

## New Strategies in Myelodysplastic Syndromes: Application of Molecular Diagnostics to Clinical Practice

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### Abstract

An increasingly complete compendium of recurrently mutated genes in myelodysplastic syndromes (MDS) has been defined, and the application of massively parallel sequencing to identify mutations in clinical practice now promises to improve the care of patients with this disease. More than 25 recurrent MDS-associated somatic mutations have been identified, involving biologic pathways as diverse as chromatin remodeling and pre-mRNA splicing. Several of these mutations have been shown to have prognostic implications that are independent of existing risk stratification systems based on clinical and pathologic parameters. Application of these recent discoveries to diagnosis, prognosis, risk stratification, and treatment selection for patients with MDS has the potential to improve patient outcomes. Here, we review recent advances in MDS and discuss potential applications of these discoveries to clinical practice. *Clin Cancer Res*; 19(7); 1637–43. ©2013 AACR.

### Disclosure of Potential Conflicts of Interest

B.L. Ebert has served as a consultant for Genoptix and Celgene. No potential conflicts of interest were disclosed by the other authors.

### CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

### Learning Objectives

After participating in this CME activity, participants should have a better understanding of recurrent genetic lesions identified in myelodysplastic syndromes (MDS), as well as their relevance to diagnosis, prognosis, and treatment response in MDS.

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### Background

The myelodysplastic syndromes (MDS) comprise a clinically and pathologically heterogeneous group of hematologic neoplasms, collectively characterized by clonal hematopoiesis, aberrant myeloid differentiation, ineffective hematopoiesis leading to cytopenias, and a risk of progression to acute myeloid leukemia (AML; ref. 1). The risk of developing MDS is increased both by exogenous factors, such as exposure to cytotoxic chemotherapy, radiation, or benzene, and by inherited genetic predisposition, including that associated with congenital bone marrow failure disorders or several nonsyndromic familial disorders that predispose to MDS/AML.

The diagnosis of MDS is based on persistent cytopenias in association with characteristic dysplastic findings in

the peripheral blood and bone marrow, excess blasts, or a cytogenetic or molecular marker proving clonality, as well as exclusion of other diseases (2). The 2008 World Health Organization (WHO) classification of hematologic neoplasms includes several MDS subtypes, defined by the proportion of blood and marrow blasts, the presence or absence of monocytosis or ring sideroblasts, cytogenetic findings, and the specific cell lineages involved by dysplasia (3). Despite the clinical use of WHO subgrouping, significant heterogeneity in phenotype and outcome persists within WHO-defined MDS categories (4, 5) and there are also challenging cases where a diagnosis of MDS is possible but minimal diagnostic criteria are not met (6).

Because of wide variation in the clinical course of MDS, treatment algorithms for MDS rely heavily on accurate prognostic stratification. Investigators have made multiple attempts to incorporate clinical and pathologic information into risk models for patients with MDS to guide treatment recommendations. The International Prognostic Scoring System (IPSS), published in 1997, was developed to assess prognosis of untreated adult patients with primary MDS (7). Since then, a number of other classification and prognostic scoring schemata have been developed with the goal of more accurately identifying disease subtypes with

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similar outcomes and risks of evolution to AML (8–12). Most recently, the revised IPSS (IPSS-R) defines 5 major prognostic categories based on 5 cytogenetic risk subgroups (including a broader range of karyotypic abnormalities than the original IPSS), bone marrow blast percentage, and number and severity of cytopenias (4).

Clinicians currently tailor therapeutic recommendations to each patient's situation, depending on the predicted risk of disease and the patient's age, comorbid conditions, and functional status (13). Integration of somatic mutation assays into MDS diagnosis, risk stratification, and prediction of therapeutic response has the potential to greatly improve treatment outcomes for this patient population. In addition, better understanding of the biologic basis of MDS will aid in the design of targeted, rationally designed novel therapies.

