SF3B1-mutant myelodysplastic syndrome/myeloproliferative neoplasms: a unique molecular and prognostic entity

Molecular abnormalities are prognostically relevant in morphological subtypes of myelodysplastic/myeloproliferative neoplasms (MDS/MPN), giving rise to contemporary molecularly integrated prognostic models.[1-3] Established adverse prognostic associations include truncating *ASXL1* mutations in chronic myelomonocytic leukemia (CMML)[1] and MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T),[3] *TP53* and *CBL* mutations in unclassifiable MDS/MPN (MDS/MPN-U),[4] and *TET2* mutations in *BCR-ABL1*-negative atypical chronic myeloid leukemia.[2] Recently, molecular signatures have been used to further stratify MDS/MPN-U patients into CMML-like (*ASXL1*, *SRSF2*, *RUNX1*, and/or *NRAS* mutant), MDS/MPN-RS-T-like (*JAK2* and/or *SF3B1* mutant), atypical chronic myeloid leukemia-like (*SETBP1* and/or *ASXL1* mutant), *TP53* mutant and an "others" category.[5,6] Despite their prognostic impact, these mutations are not specific for underlying disease entities. Recently, *SF3B1* mutations were shown to be disease-defining in a subset of patients with MDS[7,8] and CMML.[9] Whether *SF3B1* mutations are similarly disease-defining in other myeloid subgroups is not known. Given the relative rarity of MDS/MPN patients, we assembled a large, molecularly annotated cohort of MDS/MPN patients to assess the clinical and prognostic impact of *SF3B1* mutations, agnostic of disease morphology.

After Mayo Clinic institutional review board approval, clinical data from adult (age at diagnosis >18 years) patients with a World Health Organization (WHO)-defined diagnosis of MDS/MPN (CMML, MDS/MPN-U and MDS/MPN-RS-T), from 1994 to 2020, were included in the analysis. Patients with atypical chronic myeloid leukemia were excluded due to lack of uniform genetic annotation, limited *SF3B1* mutations (n=2), and patient numbers (n<50). A separate cohort of *SF3B1*-mutant MDS patients diagnosed between 1994 to 2017 was included for comparison. An external cohort of patients from H. Lee Moffitt Cancer Center (Tampa, FL, USA) was used for independent validation after institutional review board approval. Next-generation sequencing for myeloid relevant genes was done at diagnosis or first referral, using institutional or commercially available myeloid malignancy-specific gene panels according to previously published methods.[4] The distribution of continuous variables was statistically compared using nonparametric (Mann-Whitney or Kruskal-Wallis) tests, while nominal variables were compared using the χ^2 test. Time-to-event analyses (for overall [OS] and acute myeloid leukemia-free survival [LFS]) were performed using the method of Kaplan-Meier, with death (for OS), transformation to acute myeloid leukemia (for LFS), and allogeneic hematopoietic stem cell transplantation (for both OS and LFS) used as censors.

