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ORIGINAL ARTICLE

Clinical, biological, and prognostic implications of *SF3B1* co-occurrence mutations in very low/low- and intermediate-risk MDS patients

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Abstract

SF3B1 is a highly mutated gene in myelodysplastic syndrome (MDS) patients, related to a specific subtype and parameters of good prognosis in MDS without excess blasts. More than 40% of MDS patients carry at least two myeloid-related gene mutations but little is known about the impact of concurrent mutations on the outcome of MDS patients. In applying next-generation sequencing (NGS) with a 117 myeloid gene custom panel, we analyzed the co-occurrence of *SF3B1* with other mutations to reveal their clinical, biological, and prognostic implications in very low/low- and intermediate-risk MDS patients. Mutations in addition to those of *SF3B1* were present in 80.4% of patients (median of 2 additional mutations/patient, range 0–5). The most frequently mutated genes were as follows: *TET2* (39.2%), *DNMT3A* (25.5%), *SRSF2* (10.8%), *CDH23* (5.9%), and *ASXL1*, *CUX1*, and *KMT2D* (4.9% each). The presence of at least two mutations concomitant with that of *SF3B1* had an adverse impact on survival compared with those with the *SF3B1* mutation and fewer than two additional mutations (median of 54 vs. 87 months, respectively: $p = 0.007$). The co-occurrence of *SF3B1* mutations with specific genes is also linked to a dismal prognosis: *SRSF2* mutations were associated with shorter overall survival (OS) than *SRSF2*wt (median, 27 vs. 75 months, respectively; $p = 0.001$), concomitant *IDH2* mutations (median OS, 11 [mut] vs. 75 [wt] months; $p = 0.001$), *BCOR* mutations (median OS, 11 [mut] vs. 71 [wt] months; $p = 0.036$), and *NUP98* and *STAG2* mutations (median OS, 27 and 11 vs. 71 months, respectively; $p = 0.008$ and $p = 0.002$). Mutations in CHIP genes (*TET2*, *DNMT3A*) did not significantly affect the clinical features or outcome. Our results suggest that a more comprehensive NGS study in low-risk MDS *SF3B1*^{mut} patients is essential for a better prognostic evaluation.

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Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders leading to abnormal blood production and that have a variable risk of progression to acute myeloid leukemia (AML) [1–3].

In recent years, large-scale analysis using next-generation sequencing (NGS) has made it possible to identify recurrent genetic alterations, thereby improving our knowledge of MDS pathogenesis [4–8]. More than 80% of MDS patients harbor at least one mutation, affecting genes from a variety of functional groups: splicing machinery (*SF3B1*, *SRSF2*), DNA methylation (*DNMT3A*, *TET2*), transcription factors *(TP53*, *RUNX1*), chromatin modification (*ASXL1*, *EZH2*), RAS pathway (*KRAS*, *NRAS*), cohesin complex (*STAG2*, *RAD21*), kinases (*JAK2*, *FLT3*), and/or DNA repair (*ATM*, *BRCC3*) [4, 9–11].

Several clinical and biological implications of specific mutations have been demonstrated. Some of these gene mutations have been associated with morphological and clinical features such as complex karyotypes (*TP53*), excess proportions of bone marrow blast (*RUNX1*, *NRAS*) or ring sideroblasts (*SF3B1*), and with the prognosis for leukemia-free and overall survival [12, 13]. Mutations in *TP53*, *U2AF1*, *RUNX1*, *SRSF2*, *IDH2*, *CUX1*, *ASXL1*, and *BCOR* genes are associated with significantly worse leukemia-free survival [5]. Those in *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1* are predictors of poor overall survival (OS), while mutations in *SF3B1* are associated with a better outcome in MDS patients [6, 14]. Most of these studies have analyzed single mutations, but little is known about the impact of concurrent mutations on the outcome of MDS patients [15].

It is common for more than one mutation to be present in MDS. Papaemmanuil and colleagues clearly demonstrated the variability of gene mutation frequencies in a large series of MDS, whereby 40% of cases had 2 or 3 mutated genes and up to 10% of patients presented 4 to 8 oncogenic point mutations. All these features are associated with a more complex disease and have an adverse effect on OS [4, 5]. *SF3B1* encodes a core component of the RNA splicing machinery. NGS studies have revealed that approximately 30% of MDS cases have a mutation of the *SF3B1* gene, with a particularly high prevalence (> 90%) in the MDS with ring sideroblasts subtype (MDS-RS), as reflected in the most recent 2017 WHO classification [3, 13, 14]. Furthermore, *SF3B1* mutations in low-risk MDS patients were associated with good prognostic parameters [13]. Although no significant affinity of *SF3B1* with common mutational genes other than *DNMT3A* and *JAK2* has been found, various gene mutations co-existing with *SF3B1* have been described [4, 5].

