Myelodysplastic Syndromes and Myelodysplastic/Myeloproliferative Neoplasms: An Update on Risk Stratification, Molecular Genetics, and Therapeutic Approaches Including Allogeneic Hematopoietic Stem Cell Transplantation

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OVERVIEW

Myelodysplastic syndromes are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis, peripheral cytopenias, and a variable propensity for leukemic transformation. In recent years there has been an explosion of information on the molecular genetic changes underlying these disorders. This information has substantial prognostic implications, and the influence on therapeutic approaches and the treatment of patients is evolving. Allogeneic hematopoietic stem cell transplantation (alloSCT) is the only known cure for these diseases, but appropriate patient selection is of utmost importance from a risk-benefit perspective. This review focuses on the factors influencing risk stratification in MDS and optimal choice of front-line therapy in the current era, including the interplay of clinical factors and molecular genetic factors, and factors that determine eligibility for alloSCT. The myelodysplastic/myeloproliferative diseases also will be discussed, including the increasing effort to understand the molecular genetics and natural history of these disorders and treatment approaches.

The myelodysplastic syndromes are a clinically and molecularly heterogeneous group of clonal stem cell disorders with management options ranging from observation and growth-factor therapy to more intensive approaches, such as hypomethylating agent (HMA)–based therapies, intensive induction chemotherapy, and alloSCT. During the last decade, rapid developments centered around high-throughput molecular technologies have resulted in remarkable advancement toward the understanding of MDS pathogenesis. These technologies are anticipated to translate to innovative targeted agents that will radically affect the natural history of this disease. Presently, however, alloSCT remains the only curative treatment option in patients with MDS. Even if substantially reduced during the last 20 years, mortality and morbidity risks associated with alloSCT continue to represent a major limit to the feasibility of such a therapeutic strategy in the large number of patients. Therefore, the accurate selection of patients—together with optimal timing of transplantation—represent critical issues for maximum improvement of the alloSCT risk-benefit ratio in MDS.

As such, important advancements have been made toward the dissection of MDS clinical heterogeneity that have incorporated both clinical and molecular characteristics. In this review, we will discuss recent refinements to prognosis for patients with MDS based on clinical, cytogenetic, and molecular risk factors. We also will discuss the approach to the selection of front-line therapy in MDS, including a particular focus on alloSCT. Lastly, we will highlight myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), including recent insights into the molecular genetics and emerging therapeutic approaches in patients with these diseases.

PROGNOSTIC STRATIFICATION IN MYELODYSPLASTIC SYNDROMES

MDS is a disease found in older adults, with a median age at diagnosis of age 71. The estimated 3-year survival rate of patients with MDS is 45%, although there is substantial variability in outcomes even within the same French-American-British (FAB) classification system and the more recent World Health Organization (WHO) morphologic subtypes. This heterogeneity in outcomes underscores the need for accurate prognostication and has fuelled the evolution of various prognostic scoring models.
Prognostic Classification Models
The international prognostic scoring system (IPSS), a widely validated system currently in use, originally was created from data from 816 patients. The IPSS encompasses karyotype, blast percentage, and the number of cytopenias, thus stratifying patients with de novo MDS into four risk categories. These include lower risk MDS comprising low and intermediate-1 risk categories, and higher risk MDS comprising intermediate-2 and high-risk categories; with median survivals ranging from 5.7 to 0.4 years.15

Limitations associated with the IPSS include the lack of consideration of the severity of cytopenias, and transfusion dependency; underweighting of the prognostic import of karyotype relative to marrow blast–percent; and the fact that this model is applicable only at diagnosis. Several of these limitations were addressed by the development of other models, including the WPSS,16 which incorporates transfusion dependency and WHO subtype, among other factors. It is a dynamic model that can be applied at diagnosis and follow-up. The University of Texas MD Anderson Cancer Center (MDACC) prognostic model includes a broader population of patients, such as patients with therapy-related MDS and patients with chronic myelomonocytic leukemia (CMML).17 An additional prognostic model focused on lower-risk disease (LR-PSS) has been developed at MDACC.18

The revised IPSS (IPSS-R),19 which was created from an evaluation of more than 7,000 patients, incorporates a comprehensive 5-tiered cytogenetic risk stratification system that comprises 15 cytogenetic subtypes (Table 1).20 It also accounts for the degree of cytopenias and provides a more discriminant assessment of bone marrow–blast percentage. The predictive value of cytogenetics in MDS has long been recognized, and the comprehensive cytogenetic strata included in the IPSS-R assure that chromosomal aberrations of prognostic importance are accorded appropriate weight in the model relative to bone marrow blast–percent. Five risk groups are identified—very low, low, intermediate, high, and very-high with median survival ranging from 8.8 to 0.8 years. Similar to the IPSS, the IPSS-R was generated on data from patients evaluated at diagnosis and censored at the time of receipt of disease-modifying therapy. Despite these issues, the IPSS-R has been rapidly validated by several groups to be more discriminant than the IPSS and the WPSS,21 and to retain its predictive value even in patients treated with disease-modifying agents.22,23 Therefore, in just a few short years since its introduction, IPSS-R is rapidly being adopted as the preferred clinical prognostic tool for risk stratification in MDS.

