Frequency and prognostic impact of mutations in *SRSF2*, *U2AF1*, and *ZRSR2* in patients with myelodysplastic syndromes

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Mutations in genes of the splicing machinery have been described recently in myelodysplastic syndromes (MDS). In the present study, we examined a cohort of 193 MDS patients for mutations in *SRSF2*, *U2AF1* (synonym *U2AF35*), *ZRSR2*, and, as described previously, *SF3B1*, in the context of other molecular markers, including mutations in *ASXL1*, *RUNX1*, *NRAS*, *TP53*, *IDH1*, *IDH2*, *NPM1*, and *DNMT3A*. Mutations in *SRSF2*, *U2AF1*, *ZRSR2*, and *SF3B1* were found in 24 (12.4%), 14 (7.3%), 6 (3.1%), and 28 (14.5%) patients, respectively, corresponding to a total of 67 of 193 MDS patients (34.7%). *SRSF2* mutations were associated with *RUNX1* ($P < .001$) and *IDH1* ($P = .013$) mutations, whereas *U2AF1* mutations were associated with *ASXL1* ($P = .005$) and *DNMT3A* ($P = .004$) mutations. In univariate analysis, mutated *SRSF2* predicted shorter overall survival and more frequent acute myeloid leukemia progression compared with wild-type *SRSF2*, whereas mutated *U2AF1*, *ZRSR2*, and *SF3B1* had no impact on patient outcome. In multivariate analysis, *SRSF2* remained an independent poor risk marker for overall survival (hazard ratio = 2.3; 95% confidence interval, 1.28-4.13; $P = .017$) and acute myeloid leukemia progression (hazard ratio = 2.83; 95% confidence interval, 1.31-6.12; $P = .008$). These results show a negative prognostic impact of *SRSF2* mutations in MDS. *SRSF2* mutations may become useful for clinical risk stratification and treatment decisions in the future. (*Blood*. 2012;119(15):3578-3584)

Introduction

Myelodysplastic syndromes (MDS) are neoplastic disorders of the hematopoietic stem cell that are characterized by peripheral cytopenias and a variable propensity for transformation to acute myeloid leukemia (AML).[1] The current concept of the disease considers gene mutations, deregulated gene expression, and epigenetic changes as key steps in the pathogenesis of the disease.[2] Several mutations have been described to occur in MDS, including mutations in *TET2*, *ASXL1*, *RUNX1*, *EZH2*, *TP53*, *NRAS*, *KRAS*, *CBL*, *ETV6*, *IDH1*, and *IDH2*, with the recent addition of *DNMT3A*.[2-4] However, these mutations do not appear to be specific for MDS because they also occur in other myeloid malignancies at variable frequencies.[5] Moreover, little is known about the mechanisms leading to the disease-defining features of MDS. Recently, mutations in *Splicing factor 3B subunit 1* (*SF3B1*) were discovered that appear to be not only specifically associated with MDS but even with the subtypes of MDS that show ring sideroblasts (RARS and RCMD-RS).[6] *SF3B1* encodes a protein involved in the spliceosome pathway. Simultaneously, other splicing genes such as *U2 small nuclear RNA auxiliary factor 1* (*U2AF1*, synonym *U2AF35*), *serine/arginine-rich splicing factor 2* (*SRSF2*), and *U2 small nuclear ribonucleoprotein auxiliary factor 35 kDa subunit-related protein 2* (*ZRSR2*) involved in the spliceosome pathway have also been reported to be frequently mutated in MDS.[7] However, although these gene mutations also appear to be most prevalent in MDS, they are not associated with the presence of ring sideroblasts.[7] The mechanism of how mutations in genes belonging to the splicing machinery can lead to the pathogenesis of MDS is still unknown. Whereas some data suggest a positive or no prognostic impact of *SF3B1* mutations,[6,8-10] the prognostic impact and clinical characteristics of patients with mutations in other splicing genes such as *SRSF2*, *U2AF1*, and *ZRSR2* are unknown.

In the present study, we investigated the frequency and prognostic influence of mutations in *SRSF2*, *U2AF1*, and *ZRSR2* in the context of other prognostic clinical and molecular markers in a cohort of 193 MDS patients.

