**SF3B1** mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts

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**Key Points**

- In MDS with ring sideroblasts, **SF3B1** mutation defines a homogeneous subgroup with isolated erythroid dysplasia and favorable prognosis.
- MDS with ring sideroblasts and wild-type **SF3B1** is mainly characterized by multilineage dysplasia and unfavorable prognosis.

**Introduction**

Ring sideroblasts characterize a group of myelodysplastic syndrome (MDS) first described in 1956 by Bjorkman. These disorders were categorized as idiothetic acquired sideroblastic anemia in the French-American-British (FAB) classification, and then as refractory anemia with ring sideroblasts (RARS) or refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS) in the World Health Organization (WHO) classification, according to the presence of 15% or more bone marrow ring sideroblasts and dysplasia in 1 or more myeloid lineages.

A high prevalence of somatic mutations in **SF3B1** was reported in MDS with ring sideroblasts. Mutations of **SF3B1** were found in about 30% of patients with MDS and 20% of patients with myelodysplastic/myeloproliferative neoplasm (MDS/MPN), but a significantly higher mutation prevalence was noticed in sideroblastic categories, including RARS, RCMD-RS, and the provisional entity defined as RARS associated with marked thrombocytosis (RARS-T).

However, in patients with MDS or MDS/MPN, the association between ring sideroblasts and **SF3B1** mutations is even stronger than suggested by the higher prevalence of these mutations in WHO categories with ring sideroblasts. In fact, ring sideroblasts can be detected at variable percentages also in patients assigned to WHO categories that are not defined by this morphologic feature.

In a group of myeloid disorders classified on the basis of morphologic criteria, identifying specific associations between genotype and disease


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There is an Inside Blood Commentary on this article in this issue.

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phenotypes is essential to defining disease entities according to their distinctive genetic profiles.11 Recently, based on a comprehensive mutation analysis,12 we sought for genotype-phenotype correlations adopting unsupervised hierarchical clustering analyses including the WHO classification criteria and somatic mutations.13 The results of these analyses suggested that mutation patterns may improve the classification of these neoplasms, and 2 disease subsets were recognized within MDS with ring sideroblasts according to SFS3B1 mutation status.13 However, further investigations are needed to identify the sources of variability of disease features and clinical outcome in MDS with ring sideroblasts.

In this study, we performed a comprehensive mutation analysis of genes implicated in myeloid disorders in a large and well-characterized cohort of myeloid neoplasms with 1% or more ring sideroblasts with the aim of identifying mutation patterns that affect disease phenotype and clinical outcome.

### Patients and methods

#### Patients and clinical procedures

These investigations were approved by the ethics committee of the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, the Karolinska Institutet, Stockholm, Sweden and other local Institutional Review Boards. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000, and samples were obtained after subjects provided informed consent.

We studied patients with myeloid neoplasms with 1% or more ring sideroblasts followed at the Department of Hematology Oncology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo (Pavia, Italy); the Department of Medicine, Division of Hematology, Karolinska University Hospital (Stockholm, Sweden); and the Department of Hematology, Copenhagen University Hospital (Copenhagen, Denmark). The study population consisted of 293 patients, including 243 patients with MDS (of whom 159 assigned to sideroblastic categories [RARS, RCMD-RS] and 84 to other WHO categories) and 50 with MDS/MPN (Table 1).

Diagnostic procedures were performed according to the recommendations of the European LeukemiaNet.14 To classify patients, peripheral blood and bone marrow specimens were analyzed by 2 independent hematopathologists who were blinded to clinical data. The diagnostic criteria of the WHO classification of tumors of hematopoietic and lymphoid tissues were adopted.15,16 Quantitative enumeration of myeloblasts, ring sideroblasts, and monocytes and their precursors was performed using recently established consensus criteria.17,18 Patients were studied at diagnosis or during follow-up before any disease-modifying treatment (ie, allogeneic stem cell transplantation, intensive chemotherapy, or hypomethylating agents). A portion of these patients were included in our recent studies of targeted gene sequencing in myelodysplasia.12,13

#### Sample collection and cell separation

Mononuclear cells were separated from bone marrow samples by standard density gradient centrifugation, and granulocytes were isolated from peripheral blood as previously described.19 Genomic DNA was obtained from bone marrow mononuclear cells or peripheral blood granulocytes by following standard protocols for human tissue.