The Evolving Role of Molecular Genetics on Risk Prediction
A comprehensive evaluation of the prognostic relevance of point mutations in refining risk stratification in MDS was first established in a study of 439 patients with MDS.24 Using next-generation DNA sequencing and mass spectrometry genotyping to evaluate 111 genes, mutations in five genes (TP53, EZH2, ETV6, RUNX1, and ASXL1), which collectively occurred in approximately one-third of patients, were found to confer poor risk prognosis, independent of established clinical risk factors such as IPSS. Within each IPSS subgroup, the presence of one or more of these mutations was demonstrated to result in a decline in overall survival, which approximated that of the next-highest IPSS risk group.24

The same group has since validated the prognostic effect of these mutations (EZH2, RUNX1, TP53, and ASXL1) in patients with lower-risk MDS. The presence of these mutations was found to be associated with a shorter overall survival independent of the LR-PSS.25 In a multivariate analysis that included LR-PSS and other mutations, only EZH2 mutations retained independent prognostic significance. The clinical implication of upstaging for the risk group is recognition that patients in lower-risk IPSS categories have a worse prognosis, which may result in consideration of more intensive treatment approaches for such patients.24,26

It is now evident that more than 40 genes are mutated in MDS.4,27-29 Approximately 90% of patients with MDS harbor at least one mutation, with a median of two or three mutations detected per patient (range, 1 to 12). Mutations could be categorized into subgroups that affect specific functional pathways (Fig. 1), including the spliceosome machinery (SF3B1, SRSF2, U2AF1, U2AF2, ZRSR2); DNA methylation (TET2, DNMT3A, IDH1, IDH2); chromatin modification (ASXL1, EZH2); RAS and other kinase signaling pathways (NRAS, KRAS, CBL, JAK2); transcription factor (TP53, RUNX1, EVI, GATA2); cohesin; and DNA repair pathways. Of these, mutations affecting RNA splicing and epigenetic dysregulation (DNA methylation and chromatin modification) were commonly observed, underscoring the critical importance of these pathways in MDS pathogenesis. In particular, more than 50% of patients had a mutation in a component of the spliceosome machinery, identifying premRNA splicing as the most frequently altered biologic pathway in these diseases.

KEY POINTS
- Myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative neoplasms are clinically and molecularly heterogeneous.
- The choice of optimal front-line therapy in MDS depends on accurate risk stratification; the revised international prognostic scoring system (IPSS), IPSS-R, is the most contemporary system in use.
- Comprehensive molecular profiling has the potential to improve prognostication, risk stratification, and diagnosis.
- Allogeneic hematopoietic stem cell transplant is increasingly being used in older adults with MDS, and it should be considered early in patients with higher risk disease.
- The identification of biologically relevant pathways is anticipated to ultimately lead to targeted therapeutic agents.
These studies also provided several additional insights into the role of the molecular genetic profile in determining clinical and phenotypic heterogeneity associated with the disease. For example, the high frequency (75%) of SF3B1 mutations in MDS subtypes associated with ring sideroblasts was confirmed, implying that this mutation is a significant predictor for the presence of ringed sideroblasts in the marrow. Only mutations in SF3B1 were associated with a better clinical outcome. In contrast, the average number of mutations was higher per patient in the higher-risk WHO morphologic categories (i.e., refractory anemia with excess blasts (RAEB1 and RAEB-2), which is consistent with a higher degree of clonal evolution in these subtypes). Leukemia-free survival in patients with MDS was found to negatively correlate with the combined number of oncogenic and cytogenetic lesions. The number ranged from 49 months in patients with one lesion to 4 months in patients with six or more mutations. Furthermore, the number of oncogenic mutations provided independent prognostic information after stratification by the IPSS.4

Insights into clonal evolution and clonal architecture of MDS also have been obtained from studies with mutations in genes involved in RNA splicing or epigenetic regulation with a higher variant allele fraction, which suggests that these mutations occurred earlier during clonal evolution. Both clonal and subclonal events within the same genes, however, retained similar prognostic significance. This suggests that the finding of a mutation in a minor subclone is clinically relevant, thus emphasizing the potential utility of a targeted deep-sequencing approach that is sensitive enough to detect minor subclonal events.

Mutations within the same functional pathways often were mutually exclusive (e.g., TET2 and IDH1/2 mutations, which both affect DNA hydroxymethylation). Similarly, mutations involving the spliceosome machinery rarely co-occur. Biologically relevant interactions also were found in patterns of co-occurrence. For example, SF3B1, which is associated with good prognosis, was mutually exclusive with ASXL1 and IDH2, which both confer a poor prognosis. However, SRSF2 showed a clear propensity to associate with TET2 and its co-mutation is highly associated with monocytosis.4,27,31 It is plausible that such biologic insights also will be of clinical value in the design and use of targeted therapies.