Overall, 778 consecutive WHO-defined MDS/MPN patients were included in the primary cohort (CMML, n=578 [74%]; MDS/MPN-RS-T, n=79 [10%] and MDS/MPN-U, n=121 [16%]). The median age was 72 (range, 18-95) years with 511 (66%) males (Table 1). Four (3%) patients in the MDS/MPN-U group met proposed criteria for oligomonocytic CMML and had an absolute monocyte count between 0.5 to 0.9 x 10^9/L, with monocytes constituting >10% of the total white blood cell count.[10] Cytogenetic abnormalities (excluding sole -Y) were present in 197 (28%) of 695 assessable patients; 138 (70%) patients with a single karyotypic abnormality, 35 (18%) with a complex karyotype (defined as ≥3 independent structural/numerical abnormalities, excluding autosomal monosomies) and 26 (13%) with monosomal karyotypes, with frequent cytogenetic abnormalities including 51 (26%) +8, 49 (25%) -7/7q-, 23 (12%) 20q-, 12 (6%) 5q- (2 as sole abnormalities, classified as MDS/MPN based on morphology), 11 (6%) 13q-, 6 (3%) inv(3)/3q26 (3 *GATA2-EVI1* fusion), and 5 (3%) with-11/11q23 (*KMT2A*). Cytogenetic risk stratification as per the CMML-specific scoring system (CPSS) cytogenetic stratification[11] was predictive of OS (P<0.0001) in our cohort with 498 (72%) in the low-risk category (median OS 41 months [95% CI: 32-50]), 120 (17%) in the intermediate-risk category (median OS 21 months [95% CI: 16-33]), and 77 (11%) in the high-risk category (median OS 16 months [95% CI: 11-23]). Next-generation sequencing information at diagnosis was available for 444 (57%) patients with frequent molecular abnormalities being *ASXL1* (n=235; 45%), *SRSF2* (n=179; 40%), *TET2* (n=155; 39%), *SF3B1* (n=78, 15%), and *DNMT3A* (n=30, 7%) mutations (Table 1). At last median follow-up of 44 (95% CI: 37-50) months, transformation to acute myeloid leukemia had occurred in 123 (16%) patients, and 414 (53%) deaths had been documented. The Kaplan-Meier estimate of median OS was 32 (95% CI: 28-38) months (CMML 31 [95% CI: 27-37] months, MDS/MPN-RS-T 67 [95% CI: 43-101] months, and MDS/MPN-U 25 [95% CI 21-36] months), while the median was not reached for LFS. In the MDS/MPN cohort, there were 78 patients with *SF3B1* mutations: 18 (23%) with CMML, 45 (58%) with MDS/MPN-RS-T, and 15 (19%) with MDS/MPN-U. There were 15 *SF3B1* mutation hotspots (evaluable in 53 patients) with the most common abnormalities being K700E (n=24, 45%), H662Q (n=8, 15%), and K666R (n=6, 11%). The clinical and genomic characteristics are outlined in *Online Supplementary Table S1*.

We then combined all *SF3B1*-mutant MDS/MPN patients into one category (n=78) and compared them to their wild-type counterparts (n=446) (Table 1). The two groups had significant differences in clinical and molecular features as highlighted in Table 1. The median variant allele frequency (VAF) of mutant *SF3B1* was 43% (range, 8-65) overall, being 43% (range, 8-65) in CMML patients, 43% (range, 12-50) in MDS/MPN-RS-T patients, and 40% (range, 16-52) in MDS/MPN-U patients (P=0.9), and was comparable to the median variant allele frequency of mutant *ASXL1* at 37% (range, 11-52): CMML 37% (range, 27-37), MDS/MPN-RS-T 32% (range, 18-52), and MDS/MPN-U 29% (range, 11-43). As expected, a higher frequency of *SF3B1*-mutant *versus* *SF3B1*-wild type MDS/MPN patients (21% *vs.* 2%, P<0.0001) were treated with lenalidomide and erythropoiesis-stimulating agents (64% *vs.* 39%, P<0.0001), but the frequency of hypomethylating agent therapy use was similar (21% *vs.* 32%, P=0.1) (Table 1). The *SF3B1* mutant cohort had a lower rate of transformation to acute myeloid leukemia (5% *vs.* 18%, P=0.0006) in comparison to the *SF3B1*-wild type cohort. The Kaplan-Meier estimates of LFS (median not reached in both groups, P=0.0002) and OS (57 *vs.* 31 months, P=0.03) were higher in the *SF3B1*-mutant MDS/MPN patients (Table 1 and Figure 1). These findings were validated in an external MDS/MPN cohort from Moffitt Cancer Center comprising 380 patients, 253 with CMML, 80 with MDS/MPN-RS-T, and 47 with MDS/MPN-U. The validation cohort was similar to the Mayo Clinic cohort in terms of age (P=0.4) and median follow-up (P=0.1). Importantly, *SF3B1*-mutant VAF was not predictive of OS in either the Mayo Clinic cohort

Table 1. Comparison of the clinical and morphological characteristics of patients with *SF3B1* mutant or wild-type myelodysplastic syndromes/myeloproliferative neoplasm compared with patients with *SF3B1*-mutant myelodysplastic syndromes.