Methods

Patients

Cell samples from 193 MDS patients were collected at the time of enrollment in clinical trials. MDS patients were enrolled in multicenter treatment trials investigating the use of all-*trans* retinoic acid,[11] antithymocyte globulin (NCT00004208),[12,13] deferasirox,[14] lenalidomide, or thalidomide for the treatment of MDS; demethylating agents were not used for this patient cohort. DNA from 50 healthy blood donors (age, 18-60 years) was

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Table 1. Comparison of clinical and molecular characteristics of 193 MDS patients according to *SRSF2* mutation status

Characteristic	*SRSF2* mutated (n = 24)	*SRSF2* WT (n = 169)	*P*
Age, y			.12
Median	69	65	
Range	56-83	36-92	
Sex, n (%)			.059
Male	19 (79)	100	
Female	5 (21)	69	
WHO subtype, n (%)			.86
RA	7 (29)	31 (18)	
RARS	2 (8)	18 (11)	
del (5q)	1 (4)	17 (10)	
RCMD	3 (13)	18 (11)	
RCMD-RS	1 (4)	8 (5)	
RAEB-1	1 (4)	21 (12)	
RAEB-2	5 (21)	26 (15)	
MDS-U	1 (4)	6 (4)	
Missing data	3 (13)	24 (14)	
Karyotype risk, n (%)			.67
Good	16 (67)	93 (55)	
Intermediate	2 (8)	18 (11)	
Poor	2 (8)	21 (12)	
Missing data	4 (17)	37 (22)	
BM blasts, n (%)			.35
< 5%	15 (62)	93 (55)	
5%-9%	1 (4)	23 (14)	
10%-19%	5 (21)	25 (15)	
Missing data	3 (13)	28 (16)	
Hemoglobin, n (%)			.46
< 8 g/L	5 (21)	30 (18)	
8-10 g/L	8 (33)	70 (41)	
> 10 g/L	8 (33)	36 (21)	
Missing data	3 (13)	33 (20)	
IPSS, n (%)			.37
Low-risk	4 (16)	35 (21)	
Intermediate-1	10 (42)	47 (28)	
Intermediate-2	3 (13)	35 (21)	
High	3 (13)	10 (6)	
Missing data	4 (16)	39 (24)	
Transfusion dependence, n (%)			.96
Yes	17 (71)	107 (63)	
No	4 (16)	26 (16)	
Missing data	3 (13)	36 (21)	
Transformation into AML, n (%)			.013
Yes	11 (46)	34 (20)	
No	10 (41)	98 (58)	
Missing data	3 (13)	37 (22)	
***SF3B1* mutations, n (%)**			.75
Mutated	4 (17)	24 (14)	
WT	20 (83)	145 (86)	
Missing data	0 (0)	0 (0)	
***U2AF1* mutations, n (%)**			.143
Mutated	0 (0)	14 (8)	
WT	24 (100)	155 (92)	
Missing data	0 (0)	0 (0)	
***ZRSR2* mutations, n (%)**			.35
Mutated	0 (0)	6 (4)	
WT	24 (100)	163 (96)	
Missing data	0 (0)	0 (0)	
***ASXL1* fs mutations, n (%)**			.58
Mutated	6 (25)	34 (20)	
WT	18 (75)	135 (80)	
Missing data	0 (0)	0 (0)	
***RUNX1* mutations, n (%)**			< .001
Mutated	7 (29)	**7 (4)**	
WT	17 (71)	162 (96)	
Missing data	0 (0)	0 (0)	
***TP53* mutations, n (%)**			.7
Mutated	0 (0)	4 (2)	
WT	24 (100)	164 (97)	
Missing data	0 (0)	1 (0)	
IDH1 mutations, n (%)			.013
Mutated	3 (13)	4 (2)	
WT	21 (87)	165 (98)	
Missing data	0 (0)	0 (0)	
***DNMT3A* mutations, n (%)**			.39
Mutated	0 (0)	5 (3)	
WT	24 (100)	164 (97)	
Missing data	0 (0)	0 (0)	
***NRAS* mutations, n (%)**			.31
Mutated	0 (0)	7 (4)	
WT	24 (100)	162 (96)	
Missing data	0 (0)	0 (0)	

RA indicates refractory anemia; RARS, refractory anemia with ring sideroblasts; del(5q), MDS with isolated del(5q); RCMD, refractory cytopenia with multilineage dysplasia; RAEB-1, refractory anemia with excess blasts-1; RAEB-2, refractory anemia with excess blasts-2; MDS-U, MDS-unclassifiable; and fs, frameshift.

obtained from the Institute of Transfusion Medicine, Hannover Medical School (Hannover, Germany). Clinical and hematologic data were recorded after MDS patients gave their informed consent in accordance with the Declaration of Helsinki, and the scientific analysis of the samples was approved by the institutional review board of Hannover Medical School (ethical vote 2467). The distribution of World Health Organization (WHO) subtypes, International Prognostic Scoring System (IPSS) risk groups, and cytogenetic risk groups is shown in Table 1 and supplemental Tables 2 and 3 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). The study cohort included more low-risk (n = 96) than high-risk (n = 51) MDS patients. Patients with chronic myelomonocytic leukemia were excluded. Follow-up information was available for 154 of the 193 MDS patients and was updated through documented clinic visits as well as telephone calls to patients, their doctors, or local registry offices.

