How I use molecular genetic tests to evaluate patients who have or may have myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) can be difficult to diagnose, especially when morphological changes in blood and marrow cells are minimal, myeloblast proportion is not increased, and the karyotype is normal. The discovery of >40 genes that are recurrently somatically mutated in MDS patients raised hope that molecular genetic testing for these mutations might help clarify the diagnosis in ambiguous cases where patients present with cytopenias and non-diagnostic marrow morphological findings. However, many older healthy individuals also harbor somatic mutations in leukemia-associated driver genes, especially in DNMT3A, TET2, and ASXL1, and detection of common aging-associated mutations in a cytopenic patient can cause diagnostic uncertainty. Despite this potential confounding factor, certain somatic mutation patterns when observed in cytopenic patients confer a high likelihood of disease progression and may allow a provisional diagnosis of MDS even if morphologic dysplasia and other diagnostic criteria are absent. A subset of acquired mutations also influences risk stratification of patients with an established MDS diagnosis and can inform treatment selection. Many unanswered questions remain about the implications of specific mutations, and clinicians also vary widely in their comfort with interpreting sequencing results. Here, I review the use of molecular genetic assays in patients with possible MDS or diagnosed MDS. (Blood. 2018;132(16):1657-1663)

Introduction

Since the first formal classification of myelodysplastic syndromes (MDS) by the French-American-British cooperative group in 1976 and 1982, MDS have been defined by the presence of persistent blood cytopenia(s) associated with certain characteristic morphologic changes in blood and marrow cells.1 Searching for these “dysplastic” hematopoietic cell morphologic changes is still important in diagnostic evaluation of the patient with unexplained cytopenias.2 MDS-associated dysplastic changes in the erythroid lineage include megaloblastoid erythroid maturation, multinucleated erythroid precursors, karyorrhexis, and ring sideroblasts; in the granulocytic lineage, they include hypogranular or hypolobated (pseudo-Pelger-Huet) neutrophils; and in the megakaryocyte lineage, hypolobated, hypersegmented, or micromegakaryocytes.3

Unfortunately, none of these morphologic findings is specific for MDS. Cytopenias and morphologic dysplasia can also be caused by various “MDS mimics” that may be mistaken for MDS or may coexist with MDS, such as nutritional deficiency, viral infection, medications, or congenital or acquired marrow failure syndromes.4 Therefore, in order to diagnose MDS, the current 2016 World Health Organization (WHO) classification continues to require finding at least 10% dysplastic cells in at least 1 hematopoietic lineage, an abnormal karyotype (exclusive of certain nonspecific findings such as loss of the Y chromosome), or an increase in myeloblasts (5% to 19%), with ≥20% defined as acute myeloid leukemia [AML].5 Alternative minimal diagnostic criteria for MDS were recently proposed by an international working group (Table 1).6

In addition, dysplasia is to some extent subjective, and there is a high degree of interobserver variability in dysplasia assessment among morphologists.7-10 Many healthy persons over the age of 50 with normal blood counts have minor dysplastic changes in the marrow aspirate.11 As a result of this subjectivity and lack of specificity of dysplastic morphology, better diagnostic tests to evaluate for the possibility of MDS in the cytopenic patient are needed.10,12

In the last 10 years, >40 different recurrent gene mutations have been associated with MDS, including alterations in genes encoding factors important for messenger RNA splicing, epigenetic patterning and chromatin remodeling, DNA repair, signal transduction, transcription factors, and cohesins.13,14 Although no single mutation is detectable in more than 25% to 30% of patients, collectively almost all patients with MDS defined by WHO criteria will have at least 1 of these somatic mutations detectable.

Next-generation sequencing (NGS) assays that test for subsets of these MDS-associated gene mutations at the DNA level are now widely available in clinical practice and are frequently employed in diagnostic evaluation.15,16 As a hematology community, however, we are still learning how best to use these assays, both for the evaluation of patients with cytopenias who might have MDS and in patients with established MDS. There is considerable...
Table 1. Proposed minimal diagnostic criteria for MDS

