**SF3B1** mutated MDS: Blast count, genetic co-abnormalities and their impact on classification and prognosis

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Recenty, MDS with mutated **SF3B1** and blast count <5% was proposed as distinct entity with favorable prognosis by the international working group for the prognosis of MDS (IWG-PM), the 5th edition of the WHO classification and the International Consensus Classification. To further characterize this entity with respect to the genomic landscape, AML transformation rate and clinical outcome, we analyzed 734 MDS patients by whole genome sequencing. **SF3B1** mutations were identified in 31% (n = 231), most frequently accompanied by **TET2** mutations (29%). 144/231 (62%) **SF3B1** mutant samples fulfilled entity criteria proposed by IWG-PM (**SF3B1**ent). These cases were associated with longer survival, lower AML transformation rate, normal karyotypes and harbored less accompanying mutations compared to **SF3B1** mutant samples not falling into the proposed **SF3B1** entity (**SF3B1**ent). Of **SF3B1** mutant cases 7% (15/231; **SF3B1**ent: 3/144 [2%]; **SF3B1**ent: 12/87 [14%]) progressed to AML compared to 15% **SF3B1** wild-type patients (75/503). Of these 15 **SF3B1** mutant cases, 10 (67%) showed **RUNX1** mutations at MDS or AML stage. Multivariate analysis revealed that del(5q) and **RUNX1** mutations were independent negative prognostic factors for overall survival, while blast count >5% was not. In conclusion, **SF3B1** mutant MDS has a favorable prognosis independent of blast count if karyotype and **RUNX1** mutations are considered.

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**INTRODUCTION**

Myelodysplastic neoplasms (MDS) are clonal disorders characterized by peripheral cytopenias, morphologic dysplasia in hematopoietic cells and ineffective hematopoiesis [1, 2]. The currently used revised 4th edition of the WHO classification (WHO 2017) in MDS is mainly based on the number of cytopenias, dysplastic lineages, and the percentage of ring sideroblasts (RS) and blasts detected in bone marrow and peripheral blood samples [3]. Within the last years, the use of next generation sequencing (NGS) enabled the identification of driver genes in MDS providing insights into the underlying heterogeneous genetic landscape [4–6]. In this line, about half of MDS patients harbor somatic mutations in splicing pathway genes. Of these, **SF3B1** is the most commonly mutated gene and if mutated shown to be associated with RS, higher white blood cell counts and lower bone marrow blasts [6–9]. Moreover, **SF3B1** mutations define a distinct MDS subset showing favorable prognosis and indolent disease course [10]. Thus, in the WHO 2017 the **SF3B1** mutation is integrated into the diagnosis of MDS-RS (diagnostic criteria: RS ≥ 15% or RS ≥ 5% if **SF3B1**mut) [1].

Following up on this, the international working group for the prognosis of MDS (IWG-PM) proposed MDS with mutated **SF3B1** as a distinct entity if certain criteria are fulfilled (Supplementary Table S1) [11]. These criteria included: (1) cytopenia as defined by standard hematologic values, (2) somatic **SF3B1** mutation, (3) morphologic dysplasia (with or without RS), (4) bone marrow blasts <5% and peripheral blood blasts <1%, and (5) WHO 2017 criteria for MDS 5q-, MDS/MPN-RS-T, or other MDS/MPN or MPN are not met. Further exclusion criteria were: (1) poor-risk cytogenetics comprising monosomy 7, inv(3) or abnormalities of chromosome 3q26, and complex karyotype (≥3 chromosomal abnormalities); and (2) accompanying mutations in **RUNX1** and/or **EZH2**. The presence of **JAK2** V617F, **CALR**, or **MPL** mutations would strongly support the diagnosis of MDS/MPN-RS-T.