#### Targeted gene sequencing

A core panel of 42 genes selected on the basis of prior implication in the pathogenesis of myeloid disease was analyzed in the whole study cohort (supplemental Table 1, see supplemental Data available at the Blood Web site).

One hundred eighty-three patients were studied using a panel of 111 genes selected on the basis of prior implication in the pathogenesis of myeloid disease using 2 lanes of Illumina HiSeq (Illumina Inc), as previously reported.12

One hundred ten patients were analyzed for 42 genes recurrently mutated in myeloid disorders using the Illumina HiSeq 2000 system at the Sci-Life laboratory (Stockholm, Sweden). Additional information is provided in supplemental Methods. Variant allele frequency (VAF) was calculated for each mutation identified as number of variant reads divided by total reads.

**Table 1. Clinical features of patients with myeloid neoplasms and ring sideroblasts included in the study**

<table>
<thead>
<tr>
<th>Features</th>
<th>No. of patients</th>
<th>Median age, (range), y</th>
<th>Sex, M/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS</td>
<td>243</td>
<td>70 (18-96)</td>
<td>153/140</td>
</tr>
<tr>
<td>RARS</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCMD-RS</td>
<td>69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCUD (RA)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RARS-T</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAEB-1</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAEB-2</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS/MPN</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMML</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median hemoglobin, (range), g/dL</td>
<td>9.9 (5.6-15.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC transfusion dependency (%)</td>
<td>108/293 (37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median WBC count, (range), ×10⁹/L</td>
<td>4.9 (1.0-13.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median ANC, (range), ×10⁹/L</td>
<td>2.5 (0.1-12.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median platelet count, (range), ×10⁹/L</td>
<td>263 (7-1130)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median serum ferritin, (range), ng/mL</td>
<td>541 (30-5745)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median bone marrow blasts, (range), %</td>
<td>2 (0-19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median bone marrow ring sideroblasts, (range), %</td>
<td>32 (1-95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosomal abnormalities (%)</td>
<td>83/277 (30)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANC, absolute neutrophil count; CMML, chronic myelomonocytic leukemia; F, female; M, male; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; RARS-T, RARS associated with marked thrombocytosis; RBC, red blood cell; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD and ring sideroblasts; RCUD, refractory cytopenia with unilineage dysplasia; WBC, white blood cell.

**JAK2 and CALR mutation analysis**

JAK2 (V617F) mutation was analyzed using a quantitative real-time polymerase chain reaction (qRT-PCR)-based allelic discrimination assay to obtain a precise quantification of mutant allele burden.19 Mutations of CALR, not included in the original panel of 111 genes, were analyzed by sequencing, as previously described.20

**Hematopoietic progenitor colony assay**

Hematopoietic progenitor colonies (colony-forming unit–erythroid, burst-forming unit–erythroid, colony-forming unit–granulocyte macrophage) were obtained from bone marrow mononuclear cells and individual colonies were isolated and genotyped, as previously reported.19

#### Statistical analysis

Comparison of numerical variables between groups was performed using a nonparametric approach (Mann-Whitney test or Kruskal-Wallis analysis of variance). Comparison of the distribution of categorical variables in different groups was performed with either the Fisher exact test or the χ² test. VAF estimates were used to evaluate clonal and subclonal variant relationships within each sample, as previously reported.12

 Survival analyses were performed by means of Cox proportional hazards regression. The cumulative incidence (CI) of disease progression was estimated with a competing risk approach, considering death for any cause as a competing event. The comparison of CI curves was carried out using the Pepe-Mori test,21 whereas the effect of quantitative covariates was estimated by applying the Fine-Gray regression model.22 All analyses accounted for
left censoring of the observations at the time of mutation assessment. Analyses were performed using Stata SE 12.1 software (StataCorp LP; http://www.stata.com).