Variable; N (%) or median (range)	*SF3B1* mutant MDS/MPN patients (n=78)	*SF3B1* wild-type MDS/MPN patients (n=446)	*SF3B1* mutant MDS (n=75)	*P* value (*SF3B1* mutant *vs.* wild-type MDS/MPN)	*P* value (*SF3B1* mutant MDS/MPN *vs.* *SF3B1* mutant MDS
Age, years	74 (43-93)	72 (18-95)	74 (41-94)	0.3	0.8
N. of males	42 (54)	294 (66)	48 (64)	<0.0001*	<0.0001*
Hemoglobin, g/dL	9.4 (6.4-13.3)	10.6 (4.2-16.9)	9.5 (7-13.5)	<0.0001*	0.7
WBC count x 10^9/L	7.6 (1.8-96.1)	13 (1-265)	5.2 (1.5-13.1)	<0.0001*	<0.0001*
ANC x 10^9/L	4 (0.4-54.7)	6.5 (0-151)	2.9 (0.4-9.4)	0.001*	0.0004*
AMC x 10^9/L	0.7 (0.1-11.5)	2.3 (0-40)	0.4 (0.06-1)	<0.0001*	<0.0001*
Platelet count x 10^9/L	521 (63-1243)	98 (8-1778)	268 (62-599)	<0.0001*	<0.0001*
BM RS, %	50 (0-90)	0 (0-80)	40 (5-80)	<0.0001*	0.4
PB blasts ≥1%	9 (12)	130 (29)	-	0.0005*	0.0003*
BM blasts ≥5%	8 (10)	150 (34)	-	<0.0001*	<0.0001*
Abnormal karyotype (except -Y, %), Evaluable=695	11 (15)	123 (30)	11 (15)	0.0004*	0.7
Treatment (total evaluable=619)					
Hydroxyurea	33 (47)	170 (55)	1 (2)	0.4	<0.0001*
ESA	46 (64)	120 (39)	52 (84)	<0.0001*	<0.0001*
Lenalidomide	15 (21)	6 (2)	9 (15)	<0.0001*	0.006*
HMA therapy	15 (21)	94 (32)	10 (16)	0.1	0.03*
Allogeneic HCT	-	25 (6)	-	0.01*	<0.0001*
Investigational agents (clinical trial)	2 (3)	27 (9)	-	0.1	<0.0001*
Outcomes					
Transformation to AML	4 (5)	79 (18)	2 (3)	0.4	0.4
AML-free survival, months	Median NR	Median NR	Median NR	0.0002*	0.3
Overall survival, months; median (95% CI)	57 (30-68)	31 (26-36)	65 (43-85)	0.03*	0.2

MDS: myelodysplastic syndromes; MPN: myeloproliferative neoplasms; WBC: white blood cell; PB: peripheral blood; BM: bone marrow; ANC: absolute neutrophil count; AMC: absolute monocyte count; RS: ring sideroblasts; BM: bone marrow; ESA: erythropoiesis-stimulating agent; HMA: hypomethylating agent; HCT: hematopoietic stem cell transplant; AML: acute myeloid leukemia; NR: not reached; 95% CI: 95% confidence interval.. *Statistically significant differences.

