

TO THE EDITOR:

SF3B1-mutant CMML defines a predominantly dysplastic CMML subtype with a superior acute leukemia-free survival

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Malcovati et al¹ recently proposed *SF3B1*-mutant (*SF3B1*^{MT}) myelodysplastic syndrome (MDS) to be a distinct nosologic entity defined by cytopenias, somatic *SF3B1* mutation, morphologic dysplasia (with or without ring sideroblasts [RSs]), bone marrow (BM) blasts <5%, and peripheral blood (PB) blasts <1%. Select concomitant genetic exclusion criteria are del(5q), monosomy7, inv(3)/abnormal 3q26, complex karyotype, or mutations involving *RUNX1* and/or *EZH2*. Chronic myelomonocytic leukemia (CMML) is an MDS/myeloproliferative neoplasm (MPN) overlap syndrome characterized by sustained PB monocytosis ($\geq 1 \times 10^9/L$, $\geq 10\%$ of white blood cell count [WBC]) further categorized into proliferative (WBC $\geq 13 \times 10^9/L$) and dysplastic (WBC $< 13 \times 10^9/L$) subtypes on the basis of the presenting WBC count.² Although mutations involving pre-messenger RNA splicing are seen in ~70% of CMML patients, these mostly involve *SRSF2* (50%), with *SF3B1* mutations accounting for <10% with no clear prognostic impact.³ We performed this study to compare and contrast *SF3B1*^{MT} CMML with *SF3B1*-wild-type (*SF3B1*^{WT}) CMML and *SF3B1*^{MT} MDS-RS, focusing on prevalence, phenotypic correlates, epistasis with other mutations or splicing mutations, and survival outcomes.

After approval by the Mayo Clinic (Rochester, MN) and the Moffitt Cancer Center (Tampa, FL) institutional review boards, adult patients with World Health Organization (WHO)-defined CMML and MDS-RS (Mayo Clinic only) were included in the study.² BM morphology, percentage of RSs, cytogenetics, and 2016 WHO diagnoses were retrospectively reviewed, and all patients underwent targeted next-generation sequencing for myeloid-relevant genes, which were obtained on BM mononuclear cells at diagnosis by previously described methods.⁴ CMML prognostication was performed by the Mayo Molecular Model and the Groupe Francophone des Myelodysplasies (GFM) model, and MDS-RS prognostication was performed by using the Revised International Prognostic Scoring System (IPSS-R).⁵⁻⁷ The Clinical/Molecular CMML-Specific Prognostic Scoring System (CPSS-Mol) scores could not be calculated because of incomplete data with regard to transfusion dependency. Statistical analyses considered clinical and laboratory parameters obtained at the time of diagnosis or first referral (usually within 6 months of diagnosis). Median time from diagnosis to molecular testing in the Mayo Clinic cohort was 4 months (range, 0-7 months). The Mann-Whitney *U* test and Fisher's exact test were used to compare quantitative and qualitative data in subgroups. Kaplan-Meier overall survival (OS) and AML-free survival (AML-FS) estimates and Cox regression models were used for survival analysis. The Benjamini-Hochberg controlling method was used for multiple hypothesis testing.

In all, 819 patients with CMML (40 [5%] with *SF3B1*^{MT}) and 83 with *SF3B1*^{MT} MDS-RS were included in the study (Table 1; supplemental Table 1). Patients with *SF3B1*^{MT} CMML compared with their WT counterparts were more likely to have lower hemoglobin levels ($P = .01$), lower WBC counts ($P = .009$), lower absolute monocyte counts (AMC; $P = .007$), and higher platelet counts ($P = .006$). They were also more likely to have BM RSs ($P < .001$) and *JAK2*^{V617F} mutations ($P = .03$) (Figure 1A) and less likely to have *ASXL1* ($P = .03$) and *SRSF2* ($P = .02$) mutations. Thirty *SF3B1*^{MT} CMML patients (75%)

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Requests for data sharing may be submitted to the corresponding author (Mrinal M. Patnaik; e-mail: patnaik.mrinal@mayo.edu).