Nevertheless, and given that the majority of MDS patients carry multiple genetic alterations, the well-known, better OS and leukemia-free survival referred to *SF3B1* mutations in low-risk MDS patients may be worse. Recent studies in low-risk MDS–RS patients have highlighted the adverse influence of coexisting *DNMT3A* and *ASXL1-SF3B1* mutations on clinical outcome [15, 16].

However, information about the influence of other gene mutations co-existing with $SF3B1^{mut}$ in MDS patients is scarce. Detailed molecular characterization of these groups would allow a better stratification of patients within the low-risk MDS categories as well as a better choice of treatment for these patients. The aim of this study was to analyze, by means of NGS, the presence of mutations associated with $SF3B1^{mut}$ and to evaluate the prognostic value in a large series of very low/low/intermediate-risk MDS patients.

Materials and methods

Patients

The mutational profiles of 324 MDS patients, diagnosed in our center between 1999 and 2017, were analyzed. Diagnosis was based on the World Health Organization (WHO) criteria [3, 17]. Patients with refractory anemia with excess of blasts (RAEB) and MDS associated with isolated del(5q) were excluded from the study.

SF3B1 mutations were detected in 135 patients (42%). Detailed analysis of clinical parameters and cytogenetic findings was performed to facilitate risk stratification, according to the International Prognostic Scoring System (IPSS) and the revised IPSS (IPSS-R) [18, 19]. Finally, a total of 102 MDS cases with *SF3B1* mutations and very low, low, and intermediate IPSS-R scores were included. The study was approved by the Local Ethical Committee (Comité Ético de Investigación Clínica, Hospital Universitario de Salamanca), and written informed consent was obtained for each patient according to the guidelines of the Declaration of Helsinki.

Mutational analysis

Mutational screening of genomic non-amplified DNA from bone marrow (BM) or peripheral blood (PB) cells was performed. A customized myeloid panel of 117 MDS-related genes was applied (Supplementary Table S1). NGS was carried out on a NextSeq sequencing platform (Illumina, San Diego, CA, USA) following Illumina's standard protocol for Nextera Rapid Capture Enrichment. Sequencing data were analyzed by applying an in-house informatic pipeline that uses

different software tools to perform quality assessment, alignment, and variant calling (Trimmomatic, FastQC, NGSQCToolkit, BWA, GATK, VarScan, SAMTools, ANNOVAR). The Integrative Genomics Viewer (IGV, Broad Institute) was used to evaluate variants visually.

After analysis, only those variants of good quality ($Q > 30$), supported by ≥ 100 total and ≥ 10 mutated reads, with a variant allele frequency (VAF) ≥ 3%, located in exonic or splicing regions, and which generate an amino acid change were considered. In addition, already reported polymorphisms (SNPs) (dbSNP144, 1000-genomes Project, ExAC, ESP-6500, when MAF ≥ 1%) and sequencing artifacts (internal laboratory database where distortions in data processing caused by a measuring instrument, already seen in previous NGS analysis in our laboratory, are annotated and stocked as a reference for further analysis) were discarded. For variant interpretation and oncogenic potential evaluation, COSMIC and ClinVar databases, SIFT, PolyPhen-2, and Mutation Taster predictors were used (Supplementary Fig. 1).

Statistical analysis

Numerical variables were summarized as the median and range; categorical variables were summarized as the frequency and percentage of subjects in each category. Group differences were examined with Student's *t* test or the Mann-Whitney *U* test, respectively, for normally and non-normally distributed continuous variables. Overall survival was measured from the time of diagnosis to the time of last follow-up or death from any cause. Survival curves were generated using the Kaplan-Meier method and differences were assessed with the log-rank test. For multivariate analysis, Cox proportional hazards models were constructed, adjusting for potential confounding covariates. Significance of all statistical tests was defined as a value of $p < 0.050$. Analyses were carried out using the IBM SPSS version 22.0 statistical package (IBM Corp., Armonk, NY).

Results

One hundred and two patients with a mutation in *SF3B1* gene were included as lower-risk cases according to the IPSS-R: very low (34.4%), low (60.4%), or intermediate (5.2%). Regarding the WHO 2017 classification, 1% of patients had MDS with single-lineage dysplasia (MDS-SLD), 5.2% had MDS with multilineage dysplasia (MDS-MLD), 40.6% had MDS with ring sideroblasts (MDS-RS) with single-lineage dysplasia (MDS-RS-SLD), and the remaining 53.1% had MDS-RS with multilineage dysplasia (MDS-RS-MLD). The median age was 76 years (range: 41–90 years). More detailed clinical features of the cohort are summarized in Table 1.

Characterization of *SF3B1* mutations in MDS patients

One hundred and seven *SF3B1* mutations were found in 102 MDS patients. The median VAF was 33.6% (range 5.72–64.00%). All mutations were missense, heterozygous, and, except for three (K748E, Y623F, and L536V), had been previously reported. The most frequent mutation was K700E (47/107, 43.9%) followed by K666R (12/107, 11.2%) and E622D (9/107, 8.4%). All *SF3B1* mutational variants detected are illustrated in Fig. 1. Notably, five of the 102 MDS patients presented two *SF3B1* mutations. The double variants of four patients were located in exon 14 (E622D and Y623F; H662Q and K666T; and two cases with E622V and T663I), while the double *SF3B1* mutation in the other patients affected exons 14 and 15 (R625H and K700E) (detailed information about all mutations is shown in Supplementary Table S2).