Results

Spectrum of mutation in MDS with ring sideroblasts

Within the whole cohort of MDS with ring sideroblasts (number of patients: 243), the median number of mutation per patients was 1, ranging from 0 to 7. Overall, 18 MDS patients did not have evidence of genetic or cytogenetic abnormality, including 4 with RARS and with 3 RCMD-RS. No significant difference in mutation prevalence was observed among MDS categories, whereas a significantly lower median number of mutations per patient was observed in MDS compared with MDS/MPN (1, range 0-7, vs 2, range 0-7, P = .001).

The most frequently mutated gene categories in the whole cohort of MDS with ring sideroblasts were splicing factors (181 of 243, 74.5%), DNA methylators (81 of 243, 33%), chromatin modifiers (35 of 243, 14.4%), and transcription factors (28 of 243, 11.5%). The most frequent mutations involved SF3B1 (151 of 243, 62.1%), TET2 (49 of 243, 20.1%), DNMT3A (26 of 243, 10.7%), SRSF2 (19 of 243, 7.8%), ASXL1 (19 of 243, 7.8%), RUNX1 (12 of 243, 4.9%), EZH2 (10 of 243, 4.1%), TP53 (10 of 243, 4.1%), U2AF1 (7 of 243, 2.9%), ZRSR2 (6 of 243, 2.5%) (supplemental Figure 1A).

A significantly higher incidence of mutations in splicing factors was observed in sideroblastic categories (RARS, RCMD-RS) (139 of 159, 87.4%) compared with RA and RCMD with <15% ring sideroblasts (12 of 34, 35.3%) or RAEB (23 of 43, 53.5%) (P < .001). In detail, a significantly higher rate of SF3B1 mutations was noticed in sideroblastic categories (129 of 159, 81.1%) compared with RA/RCMD (4 of 34, 11.8%) or RAEB (13 of 43, 30.2%) (P < .001). Conversely, a significantly lower prevalence of mutations in splicing factors other than SF3B1 (namely, SRSF2, U2AF1, ZRSR2) was observed in RARS/RCMD-RS (11 of 159, 6.9%) compared with RA/RCMD (8 of 34, 23.5%) (P = .008) or RAEB (11 of 43, 25.6%) (P = .001) (supplemental Figure 1B). In addition, a significantly higher rate of SF3B1 mutations was noticed in MDS with isolated del(5q) (5 of 7, 71%) compared with RA/RCMD (P = .027).

A significantly higher prevalence of mutations in genes involved in DNA methylation was observed in RARS/RCMD-RS compared with RAEB (54 of 159 vs 5 of 43 respectively, P = .004). Conversely, a significantly lower rate of mutations in chromatin modifiers and transcription factors was found in sideroblastic categories compared with RAEB (17 of 159 vs 11 of 43, P = .022, and 9 of 159 vs 13 of 43, P < .001, respectively). In addition, a significantly higher prevalence of mutations in TP53 was found in RA/RCMD (4 of 34, 11.8%) and RAEB (4 of 43, 9.3%) compared with RARS/RCMD-RS (2 of 159, 1.2%) (P = .01). Finally, a significantly higher prevalence of mutations in genes involved in signaling was observed in MDS/MPN with ring sideroblasts compared with RARS/RCMD-RS (28 of 50 vs 7 of 159 respectively, P < .001).

Clinical and genetic correlates of SF3B1 mutation in MDS with ring sideroblasts

Within sideroblastic categories, SF3B1 mutation was found in 81 of 90 cases of RARS (90%) and 48 of 69 RCMD-RS (70%). Among patients classified in other MDS categories, SF3B1 mutations were observed in 22 of 84 (26.2%). In detail, SF3B1 mutations were detected in 4 of 27 cases of RCMD showing a proportion of ring sideroblasts ranging from 3% to 9%, 5 of 7 MDS with isolated del(5q) (ring sideroblasts from 21% to 69%), and 13 of 43 with RAEB-1 or -2 (ring sideroblasts from 7% to 78%).