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<th>Diagnosis of MDS requires:</th>
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<tr>
<td>(A) Persistent blood cytopenia(s) as defined by local laboratory ranges (with consideration of patient factors, such as ethnic background, altitude of residence, etc), without another reversible cause, such as nutritional deficiency or the effect of a drug, and</td>
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<td>(B1) Increased myeloblasts (5%-19%), or</td>
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<td>(B2) Extensive dysplasia (&gt;10% of marrow cells in at least 1 lineage: erythroid, granulocytic, or megakaryocytic), or</td>
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<td>(B3) Karyotypic evidence of clonality with a typical MDS-associated alteration, such as del(5q) or monosomy 7 (excluding nonspecific alterations, such as trisomy 8, loss of the Y chromosome, isolated del(20q), or trisomy 15)</td>
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<th>Supplemental “co-criteria” include</th>
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<td>(C1) Abnormal findings on histologic or immunohistochemical studies of marrow biopsy that could be consistent with MDS, such as abnormally localized immature precursors, clusters of CD34-positive blast cells, or &gt;10% dysplastic micromegakaryocytes detected by immunohistochemistry</td>
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<td>(C2) Abnormal immunophenotype of marrow cells by flow cytometry with multiple MDS-associated phenotypic aberrations</td>
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<td>(C3) Evidence of a clonal population of myeloid cells by molecular genetic testing, which is the subject of this article</td>
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If (A) is present, but not (B1-B3), then the case might be termed “idiopathic cytopenias of undetermined significance” (ICUS): a term that is agnostic about clonality

C1-C3 alone are generally not yet considered specific enough by themselves to be confident about the diagnosis of MDS, but can help confirm the diagnosis if other criteria are present

Below, I describe 4 of my patients who illustrate some of the variability in the use and interpretation of such panels among hematologists.15,17

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### Patient 1

A 72-year-old woman was found to have macrocytic anemia (hemoglobin [Hb] 10.9 g/dL, mean cell volume [MCV] 101 fL) and mild thrombocytopenia (platelet count 135 × 10^9/L) after she saw a new primary care physician and mentioned increased fatigue. The white blood cell (WBC) count and absolute neutrophil count were within normal limits, and the blood smear showed no abnormal leukocytes. She had not had a blood count in several years, but her previous counts had all been unremarkable. There was no obvious explanation for her cytopenias based on her medications (only lisinopril and sertraline) or basic laboratory studies, including serum chemistries, vitamin B12 levels, and red cell folate levels, and a serum protein electrophoresis.

Although it was proposed to the patient that she could be observed with serial blood counts given the relatively mild cytopenias, she was quite anxious about the potential diagnoses, and her primary care physician also requested that a marrow aspiration and biopsy be performed. Marrow morphology was nondiagnostic: 30% cellular (normocellular for age), with only rare dysplastic megakaryocytes representing <10% of cells in that lineage. There was no abnormal localization of immature precursors, increase in reticulin fibrosis, or aberrant immunohistochemistry pattern. Flow cytometry was reported as unremarkable (“too few blasts to enumerate”), and the karyotype was normal female metaphase in 20 metaphases.

A 95-gene NGS panel18 with average amplicon coverage of >1000× showed no pathogenic single nucleotide variants or small insertions/deletions, and read count analysis showed no copy number alterations. The patient was told that she had ICUS, and observation was again recommended.

### Discussion: patient 1

NGS panels can be useful for helping rule out a clonal disorder because they have a relatively high negative predictive value for MDS. In recently reported series, mutations were detected in 74% to 91% of patients with WHO-defined MDS, using gene panels that included 17 to 111 genes associated with hematological neoplasms.13,19,20 Carl Sagan, the noted astronomer and popularizer of science (who died in 1996 of complications of MDS and allogeneic hematopoietic cell transplant), was fond of saying, “Absence of evidence is not evidence of absence,” and a small proportion of patients with MDS will have negative results on an NGS panel. However, the finding of a negative result on a well-designed panel in an ambiguous case such as this should prompt consideration of an alternative diagnosis.

In time, a specific diagnosis, excessive alcohol consumption, became clear in patient 1, who experienced pathologic grief after the loss of her husband. After the patient was provided with appropriate psychosocial support and discontinued use of alcohol, her blood counts returned to normal.

It certainly can be argued that a patient like this woman could be followed serially over time, sparing the expense of an NGS panel (and of a marrow biopsy). However, especially for patients with >1 cytopenia, there is often considerable worry about an evolving clonal disorder, which may coexist with other potential causes of cytopenias, including alcohol use. When NGS panels are employed, they must be interpreted in the context of the information obtained from morphology and conventional cytogenetic testing. As NGS panels become less expensive, it seems likely that they will move earlier in the diagnostic testing algorithm for patients with cytopenias.