Co-occurrence of *SF3B1* mutations with other gene mutations

Co-occurrence of *SF3B1* mutations with those of other genes was observed in 82 of 102 patients (80.4%). A total of 192 concomitant mutations, involving 51 genes, were found in the 102 $SF3B1^{mut}$ patients, with a median of two additional mutations per sample (range: 0–5), (Supplementary Table S2). Patients with one mutation in addition to that of *SF3B1* were the most frequent type ($n = 26$, 25.5%), followed by two and three concurrent mutations ($n = 23$, 22.5%, and $n = 19$, 18.6%, respectively). Cases with four and five concomitant *SF3B1* mutations were infrequent ($n = 7$, 6.9% and $n = 8$, 7.8%, respectively). The most frequently mutated genes co-occurring with *SF3B1* were as follows: *TET2* ($n = 40$, 39.2%), *DNMT3A* ($n = 26$, 25.5%), *SRSF2* ($n = 11$, 10.8%), *CDH23* ($n = 6$, 5.9%), and *ASXL1*, *CUX1*, *KMT2D* ($n = 5$, 4.9% each). Mutations were also present in the *BCOR*, *NUP98*, *SMC3*, *SETBP1*, and *STAG2* genes, although at low frequencies ($n = 3$, 2.9% each) (Fig. 2).

The great heterogeneity of the overall distribution of concomitant mutations is illustrated in Fig. 3. The addition of a single mutation to the *SF3B1* mutation usually involves the *TET2* or *DNMT3A* genes. However, the addition of two mutations concomitant with *SF3B1* mutations did not show a clear pattern of distribution or evolution.

The effect of the frequency of co-occurring *SF3B1* mutations

In the next step of the study, the influence of the number of concurrent mutations was analyzed, taking the clinical characteristics and overall survival (OS) into account. In general, no differences were observed in the clinical characteristic of the patients affected by between one and five of the additional *SF3B1* mutations, (Supplementary Table S3). However, in the

Table 1 Clinical features of 102 MDS patients

Variable	$N = 102$	%	Median (range)	p10	p90
Gender					
Male	56	55.4			
Female	45	44.6			
Age			76 (41–90)	63	84
WHO 2008					
RARS	36	35.3			
RCUD	6	5.9			
RCMD	60	58.8			
WHO 2017					
MDS-SLD	1	1			
MDS-MLD	5	5.2			
MDS-RS-SLD	39	40.6			
MDS-RS-MLD	51	53.1			
Blasts			0.8 (0–4.2)	0	3
≤ 2	84	82.4			
> 2 < 5	12	11.8			
NA (< 5)	6	5.9			
Ring sideroblasts			41.5 (0–95)	7.5	73.7
< 5	6	5.9			
≥ 5 < 15	5	4.9			
≥ 15	85	83.3			
NA (< 15)	6	5.9			
Cytogenetic risk					
Very good	7	6.9			
Good	88	87.2			
Intermediate	6	5.9			
IPSS					
Low	68	67.3			
Int-1	18	17.8			
NA (low/Int-1)	15	14.9			
IPSS-R					
Very low	33	33			
Low	58	58			
Intermediate	5	5			
NA (Very low/low)	2	2			
NA (Low/Intermediate)	2	2			
AML transformation					
No	66	81.5			
Yes	15	18.5			
Status					
Alive	41	40.2			
Death	61	59.8			
Hb (g/dL)			9.6 (5.4–13)	7.9	11.6
ANC ($\times 10^9$/L)			2.7 (0.6–8.7)	1.5	6.1
Platelets ($\times 10^9$/L)			251 (34–878)	150	430

MDS, myelodysplastic syndromes; *RARS*, refractory anemia with ring sideroblasts; *RCUD*, refractory cytopenia with unilineage dysplasia; *RCMD*, refractory cytopenia with multilineage dysplasia; *MDS-SLD*, MDS with single lineage dysplasia; *MDS-MLD*, MDS with multilineage dysplasia; *MDS-RS*, MDS with ring sideroblasts; *MDS-RS-SLD*, MDS-RS with single lineage dysplasia; *MDS-RS-MLD*, MDS-RS with multilineage dysplasia; *NA*, not analyzable; *IPSS*, International Prognostic Scoring System; *Int-1*, intermediate-1; IPSS-R, revised IPSS; *Hb*, hemoglobin; *ANC*, absolute neutrophil count

univariate analysis of the series, after a median follow-up of 54 months and a median OS of 70 months, we found that the presence of at least two mutations concomitant with *SF3B1* had a more adverse effect on survival compared with those with *SF3B1* + fewer than two additional mutations (median of 54 vs. 87 months, respectively, $p = 0.007$) (Fig. 4A). The negative impact of at least two additional *SF3B1* mutations remained a significant factor in the multivariate analysis (Table 2).