### On the Horizon

#### Identification of recurrent somatic mutations in MDS

Genetic lesions that drive the phenotype of MDS contribute to enhanced or acquired self-renewal and impaired differentiation. Additional abnormalities in the bone marrow microenvironment and in the epigenetic state of malignant cells may independently contribute to disease pathogenesis (14, 15). The compendium of genes with recurrent somatic mutations in MDS has grown dramatically over the last few years with the application of single-nucleotide polymorphism (SNP) arrays, whole-exome sequencing, and whole-genome sequencing.

The genes mutated in MDS can be grouped into several categories: (i) transcription factors (e.g., *TP53*, *RUNX1*, *ETV6*; refs. 16–18), (ii) epigenetic regulators and chromatin-remodeling factors (e.g., *TET2*, *DNMT3A*, *ASXL1*, *IDH1/2*, *EZH2*; refs. 19–23), (iii) pre-mRNA splicing factors (e.g., *SF3B1*, *U2AF1*, *SRSF2*; refs. 24–26), and (iv) signaling molecules (e.g., *NRAS*, *JAK2*, *NPM1*; refs. 16, 27; summarized in Table 1). More than 70% of patients with MDS harbor somatic mutations or clonal cytogenetic abnormalities and more than 50% of patients with MDS carry at least one somatic mutation (28). Somatic mutations in *SF3B1*, *TP53*, *TET2*, and *ASXL1* are among the most commonly identified changes, whereas those in *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1* have been found to predict clinical phenotype and survival independent of other variables such as the IPSS score (ref. 16; Table 1). Similarly, a number of chromosomal aberrations, such as deletions of chromosomes 5q, 7 or 7q, Y, or 20q; trisomy 8; and recurrent translocations and inversions involving chromosome 3q, among others, have prognostic relevance (Table 2).

The phenotypic heterogeneity within MDS—as highlighted by the presence of variable number and severity of cytopenias, blast proportion, rate of progression to AML, response to treatment, and overall survival (OS)—is likely driven, to a great extent, by the specific pattern of these genetic changes and their interaction with therapeutic choices. As many patients have several detectable genetic changes, it will be important to understand the interaction of these mutations with each other, as well as the

impact of varying allele burden (i.e., degree of clonality) and its influence on the clinical phenotype.

#### Decoding the epigenetic landscape of MDS

Alteration in epigenetic mechanisms of regulation of gene expression are key drivers of malignant phenotype in different cancer types, including hematologic malignancies (29). Given the success of DNA methyltransferase inhibitors azacitidine and decitabine in the treatment of MDS, multiple studies have been undertaken to better define the epigenetic landscape of MDS, even though it is still unclear whether these agents exert their clinical effect entirely via DNA hypomethylation (30).

Promoter CpG island hypermethylation studies in MDS primary cells have identified widespread promoter hypermethylation of specific genes, including cell-cycle and apoptosis regulators, and adhesion and motility genes, although the methylation status correlates poorly with treatment response (29). In addition, both MDS and AML have widespread promoter hypermethylation, with epigenetic deregulation of both cancer- and non-cancer-associated genes (31), which has also been linked to prognostic relevance (32, 33). It is still unclear whether abnormal methylation patterns are usually driven by somatic mutations that alter the epigenetic state, such as the mutations in *DNMT3A*, *IDH1/2*, and *TET2* that affect DNA methylation status, or whether epigenetic abnormalities are themselves primary drivers of the disease in some cases.

#### Moving toward improved diagnostics

The diagnostic evaluation of MDS has not changed significantly in the last 30 years and still relies on peripheral blood findings, morphologic evaluation, and G-banded metaphase karyotyping of a bone marrow sample (34). However, morphologic diagnosis of MDS remains challenging and often lacks interobserver concurrence, with a 12% discordance rate among different pathologists with respect to diagnosing dysplasia and even greater discrepancy when assigning a specific MDS subtype (35–37). Uncertainty about MDS diagnosis impairs prognostic assessment and therapeutic decision making.