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Table 1. Clinical and laboratory features and subsequent events in *SF3B1*^{MT} CMML, *SF3B1*^{WT} CMML, and *SF3B1*^{MT} MDS-RS patients

Variable	CMML with <i>SF3B1</i> ^{MT} (n = 40)	CMML without <i>SF3B1</i> ^{MT} (n = 819)	P (CMML with vs without <i>SF3B1</i> ^{MT})	<i>SF3B1</i> ^{MT} MDS-RS (n = 83)	P (CMML with <i>SF3B1</i> ^{MT} vs <i>SF3B1</i> ^{MT} MDS-RS)
Median age (range), y	74.5 (43-95)	71.0 (2-95)	.07	74 (42-94)	.98
Male sex	26 (61.9)	556 (67.9)	.44	53 (63.9)	1.00
Median hemoglobin (range), g/dL	9.4 (6.8-13.5)	10.9 (1.4-16.9)	.01	9.5 (7-13.5)	1.00
Median WBC (range), × 10 ⁹ /L	8.0 (2.5-96.1)	13.3 (1.3-288.6)	.009	5 (1.5-13.1)	.007
Median ANC (range), × 10 ⁹ /L	3.3 (0.4-54.7)	6.5 (0.0-155.6)	.009	2.7 (0.4-9.4)	.14
Median AMC (range), × 10 ⁹ /L	1.6 (1.2-11.5)	2.7 (1-84.0)	.007	0.4 (0-1.0)	.005
Median platelets (range), × 10 ⁹ /L	138.0 (12-840)	98.0 (2-1945)	.006	259.0 (13-599)	.03
Presence of IMCs	16 (41.0)	478 (60.4)	.06	NA	.005
Median PB blast (range), %	0 (0-3)	0 (0-19)	.07	0 (0-0)	.009
Median BM blast (range), %	3 (0-16)	3 (0-19)	.9	1 (0-4)	.004
Presence of RSs*	15/17 (88)	68/482 (14)	<.001	83 (100)	1.00
Median VAF <i>SF3B1</i> (range), %	40.5 (8-48)	NA	NA	41 (9-49)	1.00
Total no. of evaluable patients with FAB CMML subtypes	40	817	.04	NA	NA
dCMML	30 (75)	399 (48.8)		NA	
pCMML	10 (25)	418 (51.2)		NA	
Total no. of evaluable patients with WHO 2016 CMML subtypes	39	778	.25	NA	NA
CMML-0	29 (74.4)	461 (59.3)		NA	
CMML-1	7 (17.9)	184 (23.7)		NA	
CMML-2	3 (7.7)	133 (17.1)		NA	
Total no. of evaluable patients with CPSS-Mol cytogenetic risk stratification	39	752	.03	83	1.00
Low	29 (74.4)	555 (73.8)		66 (79.5)	
Intermediate	9 (23.1)	85 (11.3)		13 (15.7)	
High	1 (2.6)	112 (14.9)		4 (4.8)	
Total no. of evaluable patients with NGS analysis	40	567		83	
Epigenetic regulators					
<i>TET2</i>	17 (42.5)	295 (52.0)	.39	21 (25.6)	.15
<i>IDH1</i>	2 (5.0)	5 (0.9)	.14	0 (0)	.23
<i>IDH2</i>	2 (5.0)	29 (5.1)	1.00	1 (1.2)	.50
<i>DNMT3A</i>	7 (17.5)	34 (6.0)	.05	16 (19.3)	1.00
Chromatin regulators					
<i>ASXL1</i>	4 (10.0)	277 (48.9)	.03	11 (13.3)	1.00
<i>EZH2</i>	1 (2.5)	45 (7.9)	.50	2 (2.4)	1.00
Transcription factor					
<i>RUNX1</i>	8 (20.0)	78 (13.8)	.41	2 (2.4)	.007
Spliceosome component genes					
<i>SRSF2</i>	5 (12.5)	253 (44.6)	.02	2 (2.4)	.09
<i>U2AF1</i>	0 (0)	42 (7.4)	.18	0 (0)	1.00
<i>ZRSR2</i>	1 (2.5)	36 (6.3)	.66	NA	NA

All data are no. (%) unless otherwise specified. Bold indicates statistically significant P values ($P < .05$) provided only if data are available for all 3 cohorts.

AMC, absolute monocyte count; ANC, absolute neutrophil count; CPSS-Mol, Clinical/Molecular CMML-Specific Prognostic Scoring System; dCMML, dysplastic CMML; FAB, French-American-British; GFM, Groupe Francophone des Myelodysplasies; IMC, immature myeloid cell; IPSS-R, Revised International Prognostic Scoring System; NA, not available; pCMML, proliferative CMML.