Several co-occurring *SF3B1* gene mutations reduce OS of MDS patients

Frequently mutated genes overlapping with *SF3B1* were analyzed (Fig. 2). There were no differences in the clinical features between groups of $SF3B1^{mut}$ patients with wild type or mutated forms of the most frequently mutated genes (data not provided). We observed that the presence of somatic mutations in other genes was able to modify the good prognosis of patients with isolated $SF3B1^{mut}$. Thus, in the univariate analysis, co-occurrence of *SF3B1* with *SRSF2* mutations was associated with shorter OS than isolated *SF3B1* mutations with *SRSF2* wild type (median of 27 vs. 75 months, respectively; $p = 0.001$) (Fig. 4B). However, the negative impact of *SRSF2* mutation was not retained as a significant term in the multivariate analysis (data not shown). Furthermore, although small groups of patients are involved, a similar adverse effect was observed with concomitant *IDH2* mutations (median OS of 11 vs. 75 months, respectively; $p = 0.001$) (Fig. 4C) and *BCOR* mutations (median OS of 11 vs. 71 months, respectively; $p = 0.036$) (Fig. 4D). Interestingly, *SF3B1* with *NUP98*, and *SF3B1* with *STAG2* also had a negative effect on patient prognosis (medians of 27 and 11 vs. 71 months, respectively; $p = 0.008$ and $p = 0.002$) (Fig. 4E and F). Very few patients bore these mutations, so they were not included in the multivariate analysis.

Co-occurrence *of SF3B1* and clonal hematopoiesis indeterminate potential mutations

The presence of mutations in some genes considered CHIPs (clonal hematopoiesis indeterminate potentials) co-occurring with *SF3B1* mutations was analyzed. More than half of MDS patients (57.8%, $N = 59$) displayed mutations in *TET2*, *DNMT3A*, and/or *ASXL1* (Fig. 3). The comparison of the most relevant clinical and biological charactersitics, such as age, levels of hemoglobin, platelets and neutrophils, bone marrow percentage of blast and ring sideroblast, and OS, did not differ between the low-risk MDS patients with *TET2* or *DNMT3A* as unique mutations associated with *SF3B1* and *SF3B1* mutations as the only abnormality (median OS of 70 and 87 months, respectively). Therefore, CHIP mutations had no effect on *SF3B1*-mutated cases (Table 3 and Fig. 5).

Discussion

SF3B1 is one of the most frequently mutated genes in MDS and the presence of mutations in this gene is associated with a

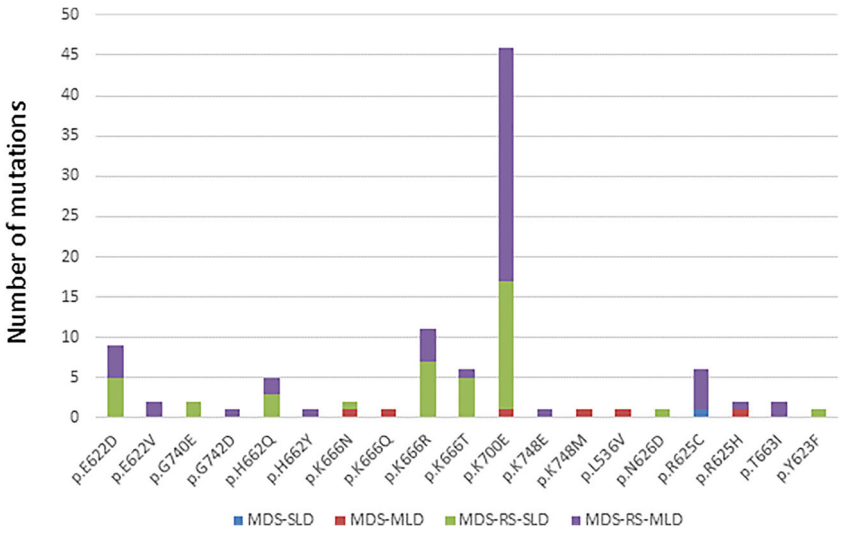

Fig. 1 *SF3B1* mutations distributed in patients included in the study and classified by WHO 2017 MDS subtype

favorable outcome in MDS patients without an excess of blasts [4, 13]. However, information about the appearance and influence of other concurrent mutations in prognosis is scarce, although they are important topics since at least 40% of patients with MDS have at least two mutations [4, 5, 20]. In the present study, we analyzed the co-occurrence of mutations in *SF3B1* and other frequent mutations in myeloid-related genes, the consequences of these concomitant mutations for the clinical and biological phenotype, and the impact on prognosis in a cohort of 102 MDS low-risk patients without an excess of blasts. Our study confirmed that a complex mutational *SF3B1* status (at least two associated mutations) is associated with a dismal prognosis in low-risk MDS patients.