The advent of multiparameter flow cytometry (MFC) in late 1980s improved identification and purification of different stem and progenitor cell populations within the context of both normal and malignant hematopoiesis. MDS cells often display an aberrant immunophenotype that can aid in their identification, and whereas standardization is still lacking and the optimal role of MFC in MDS diagnostics and risk stratification remains unclear (38, 39), addition of MFC to cytomorphology and cytogenetics can significantly increase diagnostic yield (40). MFC analysis of high- and low-risk MDS bone marrow samples show differential expansion of stem and progenitor populations, which in turn correlate with specific genetic, epigenetic, and transcriptomic alterations (41). Additional studies of highly fractionated stem and progenitor compartments in MDS and understanding of their functional and clinical relevance remain active areas of research.

**Table 1.** Summary of the currently known somatic mutations in MDS and their effect on overall survival

|  | MDS<br>(% cases) | MPN<br>(% cases) | AML<br>(% cases) | Effect<br>on OS<br>(MDS) | Independent<br>risk<br>predictor? |
|--|------------------|------------------|------------------|--------------------------|-----------------------------------|
| <b>RNA splicing</b>                        |                  |                  |                  |                          |                                   |
| SF3B1                                      | 14%–28%          | Rare             |                  | ↑/↔                      |                                   |
| U2AF1                                      | 8%               | Rare             |                  | ↓                        |                                   |
| SRSF2                                      | 15%              | Rare             |                  | ↓                        |                                   |
| ZRSR2                                      | 6%               |                  |                  | ↔/↓                      |                                   |
| SF3A1                                      | 1.3%             |                  |                  |                          |                                   |
| PRPF40B                                    | 1.3%             |                  |                  |                          |                                   |
| U2AF65                                     | <1%              |                  |                  |                          |                                   |
| SF1  | 1.3%             |                  |                  |                          |                                   |
| PRPF8                                      | Rare             |                  |                  |                          |                                   |
| LUC7L2                                     | Rare             |                  |                  |                          |                                   |
| <b>Epigenetic regulators</b>               |                  |                  |                  |                          |                                   |
| DNMT3A                                     | 10%              |                  | 22%              | ↓                        |                                   |
| TET2                                       | 19%–26%          |                  | 14%–24%          | ↔                        |                                   |
| EZH2                                       | 6%               |                  |                  | ↓                        | +                                 |
| ATRX                                       | <1%              |                  |                  |                          |                                   |
| IDH1/IDH2                                  | 4%–12%           |                  | 15%–25%          | ↓ (IDH1)                 |                                   |
| ASXL1                                      | 10%–20%          | 10%              | 5%–30%           | ↓                        | +                                 |
| UTX  | 1%               |                  |                  |                          |                                   |
| <b>Transcription factors</b>               |                  |                  |                  |                          |                                   |
| TP53                                       | 4%–14%           |                  |                  | ↓                        | +                                 |
| RUNX1                                      | 10%–20%          |                  | 12%              | ↓                        | +                                 |
| ETV6                                       | 1%–3%            |                  | 1%–3%            | ↓                        | +                                 |
| WT1  | Rare             |                  |                  |                          |                                   |
| PHF6                                       | Rare             |                  |                  |                          |                                   |
| <b>Receptor tyrosine kinases/signaling</b> |                  |                  |                  |                          |                                   |
| FLT3                                       | 2%–3%            |                  | 30%              | ↓                        |                                   |
| c-KIT                                      | 1%               |                  | 2%               |                          |                                   |
| JAK2                                       | 3%               | 50%–95%          |                  |                          |                                   |
| CBL  | 3%               |                  | 3%               |                          |                                   |
| CBLB                                       | rare             |                  |                  |                          |                                   |
| PTPN11                                     | <1%              |                  |                  |                          |                                   |
| GNAS                                       | <1%              |                  |                  |                          |                                   |
| KRAS                                       | 1%–2%            |                  |                  |                          |                                   |
| NRAS                                       | 10%              |                  | 15%              | ↓                        |                                   |
| CDKN2A                                     | <1%              |                  |                  |                          |                                   |
| BRAF                                       | <1%              |                  |                  | ↓                        |                                   |
| MPL, CSF1R                                 | rare             |                  |                  |                          |                                   |
| PTEN                                       | <1%              |                  |                  |                          |                                   |
| <b>Others</b>                              |                  |                  |                  |                          |                                   |
| NPM1                                       | 2%–3%            |                  |                  |                          |                                   |