Cytogenetic and molecular analysis

Cytogenetic analysis was performed centrally by G- and R-banding analysis. Mutation analysis was performed as described previously.[15] Mononuclear cells from patient samples were enriched by Ficoll density gradient centrifugation and were stored at −196°C in liquid nitrogen until use. Genomic DNA was extracted from samples using the All Prep DNA/RNA Kit (QIAGEN) according to the manufacturer's recommendations. Mutation analyses were performed for *ASXL1*,[16] *DNMT3A*,[17] *IDH1*,[18] *IDH2*,[19] *RUNX1*,[20] *NRAS*,[21] *TP53*,[22] *NPM1*,[21] and *SF3B1*,[9] as described previously. PCR fragments were sequenced directly and analyzed using Sequencing Analysis Version 5.3.1 software (Applied Biosystems) and Vector NTI Advance 10 software (Invitrogen). All mutations were confirmed in an independent experiment.

Analysis of mutations in genes of the splicing machinery

Preparation of mononuclear cells and genomic DNA was performed as described previously.[15,18] Genomic DNA was amplified using a whole-genome amplification kit following the manufacturer's recommendations (Sigma-Aldrich). All 11 exons of *ZRSR2* and the mutational hotspots of *U2AF1* (exon 2 and exon 6) and *SRSF2* (exon 1) were sequenced in all MDS patients using primers as described in supplemental Table 1. Mutational hotspots of *U2AF1* and *SRSF2* were also sequenced in 50 healthy volunteers. Patient DNA was amplified using the following PCR conditions: 95°C for 10 minutes and 39 cycles of 95°C for 1 minute, 56°C for 1 minute, and 72°C for 70 seconds, followed by 1 cycle of 72°C for

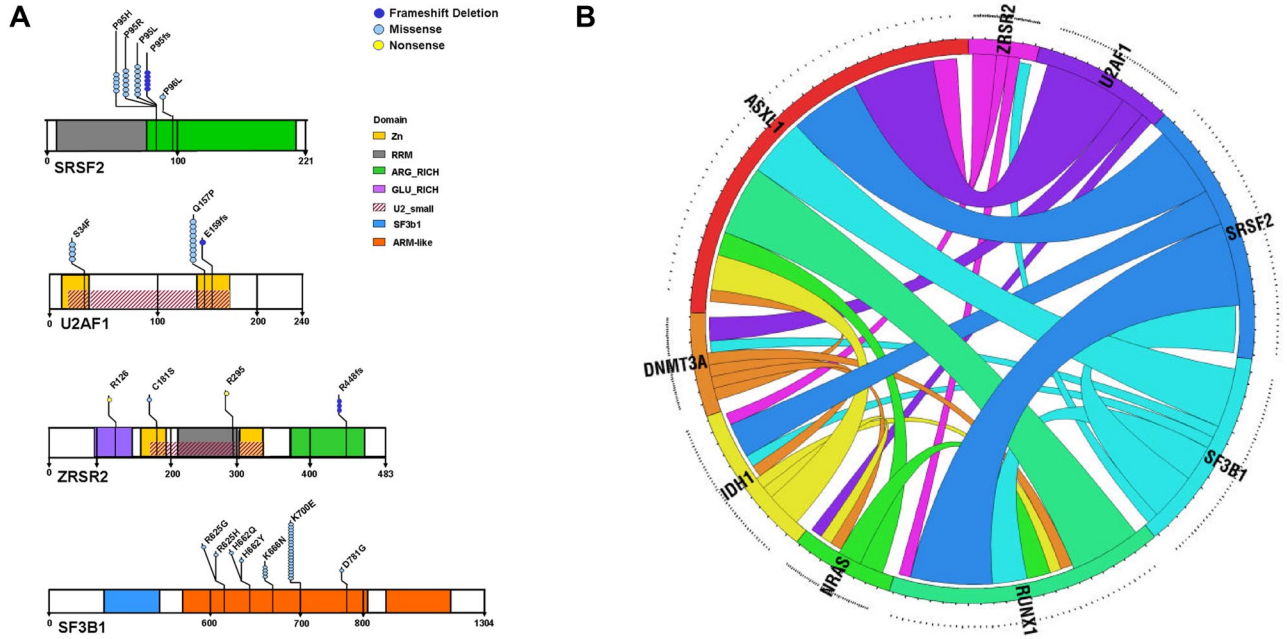

Figure 1. Gene mutations in patients with MDS. (A) Location and type of mutations in genes of the splicing machinery in 193 patients with MDS. (B) Associations of gene mutations in our MDS patient cohort outlined by a Circos diagram.

