genetic data in prognostication for patients with MDS, but its ultimate role in clinical practice, especially in the context of other clinical parameters, continues to evolve.

THERAPEUTIC IMPLICATIONS OF GENOMIC DATA IN MDS

Traditionally, therapeutic decision-making in MDS has been guided by individual risk assessment performed using the IPSS or similar tools.[51] Given the central role of somatic mutations in MDS pathogenesis, genetics also holds potential to inform treatment decisions. Proof of principle for a genetically-targeted therapeutic approach in MDS has been illustrated in del(5q) MDS. In these patients, treatment with lenalidomide resulted in cytogenetic complete remissions and lower transfusion requirements.[52,53] Lenalidomide binds to the $CRL4^{CRBN}$ E3 ubiquitin ligase, altering its substrate affinity to induce the selective degradation of casein kinase 1A1 (CK1α). CK1α is encoded by a gene in the commonly deleted region of chromosome 5 in del(5q) MDS; this creates a therapeutic window for lenalidomide-mediated degradation, because further loss of CK1α in the setting of baseline haploinsufficiency leads to p53-mediated apoptosis.[54] Consistent with this, mutations in *TP53* are associated with poor response to lenalidomide in patients with del(5q) MDS, with treatment resulting in the expansion of *TP53*-mutant subclones.[55-57]

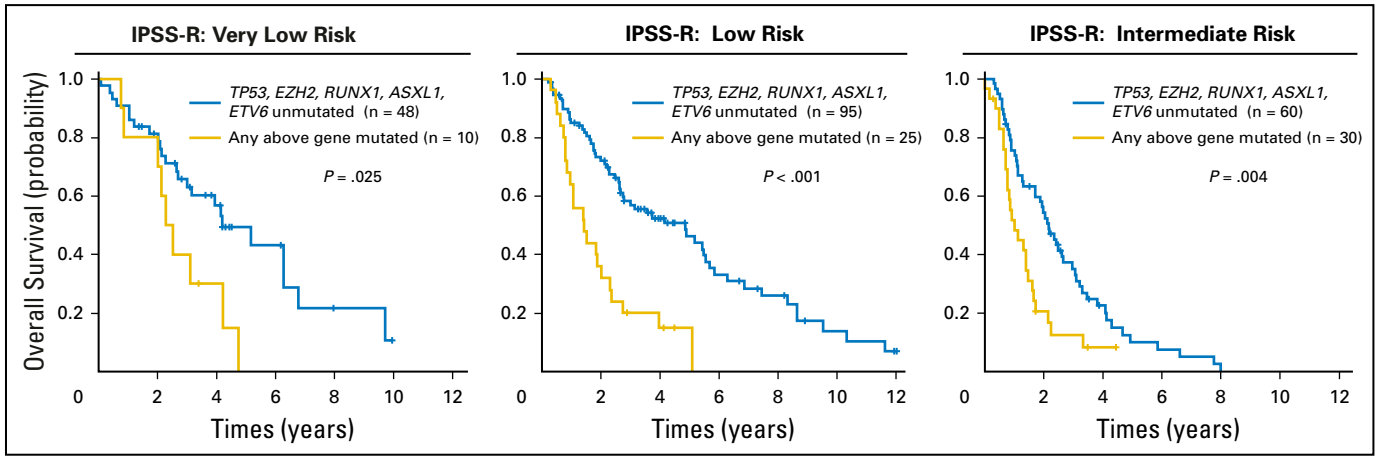

Fig 3. Somatic mutations in any of *TP53*, *EZH2*, *RUNX1*, *ASXL1*, or *ETV6* identify patients with reduced overall survival within each of the IPSS-R lower risk categories. Patients and mutational data were originally described in Bejar et al.[10] Survival curves compare patients with one or more mutations in *TP53, EZH2, RUNX1, ASXL1*, or *ETV6* (yellow lines) with patients within the same IPSS-R category without mutations in these five genes (blue lines). Adapted from Bejar et al[3] and used with permission. IPSS-R, International Prognostic Scoring System, Revised.

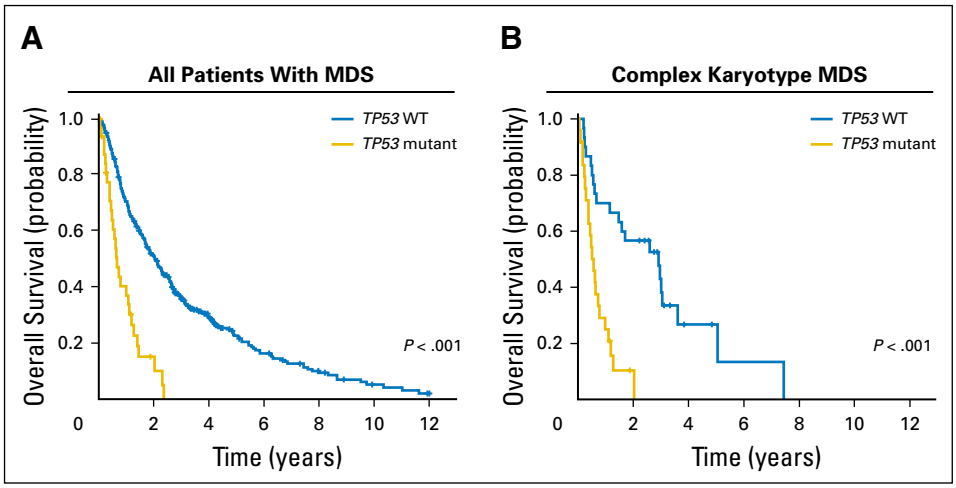

Fig 4. *TP53* mutations are associated with reduced overall survival in MDS and identify a subset of patients with complex karyotypes who have poor outcomes. (A) Survival analysis of 439 patients with MDS[10] comparing those with somatic *TP53* mutations (yellow line; n = 33) with those without *TP53* mutations (blue line, n = 406). (B) Overall survival of patients with complex cytogenetics who have *TP53* mutations (yellow line; n = 26) compared with those who lack *TP53* mutations (blue line; n = 31). Adapted from Bejar et al[10] and used with permission. MDS, myelodysplastic syndrome.