*Sideroblasts were evaluable in 17 *SF3B1* mutant CMML patients and 482 *SF3B1* wildtype CMML patients, of whom, 15 and 68 had RS, respectively.

Table 1. (continued)

Variable	CMML with <i>SF3B1</i> ^{MT} (n = 40)	CMML without <i>SF3B1</i> ^{MT} (n = 819)	<i>P</i> (CMML with vs without <i>SF3B1</i> ^{MT})	<i>SF3B1</i> ^{MT} MDS-RS (n = 83)	<i>P</i> (CMML with <i>SF3B1</i> ^{MT} vs <i>SF3B1</i> ^{MT} MDS-RS)
Cell signaling					
<i>NRAS</i>	5 (12.5)	90 (15.9)	.72	NA	NA
<i>KRAS</i>	0/17 (0)	19/313 (6.1)	.75	NA	NA
<i>CBL</i>	3 (7.5)	88 (15.5)	.28	2 (2.4)	.62
<i>PTPN11</i>	0/17 (0)	9/313 (2.9)	.66	NA	NA
<i>JAK2</i>	7 (17.5)	40 (7.1)	.03	1 (1.2)	.01
<i>CSF3R</i>	0/17 (0)	4/313 (1.3)	.76	3 (3.6)	1.00
<i>KIT</i>	1 (2.5)	24 (4.2)	.90	NA	NA
<i>MPL</i>	0 (0)	5 (0.9)	.92	NA	NA
<i>CALR</i>	0/17 (0)	1/313 (0.3)	.92	NA	NA
Tumor suppressor gene					
<i>TP53</i>	1 (2.5)	16 (2.8)	1.00	3 (3.6)	1.00
Other					
<i>SETBP1</i>	0 (0)	62 (10.9)	.10	2 (2.4)	1.00
No. of assessable patients for stratification by the Mayo Molecular Model	40	809	.02	NA	NA
Low risk	6 (15.0)	93 (11.5)		NA	
Intermediate-1 risk	20 (50.0)	265 (32.8)		NA	
Intermediate-2 risk	12 (30.0)	237 (29.3)		NA	
High risk	2 (5.0)	214 (26.5)		NA	
No. of assessable patients for stratification by the GFM Model	40	809	.04	NA	NA
Low risk	27 (67.5)	358 (44.3)		NA	
Intermediate risk	11 (27.5)	320 (39.6)		NA	
High risk	2 (5.0)	131 (16.2)		NA	
No. of assessable patients for stratification by the IPSS-R	40	748	.14	83	.43
Very low	7 (17.5)	171 (22.9)		26 (31.3)	
Low	23 (57.5)	274 (36.6)		46 (55.4)	
Intermediate	5 (12.5)	174 (23.3)		7 (8.4)	
High	5 (12.5)	88 (11.8)		4 (4.8)	
Very high	0 (0.0)	41 (5.5)		0 (0.0)	
Deaths	18 (45.0)	461 (56.2)	—	56 (67.5)	—
Leukemic transformation	6 (15.0)	159 (19.4)	—	2 (2.4)	—

All data are no. (%) unless otherwise specified. Bold indicates statistically significant *P* values (*P* < .05) provided only if data are available for all 3 cohorts.

AMC, absolute monocyte count; ANC, absolute neutrophil count; CPSS-Mol, Clinical/Molecular CMML-Specific Prognostic Scoring System; dCMML, dysplastic CMML; FAB, French-American-British; GFM, Groupe Francophone des Myelodysplasies; IMC, immature myeloid cell; IPSS-R, Revised International Prognostic Scoring System; NA, not available; pCMML, proliferative CMML.

*Sideroblasts were evaluable in 17 *SF3B1* mutant CMML patients and 482 *SF3B1* wildtype CMML patients, of whom, 15 and 68 had RS, respectively.

were classified as having a dysplastic CMML subtype compared with 399 *SF3B1*^{WT} patients (49%) (*P* = .04). Although there were fewer high-risk karyotypic abnormalities in *SF3B1*^{MT} CMML patients, there was no difference between the 2 groups with regard to WHO CMML subtypes. In the Mayo Clinic cohort, 68 *SF3B1*^{WT} CMML patients (14%) had BM RSs (>5%). Compared with *SF3B1*^{MT} MDS-RS patients, *SF3B1*^{MT} CMML patients were more likely to have higher WBC (*P* = .007), higher AMC (*P* = .005), lower platelet counts (*P* = .03), higher PB blast percent (*P* = .009), and higher BM blast percent (*P* = .004) and

were more likely to have *RUNX1* (*P* = .007) and *JAK2*^{V617F} (*P* = .01) mutations (Figure 1B).

