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COUNTERPOINT Do next-generation sequencing results drive diagnostic and therapeutic decisions in MDS?

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This article has a companion Point by Thol and Platzbecker.

Introduction

Multiple studies of next-generation sequencing (NGS) have shown that up to 90% of patients with myelodysplastic syndromes (MDS) carry 1 or more somatic oncogenic mutations in genes involved in RNA splicing, DNA methylation, chromatin modification, transcription regulation, DNA repair, or signal transduction.[1] The advances that these and other molecular techniques are bringing are so great that many might think we have become crazy for accepting this counterpoint role. We would like to start by paraphrasing a sentence from the famous Twin Peaks television series: "Nothing is what it seems." In this article, we advise that it is still premature to incorporate the results of NGS techniques into the diagnostic work-up or decision-making process of patients with MDS.

Main expected benefits for patients with MDS by using NGS results in daily practice

To make a strong recommendation for the use of NGS results in the clinical daily routine regarding patients with MDS, they should be reproducible, have diagnostic value, have an independent prognostic effect on outcomes (in both untreated and treated patients), be useful for choosing among different treatments, and be capable of improving outcomes.

Reproducibility of NGS results in MDS

NGS techniques are not standardized. Each laboratory sets up their criteria for considering a variant allele as pathogenic and for setting the variant allele frequency threshold. In some instances, true somatic mutations may be missed, whereas in others, a polymerase chain reaction/sequencing artifact may be reported as mutations. The latter has been already shown for *ASXL1* mutations.[2] Moreover, the molecular report could be misinterpreted. The Association for Molecular Pathology and the College of American Pathologists have stated that there is a high variability in how the molecular genetics community establishes and validates bioinformatics pipelines, and that improperly developed and validated techniques may generate inaccurate results, which may have negative consequences for patient care.[3] Others have been even more critical.[4] Further, a careful assessment of bone marrow and peripheral blood smear morphology is essential. Patients may coincidentally carry on 2 different blood disorders, such as chronic lymphocytic leukemia and MDS, and a particular mutation (eg, *SF3B1*) may portray a completely different prognosis depending on whether it is present in the chronic lymphocytic leukemia or MDS cells. To date, there has been no study showing the reproducibility of NGS results in MDS among different laboratories, and only 1 consensus guidelines publication (in Spanish) is available.[5] Thus, the risk that a patient be assigned to a wrong mutational category, with deleterious implications, is high.

Value of NGS results for the diagnosis of MDS

None of the more than 40 different somatic mutations found in patients with MDS is pathognomonic of MDS.[6] Further, given the relatively common finding of somatic mutations in blood cells of elderly healthy people,[7,8] a condition termed clonal hematopoiesis of indeterminate potential,[9] the presence of a somatic mutation is insufficient for the diagnosis. Individuals with clonal hematopoiesis of indeterminate potential are at increased risk of progressing to different hematologic malignancies; however, the rate of progression is only 0.5% to 1% per year. Moreover, the presence of clonal cytopenias of undetermined significance (idiopathic cytopenias of undetermined significance plus clonal hematopoiesis of indeterminate potential) does not inevitably lead to MDS. In a recent series, only patients with clonal cytopenias of undetermined significance showing on peripheral blood granulocyte mutation

patterns highly predictive of myeloid neoplasms, defined as the presence of spliceosome gene mutations (especially *SF3B1*) and mutations in *TET2*, *ASXL1*, or *DNMT3A* with additional mutations, had a risk for progression to myeloid neoplasms close to 100% at 6 years.[10] The number of mutations and a variant allele frequency above 10% also showed a high correlation with the presence of a myeloid malignancy. These findings are encouraging and suggest that mutational analysis would be valuable for improving the diagnosis of subjects with unexplained cytopenias, but require confirmation. Until now, the only somatic mutation correlated with a MDS subtype, MDS with ring sideroblasts (RS), is *SF3B1*.[11] Therefore, in the current World Health Organization classification of myeloid neoplasms, the threshold in the bone marrow proportion of RS for the diagnosis of MDS with RS can be lowered from 15% to 5% if the *SF3B1* mutation is present. However, whether the outcomes of these 2 cohorts of differently defined patients is the same remains unproven. Thus, the diagnostic value of NGS results is scarce.