NOTE: Independent risk predictor (predictor of OS independent of IPSS score, age, and other mutations), ↑ (increased OS), ↓ (decreased OS), ↔ (no effect on OS), + (mutation determined to be an IPSS-independent risk predictor based on multivariable analysis; ref. 16). Abbreviation: MPN, myeloproliferative neoplasms.

Identification of somatic mutations aids in diagnosis of uncertain cases of MDS and provides additional IPSS-independent information about prognosis. Therefore, testing for the presence of these mutations in peripheral blood or bone marrow using deep sequencing is likely going to become routine, similar to our current practice of testing for *JAK2* mutations or *BCR/ABL* fusion in patients with mye-

loproliferative neoplasms. Given the remarkable advances in DNA sequencing technologies over the last few years and ever dropping costs, multiple platforms are now becoming widely available for mutational profiling in clinical practice (42). Although whole-genome sequencing provides the most comprehensive analysis of somatic mutations, high cost and our lack of understanding of clinical relevance

**Table 2.** Summary of the most common chromosomal aberrations in MDS and their effect on overall survival

|                                | MDS<br>(% cases) | Effect<br>on OS |
|--------------------------------|------------------|-----------------|
| <b>Chromosomal aberrations</b> |                  |                 |
| Del(5q)                        | 10%–15%          | ↑               |
| Monosomy 7, del 7q             | 10%              | ↓               |
| Trisomy 8                      | 8%               | ↔               |
| t(3q), inv(3q)                 | <5%              | ↓               |
| i(17q)                         | Rare             | ↔               |
| Del(20q), Del(12p)             | <5%              | ↑               |
| Del Y, Del(11q)                | <5%              | ↑↑              |

NOTE: ↑ (increased OS), ↑↑ (significantly increased OS), ↓ (decreased OS), ↔ (no effect on OS).

of noncoding DNA changes impede its adaptation into routine clinical practice at this time. Targeted sequencing, such as hybrid capture of selected exons or whole-exome sequencing, can yield much higher coverage of relevant parts of the genome at a lower cost. Hybrid capture platforms currently offer excellent coverage at a reasonable cost and turnaround time (~2 weeks).

It remains to be determined whether the natural history of MDS diagnosed primarily on the basis of mutation testing has a similar natural history to MDS diagnosed using conventional criteria. The presence of an MDS-associated somatic mutation can at least confirm the presence of clonally restricted hematopoiesis. For example, it has been shown that elderly individuals without any evidence of hematologic malignancy can acquire clonal hematopoiesis with recurrent somatic *TET2* mutations (43). The contribution of such mutations to oncogenic transformation remains an area of active research.

Finally, it is possible that a broad range of clinicians, including primary care providers, will eventually be able to evaluate their patients with new-onset cytopenias via peripheral blood testing for the presence of MDS-defining mutations, once a few common nonclonal causes of cytopenias such as nutritional deficiencies have been excluded. Having a noninvasive option for diagnosis of MDS would be predicted to lower the threshold for testing and result in earlier identification of cases. Furthermore, although the initial diagnostic confirmation would most likely still involve a bone marrow biopsy with a karyotype analysis even in patients with documented somatic mutations in their peripheral blood, serial repeat bone marrow biopsies could become less critical for monitoring treatment response and disease progression.

