Pathologic Spectrum and Molecular Landscape of Myeloid Disorders Harboring *SF3B1* Mutations

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ABSTRACT

Objectives: SF3B1 *mutations are the most common mutations in myelodysplastic syndromes (MDS). The International Working Group for the Prognosis of MDS (IWG-PM) recently proposed* SF3B1*-mutant MDS (*SF3B1^{-mut}*-MDS) as a distinct disease subtype. We evaluated the spectrum and molecular landscape of* SF3B1*-mutated myeloid disorders and assessed the prognostication in MDS harboring* SF3B1 *mutations (MDS-*SF3B1*).*

Methods: *Cases were selected by retrospective review. Clinical course and laboratory and clinical findings were collected by chart review.* SF3B1^{-mut}*-MDS was classified following IWG-PM criteria.*

Results: SF3B1 *mutations were identified in 75 of 955 patients, encompassing a full spectrum of myeloid disorders. In MDS-*SF3B1*, Revised International Prognostic Scoring System (IPSS-R) score greater than 3 and transcription factor (TF) comutations were adverse prognostic markers by both univariate and multivariate analyses. We confirmed the favorable outcome of IWG-PM-defined* SF3B1^{-mut}*-MDS. Interestingly, it did not show sharp prognostic differentiation within MDS-*SF3B1.

Conclusions: SF3B1 *mutations occur in the full spectrum of myeloid disorders. We independently validated the favorable prognostication of IWG-PM-defined* SF3B1^{-mut}*-MDS. However it may not provide sharp prognostication within MDS-*SF3B1 *where IPSS-R and TF comutations were prognostic-informative. Larger cohort studies are warranted to verify these findings and refine MDS-*SF3B1 *prognostication.*

Key Points

- *SF3B1* mutations occur in the full spectrum of myeloid disorders, ranging from clonal cytopenia with undetermined significance to acute myeloid leukemia.
- Revised International Prognostic Scoring System score greater than 3 and presence of transcription factor comutations are adverse prognostic markers in myelodysplastic syndromes (MDS) harboring *SF3B1* mutations (MDS-*SF3B1*).
- We validated the favorable clinical outcome of *SF3B1*-mutant MDS as defined by the International Working Group for the Prognosis of MDS; however, this designation, by its proposed exclusion criteria, may not provide sharp prognostication within MDS-*SF3B1*.

The messenger RNA (mRNA) spliceosome is composed of a complex of small nuclear ribonucleoproteins (snRNP) U1, U2, U4, U5, and U6 and plays critical roles in regulating mRNA splicing and subsequent protein expression. It is assembled through binding of U1 snRNP to the 5′ splice site of the pre-mRNA, recruitment of U2snRNP to the 3′ splicing site at the intron–exon junction guided by the branch region sequence, and joining of the tri-snRNP U4/U5/U6.[1-3] SF3B1, an essential protein in the U2snRNP complex, facilitates U2snRNP binding at the branch point near the 3′ splicing site. The critical role of SF3B1 and other spliceosome components (SRSF2, U2AF1, and ZRSR2) in hematopoiesis was initially recognized from the discovery of their frequent mutations in myelodysplasia by whole-exome sequencing studies.[4-6] SF3B1 mutants were shown to alter U2snRNP function by promoting alternative branch-point usage and induction of cryptic

3′ splice site selection, thereby generating aberrantly spliced mRNA transcripts subject to nonsense-mediated decay and downregulation of target transcripts and protein expression.[7,8] Morphologically, mutations in *SF3B1* show a strong association with ring sideroblasts (RS) in myelodysplastic syndromes (MDS) with RS (MDS-RS) and MDS/myeloproliferative neoplasm with RS and thrombocytosis (MDS/MPN-RS-T).[5,6,9] In MDS harboring *SF3B1* mutations (MDS-*SF3B1*), aberrant splicing was seen in genes involved in heme metabolism and iron homeostasis, such as the iron transporter *ABCB7*, whose downregulation led to impaired erythroid differentiation, accumulation of mitochondrial ferritin, and presentation of RS.[10-16] Besides MDS-RS and MDS/MPN-RS-T, *SF3B1* mutations have also been reported in other myeloid neoplasms such as MPN[17] and acute myeloid leukemia (AML),[18] as well as chronic lymphocytic leukemia[4] and solid tumors,[19-22] albeit at lower frequencies. However, the molecular landscape of the full spectrum of *SF3B1*-mutated myeloid disorders has not been thoroughly characterized because most published studies focus on one specific myeloid neoplasm (eg, MDS or MDS/MPN-RS-T).

Furthermore, the majority of studies, although not all, reported an independent association of *SF3B1* mutations with favorable clinical outcomes in MDS.[5,9,23-28] The International Working Group for the Prognosis of MDS (IWG-PM) recently proposed *SF3B1*-mutant MDS (*SF3B1*$^{\text{-mut}}$-MDS) as a distinct disease subtype,[29] based on cumulative data supporting somatic *SF3B1* mutation as a disease-defining genetic abnormality, demonstrated by the following characteristics: (1) it commonly represents a founding genetic lesion, (2) it is a major determinant of disease phenotype characterized by erythroid dysplasia with RS and ineffective erythropoiesis, (3) it showed independent prognostic value on survival and risk of AML progression in the majority of studies, and (4) it may predict response to specific agents such as transforming growth factor-β ligand trap luspatercept.[5,9,23-28,30] The proposed *SF3B1*$^{\text{-mut}}$-MDS diagnostic criteria included (1) cytopenia by standard hematologic values; (2) somatic *SF3B1* mutation; (3) morphologic dysplasia with or without RS; (4) bone marrow blasts less than 5% and peripheral blood blasts less than 1%; and (5) not meeting World Health Organization (WHO) criteria for MDS with isolated del(5q), MDS/MPN, and MPN. Additional exclusion criteria included adverse genetic markers of monosomy 7, inv(3) or abnormalities of chromosome 3q26, complex karyotype (3 or more alterations), and comutation in *RUNX1* and/or *EZH2*. Previous studies in MDS have also described mutations in *FLT3*, *NPM1*, *NRAS*, *PTPN11*, *WT1*, *IDH1*, and *IDH2* as type 1 mutations, given their association with AML progression, and mutations in *RUNX1*, *GATA2*, *ZRSR2*, *TP53*, *STAG2*, *ASXL1*, *KRAS*, and *TET2* as type 2 mutations with enrichment in high-grade MDS.[31,32] Our study aimed to evaluate the spectrum of *SF3B1* mutation-harboring myeloid disorders, to analyze their molecular landscape and associated clinical and laboratory findings, and to further explore the prognostication in MDS-*SF3B1*, including the newly proposed classification of *SF3B1*$^{\text{-mut}}$-MDS by the IWG-PM and prognostic impacts of comutations.