The upcoming 5th edition of the WHO Classification (2022) emphasizes a genetic basis for defining diseases and has now categorized MDS into morphologically defined MDS and MDS with defining genetic abnormalities (DGA) while largely abandoning the blast cut-off between MDS and AML if AML DGA are present [2]. It has further incorporated many of the proposed IWG-PM criteria into the newly introduced entity “MDS with low blasts and **SF3B1** mutation” [2]. However, according to WHO 2022 only biallelic **TP53** inactivations are excluded besides certain cytogenetic abnormalities (Supplementary Table S1). In contrast to the WHO 2022, the International Consensus Classification (ICC) requires an **SF3B1** variant allelic frequency (VAF) ≥ 10% in the absence of certain cytogenetic abnormalities, **RUNX1** and multi-hit **TP53** (Supplementary Table S1) [12]. It further sets the blast cut-off for AML-DGA to 10%, while cases with 10–19% blasts without DGA are assigned as a new category MDS/AML. In this study, we defined the **SF3B1** entity (**SF3B1**ent) based on the first publication proposed by the IWG-PM.
but also discuss the changes in classification according to 5th edition of the WHO classification and ICC.

The aim of the study was to analyze the SF3B1 mutation and the proposed SF3B1 entity in a large cohort of 734 MDS patients with respect to the incidence, genomic landscape, AML transformation rate and clinical outcome.

MATERIAL AND METHODS

Patients cohort and samples
For this analysis, we selected 734 MDS samples with material available to perform whole genome sequencing sent to the MLL Munich Leukemia Laboratory between 09/2005 and 12/2019. Diagnoses (from peripheral blood and bone marrow) were made based on cytomorphology, cytogenetics and molecular genetics as previously published [13–15]. All cases were classified into specific subgroups according to WHO 2017 [16]. For abbreviations of entities, see Table 1. Therapy-related MDS were excluded from this study. The MDS cohort comprised 310 (42%) female and 424 (58%) male cases with a median age of 73 years (range: 23–93 years) and a median follow-up of 9.3 years. All patients gave their written informed consent for genetic analyses and to the use of laboratory results as well as clinical data for research purposes according to the Declaration of Helsinki. The study was further approved by the laboratory’s institutional review board.

Whole genome sequencing (WGS) and variant filtering
WGS analysis was performed for all patients. For this, total genomic DNA was extracted from lysed cell pellet of bone marrow or peripheral blood using the Magna Pure 96 with DNA and Viral Nucleic Acid Large Volume Kit and Cellular RNA Large Volume Kit (Roche, Basel, Switzerland). Library preparation and sequencing as well as calling and filtering of single nucleotide variants, structural variants and somatic copy number variations (CNVs) were performed as previously described [17, 18]. Copy neutral loss of heterozygosity (CN-LOH) was assessed using HadoopCNV.

Mutational analysis
In this study, we evaluated mutations in 73 genes associated with myeloid neoplasms for all patients from WGS data only or from combined WGS and targeted NGS panels for the other patients. A total of 360 samples were additionally analyzed by targeted sequencing within a recent study [6] and 87 cases were analyzed by targeted NGS during routine diagnostics [19]. WGS data confirmed all mutations detected by targeted NGS panels and was further consulted for completing the mutational analysis of the 73 genes. The presence of FLT3-ITD and KMT2A-PTD were retrieved from WGS data only.

Statistical analysis
Statistical analyses were performed using SPSS version 19.0 (IBM Corporation, Armonk, NY). Analyses for overall survival (OS) and cumulative incidence (CI) of disease progression were performed according to Kaplan-Meier and compared using two-sided log rank tests. The OS was calculated as time from diagnosis to death or last follow-up. For the CI of disease progression death was considered as a competing event. Between different groups numerical variables were compared using the Mann-Whitney-U-Test, and dichotomous variables using chi-square test. Cox proportional hazards regression model was used to identify the impact of different variables on OS or AML transformation. All results were considered significant at p < 0.05.