These molecular profiling efforts successfully demonstrate that the integration of clinical, morphologic, and cytogenetic information already encompassed by the IPSS and IPSS-R, along with the comprehensive molecular genetic profile now available via targeted deep sequencing techniques, has the potential to substantially refine risk stratification in MDS.27 In the 21st century, it is highly anticipated that such combined risk models—once validated prospectively—will enter into common clinical use to refine prognosis and potentially determine treatment approaches for patients with MDS.

**TABLE 1. MDS Cytogenetic Risk Stratification System**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Frequency (%)</th>
<th>Karyotypic Abnormalities</th>
<th>Median Survival (y)</th>
<th>Time until 25% of Patients Developed AML (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>4%</td>
<td>-Y, del(1q)</td>
<td>5.4</td>
<td>NR</td>
</tr>
<tr>
<td>Good</td>
<td>72%</td>
<td>Normal, del(5q), del(12p), del(20q), double including del(5q)</td>
<td>4.8</td>
<td>9.4</td>
</tr>
<tr>
<td>Intermediate</td>
<td>13%</td>
<td>Del(1q), +8, +19, i(17q), any other single or double independent clones</td>
<td>2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Poor</td>
<td>4%</td>
<td>-7, inv (3)/t(3q), double including -7/del(7q), complex with 3 abnormalities</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Very poor</td>
<td>7%</td>
<td>Complex (&gt; 3 abnormalities)</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Abbreviations: MDS, myelodysplastic syndromes; y, years; AML, acute myeloid leukemia; NR, not reported.

**FIGURE 1. The Mutational Complexity of MDS**

This chart illustrates the mutational complexity of MDS with mutations organized into functional pathways affecting RNA splicing (includes: SF3B1, SRSF2, ZRSR2, U2AF1/2); DNA methylation (includes: TET2, DNMT3A, IDH1/2); chromatin modification (includes ASXL1, EZH2); transcription factor (includes RUNX1, TP53, BCR, PHF6, NCOA, CEA, GATA2); receptors/kinases (JAK2, MPL, FLT3, GNAS, KIT); RAS pathway (KRAS, NRAS, CBL, NF1, PTPN11); DNA repair (ATM, BRCC3, DLK1, FANC); and cohesins (STAG2, CTCF, SMCA, RAD21). In approximately 10%, no mutation could be identified. Overlapping mutations (co-occurrence of two or more mutations in patients with MDS) are not depicted on this chart, thus percentages add up to more than 100%. Chart is created from data derived largely from targeted deep sequencing of 104 genes in a cohort of 944 patients with MDS, published by Haferlach et al.27

**FRONT-LINE THERAPY IN MYELODYSPLASTIC SYNDROMES**

The concept of individualized therapy is particularly relevant in the selection of optimal therapeutic strategies in this disease, and it should incorporate a consideration of both patient- and disease-specific elements. Higher-risk patients generally are offered more intensive therapeutic options, including HMA therapy and an early consideration of stem cell transplantation, which is discussed in substantial detail below. Lower-risk patients are offered therapies that range from observation (in patients who are asymptomatic with relatively well-preserved blood counts), to growth factors
such as erythropoietin (in patients with anemia), to immunosuppressive therapy (in patients with multiple cytopenias). Therefore, elements that factor into risk stratification are of critical therapeutic importance. Contemporary approaches to risk refinement, including the use of more discriminant prognostic models, such as the IPSS-R, are highly encouraged. Data are evolving to support the potential utility and applicability of models that combine comprehensive targeted molecular profiling with clinical models, such as the IPSS-R, in making treatment decisions, and they deserve prospective validation.

**Supportive Care/Growth Factor Therapy**

Lower-risk patients with anemia may benefit from erythropoietin (EPO) therapy. Predictors of response include lower serum EPO level (< 500 U/L) and a lower transfusion requirement (< 2 units of red cells per month). Response rates in such patients may be as high as 60%; however, it is substantially lower (less than 10%), in the absence of the above criteria. Since most responses to EPO will occur within 8 weeks, a time limited trial of 8 weeks is reasonable when EPO is being used for treatment of MDS.

**Immunosuppressive Therapy**

Immunosuppressive therapy (IST) with antithymocyte globulin (ATG)-based therapies are reasonable to consider in lower-risk patients with MDS who have multiple cytopenias, who are younger (< age 55), and who are positive for the HLA DR15 allele. These latter factors were positive predictors of response to IST. A hypocellular marrow, which suggests a disease more closely aligned with aplastic anemia, also has been found in some studies to be predictive of response, but it has not been confirmed in others. Since activated T cells are the target of IST, a predictive model that consists of a T-cell activating signature and duration of disease also has been proposed.