The median age for *SF3B1*^{MT} CMML patients was 74.5 years, and 62% were males. The median *SF3B1*^{MT} variant allele frequency (VAF) burden in *SF3B1*^{MT} CMML was 40.5% compared with 41% for *SF3B1*^{MT} MDS-RS (Figure 1C). Mutant *SF3B1*-associated amino acid changes included 23 (48%) K700E, 6 (12.5%) H662Q, 3 (6.2%) K666N, 2 (4%) G740E, 2 (4%) K554E, and 1 each (2%) for K669N, K741E, E622D, Y623C, Y768N, E283K, H516Y,

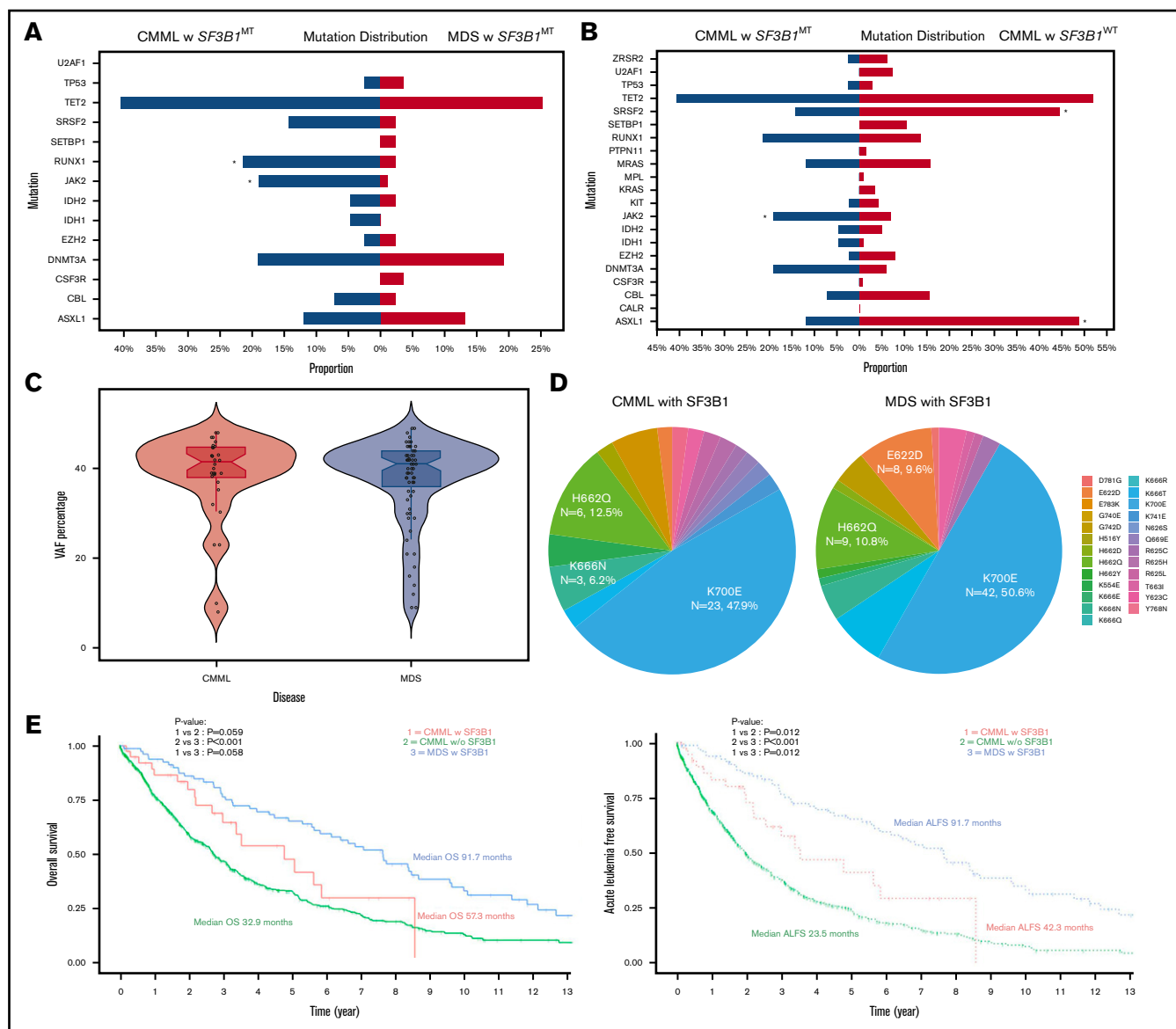


Figure 1. Phenotypic correlates, molecular features, and survival outcomes of *SF3B1*^{MT} CMML, *SF3B1*^{WT} CMML, and *SF3B1*^{MT} MDS-RS. (A-B) Mutational distribution of myeloid-relevant mutations in *SF3B1*^{MT} CMML and *SF3B1*^{WT} CMML (A) and *SF3B1*^{MT} CMML and *SF3B1*^{WT} MDS-RS (B). Asterisks denote statistically significant differences between the 2 groups. (C) Median VAF burdens of *SF3B1* mutations in *SF3B1*^{MT} CMML and *SF3B1*^{WT} MDS-RS. (D) Amino acid changes in *SF3B1* secondary to *SF3B1* mutations in CMML and MDS-RS. (E) Pertinent outcomes including OS and acute leukemia-free survival (ALFS) among *SF3B1*^{MT} CMML, *SF3B1*^{WT} CMML, and *SF3B1*^{MT} MDS-RS patients.

Y623C, Y625H/L, N626S, and Y765N (3 patients had 2 *SF3B1* mutations) (Figure 1D). Nine *SF3B1*^{MT} CMML patients (22.5%) had >1 splicing mutation (supplemental Table 2): 5 *SRSF2*^{P95H/L}, 3 *SF3B1*, and 1 *ZRSR2*. The VAFs of concurrent splicing mutations were similar (codominant) in 2 patients (*SF3B1*^{K700E} 42%/ *SRSF2*^{P95H} 45% and *SF3B1*^{G669Q} 43%/ *SRSF2*^{P95L} 42%). In 5 patients, the *SF3B1* mutations were dominant, and the second splicing mutation represented subclones (VAFs not available in 2 patients). In 3 *SF3B1*^{MT} CMML patients, no other driver mutations were identified. Common concurrent mutations included 17 (42.5%) *TET2*, 8 (20%) *RUNX1*, 7 (17.5%) *DNMT3A*, 7 (17.5%) *JAK2*^{V617F}, and 5 (12.5%) *SRSF2* (supplemental Figure 1); 10 patients (25%) had clonal cytogenetic abnormalities. *U2AF1* mutations were not