#### Moving toward improved classification and prognostication

Multiple classification and prognostic scoring systems have been developed to identify MDS subtypes or risk

groups with similar outcomes, as discussed above (4, 7–12). These schemes are based on morphology, clinical variables such as cytopenias, and cytogenetics. Somatic mutations are fundamental determinants of MDS pathophysiology but are not part of any of the existing risk stratification systems.

Future classification and prognostic scoring systems may incorporate somatic mutations into existing models. Alternatively, new classification schemes and prognostic scoring systems may be developed that are fundamentally based on molecular abnormalities. If all mutations can be detected in peripheral blood by deep sequencing, it is possible that MDS could be diagnosed, classified, and followed over time using peripheral blood without the need for repeat bone marrow biopsies (Fig. 1).

#### Identification of predictors of response to treatment

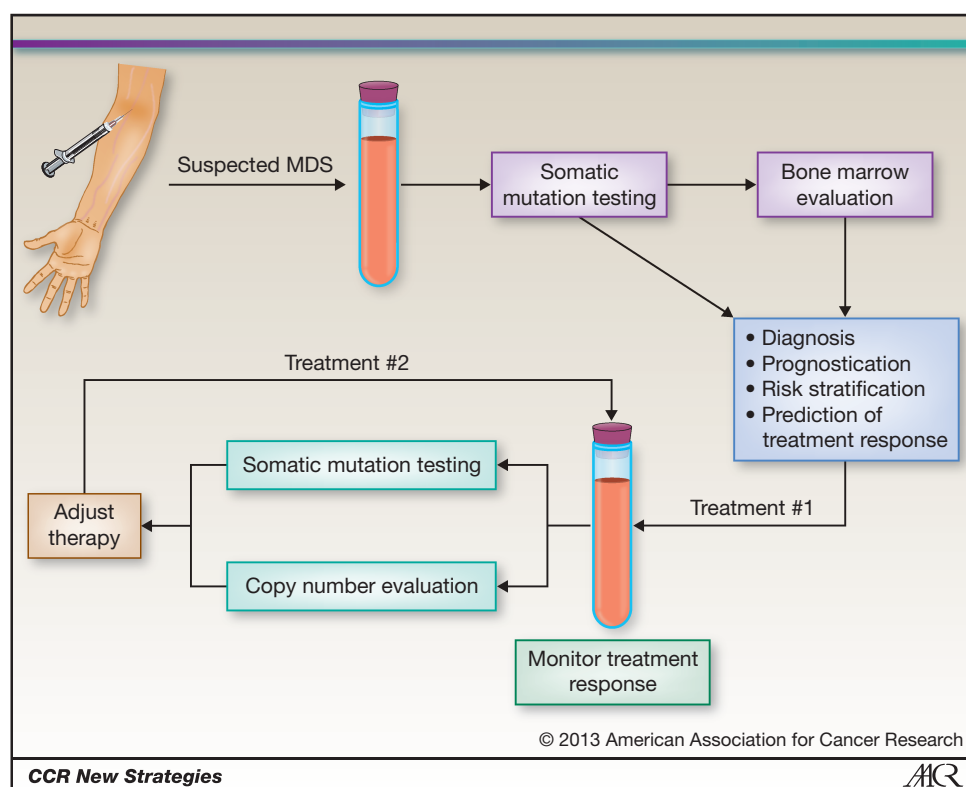
Patients with MDS with interstitial deletions in the long arm of chromosome 5, in the absence of complex cytogenetics or *TP53* mutations, show a striking response to treatment with the thalidomide derivative lenalidomide (44). In a phase III randomized double-blind study of lenalidomide in red blood cell (RBC) transfusion-dependent patients with low/intermediate-1-risk MDS with del(5q), treatment with lenalidomide resulted in RBC transfusion independence in 56% patients, a cytogenetic response in 50% of patients, and increased OS with decreased risk of transformation to AML (45, 46). The exact mechanism of response is the subject of ongoing investigation.