10 minutes. Sequencing of *SRSF2* required 2 rounds of amplification using nested primers with the same cycling conditions as above for 35 cycles with each primer pair (supplemental Table 1). Purified PCR fragments were sequenced directly using forward or reverse primers. The sequences were compared with the reference sequence available from Ensembl (transcripts *ZRSR2* ENST00000307771, *U2AF1* ENST00000291552, and *SRSF2* ENST00000392485). All mutations were confirmed in nonamplified genomic DNA in an independent experiment. The somatic or germline status of mutations in *U2AF1* and *SRSF2* was established by evaluating T cells ($CD3^+CD11b^-CD14^-CD33^-$) purified from diagnostic samples by flow cytometry.

Statistical analysis

Overall survival (OS) end points, measured from the date of first sample collection, were death (failure) and alive at last follow-up (censored). Time to AML progression was measured from date of first sample collection to the time of AML diagnosis. Progression to AML was defined according to the 2008 WHO classification. The median follow-up time for patients alive was calculated according to the method of Korn.[23] Primary analysis was performed on OS and time to AML progression. The Kaplan-Meier method, log-rank test, and Cox proportional hazards models were used to estimate the distribution of OS and time to AML progression and to compare differences between survival curves, respectively. Pairwise comparisons were performed by Mann-Whitney test or the Student *t* test for continuous variables and by 2-sided χ^2 tests for categorical variables and are provided for exploratory purposes. Associations between gene mutations are represented by a Circos diagram.[24] For multivariate analysis, a Cox proportional hazards model was constructed for OS and time to progression to AML, adjusting for potential confounding covariates.[25] Variables considered for model inclusion were IPSS, transfusion dependence, ferritin level (above or below 1000 μg/L), age (below vs above median), *SF3B1* mutation status, *ASXL1* mutation status, *RUNX1* mutation status, *IDH1* mutation status, *TP53* mutation status, *DNMT3A* and *NRAS* mutation status, and *SRSF2*, *U2AF1*, and *ZRSR2* mutation status. A limited backward-selection procedure was used to exclude redundant or unnecessary variables. Variables with $P \leq .1$ in univariate analysis for OS were included in the model. The 2-sided level of significance was set at $P < .05$. The statistical analyses were performed with the statistical software package SPSS Version 19.0 (IBM Deutschland GmbH).

Results

Mutation status of *SRSF2*, *U2AF1*, *ZRSR2*, and *SF3B1* in MDS patients

Mutations in one of the genes belonging to the splicing machinery (*SRSF2*, *U2AF1*, *ZRSR2*, and *SF3B1*) were found in 67 of 193 MDS patients (34.7%). Twenty-four patients were identified as harboring a mutation in *SRSF2* (12.4%), 14 patients showed a mutation in *U2AF1* (7.3%), and 6 patients were found to have a mutation in the coding region of *ZRSR2* (3.1%). In addition, we identified intronic mutations in 2 patients at base pair 1 and 7, respectively, of intron 7/8 of *ZRSR2*. As described previously, we identified 28 mutations of *SF3B1* in our cohort.[9] No aberrations in the mutational hotspots of *SRSF2* (exon 1), *U2AF1* (exon 2 and exon 6), or *SF3B1* (exons 13-16) were identified in any samples from healthy volunteers (n = 50). Interestingly, *SRSF2*, *U2AF1*, and *ZRSR2* mutations were mutually exclusive, with no patient having more than one of these genes affected (Table 1 and supplemental Tables 2 and 3). However, we identified 4 patients with concomitant *SF3B1* and *SRSF2* mutations and 1 patient with concomitant *SF3B1* and *ZRSR2* mutations (Table 1 and supplemental Tables 2 and 3). The majority of mutations in *SRSF2*, *U2AF1*, and *ZRSR2* were heterozygous point mutations (n = 33; Figure 1A); 9 frameshift and 2 nonsense mutations were also observed. In *SRSF2*, codon P95 located in exon 1 was most frequently mutated (n = 18), leading to a change from proline to leucine (n = 7), arginine (n = 6), or histidine (n = 5). Five patients harbored a frameshift mutation in codon P95 and 1 patient showed a heterozygous point mutation in codon P96 leading to a change from proline to leucine. In *U2AF1*, the most commonly affected codon was Q157 resulting in a glutamine to proline substitution in 9 patients. Four patients showed a heterozygous point mutation in codon S34 leading to a change from serine to phenylalanine and 1 patient had a frameshift mutation in codon E159. In *ZRSR2*, the only recurrent