When analyzing the whole MDS study population, patients with MDS carrying the SF3B1 mutation showed significantly lower incidence of multilineage dysplasia (46.3% vs 82.6%, P < .001), lower proportion of dysplastic myeloid cells and megakaryocytes (median values, 14% vs 27% and 3% vs 30%, respectively, P < .001), higher absolute neutrophil (2.62 × 10^9/L vs 1.6 × 10^9/L, P < .001) and platelet counts (269 × 10^9/L vs 123 × 10^9/L, P < .001), a higher proportion of bone marrow ring sideroblasts (40% vs 15%, P < .001) and serum ferritin level (untransfused patients: 408 vs 223 ng/mL, P < .001), as well as a significantly lower proportion of bone marrow
mutations showed a significant effect on overall survival (OS; hazard ratio [HR] = 2.6, P < .001) and on CI of disease progression accounting for death as a competing event (HR = 3.8, P = .007; CI of disease progression: HR = 2.2, P = .026) (Table 2; Figure 2C-D). This independent value was retained when including in the multivariable analyses including demographic and disease-related factors (OS: HR = .37, P = .003; CI of disease progression accounting for death as a competing event: HR = .31, P = .018) (Table 23-25).

Next, we performed uni- and multivariable analyses in patients stratified into WHO categories. When the analyses were focused on sideroblastic categories (RARS, RCMD-RS), the independent prognostic value of SF3B1 mutations was confirmed in both univariable (OS: HR = .25, P = .001; CI of disease progression: HR = .28, P = .032) and multivariable analyses (OS: HR = .27, P = .007; CI of disease progression: HR = .22, P = .026) (Table 2; Figure 2C-D). This independent value was retained when including in the multivariable analysis patients with RA or RCMD (OS: HR = .38, P = .009; CI of disease progression: HR = .33, P = .049). By contrast, within MDS with excess blasts (RAEB-1 and -2), the mutation did not retain significant effect on OS (P = .17) and CI of disease progression (P = .28).

Genetic determinants of disease phenotype and clinical outcome in MDS associated with SF3B1 mutation

We investigated whether the current WHO classification criteria may identify distinct subsets within SF3B1-mutated MDS. We first evaluated the impact of bone marrow blasts, and found that a percentage of bone marrow blasts ≥5% significantly affected survival and CI of disease progression (HR = 4.31, P = .02, and HR = 3.86, P = .05).
Table 2. Results of uni- and multivariable analyses in MDS with 1% or more ring sideroblasts and in sideroblastic categories (RARS, RCMD-RS)