Effect of NGS results on prognosis

Several studies have shown that NGS results are relevant for prognostication.[6,11,12] First, outcomes progressively worsen as the number of oncogenic mutations increases. In addition, several mutations predict overall survival (OS) in univariable analyses, with *TP53*, *EZH2*, *ETV6*, *RUNX1*, *ASXL1*, and *SRSF2* mutations associated with poor OS and *SF3B1* mutations with a better outcome.[6,11,12] Nonetheless, combining molecular mutations with the revised International Prognostic Scoring System (IPSS-R) and age only modestly improves the predictive value of the IPSS-R.[6] Further, as many somatic mutations are closely associated with other well-recognized prognostic variables, their independent prognostic value remains disputed and relevant issues unsolved. First, with the exception of *TP53* (high-risk) and *SF3B1* (low-risk) mutations, there is no consensus on which mutations should be assigned to a particular risk mutational category. Further, the added prognostic value offered by *TP53* and *SF3B1* mutations is scarce in clinical practice. In a large series of patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT), *TP53* mutations were present in only 13% of patients with low- or intermediate-risk IPSS at transplantation,[13] and the incidence of these mutations in lower-risk MDS is below 5% because of its strong correlation with poor-prognosis chromosomal abnormalities and bone marrow blasts percentage. Thus, only a very small fraction of lower-risk patients by the IPSS or IPSS-R would be reclassified to a higher-risk category by *TP53* mutations alone. Even the refinement in prognosis yielded by the presence of *TP53* mutations in patients with a complex karyotype (55%)[14] is meaningless for therapy planning. Regarding *SF3B1* mutations, their independent prognostic value remains disputed both in MDS with RS,[15-17] and in MDS as a whole.[18] Patients with MDS with RS carrying *SF3B1* mutations have a lower proportion of poor-risk chromosomal abnormalities and a lower platelet count.[19]

The prognostic value of somatic mutations is even more blurred after taking into account new recent and relevant findings. The case for *TP53* mutations is particularly noteworthy. The very poor prognosis of *TP53* mutations in MDS seems to be restricted to patients carrying *TP53* biallelic mutations, which are far more common in patients with complex karyotypes. In sharp contrast, monoallelic *TP53* mutations do not seem to alter the course of the disease (E. Bernard, G.F.S., M.I., and E.S., manuscript submitted October 2019).

Finally, the prognostic value of co-occurring mutations is unclear, and the results of a multinational task force supported by the MDS Foundation to develop a molecular IPSS-R are eagerly awaited. Therefore, NGS results alone are inadequate for assessing prognosis.

NGS results for predicting response to and for planning treatment

No single somatic mutation except the *TP53* mutation has been enabled to accurately predict outcomes after treatment with hypomethylating agents, with multiple studies offering discrepant results (Table 1).[20-30] Again, no single mutation has shown a clear association with response to erythropoiesis-stimulating agents (ESAs). A higher response rate to ESAs has been reported for patients harboring *SF3B1* mutations in 1 series,[31] but not in another,[32] and the higher but not statistically significant response rate after luspatercept in a clinical trial in red blood cell transfusion-dependent lower-risk MDS with RS or presence of *SF3B1* mutations[33] is pending confirmation. In fact, luspatercept has recently shown to be superior to supportive care in lower-risk transfusion-dependent patients with MDS with RS who are refractory or have lost response to ESAs.[34] The fact that another phase 3 clinical trial currently under way is comparing luspatercept with ESAs as the first line of lower-risk transfusion-dependent patients with MDS irrespective of the presence of RS or *SF3B1* mutations, however, suggests that the efficacy of this drug may not be restricted to MDS with RS.

In contrast, although the presence of *TP53* mutations in lenalidomide-treated patients does not decrease the likelihood of achieving red blood cell transfusion independence, and its effect on the cytogenetic response rate is debatable, it consistently reduces OS and increases the risk for progression to AML.[35,36] In those instances, allo-HCT should be promptly considered. Regarding the effect of somatic mutations on the outcomes after allo-HCT (Table 2), only *TP53* mutations have universally demonstrated a clear independent association with OS and relapse risk.[13,37-41] However, OS of patients with *TP53* mutations is clearly better for those without complex karyotypes,[38] suggesting that *TP53* mutational status per se should not be considered as a contraindication for allo-HCT. The relative contribution of somatic mutations for predicting OS after allo-HCT in a large series was only 8% after considering other clinical and biological prognostic characteristics.[38]