**Lenalidomide**

In patients with deletion 5q (del[5q]) and lower-risk disease who require red cell transfusions, the use of lenalidomide is associated with a transfusion independence rate in the 70% range, with a median duration of response of 2.2 years. In lower-risk patients with MDS without the del(5q), the response rate is in the 25% range and response duration is substantially shorter. High karyotypic complexity, lower platelet count, and *TP53* mutations tend to be associated with lenalidomide resistance, even in the presence of del(5q). Levaldoidimide currently is being investigated in combination with erythropoietin in a phase III trial in lower-risk patients with MDS. In this trial, it is hypothesized that the combination of lenalidomide and EPO will potentiate erythroid response in patients who have failed to respond to EPO or in whom EPO is predicted to have a low probability of being effective. The trial is based at least in part on promising results from an early phase trial that investigated the combination. Recently, a novel mechanism of action involving lenalidomide-induced degradation via cereblon—of cascin
HMA treatment, but they did not predict response. These findings require validation in prospective clinical trials. Presently, insufficient evidence exists to suggest that the decision to treat patients with HMAs can be made on the basis of mutational profiling alone, especially since responses to HMAs were observed even in patients with mutations that confer a very poor prognosis.\(^5\)

**Combination Therapies**

There remains an urgent need for the development of new drug therapies and combinations to treat patients with MDS. An early focus was the combination of HMAs with histone deacetylase inhibitors (HDACi).\(^{51-55}\) This was based on the hypothesis that inhibiting two pathways of epigenetic deregulation would be potentially synergistic, and abundant preclinical evidence of synergy. Early phase trials investigating the combination of azacitidine with the HDACi entinostat or vorinostat,\(^{52,56}\) or the immunomodulatory agent lenalidomide,\(^{57}\) yielded promising results, which has led to randomized trials conducted in the intergroup setting.

The results of randomized trials conducted in the U.S. intergroup setting (E1905) comparing azacitidine versus azacitidine plus entinostat,\(^{58}\) and preliminary results of the recently concluded three-arm randomized phase II North American intergroup trial (S1117) comparing single-agent azacitidine to azacitidine plus vorinostat and azacitidine plus lenalidomide combinations,\(^{59}\) respectively, however, have shown no significant advantage to the combination arms as to improvement in response rates. Therefore, azacitidine remains the standard of care. Survival endpoints, either progression-free or overall survival, may be more relevant primary endpoints of future large randomized efforts that compare single-agent HMA therapy to novel combinations and approaches.

**ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

Individual decision making on whether to consider alloSCT as a possible treatment option in treating patients with MDS requires the accurate assembling of several disease- and patient-characteristics.

**Disease Characteristics**

In general, MDS includes an extremely heterogeneous group of myeloid malignancies with different natural courses and life expectations. Therefore, a risk-based approach for individual decision making on treatment strategy is highly suggested, and it is mandatory when referring patients for alloSCT.\(^{5}\) Together with the nosological classification methods (such as the FAB and, more recently, the WHO classification schemes), prognostic scoring systems in MDS have been considered the most appropriate tools for treatment stratification. For almost 15 years, the IPSS has been universally recognized as the landmark reference method for risk-stratification with patients classified in high-risk categories (including intermediate-2 and high-risk groups) as generally being considered for early alloSCT. However, a different treatment plan usually is recommended for patients in the lower IPSS risk categories (i.e., low and intermediate-1). In a foremost decision-analysis study that included patients younger than age 60, delayed transplantation was shown to associate with maximal life expectancy, with an even more marked survival advantage for patients under age 40.\(^{61}\) In 2007, a time-dependent WHO classification-based prognostic scoring (WPSS) incorporating transfusion dependence was proposed for untreated patients.\(^{16}\) Different from IPSS, the WPSS identifies five risk groups of patients with different survival, and it allows for real-time assessment of prognosis in MDS. Its relevance after alloSCT was validated in a subsequent study from the GITMO group that included 406 patients,\(^{62}\) in which a multivariate Cox survival analysis also included age and sex of patient, time between diagnosis and alloSCT, year of transplantation, disease stage at transplantation, source of stem cells, type of donor, and type of conditioning. WPSS showed a prognostic significance on both overall survival and probability of relapse. The validity of WPSS in predicting outcomes after alloSCT in patients with MDS recently was confirmed in a population of 60 Southeast Asian patients.\(^{63}\)

Cytogenetics appears to be the most critical factor in determining survival in patients with MDS. However, the cytogenetic categories included in all of the proposed prognostic scoring systems were derived from large series of patients who were only treated with supportive care. Because alloSCT represents a treatment strategy that is potentially capable of eradicating the hematopoietic malignant cell clone, it could be postulated that the prognostic significance of cytogenetics would persist using this treatment approach. However, the negative effect of poor-risk cytogenetics on the outcomes of patients with MDS undergoing alloSCT has been confirmed.\(^{64-69}\) For example, a recent retrospective analysis from the European Society for Blood and Marrow Transplantation (EBMT) demonstrated that poor-risk cytogenetics, as defined by standard IPSS scores, were associated with a relatively poor survival after alloSCT from HLA-identical siblings, except in patients with low marrow blast count (i.e., RA/RARS) who were transplanted up front.\(^{69}\)

In 2012, the revised IPSS (IPSS-R) for MDS was generated by analyzing 7,012 patients in a new international collaborative effort. Based on a new comprehensive cytogenetic score,\(^{20}\) and considering severity of cytopenias by incorporating different cutoff points, the IPSS-R stratifies five risk groups with different clinical outcomes.\(^{19}\)