<table>
<thead>
<tr>
<th>Variable</th>
<th>OS (HR, P)</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
<th>P</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDS with ≥1% ring sideroblasts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SF3B1 mutation</td>
<td>0.26</td>
<td>&lt;.001</td>
<td>0.003</td>
<td>.27</td>
<td>.001</td>
<td>.018</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.02</td>
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<td>.31</td>
<td>.98</td>
<td>.03</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>Bone marrow blasts*</td>
<td>1.17</td>
<td>&lt;.001</td>
<td>0.001</td>
<td>1.24</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Multilineage dysplasia†</td>
<td>3.7</td>
<td>&lt;.001</td>
<td>2.2</td>
<td>2.91</td>
<td>.030</td>
<td>.73</td>
<td></td>
</tr>
<tr>
<td>RBC transfusion dependency‡</td>
<td>1.89</td>
<td>.02</td>
<td>.026</td>
<td>1.52</td>
<td>.31</td>
<td>.87</td>
<td></td>
</tr>
<tr>
<td>Absolute neutrophil count§</td>
<td>2.96</td>
<td>.015</td>
<td>.46</td>
<td>4.79</td>
<td>.003</td>
<td>.48</td>
<td></td>
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<tr>
<td>Platelet count§</td>
<td>1.76</td>
<td>.006</td>
<td>.61</td>
<td>1.68</td>
<td>.038</td>
<td>.14</td>
<td></td>
</tr>
<tr>
<td>Karyotype§</td>
<td>2.28</td>
<td>&lt;.001</td>
<td>0.001</td>
<td>1.77</td>
<td>.01</td>
<td>.29</td>
<td></td>
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<tr>
<td><strong>Sideroblastic categories (RARS, RCMD-RS)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SF3B1 mutation</td>
<td>0.25</td>
<td>.001</td>
<td>0.007</td>
<td>0.28</td>
<td>.032</td>
<td>.026</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.03</td>
<td>.11</td>
<td>.098</td>
<td>.99</td>
<td>.11</td>
<td>.052</td>
<td></td>
</tr>
<tr>
<td>Bone marrow blasts*</td>
<td>1.23</td>
<td>.059</td>
<td>.89</td>
<td>1.18</td>
<td>.33</td>
<td>.51</td>
<td></td>
</tr>
<tr>
<td>Multilineage dysplasia†</td>
<td>3.06</td>
<td>.005</td>
<td>.21</td>
<td>2.21</td>
<td>.17</td>
<td>.53</td>
<td></td>
</tr>
<tr>
<td>RBC transfusion dependency‡</td>
<td>2.07</td>
<td>.046</td>
<td>.085</td>
<td>1.36</td>
<td>.56</td>
<td>.55</td>
<td></td>
</tr>
<tr>
<td>Absolute neutrophil count§</td>
<td>1.19</td>
<td>.86</td>
<td>.77</td>
<td>2.77</td>
<td>.29</td>
<td>.36</td>
<td></td>
</tr>
<tr>
<td>Platelet count§</td>
<td>2.20</td>
<td>.011</td>
<td>.51</td>
<td>1.64</td>
<td>.63</td>
<td>.39</td>
<td></td>
</tr>
<tr>
<td>Karyotype§</td>
<td>2.11</td>
<td>.029</td>
<td>.069</td>
<td>1.98</td>
<td>.085</td>
<td>.46</td>
<td></td>
</tr>
</tbody>
</table>

*Bone marrow blasts were analyzed as a percentage of bone marrow nuclear cells.8
†Multilineage dysplasia was analyzed as uni- vs multilineage dysplasia according to WHO criteria.9
‡RBC transfusion dependency was defined according to Malcovati et al.23 and Such et al.24
§Cytogenetic abnormalities, absolute neutrophil count, and platelet count were categorized according to the revised international prognostic scoring system.25

Next, we assessed the effect of the proportion of bone marrow ring sideroblasts (≥15% or <15%) among SF3B1-mutated patients, and found that this threshold did not have significant impact on OS (P = .83) and CI of disease progression (P = .85). Finally, we evaluated the impact of uni- or multilineage dysplasia within SF3B1-mutated patients, and no significant effect of multilineage dysplasia was found on OS (P = .5) and CI of disease progression (P = .94) (Figure 3C-D).

Therefore, we focused on MDS associated with SF3B1 mutation, defined by SF3B1 mutation, no excess blasts, or del(5q) irrespective of the proportion of ring sideroblasts (81 RARS, 48 RCMD-RS, 4 RA or RCMD) with the aim of identifying genetic determinants of disease phenotype variability and clinical outcomes. OS and CI of disease progression of MDS associated with SF3B1 mutation are shown in Figure 3A-B. Interestingly, DNA methylation gene mutations (TET2, DNMT3A) were significantly associated with multilineage dysplasia (P = .015) but neither number of dysplasias nor presence or absence of methylation gene mutations were associated with hematologic parameters, OS (P = .78), or CI of disease progression (P = .51) (supplemental Figure 3).

A significant correlation was observed between SF3B1 mutant allele burden and proportion of bone marrow ring sideroblasts (P = .009). VAF analysis showed that in most cases (91%) SF3B1 mutation was in the dominant clone.

We then used Cox regression models in SF3B1-mutated MDS with the aim of identifying mutation patterns associated with relevant clinical outcomes, including survival, risk of disease progression, and red blood cell (RBC) transfusion dependency. We found that mutations in RUNX1 were significantly associated with worse OS (HR = 9.02, P = .004) and higher CI of disease progression (HR = 19.12, P = .002) (Figure 3E-F). The prognostic value of RUNX1 mutations was confirmed in multivariable analyses adjusted for demographic and disease-related variables (P = .018 and P = .037 for OS and CI of disease progression, respectively).