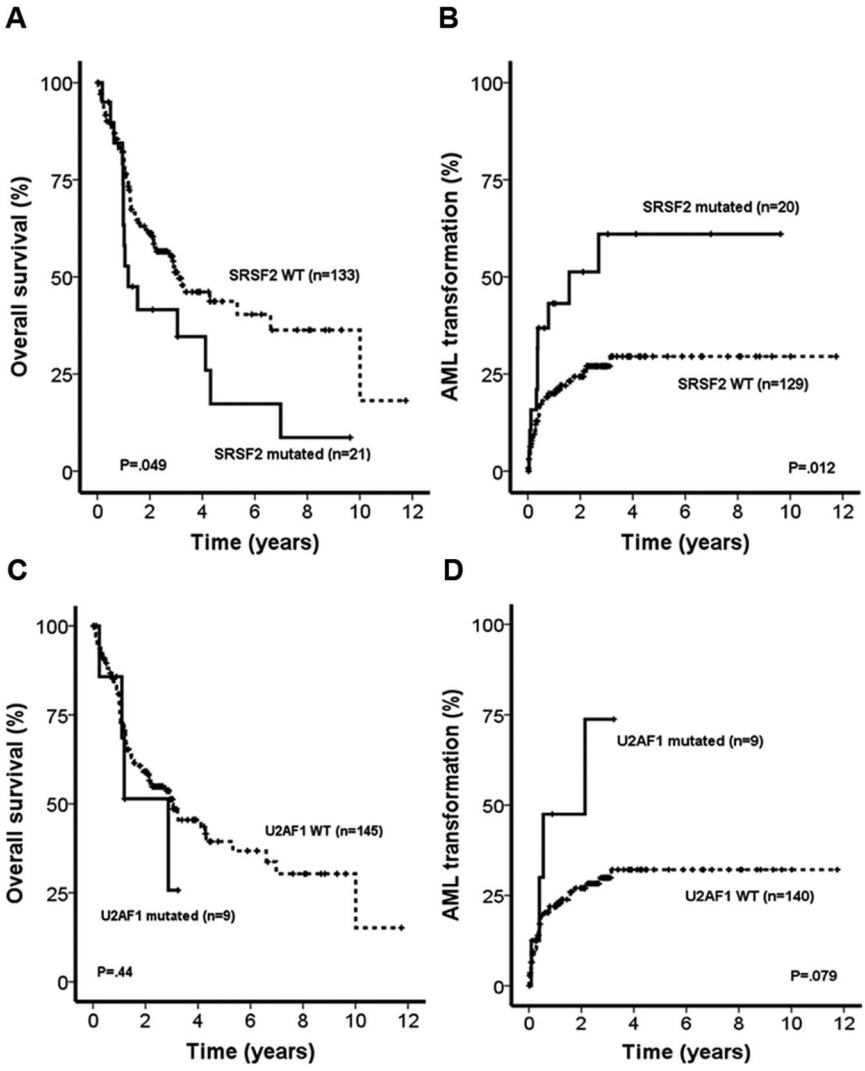

Figure 2. OS and time to AML progression in MDS patients. OS (A) and time to AML progression (B) in MDS patients according to *SRSF2* mutation status (log-rank test) are shown. OS (C) and time to AML progression (D) in MDS patients according to *U2AF1* mutation status (log-rank test) are shown.

mutation identified was a frameshift in codon R448 (n = 3). However, 2 heterozygous nonsense mutations affecting codons R126 and R295 and 1 heterozygous point mutation causing a change from cysteine to serine in codon C181 were also identified. The somatic nature of mutations in *U2AF1* and *SRSF2* was confirmed by sequencing nontumoral $CD3^+$ cells in 4 patients (2 patients with *U2AF1* Q157P mutations and 2 patients with *SRSF2* P95R mutations; supplemental Figures 1 and 2).

Patient characteristics in relation to *SRSF2*, *U2AF1*, and *ZRSR2* mutation status

The clinical and hematologic characteristics of patients with mutated versus wild-type (WT) *SRSF2*, *U2AF1*, and *ZRSR2* are shown in Table 1, supplemental Table 2, and supplemental Table 3, respectively. There were no significant differences in age, WHO classification, IPSS-based karyotype, BM blasts, hemoglobin, IPSS, or transfusion dependence between the mutated and the WT patients in any of the 3 genes. Interestingly, unlike *SF3B1*,[9] we did not identify an association between *SRSF2*, *U2AF1*, or *ZRSR2* mutation status and the presence of ring sideroblasts.

Patients with mutated *SRSF2*, but not *U2AF1* or *ZRSR2*, were found to have a higher transformation rate to AML ($P = .013$). We found a correlation between mutations in genes belonging to the splicing machinery and other mutations (Figure 1B). For example, there was an association between *SRSF2* mutations and *RUNX1* mutations ($P < .001$) and *IDH1* mutations ($P = .013$). Interestingly, *U2AF1* mutations were associated with mutations in *ASXL1* ($P = .005$) and *DNMT3A* ($P = .004$), both of which are involved in epigenetic regulation. Mutations of *ZRSR2*, which is located on the X chromosome, was found in both males (n = 4) and females (n = 2). We did not observe any association between *ZRSR2* and other mutations, which may be because of the low number of *ZRSR2*-mutated patients in our cohort. Considering mutations in all genes investigated in our study, at least 1 mutation was detected in 100 (51.8%) patients, of which 20 (10.4%) had 2 mutated genes and 11 (5.7%) had 3 mutated genes.