Methods

Case Selection and Data Collection

Following Mayo Clinic institutional review board approval, we retrospectively screened Mayo Clinic cases that had a targeted 35-gene OncoHeme next-generation sequencing (NGS) panel performed for diagnosed or suspected hematologic neoplasms by December 2016. Cases that had *SF3B1* mutations and bone marrow examination were selected. The bone marrow pathologic diagnoses were verified by at least 2 hematopathologists and were based on the 2016 revision of the WHO classification.[33] Patients' clinical courses and pertinent laboratory, pathologic, and clinical findings were collected by chart review.

Cytogenetic Analysis

Fresh bone marrow aspirate samples were cultured and harvested following standard cytogenetic methods, and chromosome preparations were stained using GTL banding with trypsin and Leishman stain. A total of 20 metaphases were analyzed and reviewed for each sample when available.

NGS Analysis

NGS testing was performed using a targeted, myeloid neoplasm–focused OncoHeme panel that interrogates 35 genes, including epigenetic modifiers *ASXL1, DNMT3A, TET2, IDH1, IDH2, EZH2*, and *WT1;* splicing factors *SF3B1, SRSF2, U2AF1*, and *ZRSR2;* transcription factors (TF) *BCOR, CEBPA, ETV6, GATA1, GATA2, NOTCH1*, and *RUNX1;* signaling and kinase factors *BRAF, CALR, CBL, CSF3R, FLT3, JAK2, KIT, KRAS, MPL, MYD88, NRAS*, and *PTPN11;* tumor suppressors *TP53* and *PHF6*; molecular chaperone *NPM1*; telomerase reverse transcriptase

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TERT; and the functionally poorly defined *SETBP1*. DNA was extracted from bone marrow and peripheral blood using Qiagen EZI. The 200-ng sheared DNA was target-enriched with a custom hybridization-capture reagent (SureSelectXT; Agilent) and sequenced on the MiSeq or HiSeq platforms (Illumina) at the Mayo Clinic Clinical Genome Sequencing Laboratory. The read length was 151 bp and 101 bp on the MiSeq and HiSeq platforms, respectively. Sequencing data were processed through a custom bioinformatics pipeline, Mayo NGS Workbench, using CLC Bio Genomics Server v6.0 (Qiagen) for alignment and variant calling. The aligned BAM files were further processed through an in-house developed breakpointSearch tool for large insertion and deletion detection. BAM files of all variant calls were manually reviewed in the genome browser Alamut Visual (Interactive Biosoftware) for confirmation. The limit of detection of the NGS assay is 5% with a minimum 250× depth of coverage. More than 95% of tested regions had greater than 1,000× depth of coverage in the clinical assay. Genetic variants were curated and annotated in the molecular hematopathology laboratory following the American College of Medical Genetics and Genomics 5-tier system.[34]

Statistical Analysis

Numerical variables were presented by median and range; categorical variables were described by count and percentage in each category. The correlation between *SF3B1* variant allele fraction (VAF) percentage and RS percentage were evaluated using the Pearson correlation test. We used the Wilcoxon rank sum or Mann-Whitney *U* test to compare the median hemoglobin level among different *SF3B1* mutations and other numerical and ordinal variables. The Fisher exact test was performed to determine the significance of associations between categorical variables. Overall survival (OS) was defined as the time from the date of diagnosis to the date of death (for patients who were deceased) or last follow-up (for patients who were censored). Progression-free survival (PFS) was defined as the time from the date of diagnosis to the date of leukemic transformation (for patients transformed to AML) or last follow-up (for censored patients). The Kaplan-Meier survival curve and the Cox proportional hazards regression model (log-rank test, both univariate and multivariate analysis) were used to identify the potential impact of different variables on OS and PFS. Statistical significance was based on $P < .05$. Statistical analyses for this study were performed by using JMP Pro software version 14 (SAS Institute).

Results

SF3B1 Mutation Distribution in Myeloid Disorders

Of the 955 patients who had undergone NGS testing for diagnosed or suspected hematologic neoplasms, we identified 75 patients (8%) harboring an *SF3B1* mutation. The mutations were all missense mutations, and the most common ones were K700E (n = 38, 51%), K666R/T/N/Q (n = 15, 20%), H662D/Q/Y (n = 6, 8%), and R625C (n = 5, 7%). Other less frequent mutations included E622D, Y623C, Q659R, K741N, G742D, and D781G. Patients' demographics and clinical and laboratory findings are summarized in **Table 1**. *SF3B1* mutations were detected across the full spectrum of myeloid disorders and comprised 7% (3/42) of clonal cytopenia of uncertain significance (CCUS), 19% (40/206) of MDS, 14% (12/89) of MDS/MPN, 4% (8/202) of MPN, 5% (11/209) of AML, and 11% (1/9) of systemic mastocytosis (SM) cases in the entire study cohort of 955 cases. *SF3B1* mutations were not detected in 121 cytopenia or cytosis cases without evidence of myeloid disorders, 16 cases of aplastic anemia or paroxysmal nocturnal hematuria, or other miscellaneous cases including 18 with low-grade B-cell lymphoproliferative disorder, 14 with plasma cell proliferative disorder, and 10 with B-lymphoblastic lymphoma/leukemia. The disease distribution within the 75 *SF3B1* mutation-positive cases was as follows: 4% CCUS (3/75), 53% MDS (40/75), 16% MDS/MPN (12/75), 11% MPN (8/75), 15% AML (11/75), and 1% SM (1%, 1/75) (Table 1).

We then focused our study on the 75 *SF3B1*-mutated cases of myeloid disorders **Figure 1**. Of the 40 cases with MDS-*SF3B1*, 83% (33/40) were MDS-RS, including 23 MDS-RS–single lineage dysplasia (SLD) and 10 MDS-RS–multilineage dysplasia (MLD). Only 10% (4/40) were high-grade MDS with excess blasts (MDS-EB) including 3 MDS-EB1 and 1 MDS-EB2. The other 3 MDS cases were MDS-MLD, MDS with fibrosis, and MDS unclassifiable. Of the 12 patients with *SF3B1*$^{-mut}$-MDS/MPN, 67% (8/12) had MDS/MPN-RS-T, besides 1 with chronic myelomonocytic leukemia and 3 with MDS/MPN unclassifiable. The 8 cases of MPN included 1 primary myelofibrosis, 1 polycythemia vera (PV), 1 post-PV myelofibrosis, 3 post–essential thrombocythemia myelofibrosis, and 2 MPN unclassifiable. Of the 11 patients with AML, 82% (9/11) had AML with myelodysplasia-related changes (AML-MRC), of whom 6 had a prior history of MDS. The remaining 2 had AML with inv(3)(q21q26.2) and AML not otherwise specified. The SM case was mast cell leukemia.