Unambiguous statistical evidence of subclonality of RUNX1 mutations was obtained in 50% of cases. In addition, we found that mutations in RUNX1 and EZH2 were associated with a significantly higher CI of RBC transfusion dependency estimated with a competing risk approach (P = .002 and P = .007, respectively) (supplemental Figure 4).

**Mutation patterns in SF3B1-negative MDS with ring sideroblasts**

We then focused on SF3B1-unmutated MDS with ring sideroblasts, no excess blast, or del(5q) (7 RA, 23 RCMD, 9 RARS, 21 RCMD-RS). A significantly higher prevalence of mutations in splicing factors other than SF3B1 (SRSF2, U2AF1, ZRSR2) was observed in SF3B1-unmutated MDS with ring sideroblasts compared with MDS associated with SF3B1 mutation (18 of 60 vs 1 of 133, P < .001) (supplemental Figure 1B). In detail, 13 patients carried mutation of SRSF2, 4 of U2AF1, and 1 in ZRSR2 (Figure 1).

Within SF3B1-negative MDS with ring sideroblasts, a significantly higher prevalence of mutations in TP53 was found (9 of 93, P = .001), with 6 of 9 cases showing disease phenotype with multilineage dysplasia and no excess blasts. No frameshift or nonsense mutations were observed. All missense mutations found in these patients were located outside of p53 DNA binding surface in the β-sandwich region.

**Clonal analysis in MDS/MPN with ring sideroblasts**

Fifty patients with ring sideroblasts met WHO criteria for classification as MDS/MPN. Nine cases were classified as CMML; 4 of 9 patients had mutations in SF3B1, which was associated with mutation in TET2 in 3 of 4. Among the remaining ones, 4 had a mutation in SRSF2, 3 having comutation of TET2. All CMML with ring sideroblasts showed a myelodysplastic phenotype according to FAB criteria, whereas a higher proportion of ring sideroblasts was observed in SF3B1-mutated vs SRSF2-mutated patients (50% vs 6.5%).
Forty-one patients were classified as RARS-T according to WHO criteria. Thirty patients of 41 (73.2%) had mutation of SF3B1, 20 of JAK2 (V617F) (48.8%), 4 of MPL (9.8%), and 6 of CALR (14.6%). Six additional patients classified in different WHO categories showed JAK2 (V617F): 1 patient had a slight increase of bone marrow blasts, 1 had proportion of ring sideroblasts <15%, whereas 4 showed at the time of diagnosis a platelet count <450 × 10^9/L precluding the diagnosis of RARS-T. Overall, the occurrence of JAK2, MPL, or CALR mutations in patients with ring sideroblasts had a positive predictive value for myelodysplastic/myeloproliferative phenotype of 82%, whereas the absence of mutations in these genes had a negative predictive value of 95%.

SF3B1 median mutant allele burden was 43% (range, 14.7%-48.7%), consistent with the presence in the majority of patients of clonal hematopoiesis characterized by a dominant clone carrying a heterozygous SF3B1 mutation (supplemental Figure 5). Patients carrying the JAK2 (V617F) mutation had a median allele burden of 7.1% (range, 0.1%-27.7%), whereas median allele burden of MPL (W515L) and CALR mutations were 27.5% (range, 25%-50%) and 46.2% (range, 10%-59%), respectively.