### Patient 2

A 67-year-old man was referred to our center after he was found to have macrocytic anemia (Hb 11.2 g/dL, MCV 100 fL) and mild thrombocytopenia (platelet count 135 × 10^9/L) at the time of initial evaluation at the referral center. His WBC count and differential were unremarkable, and his platelet count was at the lower end of the normal range (188 × 10^9/L). His previous blood counts had been normal except for mild macrocytic anemia noted at the time of a bacterial pneumonia 3 years earlier, which eventually resolved. There were no medications or comorbid conditions that were felt likely to be contributing to his cytopenias, and he did not drink alcohol. A serum chemistry group, vitamin B12 levels, and red cell folate levels were all normal. 

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The patient was a physician with extensive previous exposure to fluoroscopy that raised concern for an exposure-related MDS, and therefore, he had undergone bone marrow aspirate and biopsy under the care of his local hematologist-oncologist. This was a 40% cellular marrow with rare megaloblastoid or binucleate erythroid cells representing <10% of nucleated erythroid precursors. Flow cytometry was unremarkable, and the karyotype was normal male metaphase in all 20 metaphases tested. However, a molecular genetic panel showed a DNMT3A R882H mutation with 14.6% variant allele frequency (VAF). The patient was concerned that he was evolving MDS.

Discussion: patient 2

Somatic mutations in leukemia-associated driver genes are observed in >10% of people older than age 60 years. Clonal hematopoiesis not associated with recognized leukemia driver gene mutations, due to either unknown driver mutations or neutral age-associated clonal drift, is even more common with aging. When 1 of the known driver mutations is present at a VAF of >2%, the associated clonal expansion has been termed clonal hematopoiesis of indeterminate potential ("CHIP") and confers a 0.5% to 1.0% risk of progression to WHO-defined MDS, AML, or another hematological neoplasm. CHIP is also associated with an increased risk of cardiovascular events and mortality. The majority of patients with CHIP have a single point mutation in DNMT3A, TET2, or ASXL1 at VAF <20%, most likely resulting from a cytidine-to-thymidine transition from misrepair of a spontaneous deamination event. These mutations can be detected in patients with normal blood counts who appear healthy a decade or more before a diagnosis of AML, and certain mutation patterns predict a higher risk of AML development.

When a mutation is present in an older patient with a cytopenia, especially a mutation commonly associated with CHIP, the possibility is raised that the mutation and the cytopenia may be unrelated. Still, a recent European series has clarified that in patients with unexplained persistent cytopenias, mutations in leukemia-associated driver genes confer a substantial risk of progression to WHO-defined MDS or AML, with a hazard ratio >13.9-fold higher than the risk of MDS/AML progression in patients with ICUS without a clonal mutation. The risk of progression for the patients with "clonal cytopenias of indeterminate significance" (CCUS) was greatest for those with a higher mutation burden (VAF >20%), >1 mutation, or a mutation in a splicing factor. Among patients with CCUS, the cumulative probability of evolution to MDS/AML at 4 years was >70%, compared with <10% for unmutated ICUS patients. In the future, it is possible that patients with certain mutation patterns may be able to be diagnosed as "MDS without dysplasia," given this shortened life expectancy and a natural history that is similar to that observed with MDS diagnosed using current WHO criteria.

Faced with a diagnosis of CCUS and a relatively high possibility of subsequent evolution of WHO-defined MDS or AML, patient 2 retired from his medical practice and took a series of international trips that he had always desired but had never been able to find time for due to his consuming work. Two years after initial evaluation, he developed pancytopenia and was found to have increased blasts, and a diagnosis of MDS with excess blasts type 2 was made using WHO criteria. Despite treatment with decitabine followed by an allogeneic hematopoietic cell transplant, his MDS recurred and he died.

Patient 3

A 64-year-old woman who had not had a blood count in >5 years presented with pancytopenia (Hb 9.7 g/dL, WBC 1.4 × 10^9/L, platelets 48 × 10^9/L) and underwent marrow aspiration and biopsy, which was consistent with MDS with excess blasts type 1. Multilineage dysplasia was present, including 10% ring sideroblasts. The karyotype showed monosomy 7 in 6/20 metaphases, deletion of chromosome 20q and loss of the X chromosome in 8/20 metaphases, and 6 normal female metaphases. Mutation testing showed SF3B1 K700E (26.8% VAF) and TP53 V197M (16.0% VAF).