To ensure the consistency of our analyses, we included only those low-risk patients who were categorized as very low, low, and intermediate risk according to the IPSS-R. Clinical features did not differ from those typical of low-risk MDS patients with respect to age, gender, and WHO classification [19, 21, 22]. Patients with excess blasts were excluded from the study because the frequency of *SF3B1* mutations in this population was low and, due to an adverse outcome, predefined in this subset of patients [4, 5, 23]. In addition, and based on another recognized entity, with a clear genotype-phenotype relationship and prognosis, patients with MDS with an isolated 5q deletion were excluded from the study [24, 25].

The MDS subtype most frequently found in our cohort was MDS-RS (93.7%) according to the WHO's 2017 classification. The percentage of RS ranged between 0 and 95%, with the majority of cases (83.3%) containing more than 15% of RS [26, 27]. The 2017 WHO classification expanded the MDS-RS group to include cases with an RS level between 5 and 15% and confirmed the *SF3B1* mutation, which involved 4.9% of the cases in our cohort (Table 1) [3]. Six patients

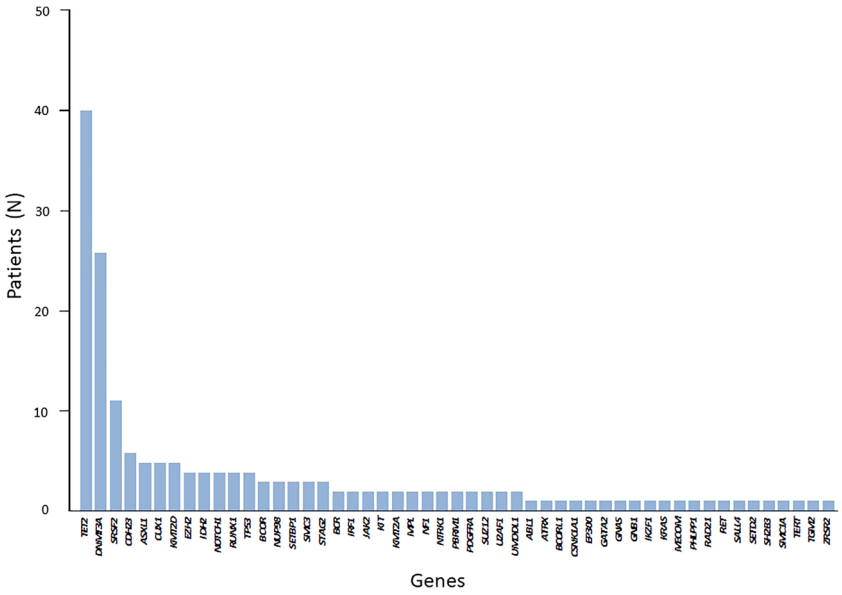

Fig. 2 Mutations in 51 genes co-occurring with *SF3B1* in 102 very low, low, and intermediate-risk MDS patients

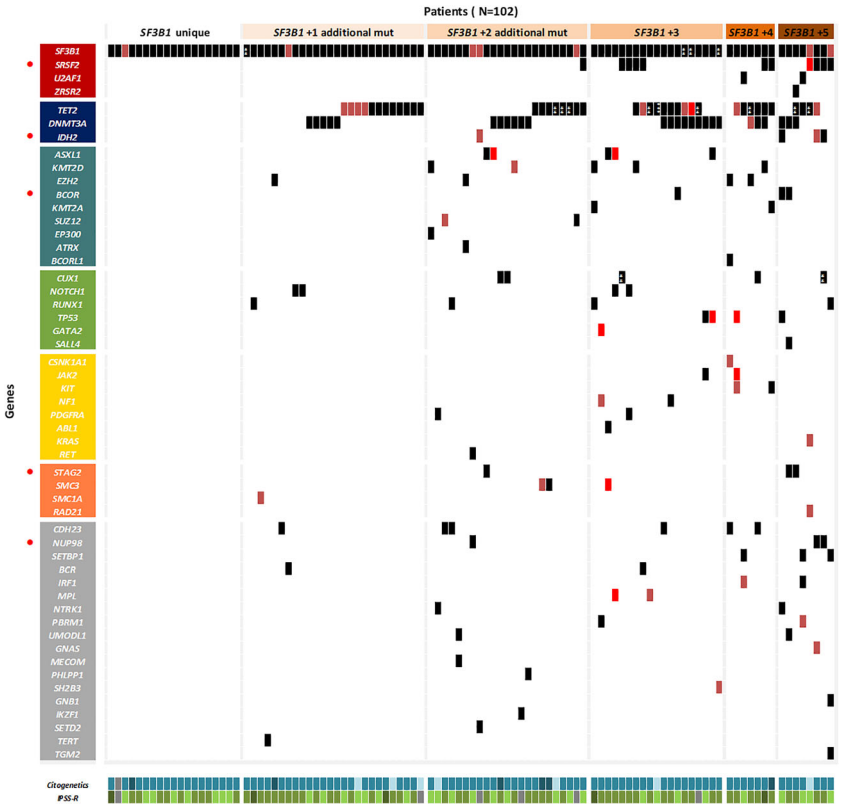

Fig. 3 Mutational landscape of 102 MDS. Each column represents one patient. Patients are grouped by number of additional *SF3B1* mutations. Each line represents one gene. Genes are grouped by function. Red circles indicate genes that reduce overall survival of MDS patients.VAF: ≥3 < 5% ≥5 < 10% > 10%. Gene function: splicing, DNA methylation, chromatin modification, transcription factors, activated signaling, cohesin complex, other. Cytogenetics: very good, good, intermediate, NA. IPSS-R: very low, low, intermediate, NA

could not be classified because their RS frequency was not known. The increase in the proportion of patients diagnosed with MDS-RS following the 2017 WHO reclassification has been noted in other studies [28].