Figure 3. OS and CI of disease progression of patients with MDS associated with SF3B1 mutation. (A-B) Respectively, OS and CI of disease progression of MDS associated with SF3B1 mutation, defined by SF3B1 mutation, no excess blasts, or del(5q) (81 RARS, 48 RCMD-RS, 4 RA, or RCMD). (C-D). Respectively, OS and CI of disease progression of patients with MDS associated with SF3B1 mutation according to uni- or multilineage dysplasia (P values from multivariable analyses, OS: P = .5; CI of disease progression: P = .94). (E-F) Respectively, OS and CI of disease progression of patients with MDS associated with SF3B1 mutation according to RUNX1 mutation status (RUNX1 unmutated = 128, RUNX1 mutated = 5) (P values from multivariable analyses, OS: P = .018; CI of disease progression: P = .037).
We genotyped individual colonies from peripheral blood in 4 patients with multiple mutations. In 3 cases, data were consistent with the existence of a dominant hematopoietic clone carrying the SF3B1 mutation with the subsequent emergence of a JAK2-mutated subclone (supplemental Figure 6A). The other patient, who was initially SF3B1-positive and JAK2-negative, had at the time of colony assay a mutant allele burden equal to 50% and 1% for SF3B1 and JAK2, respectively. Forty-three of 45 colonies were heterozygous for SF3B1 (K700E) and wild type for JAK2 mutation. The opposite pattern (JAK2-positive and SF3B1-negative) was observed in the remaining 2 colonies. All 45 colonies were found to carry a mutation of ARHGAP26 (R367Q), a GTPase activating protein that mediates the activity of the GTP binding proteins RhoA and Cdc42. These data indicate the coexistence of 2 distinct subclones originating from a founding clone, a dominant one carrying the SF3B1 mutation and a minority JAK2-positive clone (supplemental Figure 6B).

**Discussion**

In this study, we performed targeted gene sequencing in a large and well-characterized cohort of myeloid neoplasms with 1% or more ring sideroblasts. Our findings indicate that irrespective of current WHO criteria, SF3B1 mutation defines a distinct subset of MDS with homogeneous molecular/clinical features and favorable prognosis. Conversely, MDS patients with ring sideroblasts and wild-type SF3B1 have more heterogeneous features and worse outcome.

The studies published so far on the prognostic relevance of SF3B1 mutations in MDS have provided conflicting results.8 In a study including 107 MDS patients with ≥15% ring sideroblasts, SF3B1 mutation was associated with better OS and leukemia-free survival in univariable analysis, although this prognostic value was lost in multivariable analysis.26 Conversely, a recent study found that SF3B1-mutated cases had a significantly better survival compared with unmutated cases in both uni- and multivariable analysis.27 In our study, we analyzed a large cohort of 243 MDS with 1% or more ring sideroblasts, and found that SF3B1 mutations had a positive prognostic value on survival and risk of disease progression, and that this independent value was retained when the analysis was restricted to WHO subtypes without excess blasts or to sideroblastic categories.

We found that patients with MDS carrying the SF3B1 mutation showed a homogeneous phenotype characterized by a high prevalence of isolated erythroid dysplasia and a high proportion of bone marrow ring sideroblasts. Notably, current criteria adopted by the WHO to classify MDS subtypes without excess blasts failed to recognize distinct subsets within SF3B1-mutated MDS. In fact, in SF3B1-mutated patients multilineage dysplasia was sustained by mild myeloid dysplasia without significant effect on peripheral cytopenia and outcome. Occasional patients in our study had the SF3B1 mutation and a proportion of ring sideroblasts below the threshold of 15%, and no significant difference was observed in disease phenotype and outcome compared with patients having 15% or more ring sideroblasts, supporting the classification of these cases according to their SF3B1 mutation status. Conversely, the development of excess blasts significantly worsened the prognosis of SF3B1-mutated patients, suggesting that clonal evolution may overcome the positive prognostic value of the mutation. Taken together, these results suggest that SF3B1 mutation is the major determinant of disease phenotype, irrespective of current WHO classification criteria, and support the recognition of MDS associated with SF3B1 mutation as a distinct MDS subtype.

Bone marrow ring sideroblasts and SF3B1 mutation were also reported in a fraction of patients with MDS with isolated del(5q).10 In our cohort, only 7 patients with MDS with isolated del(5q) and ring sideroblasts were identified, precluding any further inference about the most appropriate classification of these cases. A recent study by Woll et al28 showed that del(5q) usually precedes recurrent driver mutations with the exception of SF3B1-mutated cases, in which the initiating event may be either del(5q) or SF3B1 mutation. Thus, additional studies of distinct myelodysplastic stem cells are needed to determine unambiguously the phylogenetic relationship among these driver lesions as a basis for a genetic ontogeny-based classification.