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Table 1

Demographic, Clinical, Pathologic, and Genetic Characteristics of *SF3B1*-Mutated Myeloid Disorders

	Total	CCUS	MDS	MDS/MPN	MPN	AML	SM
Patients, No. (%)	75	3 (4)	40 (53)	12 (16)	8 (11)	11(15)	1 (1)
Female sex, No.	25	0	15	3	2	4	1
Male sex, No.	50	3	25	9	6	7	0
Age, median (range), y	73 (35-89)	73 (72-76)	74 (47-85)	73 (53-89)	71 (57-80)	69 (35-85)	68
Progression to AML, No.	5	0	5	0	0	NA	0
Death, No.	25	1	11	5	2	6	0
Hemoglobin, median (range), g/dL	NA	9.3 (7.5-9.5)	8.7 (6.0-12.6)	9.0 (6.1-11.4)	9.7 (7.1-17.6)	8.1 (6.5-11.6)	11.8
RDW, median (range), %	NA	15.6 (14.7-16.9)	18.4 (14.1-23.4)	22.6 (16.9-22.8)	19.9 (17.2-25.6)	19.3 (15.7-26.9)	21.3
WBC, median (range), $\times 10^9$/L	NA	3.5 (3.0-33.7)	4.5 (1.2-103.2)	7.1 (2.5-23.0)	10.8 (3.5-41.7)	2.7 (0.2-27.5)	11.2
Platelet count, median (range), $\times 10^9$/L	NA	63 (38-73)	199 (18-632)	435 (156-791)	191 (32-664)	79 (21-274)	32
Ring sideroblasts, median (range), %	NA	0 (0)	16 (0-90)	50 (15-75)	0 (0)	15 (0-30)	0
SF3B1 VAF, median (range), %	NA	26 (6-27)	38 (6-48)	36 (13-48)	34 (6-48)	35 (14-46)	5
SF3B1 mutation position							
p.E622	1		1				
p.Y623	1			1		1	
p.R625	5		4	1			
p.N626	1					1	
p.Q659	1		1				
p.H662	6	1	2		2	3	
p.K666	15		6	4	3	1	1
p.K700	38	1	23	6	3	5	
p.K741	1		1				
p.G742	1		1				
p.D781	1	1	1				
No. of comutations, median (range)	NA	0 (0-1)	1 (0-5)	1 (0-3)	3 (1-5)	2 (1-5)	0
Abnormal karyotype, No.	27	0	8	6	5	8	0

AML, acute myeloid leukemia; CCUS, clonal cytopenia with undetermined significance; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NA, not applicable; RDW, red cell distribution of width; SM, systemic mastocytosis; VAF, variant allele frequency.

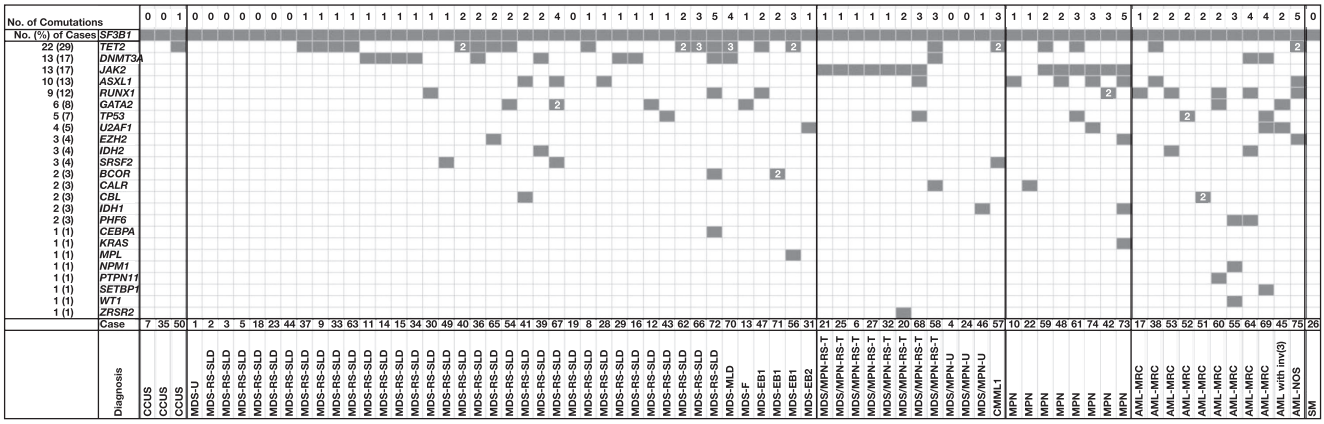

Figure 1 Summary of molecular landscape and pathology diagnoses in 75 patients with *SF3B1*-mutated myeloid disorders. The information of each patient is present in a column. Black boxes indicate the presence of mutations. The numbers in the box indicate the number of mutations of the corresponding gene. AML, acute myeloid leukemia; AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; AML-NOS, acute myeloid leukemia not otherwise specified; CCUS, clonal cytopenia with undetermined significance; CMML1, chronic myelomonocytic leukemia 1; MDS-EB, myelodysplastic syndrome with excess blasts; MDS-F, myelodysplastic syndrome with fibrosis; MDS-MPN-RS-T, myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; MDS/MPN-U, myelodysplastic/myeloproliferative neoplasm, unclassifiable; MDS-RS-MLD, myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia; MDS-RS-SLD, myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia; MDS-U, myelodysplastic syndrome, unclassifiable; MPN, myeloproliferative neoplasm; SM, systemic mastocytosis.

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SF3B1 Mutations and Association With Laboratory Findings

The VAF of *SF3B1* mutations showed no statistically significant differences among various myeloid disorders ($P > .05$). It also did not demonstrate statistically significant associations with patient age, sex, hemoglobin level, RBC distribution width, neutrophil count, platelet count, number of comutations, or cytogenetic abnormalities. The distributions of *SF3B1* mutations were similar among various myeloid disorders, with K700, K666, and H662 consistently being the mutational hotspots (Table 1). Interestingly, among the 3 hotspots, cases harboring K666 (R/T/N/Q) mutations exhibited higher hemoglobin levels than those seen in K700 ($P = .13$) or H662 ($P = .033$) mutations **Figure 2**.