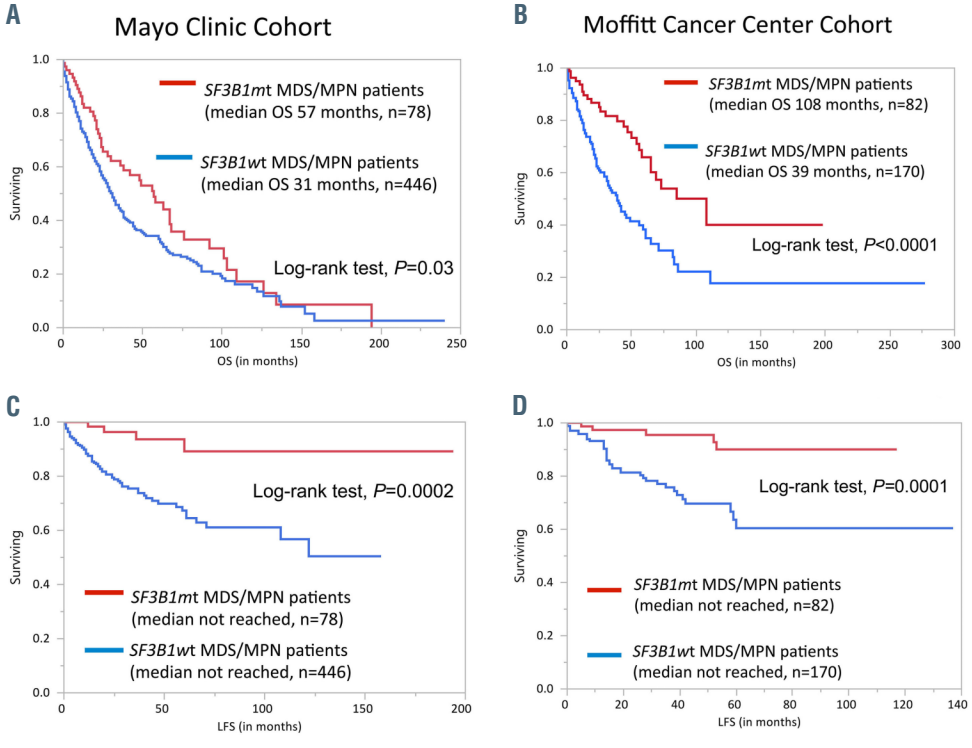

Figure 1. Differences in outcomes of patients with myelodysplastic syndrome/myeloproliferative neoplasms with or without *SF3B1* mutations. (A) Kaplan-Meier estimates of overall survival (OS) in myelodysplastic syndrome (MDS) /myeloproliferative neoplasms (MPN) patients with *SF3B1* mutations compared to *SF3B1* wild-type MDS/MPN patients (median: 57 *vs.* 31 months, *P*=0.03) in the Mayo Clinic cohort. (B) Leukemia-free survival in MDS/MPN patients with *SF3B1* mutations compared to *SF31B* wild-type MDS/MPN patients (median not reached in either group, *P*=0.0002) in the Mayo Clinic cohort. (C) Overall survival in *SF3B1* mutant MDS/MPN patients compared to *SF3B1*-wild type patients in the Moffitt Cancer Center cohort (median: 108 *vs.* 39 months, *P*<0.0001). (D) Leukemia-free survival in *SF3B1* mutant MDS/MPN patients compared to *SF3B1*-wild-type patients in the Moffitt Cancer Center cohort (median not reached in either group, *P*=0.0001).

(*P*=0.3) or the Moffitt Cancer Center cohort (*P*=0.7). In addition, there were no differences in OS between patients with mutations in the K700E hotspot and non-K700E sites in either the Mayo Clinic cohort (median OS 49 [95% CI: 22-109] months *vs.* 67 [95% CI: 36-126] months, *P*=0.5) (*Online Supplementary Figure S1A*) or the Moffitt Cancer Center cohort (median OS 85 [95% CI) months *vs.* not reached, *P*=0.9) (*Online Supplementary Figure S1B*).

We then compared *SF3B1*-mutant MDS/MPN patients (n=78) with *SF3B1*-mutant MDS patients (n=75) (Table 1). *SF3B1*-mutant MDS/MPN patients had a higher frequency of *JAK2* V617F mutations (25% *vs.* 1%, *P*<0.0001; 10% *vs.* 1%, *P*=0.002 when MDS/MPN-RS-T patients were excluded) (Figure 2A, Table 1). When the *SF3B1* mutation hotspots were compared between the two groups, *SF3B1* K700E was the most common hotspot in both categories and was present in 24 (47%) MDS/MPN patients and 39 (53%) *SF3B1*-mutant MDS patients (*P*=0.5) (*Online Supplementary Figure S1C, D*). Overall, there were seven patients with co-occurring *SF3B1* and *SRSF2* mutations (4 with CMML, 3 with *SF3B1*-mutant MDS). Mutation details were available for two CMML patients; *SF3B1* Y623C (42%)/*SRSF2* P95H (45%) and *SF3B1* K700E (45.2%)/*SRSF2* P95H (2.8%), and two *SF3B1*-mutant MDS patients; *SF3B1* K666Q (29%)/*SRSF2* P95T (48%) and *SF3B1* K700E (9%)/*SRSF2* P95R (29%). At last median follow-up of 102 (95% CI: 63-141) months, there were no significant differences in rates of transformation to acute myeloid leukemia (5% *vs.* 3%, *P*=0.4), Kaplan-Meier estimates of median LFS (median not reached, *P*=0.3) or median OS (median, 57 *vs.* 65 months, *P*=0.2) between the two cohorts (Figure 2B, Table 1).

