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EXPERT REVIEWS

Genome sequencing in myelodysplastic syndromes: can molecular mutations predict benefit from hypomethylating agent therapy?

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Evaluation of: Bejar R, Lord A, Stevenson K, *et al.* TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood 2014 Oct 23; 124(17):2705-12.

Patients with myelodysplastic syndromes (MDS) have clinically variable courses even within the same prognostic subgroups. Although hypomethylating agents (HMAs) have been shown to improve outcomes in patients with high-risk MDS, many patients do not derive benefit. There is an urgent clinical need to identify patients with low probability of benefiting from HMAs but no reliable clinical predictors or biomarkers have been discovered to date. Although some recurrent molecular mutations in MDS carry independent prognostic value, their ability to predict benefit from HMAs is not clear. Here, we discuss an important article in which sequencing from samples of 213 patients identified recurrent mutations associated with response to HMAs. Although an important step in the right direction, the clinical implications of these findings are far from optimal and identification of biomarkers that can reliably predict benefit from HMAs and other therapies in patients with MDS remains a top clinical and a research priority.

KEYWORDS: biomarkers • genome sequencing • hypomethylating agents • myelodysplastic syndromes • prognostication • TET2 mutation

Myelodysplastic syndromes (MDS) are a group of clonal neoplasms of the hematopoietic stem cell characterized by ineffective hematopoiesis leading to variable degrees of peripheral blood cytopenias and risk of transformation to acute myeloid leukemia [1]. Currently, the most widely used prognosticating system for patients is the International Prognostic Scoring System (IPSS) which divides patients into four risk groups (low, intermediate-1, intermediate-2, and high) based on number of cytopenias, bone marrow (BM) blast percentage and recurrent prognostic karyotypic abnormalities to predict survival and evolution to acute myeloid leukemia [2]. Those with higher risk MDS (intermediate-2, high) have a median survival of <1 year, whereas those with lower risk MDS (intermediate-1, low) have a median survival of 3–8 years [3]. Prediction of outcomes is important in management as this impacts risk/benefit estimations for initiation of treatment, including hypomethylating agents (HMAs) and allogeneic hematopoietic cell transplantation (AlloHCT) [4]. Although HMAs improved outcomes for patients with higher risk MDS, not all patients benefit from this therapy and no reliable methods have been discovered to predict differential likelihood of benefit from HMAs. The IPSS, as well as other newer and improved scoring systems such as the WHO classification-based Prognostic Scoring System and the revised

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IPSS (IPSS-R), were not designed to predict response to specific treatments and do not include the recently discovered recurrent molecular mutations [4].

Technological advances have allowed for substantial decreases in time and cost of DNA sequencing, thus enabling investigation to explore the role of novel mutations in this disease [5]. Gene mutations are considered among the key steps in development of MDS and the diversity of clinical phenotypes is thought in part to be due to the variety of genetic mutations [6]. In addition, these genetic alterations were found to provide additional prognostic information [7,8]. For example, mutations in *TP53, EZH2, ETV6, RUNX1* and *ASXL1* were independently found to be associated with shorter overall survival (OS) [9]. However, how the presence of the recurrent molecular mutations correlates with the achievement of clinical benefit from HMA therapy is not clear.

Summary of methods & results

In a multicenter prospective study, Bejar *et al.* [10] collected tumor DNA from peripheral blood or BM samples of 213 patients with MDS before treatment with HMA to evaluate the predictive potential of 40 genes that are recurrently mutated in MDS. Of the patients, 5, 40, 36, 17 and 1% had IPSS low, intermediate-1, intermediate-2, high risk, and unknown; 14, 11, 59, 3, 10 and 3% had refractory anemia (RA), RA with ringed sideroblasts, RA with excess blasts, RA with excess blasts in transformation, chronic myelomonocytic leukemia, and other, respectively. Whole genome amplification was performed on genomic DNA with generation of a genotype fingerprint of 22 common single nucleotide polymorphisms for each sample. Target enrichment was performed, followed by sequencing using Illumina Hi Seq 2000. Logistic regression models were used to predict response to therapy, adjusting for age, sex, IPSS risk group and treatment. OS was calculated from the time of treatment to the time of death from any cause or date last known alive.