seen in either *SF3B1*^{MT} CMML or in *SF3B1*^{MT} MDS-RS patients. Seven *SF3B1*^{MT} CMML patients had concurrent *JAK2*^{V617F} mutations, with 4 (57%) having a proliferative CMML subtype. None of these patients had *ASXL1* or oncogenic RAS pathway mutations, and the median VAF for *JAK2*^{V617F} was 20% (range, 5%-52%). Within the limitations of a smaller data set, *JAK2*^{V617F} and *SF3B1* co-mutated CMML patients had a trend toward higher WBC and higher platelet counts without any significant differences in OS and AML-FS compared with *SF3B1*^{MT} CMML patients. In *SF3B1*^{MT} MDS-RS, frequencies of common hotspot amino acid changes included K700E, 50.6%; H662Q, 10.8%; E662D, 9.6%; and K666N, 4.8% (Figure 1D), with common concurrent mutations being *TET2* (25%), *DNMT3A* (20%), and *ASXL1* (13%).

At the last follow-up, among *SF3B1*^{MT} CMML, *SF3B1*^{WT} CMML, and *SF3B1*^{MT} MDS-RS patients, 18 (45%), 461 (56%), and 56 (68%) deaths and 6 (14%), 159 (18%), and 2 (2.4%) leukemic transformations were documented, respectively. The median OS for *SF3B1*^{MT} CMML patients was 57.3 months (95% confidence interval [CI], 35.8 months to not reached) compared with 32.9 months for *SF3B1*^{WT} CMML patients (95% CI, 30-37.3 months; *P* = .059). *SF3B1*^{MT} MDS-RS patients had a trend toward a better median OS (median OS, 91.7 months; 95% CI, 69.8-115.6 months; *P* = .058) compared with *SF3B1*^{MT} CMML patients and a clear survival advantage over *SF3B1*^{WT} CMML patients (*P* < .001; Figure 1E). The median AML-FS for *SF3B1*^{MT} CMML patients was 42.3 months (95% CI, 30.5 months to not reached) compared with 23.5 months (95% CI, 20.8-26.4 months; *P* = .012) for *SF3B1*^{WT} CMML patients (Figure 1E). *SF3B1*^{MT} MDS-RS patients had a better AML-FS than CMML patients in both categories (91.7 months; 95% CI, 69.8-115.6 months; *P* = .012 for both categories).