The ability of the new cytogenetic risk classification to predict post-transplant outcome was promptly confirmed in a series of 1,007 patients who underwent alloSCT at the Fred Hutchinson Cancer Research Center in Seattle. A substantially higher rate of relapse and mortality rate was found in patients with very poor cytogenetics compared to patients with good-risk cytogenetics.\(^{70}\)

An additional disease characteristic recently has been shown to negatively affect the prognosis of patients with
MDS. Not included in any of the previously described scoring systems is the presence of a monosomal karyotype (MK) (i.e., the presence of two or more distinct autosomal monosomies or a single monosomy associated with a structural abnormality). However, an association of MK with lower survival, higher relapse incidence, and overall mortality after alloSCT—even among patients with a complex karyotype—has been reported consistently. The effect of the IPSS-R on alloSCT outcomes recently was demonstrated in an analysis from the Italian GITMO cooperative group that included 374 patients with primary MDS. Both IPSS-R and monosomal karyotypes were independently associated to a lower overall survival and a higher relapse probability by multivariate analysis. In this study, a predictive model of post-transplant outcome in patients with MDS (Transplantation Risk Index) was originated based on the age of the patient, IPSS-R category, monosomal karyotype, hematopoietic cell transplantation (HCT)-specific comorbidity index, and refractoriness to induction chemotherapy. In addition to cytogenetics, other disease characteristics have been associated with a poor prognosis in patients with MDS. These include severe bone marrow fibrosis, refractory life-threatening cytopenias, and gene mutations. As to the effect on alloSCT outcome, bone marrow fibrosis, a recent EBMT retrospective analysis of 721 patients with MDS demonstrated that only severe fibrosis was shown to affect survival, whereas patients with mild or moderate fibrosis had an alloSCT outcome comparable to patients without bone marrow fibrosis. For gene mutations, an independent association with a shorter post-transplant overall survival, after adjusting for clinical variables and complex karyotype status, recently has been reported for mutations in TP53 and TET2.

**Patient Characteristics**

Apart from disease characteristics, host-specific risk assessment in determining indications for alloSCT always should be grounded on essential patient-related factors, including age, comorbidities, and donor availability. Although usually considered as a treatment option for patients younger than age 60, over the last 2 decades the development of reduced-intensity conditioning (RIC) regimens, together with substantial progress in supportive care measures, have resulted in an increase in the upper age limit to age 70 (occasionally even older) in carefully selected very-fit patients. Because MDS are much more common in older people (median age at diagnosis, over age 70), with only 10% of patients younger than age 50, this age-limit extension has been shown to provide additional prognostic information on patients treated with alloSCT in Italy over 2 years, including 199 patients with MDS. In 2011, a time-dependent MDS-specific Comorbidity Index (MDS-CI) was developed within the European group of myeloablative conditioning strategies. The HCT-CI also was prospectively validated by the GITMO group in a consecutive cohort of 1,937 patients receiving alloSCT in Italy over 2 years, including 199 patients with MDS. Since it has been shown to provide additional prognostic information on patients stratified according to the IPSS-R, the MDS-CI may represent a valuable tool in support of the HCT-CI when evaluating patients for possible transplant indication.

When to Transplant

Although very heterogeneous, the natural course of MDS is typically characterized by a disease progression with patients exhibiting gradual worsening of peripheral blood cytopenias, which eventually lead to transfusion dependency. Sequential marrow examination, especially in the presence of myeloblast excess (i.e., ≥5%), often can demonstrate an increase in marrow blast count culminating in AML evolution. Cytogenetics tend to remain stable, even though the occurrence of chromosome aberrancies (or additional ones, when already present at diagnosis) occasionally may be observed. Time-dependent disease modifications are outlined...
as phase progression by the WPSS and the IPSS/IPSS-R classification system, IPSS-R.\textsuperscript{15,16,19}

The Markov retrospective analyses cited above have shown a substantial survival advantage for patients who are in high-risk categories of MDS (intermediate-2 and high-risk, according to IPSS) undergoing early alloSCT from HLA-matched donor, whereas a transplant deferment until disease progression seems to confer longer life expectancies in patients with lower-risk MDS (low- and intermediate-1 risk, according to IPSS).\textsuperscript{61,94} In general, because the status of the disease at transplant has a major effect on all outcomes, better long-term results seem to be achieved when transplantation is performed earlier during the course of the disease.\textsuperscript{62,111,112}

In addition to disease progression, risk associated with transplant delay may include occurrence of infectious complications, acquisition of transfusion refractoriness, iron overload secondary to transfusion history, performance status decline, and acquisition of additional comorbidities, which lead to a substantially higher risk of nonrelapse mortality following transplantation. Nonetheless, the question of the optimal time for alloSCT in individual patients may be difficult, because of the unavoidable morbidity and nonrelapse mortality risks associated with the transplantation procedure and to the current availability of alternative treatment options, such as HMAs. In a recent GITMO decision study that included 660 patients with MDS who received best supportive care and 449 who underwent transplantation, a continuous-time multistate Markov model was applied to describe the natural history of the disease and to evaluate the effect of alloSCT on survival.\textsuperscript{113} Results of this study indicate that a delayed transplantation is advisable only for patients with early disease (low-risk by IPSS, very low- and low-risk by WPSS), with the best survival benefit deriving from alloSCT for patients classified in the intermediate-1, according to IPSS (in the presence of multilineage dysplasia and/or transfusion dependency) or the intermediate WPSS risk category.