A mutation in at least one of the targeted 40 genes was found in 94% of patients with the following being the most commonly mutated: *ASXL1* (46%), *TET2* (27%), *RUNX1* (20%), *TP53* (18%), *DNMT3A* (16%), *SRSF2* (16%), *SF3B1* (15%) and *U2AF1* (14%). As expected, mutations of *SF3B1* were found mainly in patients with RA with ringed sideroblasts. The overall response rate (ORR, defined as complete response + partial response + hematologic improvement) was 47 with 31% achieving complete response. Of the patients, 20, 68 and 13% were treated with azacitidine alone, decitabine alone, and decitabine along with an unspecified agent, respectively, and there was no significant difference in response based on treatment regimen. No mutation was associated with significantly improved ORR but *TET2* mutant patients had a trend toward increased ORR compared with wild type (55 vs 44%; $p = 0.14$).

In a revised analysis wherein mutations with a variant allele fraction of ≥10% only were considered mutated, *TET2* mutations were associated with a significantly higher ORR compared with wild type (60 vs 43%, odds ratio [OR]: 1.99, $p = 0.036$; adjusted OR 1.98, $p = 0.044$). Using logistic regression analysis to look at association of gene mutations with ORR, those with mutated *TET2* and unmutated *ASXL1* had an increased ORR compared with all other combinations of mutations. Of the 146 patients with survival data, 119 died in follow-up, with median follow-up being 3.8 years. *TET2* mutation status was not associated with OS, whereas *TP53* and *PTPN11* mutations were associated with lower OS. Those with complex karyotype and *TP53* mutation had a median survival of 0.9 years compared with an OS of 1.3 years for patients with complex karyotype and no *TP53* mutation.

In an *in vivo* model of murine competitive BM transplantation, donor Tet2-null and wild-type mice were treated with plpC to induce deletion of exon 3 of Tet2. BM was then harvested and combined in a 1:2 ratio with marrow from wild type donors for transplantation into a murine recipient. At 2.5 weeks after transplant, there was greater engraftment of Tet2-null cells. Seven of the transplanted mice were treated with azacitidine and seven were treated with control. Those treated with azacitidine had significant decreases in white blood cell counts and hematocrit as well as significantly decreased presence of Tet2 null cells in the blood [10].

Discussion & five-year view

An important focus for current research in MDS revolves around predicting benefit (or lack thereof) to HMA therapy to avoid exposing patients with low probability of benefit to prolonged and potentially toxic ineffective treatment, increased costs, and to allow earlier consideration of clinical trials that can alter the natural history of the disease with potentially more time for benefit [11]. Unfortunately, all efforts to date have failed in discovering clinical and/or biomarkers that allow reliable prediction of clinical benefit from HMA therapy [4,11]. A French prediction tool was developed to separate higher risk MDS patients based on OS advantage achieved with azacitidine using readily available clinicopathologic parameters [12], but it was subsequently found to offer no prognostic utility beyond that of the more commonly used IPSS-R [13]. After the disappointment of methylation predictive studies, the recent discoveries of >40 genes with recurrent somatic mutations in the vast majority of patients have renewed hope of identifying that evasive biomarker(s) [7,8,10]. The recurrent mutations were observed most commonly in genes involved in important biologic pathways that contribute to the pathogenesis of MDS such as DNA methylation (*DNMT3A, TET2, IDH1 and IDH2*), chromatin modification (*EZH2 and ASXL1*), transcriptional regulation (*TP53, RUNX1 and GATA2*), RNA splicing (*SF3B1, U2AF1, SRSF2 and ZRSR2*) and signal transduction (*JAK2, KRAS* and *CBL*) [14]. Several of these mutations were found to have an independent prognostic value and, interestingly, there appears to be no significant difference in survival impact of a specific mutation if present in a dominant clone versus a subclone [8]. Despite the growing literature supporting the independent significance of molecular mutations in MDS, none have yet been incorporated formally into validated prognostic scoring systems

(in contrast to chromosomal lesions) nor used to make treatment decisions or predict responses to specific therapies [8,9,15].