Prognostic impact of *SRSF2*, *U2AF1*, and *ZRSR2* mutations

The prognostic impact of mutations was evaluated in MDS patients for whom follow-up information was available (n = 154). The median follow-up of patients was 3 years. The prognostic impact of each mutated gene was calculated separately. Univariate analysis demonstrated that patients with *SRSF2* mutations had significantly shorter OS compared with patients without the mutation (5-year OS, 17% vs 39%; hazard ratio [HR] = 1.76; 95% confidence interval [95% CI], 1.0-3.1; $P = .049$; Figure 2A and Table 2) and

Table 2. Univariate and multivariate analysis for OS in MDS patients

	OS univariate analysis			OS multivariate analysis		
	HR*	95% CI	*P*	HR*	95% CI	*P*
SRSF2 mutation status, mutated vs WT	1.76	1.0-3.1	.049	2.3	1.28-4.13	.017
ASXL1 mutation status, mutated vs WT	2.06	1.21-3.50	.008	2.01	1.13-3.57	.017
RUNX1 mutation status, mutated vs WT	2.44	1.21-4.92	.013	1.28	0.60-2.71	.53
IDH1 mutation status, mutated vs WT	3.28	1.48-7.05	.003	1.90	0.79-4.58	.16
NRAS mutation status, mutated vs WT	5.39	2.29-12.66	< .001	5.25	1.99-13.84	.001
Transfusion dependence, dependent vs independent	3.72	1.70-8.14	.001	2.79	1.23-6.36	.014
IPSS, low vs intermediate 1 vs intermediate 2 vs high	1.73	1.35-2.2	< .001	1.48	1.13-1.94	.005

*HR > 1 indicates an increased risk of an event for the first category listed.

shorter time to AML progression (5-year AML-free survival 39% vs 69%; HR = 2.5; 95% CI, 1.22-5.1; $P = .012$; Figure 2B and Table 3). The relatively high incidence of mutations in *SRSF2* in the IPSS low and intermediate-1 group (n = 14) allowed us to perform a subgroup analysis in patients with low and intermediate-1 IPSS. In this subgroup, *SRSF2* mutations were associated with an inferior OS (5-year OS, 19% vs 50%; HR = 2.23; 95% CI, 1.11-4.5; $P = .025$) and shorter time to AML progression (5-year AML-free survival, 50% vs 81%; HR = 3.42; 95% CI, 1.14-10.27; $P = .028$; Figure 3A-B).

Mutations in *U2AF1* had no impact on OS (HR = 1.49; 95% CI, 0.54-4.1; $P = .44$; Figure 2C), but showed a trend toward a more rapid transformation to AML (HR = 2.53; 95% CI, 0.9-7.13; $P = .079$; Figure 2D and Table 3). However, this analysis was hampered by the small number of mutated patients (n = 9). The low incidence of *ZRSR2* mutations precluded a formal survival analysis. Three mutated patients died after 56 days, 1.4 years, and 3.5 years, whereas 3 patients were alive at last follow up 117 days, 2.1 years, and 5.8 years after diagnosis. Multivariate analysis for OS, including mutations in *SRSF2*, *ASXL1* (frameshift mutations), *RUNX1*, *NRAS*, and *IDH1*, as well as transfusion dependence and IPSS risk group, revealed that the presence of *SRSF2* mutations was an independent unfavorable prognostic factor for OS (HR = 2.3; 95% CI, 1.28-4.13; $P = .017$; Table 2). Multivariate analysis for AML transformation, including mutations in *SRSF2*, *U2AF1*, *ASXL1* (frameshift mutations), *RUNX1*, *TP53*, *NRAS*, and *IDH1*, as well as transfusion dependence and IPSS, demonstrated that the presence of *SRSF2* but not *U2AF1* mutations was found to be an independent unfavorable prognostic factor for AML transformation (*SRSF2*, HR = 2.83; 95% CI, 1.31-6.12; $P = .008$; Table 3; *U2AF1*, HR = 1.25; 95% CI, 0.36-4.33; $P = .73$; Table 3). The OS for patients with mutations in 1 of the splicing genes not associated with ring sideroblasts (ie, *U2AF1*, *SRSF2*, and *ZRSR2*) was significantly lower compared with WT patients (HR = 1.81; 95% CI, 1.11-2.98; $P = .018$; supplemental Figure 3A) and these patients had a significantly higher rate for AML transformation (HR = 2.33; 95% CI, 1.22-4.46; $P = .011$; supplemental Figure 3B). The OS and AML transformation rate was similar between mutated and WT patients when considering all 4 investigated splicing genes (*SRSF2*, *U2AF1*, *ZRSR2*, and *SF3B1*; OS, HR = 1.26; 95% CI, 0.8-1.99; $P = .31$; supplemental Figure 4A; AML transformation, HR = 1.28; 95% CI, 0.68-2.39; $P = .48$; supplemental Figure 4B).