Furthermore, new and interesting information that could be combined for clinical decision making in patients with MDS may originate from a further retrospective GITMO study. This study was recently performed in a large population of 529 patients with MDS without a compatible family donor but with plain indication for alloSCT. A competing risk analysis unveiled high pretransplant risk of disease evolution and mortality resulting from the time spent waiting for the identification of a suitable unrelated donor, both on the entire population of patients with MDS and on specific subgroups stratified by disease risk and age (GITMO Registry, M. Della Porta, personal communication, 2015). If confirmed in other series, these findings would support selection of haploidentical donors to perform immediate alloSCT in patients with high-risk MDS without a promptly available HLA-matched donor.\textsuperscript{114,115}

Apart from obviously depending on donor availability, the decision on when to transplant in MDS often has to consider potential treatment to be administered before transplantation, especially in patients with a higher percentage of marrow to possibly downstage the disease to a lower-risk category.\textsuperscript{116–118} Because AML-like induction chemotherapy, besides being rather toxic in older patients, is frequently leading to disappointing results in MDS, especially in the presence of high-risk cytogenetics, HMAs frequently are preferred in the interim period before transplantation. Life expectancy has been shown to be very dismal for patients with loss of response to azacitidine.\textsuperscript{119,120} In patients whose disease responds, alloSCT possibly should be offered before disease progression, if a donor is available. Presently, because of the absence of randomized studies, optimal pretransplantation therapy is unknown,\textsuperscript{121} although trials with HMAs employed as a bridge to transplant are currently ongoing.

In conclusion, individual decision making on the best treatment strategy pertaining to allogeneic transplantation is usually the consequence of a difficult composite judgment that includes disease-, patient-, and transplant-characteristics together with the patient’s expectations and opinions.

**GENETICS IN MYELODYSPLASTIC SYNDROMES/MYELOPROLIFERATIVE NEOPLASMS**

MDS/MPNs represent an optimal disease model for the clinical implementation of broad spectrum mutational profiling. First, the clinical and pathologic characteristics of MDS/MPNs are challenging to recognize, and they often can change within the context of cytoreductive agents or other therapies. Second, the spectrum of mutations in MDS/MPNs is relatively well-characterized and allow for the sensitive annotation of clonality in most patients. Lastly, although substantial overlap occurs among recurrent mutations in MDS/MPNs, it could be argued that each disease entity is characterized by a unique genetic fingerprint that may aid in diagnosis. Emerging evidence suggests that these genetic lesions are independently prognostic and may aid in therapeutic decisions. Below is a disease-specific summary of recurrent mutations and their clinical relevance (Table 2).

**Chronic Myelomonocytic Leukemia**

The hematologic phenotype of chronic myelomonocytic leukemia (CMML) is defined by a peripheral monocytosis and dysplasia.\textsuperscript{122} The recurrent genetic mutations associated with this CMML phenotype converge on diverse pathways that include mutations of signal transduction (NRAS, KRAS, CBL, JAK2); DNA methylation (DNMT3A, TET2, IDH 1/2); transcriptional regulation (ETV6, RUNX1); chromatin modification (EZH2, ASXL1); and the RNA splicing machinery (SF3B1, SRSF2, ZRSR2, U2AF1).\textsuperscript{123–125} Although the pathways affected are heterogeneous, a genetic clonal event can be identified in greater than 90% of CMML cases by sequencing only nine genes. In fact, mutations in TET2, ASXL1, and SRSF2 are highly recurrent with each identified in up to 45% of patients.\textsuperscript{123–127} This allows for the CMML clinician to obtain evidence of clonality in almost all cases of suspected CMML, making it straightforward to parse malignant from reactive monocytosis. Additionally, co-mutation of SRSF2...
and TET2 is highly specific for patients with CMML, which aids in the diagnosis of the treated CMML patient when historic monocytosis may be unclear and current monocytosis may be suppressed by HMAs or cytoreductive agents.31

The prognostic significance of recurrent mutations in CMML also has been tested. Interrogation of the most common mutations in CMML has identified ASXL1 to be independently prognostic.48,73 This has led to its incorporation into two independent prognostic scoring systems. However, larger datasets will be required to fully annotate the prognostic significance of single and combination mutations in CMML.