We then stratified *SF3B1*-mutant MDS/MPN patients by morphological features such as percentage of ring sideroblasts in bone marrow and percentages of blasts in peripheral blood and bone marrow. In a univariate survival analysis, percentage of peripheral blood blasts (*P*=0.1), percentage of bone marrow blasts ≥5 (*P*=0.4), abnormal karyotype (*P*=0.3), revised International Prognostic Scoring System score (*P*=0.8) and CPSS cytogenetic group (*P*=0.5) were not predictive of OS. Molecular abnormalities (overall frequency ≥5%) such as *ASXL1* (*P*=0.3), *TET2* (*P*=0.08), *DNMT3A* (*P*=0.6), *JAK2* V617F (*P*=0.8), *U2AF1* (*P*=0.2), *SRSF2* (*P*=0.7), *ZRSR2* (*P*=0.3), *CBL* (*P*=0.3) *NRAS* (*P*=0.8), or any RAS pathway mutation (*KRAS/NRAS/CBL/PTPN11*, *P*=0.3) did not affect OS (only 1 patient each had *TP53* and *RUNX1* mutations). Additionally, WHO criteria were unable to prognostically distinguish both Mayo Clinic (*P*=0.3) and combined (Mayo Clinic and Moffitt Cancer Center) cohorts of *SF3B1*-mutant MDS/MPN patients (*P*=0.7). Furthermore, neither the standard International Prognostic Scoring System (*P*=0.3), nor the revised version (*P*=0.7) was able to stratify *SF3B1*-mutant and MDS/MPN patients into prognostically relevant subtypes.

Finally, we conducted a multivariate analysis in the combined Mayo Clinic cohort of MDS/MPN and MDS patients with known independent prognostic factors in myeloid malignancies such as hemoglobin <10 g/dL, age