Of 59 cases with adequate bone marrow aspirate that had Prussian blue iron stain performed, 55 (93%) showed the presence of RS, seen in 0 of 1 CCUS, 36 of 38 MDS, 12 of 12 MDS/MPN, 1 of 2 MPN, and 6 of 6 AML cases. The 36 MDS cases comprised 23 MDS-RS-SLD and 10 MDS-RS-MLD with 5% to 90% RS and 3 MDS-EB1 with 4% to 8% RS. All 12 MDS/MPN cases (including 8 MDS/MPN-RS-T) had 15% to 75% RS. All 6 AML cases with RS (range, 5%-30%) were AML-MRC, and 5 had a prior history of MDS. Four cases lacked RS (Supplementary Table 1; all supplemental material can be found at *American Journal of Clinical Pathology* online), including one with CCUS (case 35), one with MDS-MLD (case 70), one with MDS-EB2 (case 31), and one with MPN (PV, case 59), with the PV case showing absence of storage iron. No statistically significant numerical correlation between *SF3B1* mutational VAF percentage and RS percentage was observed (Pearson correlation coefficient, 0.25; $P = .09$). In addition, no statistically significant association between RS percentage and Revised International Prognostic Scoring System (IPSS-R) risk categories was observed (median [range]: 10% [0%-30%] vs 17% [0%-90%]; $P = .09$, IPSS-R greater than 3 vs 3 or lower).

Molecular Landscape of Comutations in *SF3B1*-Mutated Myeloid Disorders

A total of 123 comutations were detected in 26 genes in 62 of 75 *SF3B1*-mutated cases of myeloid disorders, involving *TET2*, *ASXL1*, *DNMT3A*, *EZH2*, *IDH1*, and *WT1* in epigenetic modifiers; *RUNX1*, *GATA2*, *BCOR*, and *CEBPA* in TFs; *JAK2*, *CBL*, *CALR*, *KIT*, *KRAS*, *MPL*, and *PTPN11* in signaling and kinase factors; *U2AF1*, *SRSF2*, and *ZRSR2* in splicing factors; and *TP53*

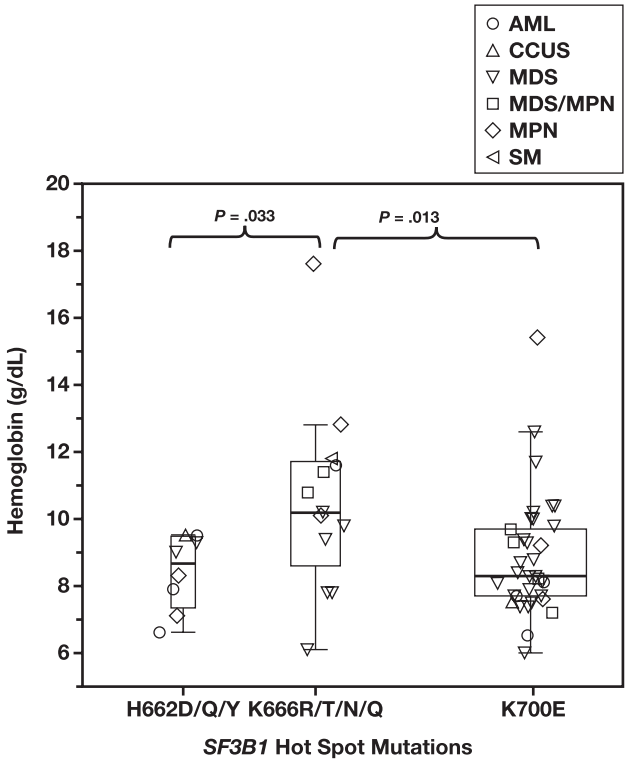

Figure 2 Association between *SF3B1* mutational hotspots and hemoglobin levels. A whisker plot of the hemoglobin levels of 3 *SF3B1* mutational hotspots K700, K666, and H662, showing range, interquartile range, and median of the hemoglobin levels. Different label shapes mark the specific disease categories of the cases.

and *PHF6* in tumor suppressors, in addition to *NPM1* and *SETBP1* (Figure 1). The most frequent comutations occurred in *TET2* (22/75, 29%), *DNMT3A* (13/75, 17%), *JAK2* (13/75, 17%), and *ASXL1* (10/75, 13%). Mutations in these 4 genes accounted for 51% of the total number of comutations. Two or more *TET2* mutations occurred in 7 of 22 cases. Although the *JAK2* mutation was observed exclusively in MPN and MDS/MPN-RS-T, *TET2*, *ASXL1*, and *DNMT3A* mutations were present across different categories of myeloid disorders (Figure 1).

In terms of disease, only 1 of 3 CCUS cases showed one comutation in *TET2*. Of the 40 MDS-*SF3B1* cases, comutations were seen in *TET2* (n = 16); *DNMT3A* (n = 10); *GATA2* (n = 4); *ASXL1* (n = 3); *RUNX1* (n = 3); *BCOR* (n = 2); and *EZH2*, *TP53*, *U2AF1*, *IDH2*, *CBL*, *CEBPA*, *SRSF2*, *MPL*, and *TERT* (n = 1 for each), in descending order of frequency. As for the 11 AML cases, comutations were detected in *RUNX1* (n = 5); *TET2* (n = 2); *DNMT3A* (n = 2); *ASXL1* (n = 2); *IDH2* (n = 2); *GATA2* (n = 2); *TP53* (n = 2); *PHF6* (n = 2); *U2AF1* (n = 2); and *EZH2*, *CBL*, *NPM1*, *PTPN11*, *SETBP1*,

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and *WT1* (n = 1 for each). Type 1 mutations[31] associated with AML progression were seen in *IDH2*, *NPM1*, *PTPN11*, and *WT1* and were present mainly in AML rather than MDS cases (5/11 vs 1/40; $P = .001$). In the 12 MDS/MPN cases, comutations were present in *JAK2* (n = 7); *TET2* (n = 2); and *CALR*, *ASXL1, TP53*, *IDH1*, *SRSF2*, and *ZRSR2* (n = 1 for each). Among the 8 MPN cases, comutations occurred in *JAK2* (n = 6); *ASXL1* (n = 4); *TET2* (n = 2); and *CALR*, *RUNX1*, *EZH2*, *TP53*, *U2AF1*, *CEBPA*, *IDH1*, and *KRAS* (n = 1 for each). The number of comutations in the 75 *SF3B1*-mutated cases of myeloid disorders ranged from 0 to 5 with a median (range) of 0 (0-1) in CCUS, 1 (0-5) in MDS, 1 (0-3) in MDS/MPN, 3 (1-5) in MPN, and 2 (1-5) in AML (Figure 1). The numbers of comutations in MDS were lower than those in MPN ($P = .015$) and AML ($P = .004$). The MDS/MPN cases also had fewer comutations than those in AML ($P = .037$). Twenty-seven of the 75 *SF3B1*-mutated cases of myeloid disorders had chromosome abnormalities and did not show association with the numbers of comutations ($P = .65$) (Supplementary Table 1).