In contrast, the presence of AML-specific mutations (*NPM1*, *FLT3*) might in the future influence the choice of treatment. *NPM1* mutations are associated with a rapid progression to AML, likely because they should be considered early-stage AML rather than MDS. Those *NMPM1*-mutated patients might have a good response to high-dose cytarabine schedules. In the same way, patients with *FLT3* mutations, infrequent in MDS (5%-10%) but present in up to 30% of patients with MDS evolved to AML, could benefit from tyrosine kinase inhibitors already approved for AML,[42] but this potential advantage remains to be proven in MDS. Whether the use of *IDH* inhibitors, particularly active in patients with AML with *IDH1* or *IDH2* mutations, could be a potentially useful treatment of patients with MDS with those mutations (5%-10%) also remains to be demonstrated.

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Table 1. Effect of somatic mutations on outcomes after treatment with hypomethylating agents

	Effect on OS		Effect on ORR	
	Number of patients with mutations/overall number of patients (reference)		Number of patients with mutations/overall number of patients (reference)	
Gene mutation	Shorter OS*	No differences	Lower ORR	No differences
TP53	39/213 (20)	21/116 (30)†	21/116 (30)†‡	39/213 (20)
	20/134 (21)			20/134 (21)
	13/107 (22)*			13/107 (22)*
	38/168 (23)			38/168 (23)
	10/114 (24)			10/114 (24)
	11/84 (25)			11/84 (27)
	38/213 (29)			38/213 (29)
TET2	13/86 (25)	58/213 (20)	58/213 (20)	26/134 (21)
		26/134 (21)	13/86 (25)	17/107 (22)*
		17/107 (22)*	17/92 (26)	29/79 (23)
		29/79 (23)		33/114 (24)
		33/114 (24)		32/84 (27)
		17/92 (26)		86/357 (28)
		32/84 (27)		58/213 (29)
		86/357 (28)		
		58/213 (29)		
DNMT3A	9/107 (23)*	34/213 (20)		34/213 (20)
		10/134 (21)		10/134 (21)
		6/168 (23)		9/107 (22)*
		7/114 (24)		6/168 (23)
		8/92 (26)		7/114 (24)
		18/84 (27)		8/92 (26)
		34/213 (29)		18/84 (27)
				34/213 (29)
ASXL1	99/213 (21)	20/107 (22)*		99/213 (20)
	29/134 (22)§	17/79 (23)		20/134 (21)
	24/92 (27)	23/114 (24)		20/107 (22)*
		11/84 (27)		17/79 (23)
		96/357 (28)		23/114 (24)
		98/213 (29)		24/92 (26)
				11/84 (27)
				96/357 (28)
				98/213 (29)
RUNX1	18/84 (27)	17/134 (21)		42/213 (20)
	42/213 (20)	12/107 (22)*		17/134 (21)
		20/79 (23)		12/107 (22)*
		16/114 (24)		20/79 (23)
		43/213 (29)		16/114 (24)
				18/84 (27)
				43/213 (29)
EZH2	17/84 (27)	10/107 (22)*		21/213 (20)
	12/134 (21)§	2/168 (23)		12/134 (21)

ORR, overall response rate.
*Azacitidine-treated patients.
†Decitabine-treated patients (type of hypomethylating agent used not specified in remaining series).
‡Higher ORR.
§Longer OS.

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Table 1. (continued)

Gene mutation	Effect on OS: Number of patients with mutations/overall number of patients (reference)		Effect on ORR: Number of patients with mutations/overall number of patients (reference)	
	Shorter OS*	No differences	Lower ORR	No differences
	21/213 (20)	5/114 (24)		10/107 (22)*
				2/168 (23)
				5/114 (24)
				17/84 (27)
RAS	12/107 (22)*	24/213 (20)	8/168 (23)	24/213 (20)
		12/134 (21)		12/134 (21)
		8/168 (23)		12/107 (22)*
		8/114 (24)		8/114 (24)
		14/84 (27)		14/84 (27)
SRSF2		35/213 (20)		35/213 (20)
		24/134 (21)		24/134 (21)
		1/107 (22)*		1/107 (22)*
		11/53** (23)		11/53** (23)
		27/114 (24)		27/114 (24)
		34/213 (24,29)		34/213 (29)
U2AF1		29/213 (20)	21/107 (22)*	29/213 (20)
		5/134 (21)		5/134 (21)
		21/107 (22)*		13/53 (23)
		13/53 (23)		6/114 (24)
		6/114 (24)		12/84 (25)
		12/84 (27)		53/357 (28)
		53/357 (28)		30/213 (29)
		30/213 (29)		

ORR, overall response rate.
*Azacitidine-treated patients.
†Decitabine-treated patients (type of hypomethylating agent used not specified in remaining series).
‡Higher ORR.
§Longer OS.