The extent of *SF3B1* mutations among MDS-RS patients was similar to that reported in previous studies. All the mutations found were missense and heterozygous, with a change from lysine to glutamic acid in codon 700 (K700E) being the most frequent [14, 29]. Apart from three variants (K748E, Y623F, and L536V), all were already known to occur in hematological cancer, and at similar frequencies (Fig. 1) [30–32]. Interestingly, five of 102 patients had double *SF3B1* mutations (E622D and Y623F; H662Q and K666T; two cases with E622V and T663I and R625H and K700E). Double mutations occur rarely in the *SF3B1*, although they have been reported [14, 20].

Co-occurrence analyses of the *SF3B1* gene with other myeloid-related genes revealed that only 20 patients (19.3%) carried a mutation of *SF3B1* as the only mutation. Previous studies had reported that more than 40% of cases of MDS had at least two mutations, which underlines the need for a detailed and complete molecular analysis to gain a better knowledge of the molecular pattern and risk to our patients, especially those with low-risk MDS [4, 5]. The isolated *SF3B1* mutation appeared as a minority group in our cohort and in other studies, with a tendency to be higher (40%) among the MDS-RS cases [4, 20]. Those discrepancies may have arisen because the studies used different NGS methods and VAF cut-offs. Martin et al. used the amplicon-based technique to analyze regions of 39 genes, while we used a custom-capture strategy to interrogate exonic regions of 117 genes. Martin et al. chose a VAF cut-off of 5% whereas the threshold was

Table 2 Univariate and multivariate Cox proportional hazard model for clinical features and number of additional mutations to *SF3B1*

	Univariate			Multivariate		
	HR	95.0% CI	*p*	HR	95.0% CI	*p*
Age ≥ 76	2.147	1.203–3.833	0.008	1.719	0.931–3.174	0.083
Gender (male)	1.925	1.120–3.310	0.015	1.712	0.988–2.969	0.055
WHO 2017	1.806	1.053–3.096	0.027	0.953	0.504–1.801	0.881
Blasts	2.528	1.258–5.081	0.006	2.271	1.106–4.664	0.025
SF3B1 + ≥2 additional mutations	1.986	1.159–3.405	0.010	1.908	1.036–3.515	0.038