RESULTS

Incidence and prognostic impact of SF3B1 mutations
SF3B1 mutations were identified in 231 of 734 (31%) MDS patients and were mainly found in MDS-RS (171/200; 86%); MDS-RS-SLD: 43/51, 84%; MDS-RS-MLD: 128/149; 86%) resulting in 74% (171/231) of all SF3B1mut cases (Table 1; Fig. 1A–C). In addition, 13% (37/300) of MDS with excess blasts (MDS-EB-1/2) and 20% (21/107) of MDS 5q- harbored SF3B1 mutations together accounting for 25% (58/231) of all SF3B1mut cases (Table 1; Fig. 1B, C). The remaining 1% of SF3B1mut cases were an MDS-SLD and an MDS-MLD sample. Of note, SF3B1 mutations were most frequently found in patients with blast count <5% (192/419; 46%).

In the total MDS cohort SF3B1 mutations were associated with better OS (median: 79 vs. 53 months; p < 0.001; Fig. 1D). Within the different MDS entities SF3B1 mutations were favorable in MDS-RS-SLD (median OS: 106 vs. 25 months; p = 0.009), MDS-RS-MLD (median: 82 vs. 64 months; p = 0.049) and MDS-EB-2 (median: 129 vs. 25 months; p = 0.011), but were associated with a shorter OS in MDS 5q- (median: 69 vs. 79 months; p = 0.044) (Fig. 1E–I). Irrespective of the SF3B1 mutation status, MDS-RS-SLD patients showed the best OS within the entire MDS cohort, while in contrast MDS with excess blasts was associated with the shortest OS (Supplementary Fig. S1A). A similar pattern was observed when focusing on SF3B1mut (SF3B1mut) patients (Fig. S1B). However, the unusual long OS for SF3B1mut MDS-EB-2 might be affected by therapy, in this regard all gene stem cell transplantation (SCT) received by 3/12 SF3B1mut MDS-EB-2 patients (Supplementary Table S2).

Within the SF3B1mut cohort 144/231 (62%) samples fulfilled the criteria proposed by IWG-PM (SF3B1ent) (Table 1; Fig. 2A; Supplementary Fig. S2A). SF3B1ent cases had a longer OS compared to SF3B1mut samples not falling into the proposed SF3B1 entity (SF3B1ent) (Fig. 2B; median: 97 vs. 63 months; p < 0.001). However, no positive effect of SF3B1 mutations on OS was observed within MDS-RS-SLD or MDS-RS-MLD if SF3B1 non-entity mutated cases were compared to wild-type cases (Supplementary Fig. S3A, B). Of note, SF3B1 mutations were associated with the presence of RS in both groups (SF3B1ent and SF3B1nonent), showing median percentages of 63 and 49, respectively (Supplementary Fig. S2B; p < 0.001).

Differences in the defining criteria for the SF3B1 entity between WHO 2022, ICC and IWG-PM lead to changes in assignment of 18 and 14 SF3B1mut cases, respectively (Supplementary Table S1; Fig. 2C). A detailed analysis of the changes is described in the supplement.
Recurrent SF3B1 mutations