In contrast to prior large studies, the report by Bejar *et al.* [10] clarifies the impact of recurrent mutations on OS among HMA-treated MDS patients as well as their potential impact on response to treatment. Interestingly, the only two genes whose mutations affected OS (*TP53* and *PTPN11*) had no association with response to HMAs [10]. Importantly, among the genes previously reported to be associated with OS in largely untreated patients [9], only *TP53* mutations remained independently prognostic of OS among HMA-treated patients, further demonstrating the challenges of incorporating molecular alterations into clinical decision-making. Several groups have reported on the negative impact of *TP53* mutations, its association with 5q- and complex cytogenetic profiles, and that *TP53* appears to be responsible for the dismal outcomes in these patients with complex karyotype [9,16]. Patients with 5q-MDS who had *TP53* mutations (~ 20%) had poor outcomes despite lenalidomide therapy [17,18]. Recent data suggest that in 5q-MDS haploinsufficiency for casein kinase 1A1 gene (*CSNK1A1*) might increase sensitivity to lenalidomide which induces the ubiquitination and consequent degradation of the kinase by activating the CRBN-CRL4 E3 ubiquitin ligase [19,20]. *TP53* mutations might confer resistance to lenalidomide by reducing the sensitivity of casein kinase 1A1 to the drug effects [19,20]. In a recent study of MDS patients who underwent alloHCT, *TP53* mutations were the most important independent predictor for mortality after alloHCT and patients with these mutations had a strikingly poor median OS of 4.6 months [21]. Taken together, these findings suggest that neither HMA therapy nor alloHCT abrogate the negative prognostic impact of *TP53* mutations and that such patients represent a subgroup with a particularly dismal prognosis in whom novel approaches and clinical trials should be strongly considered [18].

Although the current report by Bejar *et al.* [10] represents the largest and most comprehensive published experience correlating molecular mutations with benefit from HMAs, this issue has been addressed by other investigators. The French group used Sanger sequencing specifically for *TET2* on samples from 86 patients with MDS or oligoblastic acute myeloid leukemia and similarly found that *TET2* mutations predicted objective responses to azacitidine therapy compared with wild-type *TET2* (85 vs 52%) without affecting OS [22]. In another retrospective study of 92 HMA-treated patients, mutations in *TET2* and *DNMT3A* in multivariate analysis predicted improved ORR to HMAs [23]. Interestingly, mutations in *TET2* (along with *TP53* and *DNMT3A*) were also associated with shorter OS after alloHCT [21]. As the authors pointed out, *TET2* mutations might increase susceptibility to HMAs via increasing the percentage of actively cycling cells rather than by methylation-mediated mechanisms [10]; therefore potentially explaining the observed partial correlation.

Despite the importance of this study, several important limitations are noted. First, from a clinical standpoint, although the 10% of patients with *TET2* mutations (variant allele fraction ≥10%) and wild-type *ASXL1* had the highest ORR to HMAs (74%), the authors did not identify any pattern of mutations defining subset of patients with low enough ORR to HMAs that could potentially justify withholding upfront therapy with the only class of agents proven to prolong OS in higher risk MDS. Second, mutation status was assessed only before initiation of HMA therapy, whereas serial analysis can potentially discover mutations associated with evolving resistance to HMA therapy [10]. Third, the authors did not account for the potential confounding effects of the inter-patient differential expression of nucleoside-metabolizing enzymes which might affect ORR to HMAs [24]. Fourth, logistical challenges such as standardization of the conduction and the interpretation of assays, and determination of the importance of variant allele fraction levels, mutational burden of clones and subclones, and intra-tumor genomic heterogeneity need further research [8,15,18]. Fifth, the complete response rate observed with HMAs in the cohort reported by Bejar *et al.* [10] was higher than the 10–20% reported in clinical trials and retrospective analyses [4] which might affect the generalizability of the results. Finally, although *TET2* mutation status predicted response rates to HMAs, it failed to predict OS, further complicating the application of the results to the clinic.