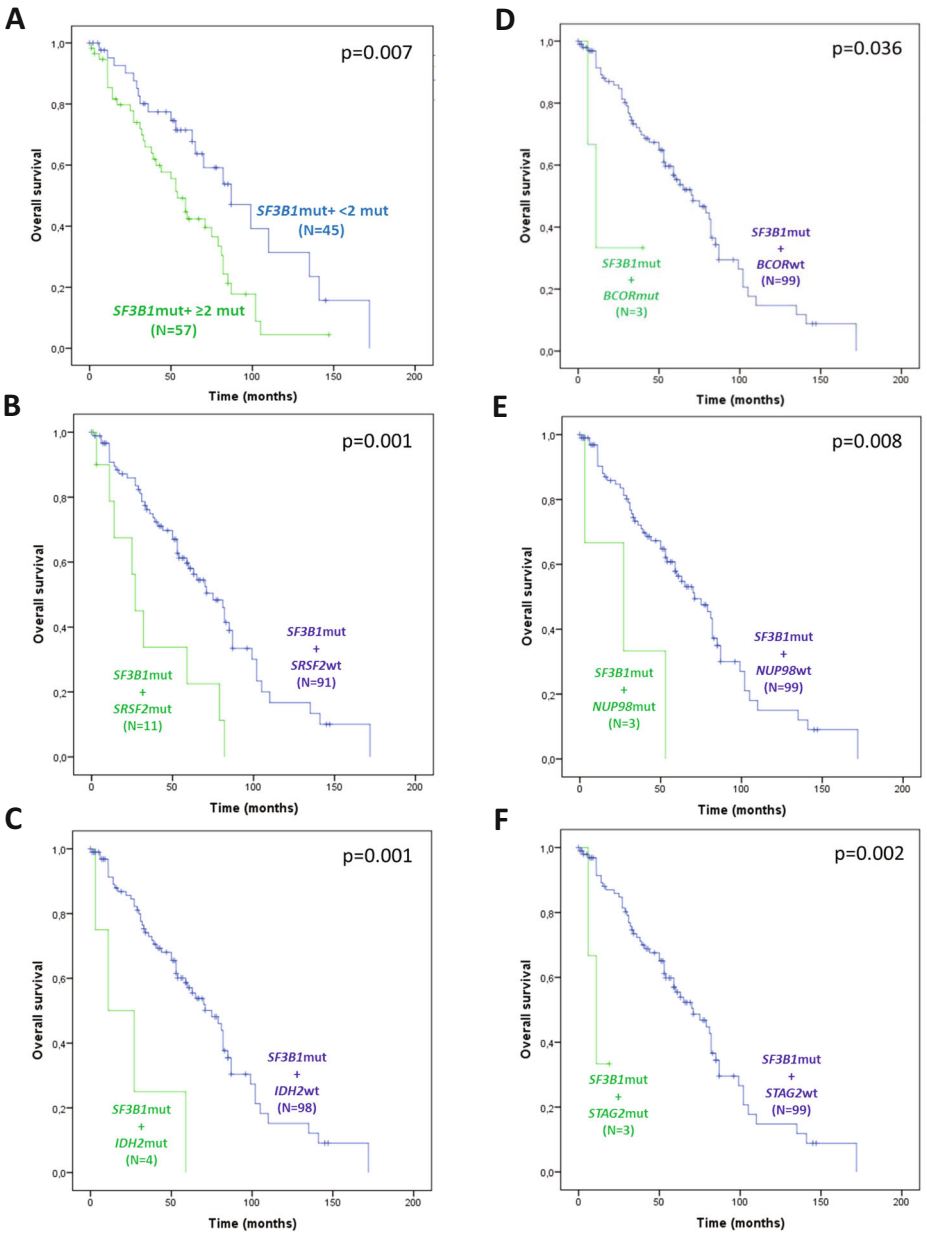

Fig. 4 Overall survival of MDS *SF3B1*^{mut} patients by frequency of concomitant mutations (median 87 vs. 54 months) (A). Co-occurrence of *SRSF2*^{mut} (median 75 vs. 27 months) (B). *IDH2*^{mut} (median 75 vs. 11 months) (C). *BCOR*^{mut} (median 71 vs. 11 months) (D). *NUP98*^{mut} (median 71 vs. 27 months) (E). *STAG2*^{mut} (median 71 vs. 11 months) (F)

3% in the present study. Nevertheless, the majority group of patients in both series bore additional mutations that coexisted with those of *SF3B1*.

The *TET2* and *DNMT3A* genes were the most frequently mutated, co-occurring with *SF3B1* in 39.2% and 25.5% of cases, respectively, similar to what was noted in previous

Table 3 Comparison of median values between patients with vs. without co-occurrence of *SF3B1* mutation with CHIP-related genes mutations

	SF3B1mut (solely) $N = 20$	*SF3B1* + *DNMT3A* or *TET2* $N = 22$	*p* value
Age	76	77.5	0.354
% blasts (BM)	0.3	0.8	0.527
% RS	46	35	0.731
Hb (g/dL)	10.05	9.6	0.642
ANC ($\times 10^9$/L)	2.9	2.7	1
Platelet ($\times 10^9$/L)	248	241	0.642