Juvenile Myelomonocytic Leukemia

Although the monocytic phenotype of juvenile myelomonocytic leukemia (JMML) is similar to that of CMML, the genetic fingerprint of JMML is distinct.128 Greater than 90% of recurrently mutated genes in JMML cluster on RAS pathway activation.129 These gene mutations include NF1, N-RAS, K-RAS, PTPN-11, and CBL.130,131 Both somatic and germ-line mutations of these genes have been identified, with the latter most associated with congenital genetic syndromes such as Noonan syndrome (PTPN11) and Neurofibromatosis type 1 (NF1).132-134 Secondary events in JAK3 and SETBP1 also have been described and suggest a poor prognosis.135,136 This genetic landscape, along with its characteristic GM-CSF hypersensitivity, has resulted in major advances in the molecular understanding of JMML.128 Further, the presence of these mutations can alter treatment decisions and management. Patients with CBL mutations and its associated congenital syndrome can manifest a JMML phenotype that is self-limited.131 Therefore, a watchful waiting strategy is often employed in these children, as opposed to allogeneic transplant in other RAS-mutated JMML cases.137

Atypical Chronic Myeloid Leukemia

Atypical chronic myeloid leukemia (aCML) Atypi is a disease characterized by severe neutrophil dysplasia and cytopenias in the absence of the BCR:ABL fusion protein. This disease has historically been difficult to diagnose given the pathologic overlap between it and other MDS/MPNs, particularly MDS/MPN Unclassifiable. Further, the diagnostic difficulty present in discerning aCML from chronic neutrophil leukemia (CNL) has made the annotation of aCML-specific mutations challenging.138 Gene mutations identified in aCML/CNL include JAK2, SETBP1, NRAS, and CSF3R. Analysis of these genes can identify a clonal event in over 50% of these diseases.139-141 CSF3R mutations are notable because they represent a potential predictive marker for targeted therapy. Preclinical data suggest that truncating CSF3R mutations predict sensitivity to SRC inhibition and CSF3R mutations affecting the membrane proximal portion for the receptor

<table>
<thead>
<tr>
<th>Mutation</th>
<th>CMML</th>
<th>JMML</th>
<th>aCML</th>
<th>MDS/MPN-U</th>
<th>RARS-T</th>
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Abbreviations: WHO, World Health Organization; MDS/MPNs, myelodysplastic syndromes/myeloproliferative neoplasms; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; aCML, atypical chronic myeloid leukemia; MDS/MPN-U, myelodysplastic syndromes/myeloproliferative neoplasms–unclassifiable; RARS-T, refractory anemia with ring sideroblast and thrombocytosis.
predicts sensitivity to JAK2 inhibition.\textsuperscript{139} The latter has been validated in clinical case reports with ruxolitinib therapy in aCML.\textsuperscript{142} However, two separate reports have failed to identify SF3B1 mutations in a stringently defined aCML cohort and were exclusively seen in CNL.\textsuperscript{143,144} Nonetheless, mutational analysis for SF3B1 should be performed if aCML is suspected, given the potential therapeutic implications.

**Myelodysplastic Syndromes/Myeloproliferative Neoplasms–Unclassifiable**

The genetic study of MDS/MPN–Unclassifiable (U) has been limited by difficulties in the diagnostic standardization of this entity. MDS/MPN–U is defined by having clinicopathologic features of myeloproliferation, dysplasia, and cytopenias but not meeting criteria for other well-defined MDS/MPNs. It is hypothesized that many MDS/MPN–U cases may be reclassified into other WHO-defined MDS/MPNs with mutational profiling. To this end, a recent retrospective study identified a clinical and genetic signature that could identify aCML among those cases initially identified as MDS/MPN–U.\textsuperscript{144} More investigation is needed to better annotate disease-specific genetic fingerprints to confidently allow for reclassification of MDS/MPN cases.

**Refractory Anemia with Ring Sideroblast and Thrombocytosis**

Refractory anemia with ring sideroblast and thrombocytosis (RARS-T) is defined by the presence of ring sideroblasts and a platelet count of greater than 450 x 10\(^9\)/dL and is included as a provisional category within the group of MDS/MPN–U by WHO.\textsuperscript{8,14} Similar to cases of MDS with ringed sideroblasts (RARS), the genetic landscape of RARS-T is dominated by the presence of mutations in SF3B1 that occur at a frequency reported between approximately 60 to 90\%.\textsuperscript{28,29,145} Other recurrent mutations identified in both RARS and RARS-T include TET2, ASXL1, and EZH2.\textsuperscript{146} However, RARS-T has been demonstrated to additionally harbor mutations in JAK2 at rates approximating 50\% and, less frequently, mutations in other signaling mutations such as CBL and CALR, which are rarely seen in RARS.\textsuperscript{28,146-149} This raises the possibility that mutations in JAK2 may be responsible for the thrombocytosis uniquely seen in RARS-T.

Although conventional wisdom states that the natural history of RARS-T is relatively benign, emerging data suggest that annotation of mutations in RARS-T may reveal a more aggressive subtype. A recent report identified SF3B1 and JAK2 mutations as favorably prognostic and associated with improved survival compared to their mutated counterpart. For example, SF3B1 wild-type cases of RARS-T had a median survival of 3.3 years compared to 6.9 years in mutated cases.