Targeting TP53 mutation could also prove beneficial in patients with MDS carrying those mutations. In a recent clinical trial with APR-246 and azacitidine (NCT03745716) in *TP53*^{mut} patients, the response rate was 100% (11 of 11 evaluable patients; 9 complete remission [CR] and 2 marrow CR), and the median OS has not been reached at a median follow-up of 7 months. The most common adverse events were grade 3/4 hematological toxicity and grade 1/2 nausea and vomiting, dizziness, headache, and neuropathy, suggesting that this combination could be better than the current standard of care.[43] However, these preliminary results need confirmation in phase 3 trials. A 10-day schedule of decitabine in a series including 21 patients with *TP53* mutation (12 AML and 9 MDS), 20 of them showing a complex karyotype, all patients responded to decitabine with both BM blast clearance to less than 5% and reduction in variant allele frequency to levels less than 5%, and median OS was 12.7 months.[30] The reproducibility of these encouraging results requires confirmation.

Thus, at this time, the presence of specific mutations for decision-making regarding therapy is small and restrained to biallelic *TP53* mutations.

Do NGS results improve patients' outcomes?

Unfortunately, the major handicap we face when treating patients with MDS is the lack of effective and harmless treatment alternatives. Allo-HCT remains the only proven curative treatment. ESAs and lenalidomide for lower-risk and hypomethylating drugs for higher-risk MDS are of certain but limited value. Until now, no single drug specifically targeting a somatic mutation has proven in phase 3 clinical trials to be of value in MDS. Further, there is no single somatic mutation that favors the use of any treatment alternative. It has been argued that early or preemptive interventions, including withdrawal of immunosuppression, infusion of donor lymphocytes, or the use of azacitidine, in transplanted patients at high risk for relapse, such as those harboring *TP53* mutations or with measurable disease by NGS techniques at early times after transplant,[44] could be valuable. However, prospective clinical trials to establish this benefit are lacking.

Conclusions

In summary, we consider that at this time, it is premature to incorporate NGS results to the diagnostic work-up or the

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Table 2. Effect of somatic mutations on outcomes after allogeneic hematopoietic cell transplantation

Gene mutation	Effect on outcomes
TP53	Shorter OS[13,37-41]
	Higher RR,[13,37-39] especially if complex karyotype present[38]
RAS pathway (including *NRAS* and *CBL* among others)	Shorter OS[13,39] (only in MDS/MPN subtypes)[38]
	Higher RR after non-myeloablative conditioning[13]
	Higher RR for *CBL*[38]
	Higher NRM for *NRAS*[38]
	Higher RR for *NRAS*[39]
JAK2	Shorter OS[13]
	Higher NRM[13]
ASXL1	Shorter OS[37]
	Higher RR[13,37]
DNMT3A	Higher RR[38]
	Lower NRM[38]
	Shorter OS[41]
RUNX1	Shorter OS[37]
	Higher RR[37]
IDH2	Shorter OS[39,40]
PPM1D (p53 regulator)	Shorter OS and more common in therapy-related MDS[13]
SBDS	Shorter OS in young adults and associated with *TP53* mutations[13]
U2AF1	Shorter OS[39]
EZH2	Higher RR[39]
TET2	Shorter OS[41]

MPN, myeloproliferative neoplasm; NRM, nonrelapse mortality; RR, relapse risk.

decision-making process regarding treatment of patients with MDS. NGS techniques are still not standardized and may yield inaccurate results, leading to misdiagnosis and wrong therapeutic decisions. Further, recent evidence, such as that concerning the prognostic value of biallelic, but not monoallelic, *TP53* mutations, clearly show that our knowledge about molecular genetics in MDS must be refined. Apart from biallelic *TP53* mutations, the value of other somatic mutations for prognostic stratification and therapy planning is quite limited. Finally, NGS results do not lead to improved outcomes. Thus, we believe that the results of NGS mutation screening should be put on hold and not be used in our daily practice until stronger evidence is available and those results can be safely and properly used.

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Authorship

Contribution: G.F.S., M.I., and E.S. wrote the manuscript and revised the final version.

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