Within the entire cohort, 25 different SF3B1 mutations, most frequently affecting amino acid K700 (53%, 123/231), were detected with a mean VAF ranging from 22% to 48% (Supplementary Fig. S4). In 5/231 patients two different SF3B1 mutations were detected resulting in 236 SF3B1 mutations in total (Table in Supplementary Fig. S4B). The VAF of each SF3B1 mutation did not exceed 50% (range: 4%–50%) (Fig. 3A). Of all SF3B1 mutations 77% (182/236) showed a VAF > 30% with SF3B1ent accounting for 66% (120/182). Moreover, 17% (39/236) showed a VAF between 15% and 29%, mainly belonging to SF3B1ent (25/39, 64%). SF3B1 VAFs <15% were seen in 15 cases, rarely in SF3B1ent (20%, 3/15). However, two of those 15 cases (one SF3B1ent; one MDS-RS-MLD) showed a second SF3B1 mutation with a VAF > 20% (Supplementary Fig. S4B). Of the
Fig. 2  Categorization and OS of SF3B1mut samples. A WHO 2017 entities of SF3B1mut samples and classification into the IWG-PM proposed SF3B1 entity (SF3B1ent) or non-SF3B1 entity (SF3B1nent). B OS of patients with mutated SF3B1 fulfilling criteria for proposed SF3B1 entity (n = 144; green) or not (n = 87; brown) vs. wild-type SF3B1 (n = 503; gray) (p < 0.001). C Comparison of SF3B1mut MDS diagnoses based on the currently used revised 4th edition of the WHO (WHO 2017) and the IWG-PM criteria (middle) to the corresponding MDS diagnoses considering the upcoming 5th edition of WHO (WHO 2022; left) and the International Consensus Classification (ICC; right).
The most frequent additional mutations in all SF3B1\textsuperscript{mut} patients were TET2 (29\%), DNM3TA (16\%) and ASXL1 (9\%) (Fig. 4; Supplementary Fig. S7A, B). The mutational frequencies of RUNX1, MPL, EZH2, and JAK2 in the total SF3B1\textsuperscript{mut} cohort were 5\% (RUNX1) and 3\% (MPL, EZH2, JAK2) and were present due to the entity criteria only in SF3B1\textsuperscript{ent} in 12, 8, and 6 cases, respectively. Of note, compared to SF3B1 wild-type samples mutations in ASXL1, RUNX1, TP53, ZRSR2, SRSF2 and STAG2 were significantly less frequent in SF3B1\textsuperscript{mut} patients, while DNM3TA mutations were more frequent (Supplementary Fig. S7A). Interestingly, within SF3B1\textsuperscript{mut} samples TP53 mutations (n = 11) were most frequently seen within MDS 5q- (3/21, 14\%). However, mutated TP53 was also seen in SF3B1\textsuperscript{ent} (6/144; 4\%), MDS-EB-1 (1/25; 4\%) and MDS-EB-2 (1/12; 8\%) (Supplementary Fig. S7B). Notably, 82\% (9/11) of TP53 mutations were monallelic events. In two samples (MDS-EB-1/2-1) both a mutation and deletion were detected affecting the TP53 gene (biallelic inactivation). In 17 SF3B1\textsuperscript{mut} samples, additional spliceosome mutations were found, namely ZRSR2 (n = 9) and SRSF2 (n = 8) (Fig. 4; Supplementary Figs. S6, S7). In SF3B1\textsuperscript{ent} patients spliceosome mutations were found in 9 cases (ZRSR2: n = 6, mean VAF: 27\% vs. 26\% of SF3B1; SRSF2: n = 3, mean VAF: 16\% vs. 47\% of SF3B1), whereas within SF3B1\textsuperscript{ent} samples showed additional ZRSR2 (n = 3, mean VAF: 26\% vs. 37\% of SF3B1) or SRSF2 (n = 5, mean VAF: 31\% vs. 29\% of SF3B1) mutations (Supplementary Fig. S8). Interestingly, additional spliceosome mutations were not detected in MDS 5q- (Fig. 4; Supplementary Fig. S7). In 5/17 (29\%) cases the SF3B1 VAF was lower than the VAF of additional spliceosome mutations (SRSF2: n = 2; all MDS-EB-1; ZRSR2: n = 3, all SF3B1\textsuperscript{ent}; Supplementary Fig. S8). In 4 of those samples the SF3B1 VAF was lower than 15\% and therefore accounted to the 13 samples of the entire MDS cohort showing only one SF3B1 mutation with a low VAF (<15\%; Fig. 3B). Thus, in 11/13 patients with a low SF3B1 VAF either deletions on chromosome 5 (n = 5), additional spliceosome (n = 4) or TP53 mutations (n = 2) were identified at MDS diagnosis.