Prognostic Factors in MDS-*SF3B1*

Among the 40 MDS-*SF3B1* cases, 36 had available cytogenetics data. Their median (range) IPSS-R score was 2.3 (1-7). With a cutoff score of 3, the group with IPSS-R score greater than 3 (intermediate, high, or very high IPSS-R risk category, n = 8) showed an adverse outcome with a median OS of 25 months, in contrast to 73 months observed in the group with a score of 3 or lower (very low or low IPSS-R risk category, n = 28; $P = .002$) **Figure 3A**. In addition, during a median (range) follow-up time of 37 months (0.3-127 months), 4 of 40 patients with MDS-*SF3B1* progressed to AML, including 1 with MDS-EB1, 1 with MDS with fibrosis, and 2 with MDS-RS-SLD **Table 2**. Median PFS for the 2 groups was 20 months vs not reached, respectively ($P = .006$, IPSS-R score greater than 3 vs 3 or lower) **Figure 3C**.

In terms of mutations, *SF3B1* mutational VAF and number of comutations showed no effect on OS or PFS (data not shown; $P > .05$). The most commonly comutated genes by pathway involved epigenetic modifiers (28/40; *TET2*, *DNMT3A*, *ASXL1*, *EZH2*, and *IDH2*) and TF (8/40; *RUNX1*, *GATA2*, *BCOR,* and *CEBPA* [coexisting with *RUNX1* and *BCOR*]) (Table 2 and Supplementary Table 1). Comutations in other pathways were infrequent, including 2 in signaling and kinase factors (*CBL, MPL*), 2 in splicing factors (*U2AF1, SRSF2*), and one in tumor suppressor (*TP53*) (Figure 1). Although the presence of comutations in epigenetic modifiers showed no impact on clinical outcomes (data not shown; $P > .05$), the presence of TF comutations demonstrated inferior OS (median, 25 vs 73 months; $P = .0003$) **Figure 3B** and PFS (median, 26 months vs not reached; $P = .0001$) **Figure 3D** in comparison to MDS-*SF3B1* cases without TF comutations.

Because the IWG-PM recently proposed *SF3B1*$^{\text{-mut}}$-MDS as a distinct disease subtype with specified inclusion and exclusion criteria,[29] we evaluated the prognostic impact of this new entity following the proposed diagnostic criteria in our study cohort with *SF3B1*$^{\text{-mut}}$ MDS. Among the 40 cases of MDS-*SF3B1*, 27 fulfilled the diagnostic criteria for IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS (Supplementary Table 1). Ten were excluded (IWG-PM–excluded MDS-*SF3B1*) according to the IWG-PM exclusion criteria, based on the presence of complex karyotype (cases 12, 43, and 54), *RUNX1* mutation (cases 30 and 72), *EZH2* mutation (case 65), and MDS-EB (cases 31, 47, 56, and 71). Three cases could not be classified because of a lack of cytogenetic data and failure to meet any exclusion criteria. The IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS cases demonstrated favorable clinical outcomes with a median OS of 73 months and unreached median PFS, similar to the data reported from the IWG-PM study.[29] Interestingly, the IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS cases did not show statistically significant differences from the IWG-PM–excluded MDS-*SF3B1* cases in either OS (median, 73 vs 54 months; $P = .22$, IWG-PM defined vs excluded) or PFS (median, both not reached; $P = .41$, IWG-PM defined vs excluded). Given the observed adverse impact of TF comutations, we explored the possible added prognostic impact of TF comutations in this context. By adding the presence of TF comutation into the exclusion criteria (*RUNX1* already in the IWG-PM exclusion criteria, *GATA2* and *BCOR* comutations added), statistically significant superior OS was seen in the refined IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS group in comparison to the refined IWG-PM–excluded MDS-*SF3B1* group (median, not reached vs 37 months; $P = .02$, n = 25 vs n = 12). PFS stayed not reached in both groups ($P = .08$).

To further define the prognostic contribution of various factors to OS in MDS-*SF3B1*, we performed univariate and multivariate analyses using a Cox proportional hazards regression model **Table 3**. Univariate analyses of hazard ratio (HR) for OS included age, sex, hemoglobin level, neutrophil count, platelet count, bone marrow blast count, RS percentage, cytogenetics risk, number of comutations, *SF3B1* mutation VAF, epigenetic modifier comutation status, TF comutation status, IPSS-R score, and IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS status. The presence of TF comutations and IPSS-R score greater than 3 were shown to be poor prognostic markers, with HRs of 9.67 ($P = .003$) and 7.88 ($P = .008$), respectively.