Other than del(5q) for lenalidomide and serum erythropoietin levels predicting response to erythropoiesis-stimulating agents, there are no widely used biomarkers to aid clinicians in selection of therapy. Somatic mutations may serve as such biomarkers if they can be shown to consistently predict response to treatment. For example, in a recent phase II study of lenalidomide and azacitidine in patients with IPSS intermediate-1, -2, and high-risk MDS, the presence of *TET2*, *DNMT3A*, *IDH1*, or *IDH2* mutations was predictive of achieving complete response, even in the presence of other somatic mutations that ordinarily carry a bad prognosis in MDS (47). In another retrospective study of 86 patients with MDS treated with azacitidine, the presence of a *TET2* mutation was associated with an 82% response rate, compared with a 45% response rate in *TET2* wild-type patients (48). Additional studies designed to identify genetic predictors of response to different treatment modalities, such as hypomethylating agents or stem cell transplantation, will be critical for tailoring appropriate therapy to each patient and minimizing toxicities from treatments with a low likelihood of therapeutic benefit.

#### Monitoring response to treatment

The ability to monitor treatment response via a noninvasive test would provide critical information to clinicians about ongoing therapeutic response and the development of resistance. Response to treatment would primarily be assessed by following the size of the malignant clone(s) over time compared with healthy cells, that is, the percentage of cells bearing particular somatic mutations.

**Figure 1.** Proposed workup of suspected MDS with incorporation of MDS-specific somatic mutation testing into clinical practice. Copy number evaluation is currently conducted by cytogenetic analysis but will most likely be captured from sequencing data in the future.



Next-generation sequencing studies have recently revealed and characterized the clonal architecture of MDS, and subsequent progression to secondary AML, in unprecedented detail. In the patients analyzed to date, more than 90% of the bone marrow cells bore clonal somatic mutations at the time of MDS diagnosis, even among patients without excess blasts (49). The percentage of cells in the bone marrow with somatic mutations does not increase significantly with progression from MDS to AML, but the genetics of the dominant clone do change. These results imply that clonal hematopoiesis involving a majority of the bone marrow is present even at early stages of MDS. Moreover, MDS is clearly a genetically complex disease, with multiple coexisting clones present in both MDS and AML, reflecting sequential acquisition of mutations and, in some cases, the parallel evolution of independent clones, some of which become extinct over time. With progression to secondary AML, the preexisting MDS-founding clone always persists, though the MDS clone is outcompeted by more aggressive daughter subclones that drive the AML phenotype.

Ascertainment of the quantitative allele burden for different somatic mutations would be achieved by determining mutant allele frequencies (adjusted for chromosomal number) by means of deep sequencing of serial peripheral blood or bone marrow samples. As the size of each clone can be evaluated by the mutant frequency allele, mutant allele frequencies would delineate each subclone. Defining clonal heterogeneity of each patient at the time of diagnosis and with treatment will be instrumental in monitoring treatment response and early evidence of resistance and could

result in much more timely change in treatment. The value of this approach will likely become apparent in prospective clinical trials.

## Conclusions

Tremendous progress has been made in defining the genetic basis of myeloid malignancies and MDS in particular. The identification of a growing compendium of recurrent genetic lesions in MDS and the development of technologies to track these mutations in a clinical setting have the potential to fundamentally alter the approach to patients with MDS, from diagnosis, prognosis and risk stratification, and treatment. We envision that assays for clinically relevant somatic mutations will soon become part of routine evaluation of patients in the clinic (Fig. 1), as well as provide fertile ground for the development of novel therapeutic strategies for the treatment of MDS.

## Authors' Contributions

**Conception and design:** Z. Tothova, D.P. Steensma, B.L. Ebert  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** Z. Tothova, D.P. Steensma, B.L. Ebert  
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