**Fig. 3** Variant allelic frequencies (VAFs) of 231 SF3B1\textsuperscript{mut} samples. A SF3B1 VAFs with respect to the different entities; n (mutations) = 236. B Characteristics of cases having only one SF3B1 mutation and a VAF below 15\%.

remaining cases having SF3B1 mutations with VAFs <15\% (n = 13), 5/13 (39\%) samples were MDS 5q-, 6 (46\%) MDS-EB-1/2 and 2 (15\%) were SF3B1\textsuperscript{ent} (Fig. 3B). Of note, no CNVs or CN-LOHs overlapping with SF3B1 were found.

**Genomic landscape of SF3B1\textsuperscript{mut} patients**

Regarding cytogenetic abnormalities, 69/231 (30\%) SF3B1\textsuperscript{mut} samples showed aberrant karyotypes (SF3B1\textsuperscript{ent}: 27/144, 19\%; SF3B1\textsuperscript{ent}: 42/87, 48\%; p < 0.001; Supplementary Fig. S5A). Notably, cytogenetic risk groups poor and very poor according to the Revised International Prognostic Scoring System (IPSS-R) were found in 11 SF3B1\textsuperscript{ent} but in none of SF3B1\textsuperscript{ent} cases (Supplementary Fig. S5B).

Within SF3B1\textsuperscript{ent} 47\% (67/144) did not harbor any additional mutation in 73 analyzed genes resulting in an average of 1.8 mutations (including SF3B1) in this group (Fig. 4), while 53\% (77/144) harbored one to four additional mutations. SF3B1\textsuperscript{ent} patients showed on average 2.6 mutations (MDS with isolated del(5q): 1.9; MDS-EB: 2.7; MDS-ES: 3.1; Fig. 4). Although SF3B1\textsuperscript{ent} samples showed in total few mutations, additional mutations (if present) were detected in 27 different genes (Supplementary Fig. S6A). Additional mutations in SF3B1\textsuperscript{ent} samples were found in 9 to 20 different genes depending on the respective entity (Supplementary Fig. S6B–F).

**Molecular genetics of SF3B1\textsuperscript{mut} patients transforming to AML**

Of SF3B1\textsuperscript{mut} patients 7\% (15/231) progressed to AML compared to 15\% (75/503) of SF3B1 wild-type patients (median follow-up: 9.3 years; Fig. 5A). In addition, time to AML was shorter in SF3B1\textsuperscript{mut} compared to SF3B1\textsuperscript{mut} patients (median: 14 vs. 27 months, p = 0.046; Fig. 5B). Notably, an AML transformation rate of 14\% (12/87) was seen in SF3B1\textsuperscript{ent} and 2\% (3/144) in SF3B1\textsuperscript{ent} (median follow-up: 122 and 112 months; Fig. 5A). A trend for
longer time to AML transformation was observed for SF3B1mut compared to SF3B1ent, however not reaching statistical significance (71 vs. 17 months, \( p = 0.0825 \)). Eleven of 15 SF3B1mut MDS cases were also analyzed for the presence of molecular mutations at their diagnosis of AML (Fig. 5A, further details are provided in the supplement). Regarding the prognostic contribution of additional gene mutations and other risk factors to AML transformation in SF3B1mut patients univariate analyses revealed bone marrow blasts <5% to be associated with lower risk (hazard ratio HR: 0.097; \( p = 0.021 \)) and RUNX1 mutations (HR: 3.518; \( p = 0.05 \)) with higher risk for AML transformation (Supplementary Table S3).

**DISCUSSION**

SF3B1 mutations are frequently detected within MDS and associated with favorable prognosis [5–7]. In our WGS-based cohort of 734 MDS patients we identified 231/734 (31%) cases with SF3B1 mutations verifying known hotspots in K700, K666 and H662 [4, 7, 8, 20, 21] and confirming a heterozygous SF3B1 mutation status with high median VAFs (35%) across all entities. VAFs >30% were observed in 77% of SF3B1mut samples. SF3B1 mutations persisted over the entire disease courses in many MDS cases were also analyzed for the presence of molecular abnormalities (<15%) were mainly found in SF3B1 non-entity cases showing excess blasts, del(5q) or TP53 mutations but also in two SF3B1ent samples harboring other splicedome mutations. In line with previous reports, SF3B1 mutations were predominantly found in MDS-RS-SLD/MLD supporting the association of SF3B1 with RS [7, 10]. Moreover, we confirmed that SF3B1 mutations in MDS were favorable with regard to OS and AML transformation [7, 10, 11].