By using this large molecularly annotated cohort of CMML and *SF3B1*^{MT} MDS-RS patients, we defined *SF3B1*^{MT} CMML to be an infrequent (5%) but unique CMML category predominantly composed of dysplastic CMML subtypes, with similarities to MDS-RS in the form of median *SF3B1* VAFs, frequencies of *SF3B1* mutational hotspots (K700E was the most common) and IPSS-R risk stratification. As seen in MDS-RS, *SF3B1* mutations were also found to occur alone in *SF3B1*^{MT} CMML and seemed to be secondary to other oncogenic mutations in a minority of cases. Although there was a higher frequency of *JAK2*^{V617F} and *RUNX1* mutations in *SF3B1*^{MT} CMML compared with *SF3B1*^{MT} MDS-RS, these mutations are not enough to fully explain biological and phenotypic differences between the 2 entities. In addition, although there was a numeric trend toward a higher frequency of *TET2* and *SRSF2* mutations (known to skew hematopoiesis toward monocytosis) in *SF3B1*^{MT} CMML, this did not reach statistical significance.

SF3B1^{MT} CMML patients had a significant AML-FS advantage compared with their WT patient counterparts, with a trend toward a better OS. *SF3B1*^{MT} MDS-RS patients had a better OS compared with *SF3B1*^{WT} CMML patients and had a clear AML-FS advantage compared with both CMML subtypes. Conversely, *SF3B1*^{MT} CMML demonstrated key differences from *SF3B1*^{WT} CMML and from CMML in general by having a lower-than-expected frequency of *ASXL1* mutations and a higher-than-expected frequency of *JAK2*^{V617F} mutations and by demonstrating frequent concurrent splicing mutations. Truncating *ASXL1* mutations are seen in 40% of CMML patients and in 10% to 15% of *SF3B1*^{MT} MDS-RS patients, and they had a negative impact on survival.^{1,6,7} Only 4 (10%) of 40 *SF3B1*^{MT} CMML patients in our cohort had an *ASXL1* mutation, suggestive of less aggressive disease biology. The association of *SF3B1*^{MT} CMML with a higher-than-expected frequency of *JAK2*^{V617F} mutations remains to be elucidated but is reminiscent of MDS-RS, in which subsequent acquisition of *JAK2*^{V617F} confers proliferative features resulting in MDS/MPN-RS thrombocytosis.⁸ Mutational co-expression of *JAK2*^{V617F} and *SF3B1* in CMML confers proliferative features to what is otherwise a predominantly dysplastic CMML subtype. Splicing mutations are frequent in myeloid neoplasms and, in general, are mutually exclusive secondary to their synthetic lethal interactions and convergent effects.⁹ We recently documented a prevalence of

0.85% for ≥ 2 splicing mutations in 4231 patients with myeloid neoplasms, with $\sim 50\%$ being in the same cell; the distribution of double mutants deviated from the single mutants with selection against the most common alleles, *SF3B1*^{K700E} and *SRSF2*^{P95H/L}.¹⁰ In our study, 9 (22.5%) of 40 *SF3B1*^{MT} CMML patients had concurrent splicing mutations, and 5 patients had coexisting *SF3B1* and *SRSF2* mutations, with 3 (75%) demonstrating involvement of the most common alleles, *SF3B1*^{K700E} and *SRSF2*^{P95H/L} (K700E 23%/P95H 45%, K700E 32%/P95L 16%, and K700E 48%/P95H 2.8%). This unique epistatic interaction deserves further exploration.

In summary, we define *SF3B1*^{MT} CMML as a CMML subtype with predominant dysplastic features, with a low frequency of *ASXL1* mutations, higher frequency of *JAK2*^{V617F} mutations, concurrent splicing mutations, and a superior AML-FS. Given its infrequent occurrence, unlike in *SF3B1*^{MT} MDS-RS because of the lack of a clear impact on OS, further validation is needed in a larger cohort of patients before this is recognized as an independent nosological entity.

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