Discussion: patient 3

Molecular testing in patients with an established diagnosis of MDS can provide information that may influence risk stratification and in some cases treatment selection. For example, a 2011 paper in the New England Journal of Medicine reported that the presence of a mutation in TP53, ETVI6, ASXL1, EZH2, or RUNX1 effectively raises an MDS patient’s International Prognostic Scoring System risk stratification by 1 risk group. Subsequently, the usefulness of molecular genetics in MDS prognostic assessment was confirmed in 1996 patients from 18 centers collected by the International Working Group on Prognosis in MDS. Among 17 genes examined in a multivariable survival model, mutations in SF3B1 were found to provide Revised International Prognostic Scoring System–independent favorable prognostic information, whereas mutations in 4 genes (TP53, RUNX1, EZH2, NRAS) were associated with poorer outcomes. More recently, among 339 patients with a complex karyotype (defined as 3 or more abnormalities, as per Revised International Prognostic Scoring System) evaluated by the International Working Group on Prognosis in MDS, 55% had TP53 mutations, and the presence of a TP53 mutation was a greater determinant of poorer overall survival than either a monosomal karyotype or a very complex karyotype (defined as 5 or more abnormalities) (Detlef Haase, Kristen E. Stevenson, Donna Neuberg, Jaroslav P. Maciejewski, Aziz Nazha, Mikael A. Sekeres, Benjamin L. Ebert, Guillermo Garcia-Manero, Claudia Haferlach, Torsten Haferlach, Wolfgang Kern, Seishi Ogawa, Yasunobu Nagata, Kenichi Yoshida, Timothy A. Graubert, Matthew J. Walter, Alan F. List, Rami S. Komrokji, Eric Padron, David Sallman, Elli Papaemmanuil, Peter J. Campbell, Michael R. Savona, Adam Seegmiller, Lionel Adès, Pierre Fenaux, Lee-Yung Shih, David Bowen, Michael J. Groves, Sudhir Tauro, Michaela Fontenay, Olivier Kosmidis, Michael Bar-Natan Zamor, D.P.S., Richard Stone, Michael Heuser, Felicitas Thol, Mario Cazzola, Luca Malcovati, Aly Karsan, Christina Garsten, Eva Hellström-Lindberg, Jacqueline Boultwood, Andrea Pellagatti, Valeria Santini, Lynn Quek, Paresh Vyas, Heinz Tüchler, Peter L. Greenberg, and Rafael Bejar for the International Working Group for MDS Molecular Prognostic Committee, manuscript submitted June 2018).

With the exception of rare targetable mutations, such as the IDH1/IDH2 mutations for which Food and Drug Administration–approved drugs are available (their regulatory approval is currently for relapsed/refractory AML, so MDS is an off-label use), NGS results have relatively little influence on drug selection outside the...
context of a clinical trial. In several series, TET2 mutations have been associated with higher likelihood of response to hypomethylating agent therapy, whereas ASXL1 mutations were associated with lower responses, but other series have reported better outcomes in ASXL1-mutated patients treated with azacitidine. Data are inconsistent, and the effect of such mutations is not strong. Given the limited number of therapies available for MDS, these results are not enough to influence choice of a specific therapy outside the context of a study.

However, NGS results may make a patient eligible for a clinical trial. For example, TET2 mutant patients are being treated with vitamin C (NCT03433781, NCT03397173), and ASXL1 mutant patients may be good candidates for bromodomain modulators. Splicing mutation mutant patients are eligible for a trial with the SF3B1 inhibitor H3B-8800 (NCT02841540), and TP53 mutant (not TP53 deleted) patients are being treated on study with the P53 protein refolding agent APR-246 (NCT03072043).

In addition, in June 2018 it was announced that the placebo-controlled MEDALIST randomized trial (NCT02631070) of luspatercept, an activin receptor ligand trap, met its primary endpoint of transfusion independence, raising the possibility that this drug will be approved for an MDS-related indication in 2019. Patients were eligible for the MEDALIST trial if they had lower-risk MDS with either ≥15% ring sideroblasts in the marrow, or ≥5% ring sideroblasts (RS) plus an SF3B1 mutation, reflecting the MDS-DS definition in the 2016 WHO classification.

In a 2-institution series, patients with higher-risk MDS who had TP53 mutations and were treated with 10-day decitabine therapy had a 100% complete response or marrow complete response rate. However, the TP53 mutant subset in other hypomethylating agent series, such as the CALGB-11002 Alliance 10-day decitabine cooperative group study (NCT01420926), has not enjoyed such a high response rate. The presence of a TP53 mutation is a risk factor for early progression in lenalidomide-treated patients.