Recently, the IWG-PM suggested MDS with mutated SF3B1 as a distinct entity [11]. In this study, we evaluated the IWG-PM proposed SF3B1 entity criteria. We confirmed the favorable clinical

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**Table 2.** Cox proportional hazards ratio analyses of variables in SF3B1mutated MDS prognostic of OS.

| Risk factor                              | Hazard ratio (HR) | 95% CI       | \( P \) |
|-----------------------------------------|-------------------|--------------|--------|
| **Univariate analysis**                 |                   |              |        |
| Sex                                     | 1.080             | 0.764–1.528  | 0.663  |
| SF3B1 VAF, <15% vs. ≥15%                | 0.705             | 0.345–1.443  | 0.339  |
| Bone marrow blast count, <5% vs. ≥5%   | 0.616             | 0.395–0.961  | 0.033  |
| Bone marrow blast count, <10% vs. ≥10% | 1.901             | 0.605–5.980  | 0.272  |
| RUNX1                                   | 4.347             | 2.325–8.128  | <0.001 |
| EZH2                                    | 1.381             | 1.054–1.781  | 0.480  |
| ASXL1                                   | 1.836             | 1.085–3.105  | 0.023  |
| DNMT3A                                  | 0.920             | 0.582–1.454  | 0.720  |
| TET2                                    | 0.979             | 0.682–1.405  | 0.907  |
| JAK2                                    | 1.115             | 0.411–3.022  | 0.831  |
| MPL                                     | 1.520             | 0.709–3.257  | 0.282  |
| TP53                                    | 1.401             | 0.652–3.008  | 0.387  |
| del(5q)                                 | 1.977             | 1.198–3.262  | 0.008  |
| Complex karyotype (≥3 abnormalities)    | 2.986             | 0.944–9.448  | 0.063  |
| MECOM rearrangement                     | 0.486             | 0.069–3.556  | 0.497  |
| Other cytogenetic abnormalities         | 1.428             | 0.930–2.194  | 0.104  |
| **Multivariate analysis**               |                   |              |        |
| Bone marrow blast count, <5% vs. ≥5%   | 0.693             | 0.418–1.148  | 0.154  |
| RUNX1                                   | 3.581             | 1.769–7.249  | <0.001 |
| ASXL1                                   | 1.157             | 0.618–2.164  | 0.649  |
| del(5q)                                 | 2.146             | 1.289–3.574  | 0.003  |

OS overall survival, CI confidence interval, VAF variant allelic frequency. \( p \)-values in bold: statistical significance (\( p < 0.05 \)).
outcome of SF3B1 entity similar to recently published studies [21, 22]. Additionally, in line with Komrokji et al. we observed a significantly longer OS of SF3B1 entity patients compared to SF3B1 non-entity patients, in contrast to Venable et al. who did not observe significant differences in OS between SF3B1ent and SF3B1nent presumably due to the small cohort size [21].

In contrast to Malcovati et al. [11], we observed that SF3B1 mutations were associated with significantly shorter OS within MDS 5q- concordant with previous reports [19, 22, 23] highlighting the adverse prognostic impact of mutated SF3B1 within this entity. Within our SF3B1mut cohort, only 5% (11/231; all SF3B1nent) had poor or very poor cytogenetic risk groups concordant with a previous report [11] adding to the reasons for the favorable prognosis of SF3B1 mutations. In this line, the lately published IPSS-M, a unique risk score, improves the risk stratification of MDS patients by including molecular genetics into their model [24], in contrast to the IPSS-R, which considers only morphological features and cytogenetics [25]. The IPSS-M model further incorporates SF3B1 mutations with different weights depending on co-abnormalities (i.e. isolated del(5q) or BCOR, BCORL1, RUNX1, NRAS, STAG2, SRSF2 mutations).