**Hypomethylating Agents**

Very limited data are available for the use of HMAs for the treatment of aCML, MDS/MPN–U, or RARS-T.\textsuperscript{155} However, robust data exist that demonstrate the activity of HMAs in CML. Several phase II studies have demonstrated that azacitadine is active in CML and associated with acceptable therapy-associated toxicity.\textsuperscript{156,157} Oral azacitadine more recently has been tested for the treatment of CML with clinical responses seen in 35\% of patients previously treated with HMA for MDS and CML, and in 73\% of patients receiving azacitadine as first-line therapy.\textsuperscript{158} Decitabine also has been examined in multiple phase II trials, with response rates ranging from 10 to 58\% in patients with CML.\textsuperscript{159} However, despite well-documented activity, no evidence exists that HMAs increase overall survival or decrease progression to AML. Therefore, we reserve this therapy for patients with CML in which cytopenias are the predominant symptom. We would not favor HMAs in rapidly proliferative patients with CMML, given the relatively long median time to HMA response. There is no CMML-specific evidence to favor one HMA over another.

**Lenalidomide and Interferon**

There has been limited data testing the activity of lenalidomide in CML and RARS-T. In CML, lenalidomide has been tested in combination with metronomic doses of melphalan. Although the study was small in number, a 25 and 33\% response rate was identified in patients with myelodysplastic and myeloproliferative CML, respectively.\textsuperscript{160} A case study also has reported a proliferative of CML cases with isolated del(5q) treated with lenalidomide that achieved blast clearance and cytoreduction. In RARS-T, three case reports have demonstrated clinical activity with respect to hematologic and splenomegaly improvement irrespective of cytogenetic abnormalities.\textsuperscript{161-163} Interferon has been tested in aCML in a limited number of cases with modest activity.\textsuperscript{164,165}
from induction chemotherapy to hydroxyurea and should be chosen based on performance status and other host-specific factors. As with HMAs, a larger clinical experience exists with cytoreductive agent in CMML compared to other MDS/MPNs. Several studies have reported the efficacy of the topoisomerase inhibitors topotecan and etoposide both as single agent and in combination with cytarabine. Other trials have examined combination with arsenic or all-trans retinoic acid (ATRA) with modest results. These two trials are notable because they represent two of the only MDS/MPN-specific studies to date. The only randomized study that has been performed in CMML randomly assigned 105 patients with CMML to receive hydroxyurea or etoposide. Surprisingly, this trial demonstrated a median overall survival of 20 months in the hydroxyurea group compared to 9 months in the etoposide arm, suggesting inferior disease-modifying capacity for etoposide in CMML. Induction chemotherapy has been used in CMML based on extrapolation from MDS. In our practice, we recommend induction chemotherapy in patients with extreme or symptomatic leukocytosis, massive splenomegaly, or severe constitutional symptoms that are refractory to hydroxyurea or other less-intensive approaches. Although it is not well addressed in the literature, a minority of patients achieves a complete response and are capable of being considered for allogeneic stem cell transplant. However, limited evidence is available that carefully weights these response rates against the substantial toxicities associated with induction-type chemotherapy.

### Future Therapies

Although other therapeutic options have the potential to improve the symptomatology of patients with MDS/MPN, the current pharmacologic landscape is limited. Ongoing studies testing JAK2 inhibitors in aCML and CMML hold promise. However, only a small number of studies currently are addressing MDS/MPN cases specifically. Hopefully, improved molecular understanding of MDS/MPNs will lead to disease-specific clinical trials with promising agents that will affect the natural history of this group of lethal diseases.

### CONCLUSION

Our burgeoning knowledge of the molecular complexity of MDS and MDS/MPNs is rapidly providing novel insights into the pathobiology of these diseases. The clinical application and relevance of these insights currently is largely limited to the refinement of prognostic risk models. Hopefully, this knowledge ultimately will translate into targeted therapies with the potential to change the natural history of these diseases. Presently, judicial selection of patients for treatment with currently available therapies, including alloSCT and nontransplant disease-modifying therapies, coupled with an improved knowledge into the best approaches to use these therapies, is of paramount importance. Given the current paucity of effective therapies for these diseases, an urgent need remains for the development of novel approaches and combinations with the potential of moving the field forward.

## Disclaimers of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “I” indicate leadership positions. Relationships marked “L” are those held by an immediate family member, those marked “B” are held by the author and an immediate family member. Institutional relationships are marked “Inst.” Relationships marked “U” are uncompensated.

### Employment

None. **Leadership Position:** None. **Stock or Other Ownership Interests:** None. **Honoraria:** Eric Padron, Incyte Corporation, Novartis. **Consulting or Advisory Role:** Olatoyosi Odenike, Algeta, Incyte, Sanofi, Spectrum Pharmaceuticals, Suneisis. **Speakers’ Bureau:** None. **Research Funding:** Olatoyosi Odenike, Astex Therapeutics (Inst), Celgene (Inst), Eisai (Inst), Incyte Corporation (Inst), NS-Pharma (Inst), S*Bio (Inst), Sanofi (Inst), Spectrum Pharmaceuticals (Inst), Suneisis (Inst), Eric Padron, Cell Therapeutics (Inst), FORMA Therapeutics (Inst), Incyte (Inst). **Patents, Royalties, or Other Intellectual Property:** None. **Expert Testimony:** None. **Travel, Accommodations, Expenses:** Francesco Onida, Celgene, Gentium, Mundipharma, Pierre Fabre. **Other Relationships:** None.

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