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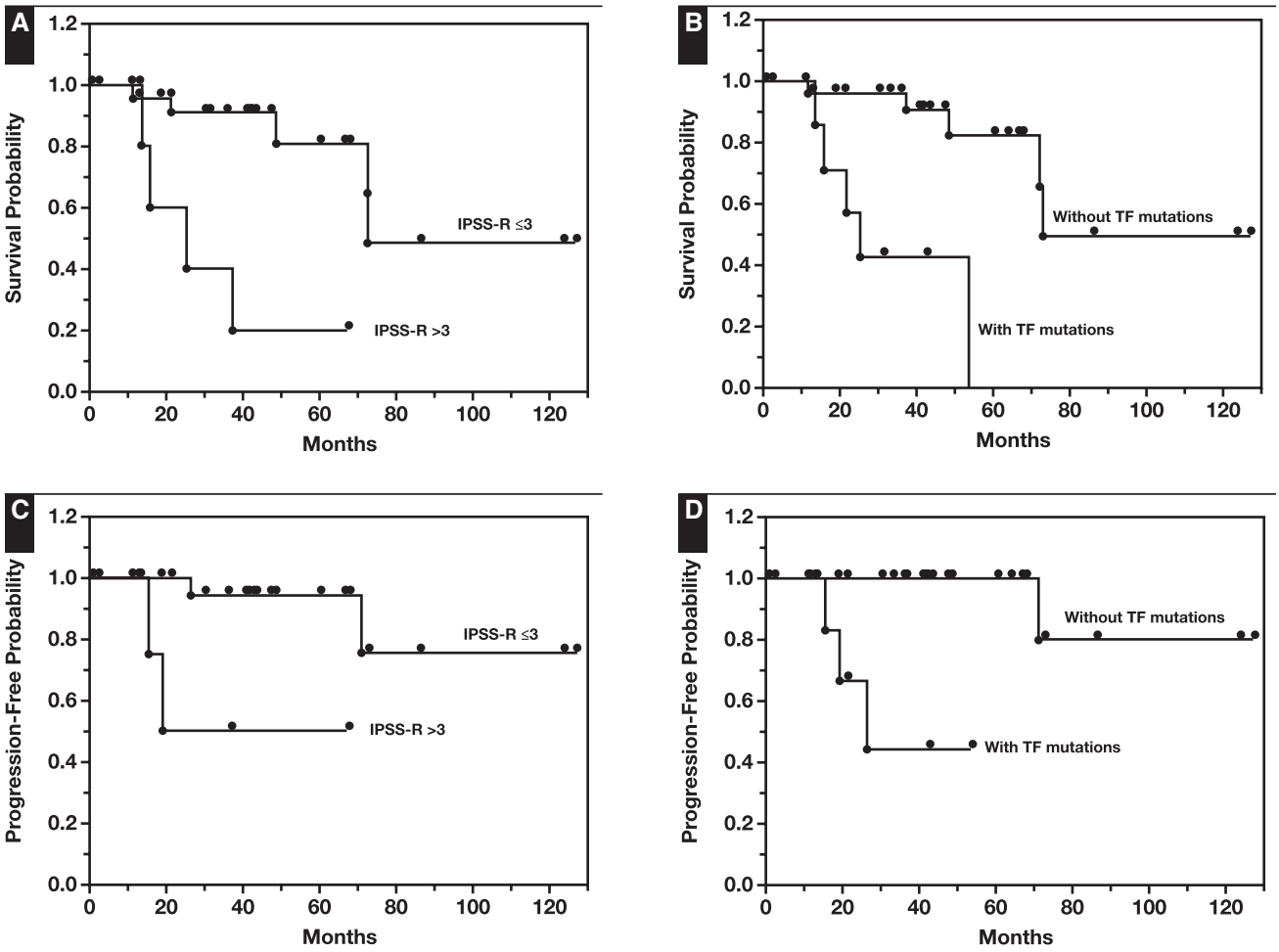

Figure 3 Clinical impact of Revised International Prognostic Scoring System (IRSS-R) and presence of transcription factor (TF) comutations on overall survival (OS) and progression-free survival (PFS) in myelodysplastic syndrome (MDS) harboring *SF3B1* mutations (MDS-*SF3B1*). **A**, OS in patients with IPSS-R scores greater than 3 vs 3 or lower ($P = .002$). **B**, OS in patients with TF comutations vs without TF mutations ($P = .0003$). **C**, PFS in patients with IPSS-R scores greater than 3 vs 3 or lower ($P = .006$). **D**, PFS in patients with TF comutations vs without TF mutations ($P = .0001$).

Neutrophil count and RS percentage showed a minimal impact (HR, 1.04 [$P = .012$] and 0.96 [$P = .04$], respectively). In the multivariate analysis, IPSS-R score greater than 3 retained its clinical validity, showing an HR of 5.12 ($P = .049$), and the presence of TF comutations demonstrated independent prognostic value even after adjustment for IPSS-R (HR, 9.14; $P = .04$).

Discussion

In this study, we evaluated the distribution of *SF3B1* mutations in 955 patients who had undergone NGS testing for hematologic neoplasms. We identified 75 cases harboring an *SF3B1* mutation, spanning the full pathologic spectrum of myeloid disorders from CCUS to AML. Consistent with previous reports, *SF3B1* mutations were highly associated with MDS-RS and MDS/MPN-RS-T (55%, 41/75), supporting a causal relationship between *SF3B1* mutations and RS formation.[5] Among the 40 MDS-*SF3B1* cases, 90% were low-grade disease; only 4 (10%) had the high-grade diagnosis of MDS-EB, concordant with previous reports.[29,35] The frequencies of *SF3B1* mutations observed in AML and MPN were also on par with previous reports,[5] supporting the representative nature of our study cohort. It was striking to see that 9 of 11 AML cases were AML-MRC, with 6 having evolved from a precedent MDS. This observation echoes the findings of Lindsley et al,[18] who showed that *SF3B1* mutation along with several other mutations in AML were more than 95% specific for secondary AML and conferred a worse clinical outcome. The diagnoses of the 3 CCUS cases were established by the presence of *SF3B1* mutations in the absence of cytogenetic or definitive

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Table 2

Genetic Findings in Patients With Myelodysplastic Syndrome Harboring *SF3B1* Mutations With TF Comutations and/or Progression to AML

Case No.	Age, y/ Sex	Diagnosis	Chromosome Results	NGS Results	Time to AML, mo
47	76/M[a]	MDS-EB1	46,XY[20]	*RUNX1*:p.R174Qln (37%) *SF3B1*: p.K666N (42%) *TET2*: p.Q1020[a] (41%)	26.4
13	79/M[a]	MDS-F	46,Y,t(X;8)(q22;q24.1)[12]/46,XY[8]	*GATA2*: p.S251Cfs[a]29 (38%) *SF3B1*:p.K700E (46%)	15.6
54	76/F[a]	MDS-RS-SLD	46,XX,-2,-20,+2mar[2]/46,XX[18]	*GATA2*:p.L321F (6%) *SF3B1*:p.K700E (10%) *TET2*: p.P1575Qfs[a]21 (10%)	19.2
67	80/F[a]	MDS-RS-SLD	46,XX[20]	*ASXL1*: p.G646Wfs[a]12 (34%) *GATA2*:p.A234Gfs[a]45 (32%) *GATA2:* p.T387_M388del (34%) *SF3B1*: p.K666N (48%) *SRSF2*: p.P95A (46%)	NA
12	59/M[a]	MDS-RS-MLD	46,XY,+1,der(1;7)(q10;p10)[6]/47,idem,+8[14]	*GATA2*: p.K389_Q394del (23%) *SF3B1*: p.K700E (28%)	NA
30	73/M[a]	MDS-RS-SLD	ND	*RUNX1*:p.G172Q (40%)*SF3B1*: p.H662Q (41%)	NA
72	77/F[a]	MDS-RS-MLD	46,XX,del(11)(q13q23)[3]/46,XX[17]	*BCOR*: p.N575Qfs[a]36 (34%) *CEBPA*: p.F31Gfs[a]72 (15%) *DNMT3A*: p.E774[a] (41%) *RUNX1*:p.R174Q (38%) *SF3B1*:p.K700E (39%) *TET2*: p.S657Hfs[a]43 (34%)	NA
71	77/M[a]	MDS-EB1	46,XY[20]	*BCOR:* p.Q1653Kfs[a]21 *(41%)* *BCOR*: p.R1661[a] (5%) *SF3B1:* p.R625C (38%)	NA
3	68/M	MDS-RS-SLD	46,XY[20]	*SF3B1*: p.K700E (38%)	47.5