Although challenging to conduct, serial and comprehensive prospective evaluation of a large cohort of HMA-treated patients to evaluate genetic, epigenetic, immunologic, and clinical parameters might be the only way to define reliable predictors of clinical benefit to HMAs in MDS patients.

Financial & competing interests disclosure

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Key issues

- Despite the growing literature on molecular mutations in MDS, they have not yet been formally incorporated into prognosticating systems or used to predict benefit from specific therapies.
- Patients with *TET2* mutations and wild-type *ASXL1* appear to have the highest response rate to treatment with hypomethylating agents among MDS patients, but only mutations in *TP53* and *PTPN11* were associated with survival.
- No pattern of recurrent somatic mutations in patients with higher risk MDS currently justifies withholding upfront therapy with hypomethylating agents.

References

1. Jadersten M, Hellstrom-Lindberg E. Myelodysplastic syndromes: biology and treatment. J Intern Med 2009;265(3):307-28
2. Greenberg P, Cox D, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89(6):2079-88
3. Sekeres MA. Epidemiology, natural history, and practice patterns of patients with myelodysplastic syndromes in 2010. J Natl Compr Canc Netw 2011;9(1):57-63
4. Zeidan AM, Linhares Y, Gore SD. Current therapy of myelodysplastic syndromes. Blood Rev 2013;27(5):243-59
5. Mendelsohn J. Personalizing oncology: perspectives and prospects. J Clin Oncol 2013;31(15):1904-11
6. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol 2011;29(5):504-15
7. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 2014;28(2):241-7
8. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 2013;122(22):3616-27; quiz 3699
9. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364(26):2496-506
10. Bejar R, Lord A, Stevenson K, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood 2014;124(17):2705-12
11. Zeidan AM, Lee JW, Prebet T, et al. Platelet count doubling after the first cycle of azacitidine therapy predicts eventual response and survival in patients with myelodysplastic syndromes and oligoblastic acute myeloid leukaemia but does not add to prognostic utility of the revised IPSS. Br J Haematol 2014;167(1):62-8
12. Itzykson R, Thepot S, Quesnel B, et al. Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood 2011;117(2):403-11
13. Zeidan AM, Lee JW, Prebet T, et al. Comparison of the prognostic utility of the revised international prognostic scoring system and the French prognostic scoring system in azacitidine-treated patients with myelodysplastic syndromes. Br J Haematol 2014;166(3):352-9
14. Voso MT, Santini V, Fabiani E, et al. Why methylation is not a marker predictive of response to hypomethylating agents. Haematologica 2014;99(4):613-19
15. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. Blood 2013;122(25):4021-34
16. Kulasekararaj AG, Smith AE, Mian SA, et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol 2013;160(5):660-72
17. Jadersten M, Saft L, Smith A, et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol 2011;29(15):1971-9
18. Zeidan AM, Gore SD, Padron E, et al. Current state of prognostication and risk stratification in myelodysplastic syndromes. Curr Opin Hematol 2015;22(2):146-54
19. Fink EC, Krönke J, Hurst SN, et al. Lenalidomide induces ubiquitination and degradation of CSNK1A1 in MDS with Del(5q). ASH Annual Meeting, San Francisco, CA; 2014
20. Schneider RK, Adema V, Heckl D, et al. Role of casein kinase 1A1 in the biology and targeted therapy of del(5q) MDS. Cancer Cell 2014;26(4):509-20
21. Bejar R, Stevenson KE, Caughey B, et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. J Clin Oncol 2014;32(25):2691-8
22. Itzykson R, Kosmider O, Cluzeau T, et al. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia 2011;25(7):1147-52
23. Traina F, Visconte V, Elson P, et al. Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms. Leukemia 2014;28(1):78-87
24. Valencia A, Masala E, Rossi A, et al. Expression of nucleoside-metabolizing enzymes in myelodysplastic syndromes and modulation of response to azacitidine. Leukemia 2014;28(3):621-8