Discussion

In this comprehensive analysis of 193 MDS patients, we identified mutations in at least 1 of the investigated genes involved in the splicing machinery (ie, *SRSF2*, *U2AF1*, *ZRSR2*, and *SF3B1*) in 34.7% of all investigated patients, suggesting that gene mutations involved in the splicing pathway are the most common mutations in MDS reported to date. Mutations in *SF3B1* and *SRSF2* were the most prevalent aberrations in MDS patients. *U2AF1* mutations were slightly less frequent and *ZRSR2* mutations were relatively rare. Unlike mutations in *SF3B1*,[6,9] mutations in *SRSF2*, *U2AF1*, and *ZRSR2* were not associated with the presence of ring sideroblasts. Instead, *SRSF2*, *U2AF1*, and *ZRSR2* mutations occurred in all different subtypes of MDS and were not associated with a specific IPSS risk profile or cytogenetic aberration. An interesting finding in our analysis is that mutations in *SRSF2* were associated with mutations in *RUNX1*, a gene coding for a transcription factor, as well as *IDH1*, a gene coding for an enzyme of the citric acid cycle. In contrast, mutations in *U2AF1* were associated with *ASXL1* and *DNMT3A* mutations, genes relevant for epigenetic regulation. The latter association is especially intriguing because chromatin and histone modifications have been shown to play a significant role in pre-mRNA splicing.[26]

SRSF2 mutations were associated with a negative prognostic impact in our study. Patients with this mutation had a significantly

Table 3. Univariate and multivariate analysis for risk of AML progression in MDS patients

	AML transformation univariate analysis			AML transformation multivariate analysis		
	HR*	95% CI	*P*	HR*	95% CI	*P*
SRSF2 mutation status, mutated vs WT	2.5	1.22-5.1	.012	2.83	1.31-6.12	.008
U2AF1 mutation status, mutated vs WT	2.53	0.90-7.13	.079	1.25	0.36-4.33	.73
ASXL1 mutation status, mutated vs WT	2.3	1.15-4.62	.019	2.34	1.12-4.89	.024
RUNX1 mutation status, mutated vs WT	3.02	1.26-7.26	.013	2.43	0.96-6.17	.062
TP53 mutation status, mutated vs WT	1.003	1.001-1.005	.009	1.01	1.002-1.008	.001
IDH1 mutation status, mutated vs WT	3.34	1.19-9.41	.023	2.33	0.75-7.21	.14
NRAS mutation status, mutated vs WT	3.23	0.98-10.68	.054	1.4	.034-5.79	.64
Transfusion dependence, dependent vs independent	6.77	1.63-28.09	.008	7.38	0.98-55.5	.052
IPSS, low vs intermediate 1 vs intermediate 2 vs high	2.84	1.99-4.06	< .001	2.89	1.93-4.31	< .001

*HR > 1 indicates an increased risk of an event for the first category listed.

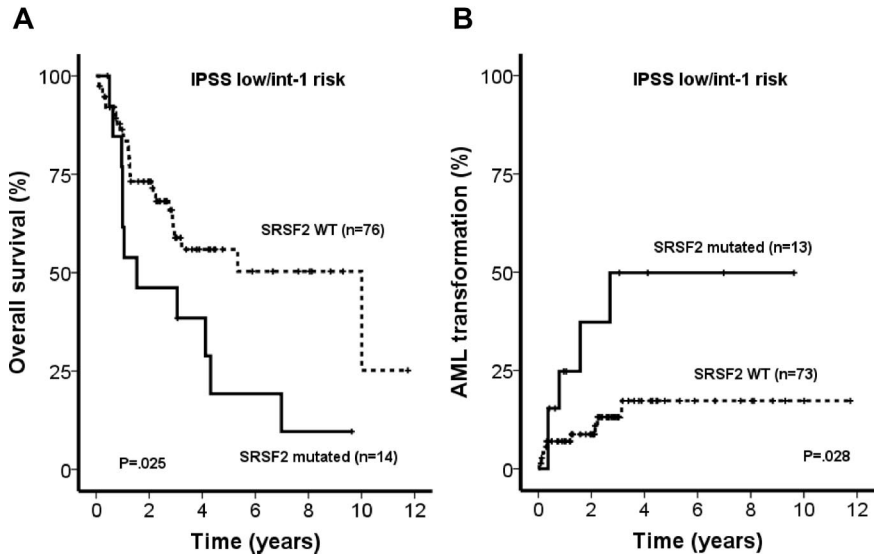

Figure 3. OS and time to AML progression in MDS patients. OS (A) and time to AML progression (B) in MDS patients with IPSS low/intermediate-1 risk according to *SRSF2* mutation status (log-rank test) are shown.