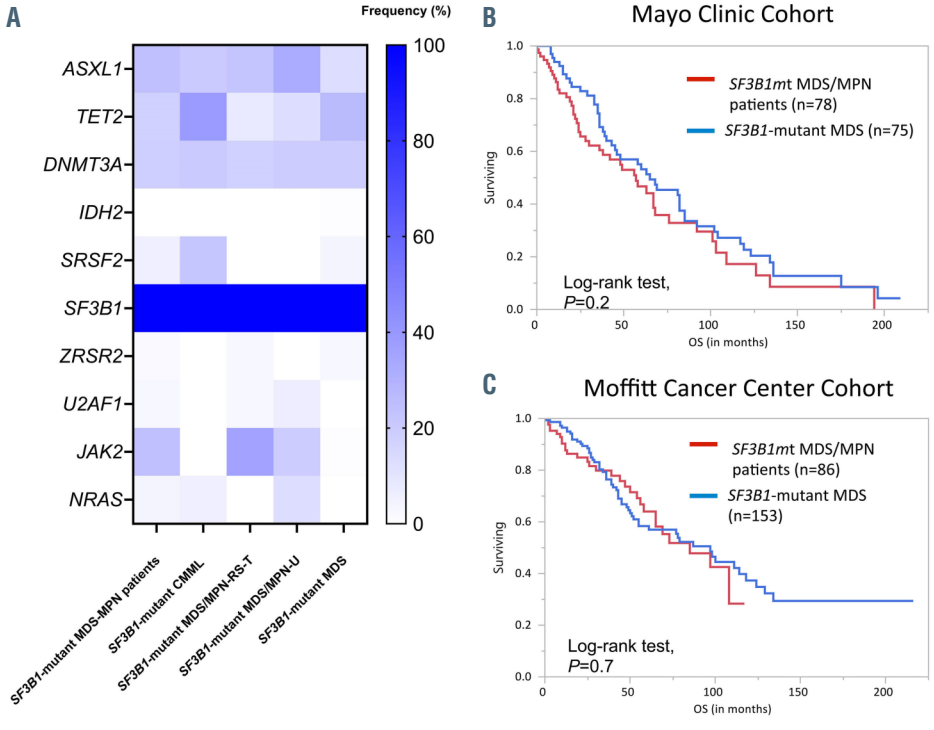

Figure 2. Differences in genomic characteristics and outcomes among patients with *SF3B1*-mutant myelodysplastic syndrome/myeloproliferative neoplasms and *SF3B1*-mutant myelodysplastic syndromes. (A) Heatmap of molecular abnormalities in the two groups (mutations in genes with a frequency of 5% or higher are included in the figure). The only statistically significant difference between the *SF3B1*-mutant myelodysplastic syndrome (MDS) /myeloproliferative neoplasms (MPN) and MDS groups was the higher frequency of *JAK2* in the former (25 *vs.* 1%, *P*<0.0001). (B) Kaplan-Meier estimate of overall survival between *SF3B1*-mutant MDS/MPN and *SF3B1*-mutant MDS (median, 57 months [95% confidence interval: 30-68] *vs.* 65 months [95% confidence interval: 43-85], *P*=0.2) patients in the Mayo Clinic cohort. (C) Kaplan-Meier estimate of overall survival between *SF3B1*-mutant MDS/MPN and *SF3B1*-mutant MDS (median, 85 months [95% confidence interval: 58-not reached] *vs.* 97 months [95% confidence interval: 55-118], *P*=0.7) patients in the Moffitt Cancer Center cohort.

≥70 years, platelet count ≥450 x 10^9/L, cytogenetic subtypes (as per CPSS stratification), *SF3B1* and *ASXL1* mutations, and bone marrow blast percentage ≥5, and found that *SF3B1* mutations retained their independent favorable prognostic impact (*P*=0.01) (*Online Supplementary Table S2*).

In summary, our data indicate that *SF3B1*-mutant MDS/MPN is a clinically and genomically distinct category within overlap myeloid neoplasms and, pending further validation, should be considered as a unique prognostic entity. Additionally, patients with this condition have distinct clinical and molecular characteristics in comparison to *SF3B1*-mutant MDS patients, arguing against a uniform classification category of *SF3B1*-mutant myeloid neoplasms.

Limitations of our study include smaller numbers of patients in certain subgroup comparisons, differential follow-up times and therapy choices, and selection biases largely due to the retrospective nature of the analyses.

Abhishek A. Mangaonkar,[1] Terra L. Lasho,[1] Christy Finke,[1] Rhett P. Ketterling,[2] Kaaren K. Reichard,[2] Kristen McCullough,[1] Naseema Gangat,[1] Aref Al-Kali,[1] Kebede H. Begna,[1] William H. Hogan,[1] Mark R. Litzow,[1] Hassan Alkhateeb,[1] Mithun Shah,[1] Animesh Pardanani,[1] Ayalew Tefferi,[1] Najla H. Al Ali,[3] Chetasi Talati,[3] David Sallman,[3] Eric Padron,[3] Rami Komrokji[3] and Mrinal M. Patnaik[1]

[1]Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN and [3]Department of Malignant Hematology, H. Lee Moffitt Cancer Center, Tampa, FL, USA.

Correspondence:

MRINAL M. PATNAIK - patnaik.mrinal@mayo.edu

RAMI KOMROKJI - Rami.Komrokji@moffitt.org

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Contributions: AM compiled the clinical and genomics data, analyzed data, and wrote all the drafts of the manuscript. TL and CF performed the genomics analysis. RPK and KKR reviewed the pathology information and edited all drafts of the manuscript. KM, NG, AAK, KHB, WHH, MRL, HA, MS, AP, and AT contributed patients and edited all drafts of the manuscript. NHA, CT, DS, EP and RK contributed data from the external (validation) cohort and edited all drafts of the manuscript. MP conceptualized the study and edited all drafts of the manuscript. All authors contributed to the writing of the manuscript.

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