AML, acute myeloid leukemia; MDS-EB1, myelodysplastic syndrome with excess blasts-1; MDS-F, myelodysplastic syndrome with fibrosis; MDS-RS-MLD, myelodysplastic syndrome with ring sideroblasts–multilineage dysplasia; MDS-RS-SLD, myelodysplastic syndrome with ring sideroblasts–single lineage dysplasia; NGS, next-generation sequencing; NA, not applicable; ND, not determined; TF, transcription factor.
[a]Cases harboring TF mutations.

morphologic abnormalities.[36,37] *SF3B1* mutation has been shown to be one of the most frequent mutations in clonal hematopoiesis of indeterminate potential (CHIP), a premalignant condition that occurs in elderly individuals without hematologic malignancies and confers an increased risk of subsequent development of hematologic malignancies.[38-41] The observed wide distribution of *SF3B1* mutations across the full spectrum of myeloid disorders, along with their frequent occurrence in CHIP, underscores the biology that *SF3B1* mutations occur early in MDS development as founder mutations.[42-44]

SF3B1 mutation hotspots involved K700E, K666, and H662, similar to previous reports.[5,6,9,12,45] We did not see disparate hotspot patterns among the various myeloid disorders. Interestingly, for the first time, an association between K666 mutations and higher hemoglobin levels was seen, suggesting that these mutations may exert less disruptive effects on erythropoiesis. Although some studies showed similar aberrant splicing patterns among mutants of the 3 hotspots, a recent study described a distinct missplicing pattern in K666N.[8,46] Further studies are required to confirm this finding and understand its clinical significance.

The molecular landscape of *SF3B1*-mutated myeloid disorders showed unique but overlapping features (Figure 1). Of 123 comutations, *TET2*, *DNMT3A*, *JAK2*, and *ASXL1* accounted for 51% of total comutations. The occurrence of epigenetic modifier *DNMT3A, TET2*, and *ASXL1* (DTA) mutations across the broad spectrum of myeloid disorders was not surprising because these mutations have also been shown to be founder mutations in the early development of myeloid neoplasms. Similarly, these genes are the most frequently mutated genes in CHIP.[32,38-42,47] Persistent DTA mutations in AML minimal residual disease monitoring also failed to predict disease relapse.[48] The enrichment of type 1 mutations in AML was in keeping with their reported association with AML progression.[31,32] *JAK2* comutations unsurprisingly and exclusively occurred in MPN and MDS/MPN-RS-T.[49-54] The lower numbers of comutations seen in MDS and MDS/MPN are likely attributable to the enrichment of lower grade diseases innately associated with *SF3B1* mutations (MDS-RS and MDS/MPN-RS-T). The higher numbers of comutations seen in AML and MPN reflect clonal heterogeneity and complexity during disease evolution.

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Table 3

Cox Proportional Hazards Ratio Analyses of Variables in Myelodysplastic Syndrome Harboring *SF3B1* Mutations Prognostic of Overall Survival

Risk Factor	Hazard Ratio	95% CI	*P*
Univariate analysis			
Age, <70 vs ≥70 y	1.80	0.38-8.56	0.46
Sex, M/F	1.08	0.29-4.03	0.91
Hemoglobin, <10 vs ≥10 g/dL[a]	3.16	0.92-14.69	0.095
Neutrophil count, <0.8 vs ≥0.8 × 10^9/L[a]	1.04	1.01-1.08	0.012
Platelet count, <50 vs 50~100 vs ≥100 × 10^9/L[a]	3.75	0.45-19.34	0.19
Bone marrow blast count[a]	0.91	0.05-5.07	0.92
Ring sideroblasts, %	0.96	0.92-1.00	0.04
Cytogenetic risks, high/very high vs intermediate/low/very low[a]	4.72	0.53-42.40	0.17
Number of comutations	1.26	0.74-1.98	0.79
SF3B1 VAF. %[b]	0.72	0.09-8.80	0.78
Epigenetic comutations[c]	0.72	0.18-2.89	0.64
TF comutations[c]	9.67	2.23-41.94	0.003
IPSS-R score, >3 vs ≤3	7.88	1.72-35.33	0.008
SF3B1^{-mut}-MDS by IWG-PM[d]	0.46	0.13-1.61	0.23
Multivariate analysis			
TF comutations[c]	9.14	1.1-76.1	0.04
IPSS-R score, >3 vs ≤3	5.12	1.0-26.1	0.049
SF3B1^{-mut}-MDS by IWG-PM[d]	1.43	0.3-6.76	0.64

IPSS-R, Revised International Prognostic Scoring System; IWG-PM, International Working Group for the Prognosis of MDS; *SF3B1*$^{-mut}$-MDS, *SF3B1*-mutant myelodysplastic syndrome; TF, transcription factor; VAF, variant allele fraction.
[a]IPSS-R categories.
[b]Range hazard ratio.
[c]Present vs absent.
[d]Yes vs no.

SF3B1 mutations are the most common mutations in MDS, as seen in approximately 20% to 25% of cases. Consequently, MDS-*SF3B1* comprises the largest MDS subgroup by genetic abnormalities. We explored the prognostication in this MDS subgroup. Significantly inferior outcomes for OS and PFS were seen in MDS-*SF3B1* with IPSS-R scores greater than 3 (intermediate, high, and very high risk) in comparison to IPSS-R scores of 3 or lower (low or very low risk); this finding is concordant with that reported in the IWG-PM cohort, for which the favorable prognostic value of *SF3B1* mutation was maintained only in the low or very low risk categories of MDS-*SF3B1*.[29] In addition, in our study, the presence of TF comutations was associated with inferior OS and PFS. The prognostic value of IPSS-R and TF comutations in MDS-*SF3B1* was confirmed in both univariate and multivariate analyses, and TF comutations were shown to be independent adverse prognostic markers even after adjustment for IPSS-R.