inferior OS and a more rapid and more frequent progression to AML. This effect was also evident when considering patients with low-risk MDS (IPSS low and intermediate-1) alone, suggesting that *SRSF2* mutations might be a molecular marker useful for risk stratification in the subgroup of lower-risk MDS patients. The negative prognostic impact was also seen in multivariate analysis both for OS and time to AML progression, further supporting the idea that *SRSF2* mutations represent an independent prognostic marker in MDS. In accordance with an earlier study, we did not find an impact on OS for *U2AF1* mutations.[27] Similarly to Graubert et al, who reported that *U2AF1* mutations were associated with an increased risk of progression to AML,[27] we observed a trend toward a more rapid progression to AML for patients with *U2AF1* mutations. However, the number of patients with *U2AF1* mutations was small, so the result requires confirmation in a larger patient cohort. Interestingly, in our analysis, point mutations in codon S34 were less frequent than mutations in codon Q157, whereas Graubert et al found more mutations in codon S34.[27] *ZRSR2* mutations had no prognostic effect, possibly related to the low mutation rate. Some studies have reported that *SF3B1* mutations are a favorable marker in MDS.[6,8] However, other studies, including our own analysis, show that *SF3B1* mutations do not represent an independent prognostic factor, but rather that the favorable prognosis of MDS patients with *SF3B1* mutations may be a consequence of the favorable prognosis for MDS patients with ring sideroblasts.[9,10] However, such a prognostic analysis of gene mutations in MDS is not only challenged by the heterogeneity of the disease, but potentially also by the different treatment strategies used. We evaluated OS in 1 uniformly treated patient group compared with all other patients; this comparison is hampered by several factors, including that patients were treated in different studies and that all lenalidomide-treated patients had 5q− syndrome, which has a better prognosis per se. Survival was similar in patients treated with antithymocyte globulin, thalidomide, or best supportive care compared with all other patients (data not shown). Lenalidomide-treated patients had an improved OS compared with all other patients ($P = .027$); however, all patients had 5q− syndrome, which has a better prognosis per se. Therefore, prospective, larger trials of uniformly treated patients are needed to confirm the effect of mutations on patient outcome.

The mechanisms of how different mutations in the same splicing pathway can have differing prognostic effects are unknown and need to be studied functionally. The presence of mutational hotspots in *SRSF2*, *SF3B1*, and *U2AF1* suggests gain-of-function mutations in these genes, all of which encode for proteins belonging to the splicing E/A complex. *U2AF1* and *SRSF2* bind to the RNA directly, whereas *ZRSR2* interacts with *U2AF1* and *SF3B1* binds to *U2snRNP*[7]. In addition to participating in the splicing process, *SRSF2* is also involved in the regulation of DNA stability.[28] It was shown previously that depletion of *SRSF2* contributes to genomic instability,[28] which is a predictive marker for adverse outcome in MDS,[29] and this could explain why *SRSF2* mutations had a strong adverse impact. It was also shown previously that mutated *U2AF1* has a growth-suppressive effect in vitro[7] and this effect might contribute to the cytopenias seen in clinical MDS patients. The fact that in the present study, only *SF3B1*, not *SRSF2*, *U2AF1*, or *ZRSR2*, were associated with ring sideroblasts, suggests that the latter 3 mutations have a different pathophysiologic effect in the biology of MDS than *SF3B1* mutations and underscores the idea that the genes in the splicing machinery have distinct biologic functions.

In summary, the results of the present study suggest that the splicing machinery is one of the most frequently affected pathways in MDS. Mutations in *SRSF2* are independently associated with a negative prognosis for OS and AML transformation. The utility of *SRSF2* mutations for treatment stratification should be assessed in future studies.

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Authorship

Contribution: F.T., A.G., and M.H. designed the research; F.T., S.K., C.S., P.L., M.M., M.W.W., B.K., M.W., K.G., and G.G. performed the research; F.T., J.K., G.B., O.O., W.-K.H., A.G., and M.H. contributed patient samples and clinical data; G.G. and B.S. performed cytogenetic studies; F.T., S.K., C.S., P.L., M.M., M.W.W., B.K., M.W., K.G., G.G., G.B., O.O., C.M.N., W.-K.H., B.S., A.G. and M.H. analyzed the data; F.T., A.G., and M.H. wrote the manuscript; and all authors read and agreed to the final version of the manuscript.

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