In a Center for International Blood and Marrow Transplant Research analysis of >1500 patients with MDS who underwent allogeneic hematopoietic cell transplant using either myeloablative or reduced-intensity conditioning, those with TP53 mutations had much poorer outcomes, and JAK2 and Ras pathway mutations also were also associated with lower long-term survival. Although there are still occasional long-term survivors with TP53 mutant disease who have undergone transplant, so that such patients should not be categorically denied the possible curative benefit of definitive therapy with transplant, such patients ideally should undergo transplant in the context of a clinical trial to try to improve on the current very poor outcomes with this genotype.

Patient 3 was started on azacitidine as a bridge to transplant and experienced a complete remission. She underwent allogeneic transplant on an experimental protocol with a posttransplant cellular vaccine, but died of complications of influenza B complicated by bacterial sepsis shortly after transplant.

Patient 4

A 72-year-old man noted left upper quadrant fullness while he was bicycling. Moderate splenomegaly was detected on physical examination and confirmed by computed tomographic imaging, which also showed liver enlargement and a few retroperitoneal lymph nodes that were also enlarged in the 2- to 3-cm range. His blood counts included Hb 11.1 g/dL, MCV 93 fl, WBC 6.65 × 10^9/L with 31% monocytes, 36% neutrophils, and 24% lymphocytes, and a platelet count of 140 × 10^9/L. Marrow biopsy was hypercellular for age with erythroid dysplasia, mild increase in reticulin fibrosis, and a monocye increase without increase in blasts or promonocytes, consistent with chronic myelomonocytic leukemia type 1 (CMML-I). Karyotype was normal, and an MDS-focused fluorescent in situ hybridization (FISH) was also unrevealing. Single-gene testing for JAK2 was wild type. Biopsy of the retroperitoneal lymph nodes demonstrated fibrosis, which was interpreted as reactive, and biopsy of the enlarged liver showed focal irregular fibrosis and areas of portal effacement. Extensive evaluation for the cause of the cryptogenic cirrhosis including viral, toxic, and immune etiologies was unrevealing. The patient developed ascites and began requiring serial large-volume paracentesis.

The patient was then referred to our center to assess whether his hepatic fibrosis might be paraneoplastic as a consequence of the CMML. Molecular typing of blood demonstrated ASXL1 G642Fs* in 70.6% of 119 sequences reads, KIT D816V in 41.8% of 593 reads, TET2 Q866* in 45.4% of 1442 reads, and TET2 Y1255* in 46.7% of 302 reads. After noting the KIT mutation, a pathologist reviewing his marrow requested outside liver biopsy and lymph node blocks for review, and immunoperoxidase studies of these samples revealed small aggregates of spindled cells within the fibrosis that were positive for mast cell tryptase and C-Kit and also aberrantly coexpressed CD25. The patient’s serum tryptase level was found to be >500 ng/mL. He had no mast cell mediator symptoms. He was then treated on a compassionate use protocol with midostaurin and experienced clinical improvement, including elimination of ascites and splenomegaly and reduction of tryptase by >50%. After he became resistant to midostaurin, he was treated with avapritinib (BLU-285), also by compassionate use, and regained response.

Discussion: patient 4

Occasionally, NGS panels may uncover an unexpected finding that may reveal an additional diagnosis or an alternative diagnosis. In patient 4 who had CMML, the discovery of a KIT D816 mutation that is commonly associated with systemic mastocytosis (SM) led to additional studies, which confirmed a diagnosis of SM with associated hematologic disease, and allowed selection of a targeted therapy that improved the patient’s symptoms.

The likelihood of finding an additional diagnosis depends on the composition of the sequencing panel. For example, for logistical and workflow reasons, our institution uses a single NGS panel with genes associated with lymphoid, plasma cell, and myeloid disorders. STAT3 mutations are associated with large granular lymphocyte leukemia (40% to 50% of cases), and in each case where we have found an STAT3 mutation, subsequent studies, including T-cell receptor gene rearrangement, demonstrated a large granular lymphocyte leukemia.

Conversely, patients who are being evaluated for a plasma cell neoplasm or lymphoproliferative disorder may be found to have a CHMP-associated mutation, which may complicate the evaluation.
because these mutations are also frequently associated with myeloid neoplasms. Thus, there may be uncertainty about whether there is concomitant MDS or another myeloid neoplasm being masked by the lymphoid or plasma cell disorder, and this uncertainty may influence eligibility for a myeloma or lymphoma treatment protocol.