With regard to the mutational landscape of SF3B1mut cases the most frequent additional mutations were DNMT3A, TET2, and ASXL1 (DTA) similar to previous reports showing that epigenetic and histone modifiers are commonly mutated in MDS, but also in aging individuals [5, 21, 26–28]. The number of additional mutations significantly impacted on OS in all SF3B1mut patients. Within SF3B1ent the number of co-mutations did not affect OS as shown in IWG-PM results [11], however the number of co-mutations was low compared to SF3B1nent cases.

Fig. 5 Genetics of MDS patients with mutated SF3B1 progressing to AML. A Molecular characterization of SF3B1mut patients progressing to AML at MDS stage (n = 15). Each column represents one patient, numbered 1–15. Number in brackets indicate that molecular data at AML stage is not available. Genes (gray: wild-type; red: mutated), WHO 2017 entities and SF3B1ent/nent are given for each patient. ent entity, nent non-entity, VAF variant allelic frequency. B Cumulative incidence of AML transformation of SF3B1 mutated (n = 15; red) vs. wild-type (n = 75; gray) patients.
In line with previous studies [5, 10], we confirmed that progression to AML occurs at a relatively low frequency in SF3B1mut patients (7%; 15/231). Furthermore, AML transformation was less frequent in SF3B1ent compared to SF3B1ent (2% vs. 14%). Progression of MDS to AML is suggested to be driven by cooperating genetic lesions (3.2 vs. 2.0). We further demonstrated that at MDS diagnosis 47% (7/15/231). Furthermore, AML transformation was less frequent in non-transforming patients. Moreover, during disease progression chromosomal aberrations were gained in two cases whereas most runs of RUNX1 mutations, significantly more frequent in AML-transforming compared to non-transforming patients. In contrast to the IWG-PM proposal, both WHO 2022 and ICC guidelines exclude biallelic mutations as independent negative prognostic factors for OS and AML transformation. In our univariate analysis, we confirmed the negative prognostic impact of del(5q) on OS of SF3B1mut cases and additionally found a negative impact of blast count >5% as well as RUNX1 and ASXL1 mutations. However, our multivariate analysis could not confirm the independent prognostic impact of blast count >5%, but showed del(5q) and RUNX1 mutations as independent prognostic markers. Thus, based on our data the threshold of <5%, which is used by IWG-PM, ICC and WHO 2022, is not required if presence of del(5q) and RUNX1 mutation are exclusion criteria for the SF3B1 entity. Of note, studies from Malcovati et al. showed a significant impact of excess blasts on the survival of SF3B1mut patients [10, 11], however, RUNX1 mutations were not included in their multivariate analysis. In conclusion, SF3B1 mutations are associated with good clinical outcome. Patients fulfilling the criteria of the SF3B1 entity proposed by the IWG-PM show an even better prognosis (longer OS, lower AML transformation rate). Our data suggest that the identification of the good prognostic subset within SF3B1mut patients can be achieved by excluding only cases with del(5q) and/or RUNX1 mutations, however completely independent of blast count.

DATA AVAILABILITY
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS
SH and CH designed the study, SH interpreted the data, SH wrote the manuscript. CH was responsible for chromosome banding and FISH analyses, MM, CB, GH, and HS for molecular and bioinformatic analyses, WK for immunophenotyping and TH for cytomorphologic analyses. All authors read and contributed to the final version of the manuscript.

COMPETING INTERESTS
CH, WK, and TH declare part ownership of Munich Leukemia Laboratory (MLL). SH, HS, MM, GH, and CB are employed by the MLL.

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