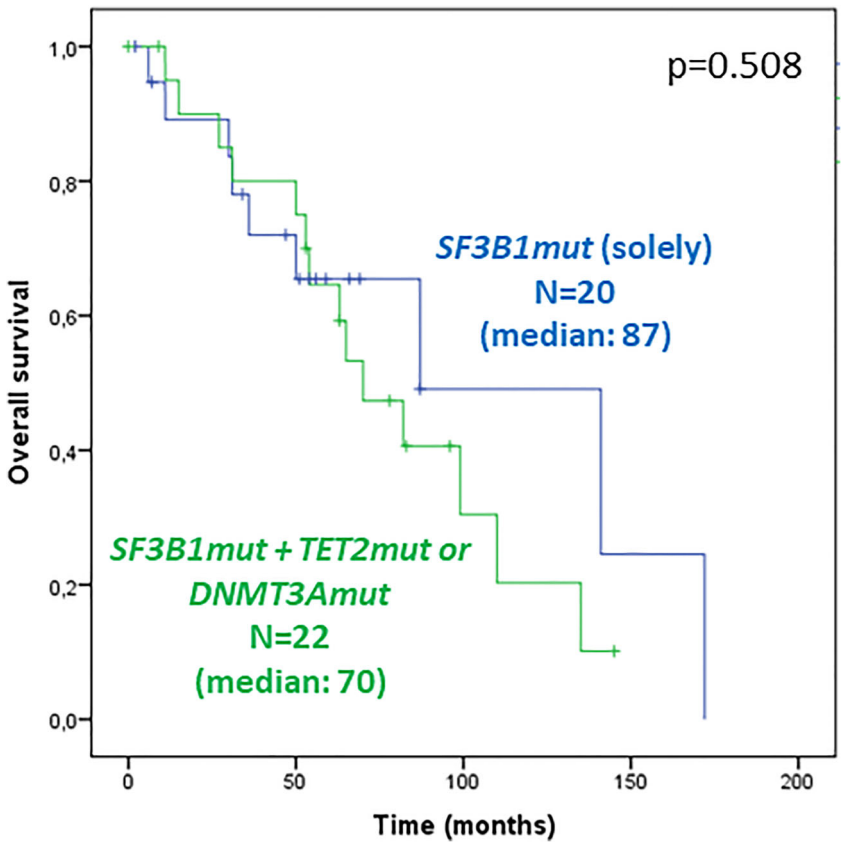

Fig. 5 Overall survival of MDS *SF3B1*^{mut} patients with respect to co-occurrence with mutations in CHIP genes (*TET2* or *DNMT3A*) (median 87 vs. 70 months)

series [4, 5, 16]. Mutations in *SRSF2* were the next most frequently observed. Although these are quite frequent in MDS or MDS-RS patients, they rarely co-occur with *SF3B1* [8, 33]. In our previous study of splicing genes in MDS-RS patients, we identified double splicing gene mutations and noted their possible influence on the outcome of patients. This prompted us to investigate in more detail the group of patients in which *SF3B1* and *SRSF2* occur jointly [14]. As a consequence, we found that patients with the *SRSF2* + *SF3B1* mutation were another of the most frequently occurring types (10.8%) in our series. This is a novel observation; double splicing gene mutations have previosuly been considered to be mutually exclusive [4, 5]. The other gene mutations known in MDS were quite similar to those previously reported in the MDS and MDS-RS subtypes, as shown in Fig. 2 [4, 5, 20].

Previous studies suggested that a higher frequency of driver mutations worsens the clinical outcome in these patients [5, 34]. Our study confirms that *SF3B1* with at least two additional mutations is an independent prognostic factor associated with shorter survival, even in these low-risk MDS patients. This could be interpreted as meaning that a highly heterogeneous mutational status gives rise to a more complex disease.

We now consider the role of the co-occurrence of particular gene mutations with *SF3B1*. Previous studies suggested that the presence of some gene mutations in low-risk *SF3B1*^{mut} MDS patients could modify clinical features and prognosis. Malcovati et al. found that in *SF3B1*^{mut} patients, *RUNX1* mutations were significantly associated with worse OS and a higher cumulative incidence of disease progression [13]. Similary, Martin et al. demonstrated the same pattern in RARS patients with *SF3B1* and *DNMT3A* mutations [15]. As far as we know, these findings have not been confirmed in other cohorts. Our own inability to corroborate these findings could be due to an insufficient number of *SF3B1*^{mut} with *RUNX1*^{mut} patients and the greater heterogeneity of our study cohort compared with other series [15]. Nevertheless, we found a negative impact on prognosis when there was co-occurrence of *SF3B1* with *SRSF2* and/or *BCOR* and/or *IDH2* and/or *NUP98* and/or *STAG2* mutations. With the exception of *SRSF2*, the small number of affected cases available to us means that these findings need to be confirmed by the analysis of a larger cohort of patients.

In 2014, NGS studies in healthy individuals revealed that around of 10–20% of older people (≥ 65 years of age) carried somatic mutations in hematopoietic disorder–related genes, making hematopoiesis clonal. The majority of the variants occurred in three genes: *DNMT3A*, *TET2*, and *ASXL1*. The clonal hematopoiesis of indeterminate potential (CHIP) of healthy people helped create an increased risk of subsequent hematological cancer and of the appearance of cardiovascular diseases [35, 36]. In the current study, *DNMT3A*, *TET2*, and *ASXL1* were among the most frequently mutated genes. No

effect of co-occurrence with *SF3B1* was seen in relation to clinical features and prognosis, although, according to previous findings, their existence could be crucial for initiating the clonal hematopoiesis that evolves to become MDS disease [35–37]. These results demonstrate the importance and utility of the NGS technique not only for monitoring patients' disease course but also for making it possible to detect molecular changes (clonal hematopoiesis) early on in otherwise healthy people, thereby increasing the opportunity to improve the follow-up of receptors and immediately determine whether the disease has evolved.

In summary, our study demonstrated that the co-ocurrence of additional myeloid mutations is very frequent (80.4%) in very low/low/intermediate-risk *SF3B1*-mutated MDS patients. The presence of at least two additional mutations is associated with shorter survival and the presence of specific mutations concomitant with those of *SF3B1* (*SRSF2*, *IDH2*, *BCOR*, *NUP98*, and/or *STAG2*). The comprehensive mutational study of low-risk MDS patients with *SF3B1* mutation is essential if they are to receive a more accurate prognosis.

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Data availability Not applicable

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval The study was approved by the Local Ethical Committee (Comité Ético de Investigación Clínica, Hospital Universitario de Salamanca) and written informed consent was obtained from each patient, in accordance with the guidelines of the Declaration of Helsinki.

Consent to participate Informed consent was obtained from all individual participants included in the study.

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