IWG-PM recently proposed *SF3B1*$^{-mut}$-MDS as a distinct disease subtype with specific inclusion and exclusion criteria, and not all MDS-*SF3B1* cases qualify as *SF3B1*$^{-mut}$-MDS. In our independent cohort of MDS-*SF3B1*, we confirmed the favorable outcome of IWG-PM–defined *SF3B1*$^{-mut}$-MDS with similar OS and PFS observed. We were interested in what a head-to-head outcome comparison in MDS-*SF3B1* would reveal between the IWG-PM–defined *SF3B1*$^{-mut}$-MDS and those excluded per IWG-PM exclusion criteria[29] (IWG-PM–excluded MDS-*SF3B1*). Interestingly, we did not observe a statistically significant difference in OS (median, 73 vs 54 months; $P = .22$) or PFS (neither median reached; $P = .41$) among the 2 subgroups. When we explored prognostic refinement of the IWG-PM–defined *SF3B1*$^{-mut}$-MDS in MDS-*SF3B1* by adding TF comutations into the exclusion criteria (*GATA2* and *BCOR* added, *RUNX1* already in the IWG-PM exclusion criteria), statistically significant favorable OS was observed in the refined IWG-PM–defined *SF3B1*$^{-mut}$-MDS group.

We cannot completely rule out that the lack of a demonstrable statistically significant prognostic differentiation among IWG-PM–defined *SF3B1*$^{-mut}$-MDS, and IWG-PM–excluded MDS-*SF3B1* may be related to the relatively small size of our MDS-*SF3B1* cohort; however, it is worth noting that such an outcome comparison between the 2 subgroups of MDS-*SF3B1* was not reported by the IWG-PM study.[29] Furthermore, consistent with the IWG-PM study, we independently confirmed the adverse prognostic value of IPSS-R greater than 3 in MDS-*SF3B1* and the favorable clinical outcome of IWG-PM–defined *SF3B1*$^{-mut}$-MDS, with similar median OS and PFS in our study. These findings support an overall representative nature of our study cohort. The constellation of findings from our study and previous studies, including the strong association between

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SF3B1 mutations and RS, support recognition of *SF3B1*$^{\text{-mut}}$-MDS as a distinct disease entity genetically, phenotypically, prognostically, and potentially therapeutically, as proposed by IWG-PM. However, based on its current diagnostic criteria, this designation may not provide sharp prognostication within MDS-*SF3B1*. This may not be surprising, as the IWG-PM exclusion criteria were based on previous reports and not all were fully validated in the IWG-PM cohort—for example, among the 3 excluding cytogenetic abnormalities monosomy 7, inv(3)/3q26 abnormalities, and complex karyotype, only monosomy 7 was fully confirmed.[29] The IWG-PM diagnostic criteria also exclude the prognostically favorable MDS with del(5q) in which *SF3B1* mutations were reported in approximately 20% of cases. In our study, IPSS-R outperformed the IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS designation in the prognostication of MDS-*SF3B1*. We also identified TF comutations as a novel risk marker with independent prognostic value in MDS-*SF3B1*, which may further refine the risk stratification of IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS. Because the TF gene *RUNX1* is already included in the IWG-PM exclusion criteria, our data confirmed its validity as an exclusion criterion and suggested the possibly improved prognostic value of expanding the exclusion criteria to additional TF markers *GATA2* and *BCOR*. Given our relatively small study cohort size, further evaluation in large, well-characterized patient cohorts is warranted to evaluate the clinical significance and biological basis of TF comutations and other informative prognostic markers in this patient cohort for improved prognostication, monitoring, and clinical management.

Biologically, it is conceivable that disturbed transcription activities secondary to TF mutations in *RUNX1, GATA2*, or *BCOR* may potentiate the deleterious effects from the aberrant transcript splicing of *SF3B1* mutants and confer worse prognosis. Somatic mutations in *RUNX1* were shown to be independent poor prognostic risk factors in MDS.[25] Consistent with our findings, comutations of *RUNX1* demonstrated negative prognostic value in MDS-*SF3B1* in subsequent studies and were included in the IWG-PM *SF3B1*$^{\text{-mut}}$-MDS exclusion criteria.[23,24,29] As a master TF, balanced *GATA2* expression is required for proper hematopoiesis, and its disruption may result in tumorigenesis. Somatic mutations in *RUNX1* and *GATA2* are considered type 2 mutations, given their association with progression from low- to high-risk MDS.[31,32] *BCOR* mutations have also been shown to be associated with inferior outcomes in MDS.[55] These findings provide supporting evidence for our observation of TF comutation as a negative prognostic marker in MDS-*SF3B1*. One *BCOR* and *RUNX1* comutated case (case 72) had a subclonal *CEBPA* mutation. Given its presence in a single case and subclonal nature, the prognostic impact of *CEBPA* was unclear in our cohort. Although germline mutations in *RUNX1*, *GATA2,* and *CEBPA* may occur in myeloid neoplasms with germline predisposition,[33] and germline mutations in *BCOR* occur in oculofaciocardiodental syndrome,[56] patients' clinical and family histories, age, and sex and/or mutational VAF of the TF comutations all supported a somatic nature of these TF comutations.

In conclusion, we report the presence of *SF3B1* mutations across the full spectrum of myeloid disorders, ranging from CCUS to AML. *SF3B1* mutations are highly associated with RS and the disease types of MDS-RS, MDS/MPN-RS-T, and AML-MRC. In MDS-*SF3B1*, IPSS-R greater than 3 and TF comutations were shown to be adverse prognostic markers. We also independently validated the favorable outcome of the new entity *SF3B1*$^{\text{-mut}}$-MDS as proposed by IWG-PM. However, the IWG-PM–defined *SF3B1*$^{\text{-mut}}$-MDS designation may not provide a sharp prognostic differentiation within MDS-*SF3B1* among those included and excluded for this new entity following the IWG-PM exclusion criteria. Pending further verification in larger cohort studies, the IWG-PM–proposed *SF3B1*$^{\text{-mut}}$-MDS exclusion criteria may benefit from refinement by other informative markers such as TF comutations in *GATA2* and *BCOR* besides *RUNX1*. Our data add to the growing body of evidence demonstrating the value of molecular analysis and incorporation of the pertinent prognostic markers into the MDS risk stratification scheme to further refine and improve personalized medicine.

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