Patient 4 was tested with an "MDS FISH panel" even though his karyotype had shown 20 normal metaphases. FISH adds very little in cases of suspected myeloid neoplasms in which at least 20 metaphases can be assessed by conventional karyotyping. For example, in a Mayo Clinic study in which 505 cases with possible MDS were assessed by both karyotyping and FISH, concordance between karyotyping and FISH was 96% in cases where cytogeneticists could count at least 20 metaphases.48 Among the small number of patients in the Mayo series in whom an additional abnormality was found by FISH that was not observed at karyotyping (mostly those with <20 metaphases), there was no impact of the FISH result on diagnosis or therapy, and only 6 patients had a change in MDS cytogenetic risk scoring as a result of a FISH-detected anomaly. The authors concluded that FISH panel testing should only be considered if <20 metaphases are examined. Of course, sometimes at the time of marrow sampling it is not yet known whether 20 metaphases will be able to be karyotyped, so some clinicians performing a marrow order both karyotyping and FISH in order to be certain. However, this is unnecessary in the vast majority of cases.

Caveats

When interpreting NGS results or considering obtaining molecular typing in a patient with MDS or possible MDS, caution is indicated. In most publications to date, mutations have been treated as a binary variable: wild type vs mutant, but many MDS-associated genes have a high degree of allelic heterogeneity, and the specific allele may influence phenotype or clinical behavior. Furthermore, mutations are often considered in isolation, but combinations of cooperating mutations may influence outcomes differently than single mutations, and mutations need to be interpreted in the context of other clinical and pathologic data. Given the combinatorial power of >40 different MDS-associated genes with allelic heterogeneity, it will take a very large series of patients to clarify these interactions.

In addition, many single nucleotide variants observed in NGS assays are of unclear significance. Despite available standards and guidelines, local implementation of rules for declaring a variant as pathogenic vs of unknown significance may differ between laboratories.49 The American Society of Hematology has convened a task force to recommend rules for variant calling in NGS assays for hematological disorders. Interpretation of variants is likely to become ever more challenging as targeted NGS panels increasingly give way to whole-exome or whole-genome sequencing approaches and as data from large-scale RNA, protein, and epigenetic assays are more widely available.

Importantly, some observed variants detected on NGS panels are germline rather than somatic, especially those with a VAF near 50%, and in genes like TP53 or RUNX1 where inherited mutations can predispose to myeloid neoplasia. The somatic vs germline distinction is important for a number of reasons, including allogeneic transplant donor selection, monitoring of patients for non–hematologic complications (for example, epithelial cancers in Li-Fraumeni syndrome), and family counseling.50 Fibroblast culture or another non–hematopoietic cell source free of blood contamination may be necessary to distinguish between germline and somatic variants.

Some variants detected during the evaluation of a patient with suspected MDS may not be in myeloid cells. For instance, it is common for older patients to have monoclonal B-cell lymphocytosis. If there is a concomitant B-cell clonal population and dysplastic myeloid cells, a detected SF3B1 variant could be present in the B-cell clone or in the MDS, and only mutation testing of lineage-sorted cells, which is not usually clinically available, can determine this with certainty.

Although the actual cost to perform molecular testing is now relatively inexpensive, clinical NGS assays in both commercial laboratories and academic institution laboratories often have a substantial billing markup and may add considerable expense to the evaluation of the patient.51 Elimination of unnecessary FISH panel testing may help reduce the cost of diagnostic evaluation, but the clinician needs to carefully consider whether the information to be gained is worth the cost of a molecular genetic panel or whether the patient can instead just be followed over time with serial blood counts, as could have been done for patient 1.

Molecular testing is not universally available, especially in the developing world. In addition, available testing panels differ in their sensitivity and in the number of genes assayed. Finally, clinicians vary widely in their comfort and skill in interpreting cancer genome tests.52 Ongoing education is important, and molecular pathologists signing out reports can help clinicians with clear, up-to-date interpretations of variants.

Conclusion

Molecular genetic testing can aid in the confirmation of a clonal disorder in a patient with unexplained cytopenias, and a negative test result can also influence diagnostic assessment, due to the high negative predictive value of a normal result for a disorder like MDS. NGS results may also aid in selecting treatment or transplant decision making for MDS patients in selected circumstances. Increasingly, DNA sequencing is likely to be part of a multilevel assessment that also involves epigenetic sequencing, RNA sequencing to look for cryptic fusions, and other assessments that will ultimately improve diagnosis, prognosis, and treatment monitoring.

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Footnote