The DNA methyltransferase inhibitors 5-azacitidine and decitabine, commonly referred to as hypomethylating agents (HMAs), are a cornerstone of treatment in high- risk MDS.[51] However, not all patients respond to these therapies, with less than 50% achieving hematologic improvement.[58] Given that genes regulating DNA methylation are recurrently mutated in MDS, there has been significant interest in the potential for genetics to identify those who may benefit from HMA therapy. Across multiple studies, *TET2* mutations have most consistently been associated with favorable responses.[59-61] Of note, this seems to be limited to patients in whom *TET2* is an early, clonal mutation as opposed to a late subclonal event,[60] providing evidence that the order of mutation acquisition, not merely the presence of a given mutation, may influence therapeutic responsiveness, as has recently been suggested for MPNs.[62] However, the power of mutational data to predict HMA response is modest at best; in particular, a set of mutations that can identify patients with response rates sufficiently low to justify withholding therapy has not been defined.[63] Moving forward, clarification of the mechanism of action and pharmacokinetics of HMAs, as well as large prospective studies that simultaneously evaluate genetic, epigenetic, and clinical contributors to treatment response are required.

At present, the only potentially curative therapy for MDS is allogeneic stem cell transplantation.[51] Accurate pretransplant risk stratification is necessary to identify patients for whom this approach has the potential for success, while preventing unnecessary morbidity in those unlikely to benefit. Although clinical factors such as cytogenetics and serum ferritin levels have been shown to influence outcomes,[64,65] there is also an emerging role for genetics in this regard. In a single-center retrospective study of patients who underwent pretransplant genetic profiling, mutations in *TP53* were associated with significantly decreased overall survival.[66] A recent study has replicated the negative prognostic impact of *TP53* mutations in the MDS transplant population, in addition to identifying *RUNX1* and *ASXL1* as potential markers of poor outcome.[67] Though evaluation of large clinical cohorts and prospective validation is required, these data suggest that genetics may help identify a subset of patients in whom outcomes with standard transplant regimens are particularly poor, and alternative therapeutic strategies should be considered.

Last, with the recent advances in our understanding of the molecular pathophysiology of MDS, novel therapeutic targets have emerged. For example, small-molecule inhibitors of mutant isocitrate dehydrogenase enzymes, present in a minority of patients with MDS, have displayed efficacy in preclinical studies of AML.[68,69] Moreover, animal models of splicing factor mutations have shown that mutant homozygosity is lethal, whereas in the setting of heterozygosity, the pattern seen in myeloid malignancies, further inhibition of the splicing machinery can drive cell death.[18,70] With the intention of exploiting the therapeutic window present in patients bearing these mutations, small-molecule spliceosome inhibitors are under development.

INTEGRATING PRECISION MEDICINE INTO PRACTICE

The detailed understanding of the genetic landscape of MDS that has emerged in recent years has revolutionized our appreciation of disease evolution from CHIP through sAML. As evidence continues to accumulate highlighting the utility of genomics to assist in MDS diagnosis, prognostication, and therapeutic decision-making, physicians face the challenge of how best to integrate mutational profiling into clinical practice. The resources, cost, and expertise required to generate these data necessitate collaboration among all stakeholders, including molecular pathologists, bioinformaticians, and front- line physicians. Other important considerations include the appropriate timing and breadth of genetic interrogation, as well as reliable approaches to distinguish pathogenic driver mutations from germline polymorphisms and passenger mutations. Last, there is a need for streamlined clinical reports that highlight actionable variants from a diagnostic, prognostic, or therapeutic perspective, facilitating interpretation by the treating physician.

A strong foundation is in place. The set of genes recurrently mutated in myeloid malignancies provide a backbone for targeted sequencing panels. Moreover, scenarios where our understanding is sufficient to inform clinical practice have begun to emerge. For example, mutations in *SF3B1*, which define a subset of patients with MDS with RSs and favorable prognosis, now form part of the WHO diagnostic criteria. At the other end of the clinical spectrum, evidence suggests that *TP53*-mutated MDS is a distinct entity, associated with adverse outcomes despite our most aggressive conventional treatment regimens. Moving forward, large, multicenter, prospective studies will enable us to build upon these and

other findings, enabling progress toward our goal of effective individualized treatment strategies on the basis of disease genotype.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Conception and design: All authors
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Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Support

Supported by grants from the National Institutes of Health (Grants No. R01 HL082945, R24 DK099808), the US Department of Defense, the Edward P. Evans Foundation, the Leukemia & Lymphoma Society, and the STARR Cancer Consortium (all to B.L.E.).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Clinical Implications of Genetic Mutations in Myelodysplastic Syndrome

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James A. Kennedy
No relationship to disclose

Benjamin L. Ebert
Consulting or Advisory Role: Genoptix, Celgene H3 Biomedicine
Research Funding: Celgene (Inst)
Patents, Royalties, Other Intellectual Property: Genoptix for an MDS genetics panel (Inst)

Acknowledgment

We apologize to those researchers whose work could not be cited because of limitations in the length of the review and the number of permitted references.