When focusing on MDS associated with SF3B1 mutation, defined by SF3B1 mutation, no excess blasts, or del(5q), irrespective of ring sideroblast percentage, we found that mutations in genes involved in DNA methylation were associated with multilineage dysplasia. Mutations in DNA methylators were reported to be early events in myeloid neoplasms,29,31 and to skew hematopoietic precursors toward myeloid differentiation.32 Consistent with these findings, mutations in TET2 and DNMT3A were reported to be associated with multilineage dysplasia in MDS.13

The recognition of a disease subset with a unique molecular basis may have potential clinical implications. We previously showed that patients with SF3B1 mutation have a high degree of ineffective hematopoiesis resulting in inappropriately low hepcidin levels and propensity to parenchymal iron loading.33 Recently, a transforming growth factor-β superfamily ligand was found to correct ineffective erythropoiesis in mice,34,35 and preliminary results from a phase 2 study in MDS showed a higher response rate in patients with the SF3B1 mutation.36 Furthermore, several compounds were reported to bind to the SF3b complex and to inhibit messenger RNA splicing,37,38 and preliminary results showed that SF3B1 modulators may induce tumor regression and increase survival in SF3B1-mutant xenografts.39

A high incidence of mutations in JAK2, as well as in MPL and CALR, was previously reported in patients with RARS-T in combination with SF3B1 mutations.10,19,20 In this study, we combined VAF analysis with genotyping of individual hematopoietic colonies in order to clarify the hierarchical relationship between mutations in these patients. Our results suggest that the occurrence of SF3B1 mutation represents an early event in patients with RARS-T, whereas the subsequent occurrence of a somatic mutation of JAK2 typically involves the emergence of minority subclones of the dominant SF3B1-positive clone. Taken together, these results suggest that the occurrence of mutations activating the JAK-STAT pathway is a recurrent pattern of evolution of SF3B1-positive clones, in accord with the hypothesis of genetic predisposition that early mutations shape the future trajectories of clonal evolution through constraints on the repertoire of cooperating genetic lesions.12

Compared with MDS associated with the SF3B1 mutation, SF3B1-unmutated MDS with ring sideroblasts showed a higher incidence of disease phenotype with multilineage dysplasia and a significantly worse prognosis, suggesting that these are indeed a different subset with clinical and hematologic features more similar to the WHO category of RCMD.13 In these patients, we observed a high prevalence of mutations in TP53, which are conversely mutually exclusive with SF3B1 mutations. Interestingly, all of the mutations found in these patients were missense and located outside of the p53 DNA binding surface in the β-sandwich region. The most characterized mutation in this region is Y220C, which forms a cavity on the p53 surface causing protein instability and aggregation,40 and several small molecules were reported to reactivate Y220C-mutated p53 with potential clinical application.41 This finding should be investigated in more
detail, and may open up a new diagnostic and therapeutic approach in treatment of these patients.

In conclusion, this study showed that SF3B1 mutation is a major determinant of disease phenotype and clinical outcome in MDS with ring sideroblasts. SF3B1-mutated MDS is characterized by homogeneous hematologic features, favorable prognosis, and restricted patterns of comutated genes and clonal evolution. Overall, these results strongly support the recognition of MDS associated with SF3B1 mutation as a distinct MDS subtype. Conversely, SF3B1-negative MDS with ring sideroblasts represents a subset with a high prevalence of TP53 mutations and worse outcome that should be taken into consideration in clinical decision-making.

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Authorship

Contribution: L.M., M.C., and E.H.L. designed the study, performed statistical analysis, and wrote the manuscript; E.P., M.K., A.G., D.P., R.R., and P.J.C. analyzed sequencing data and performed bioinformatic analysis; M. Jädersten, I.A., M. Jansson, C.E., G.W., M.G.D.P., K.R.-J., K.K., V.L., and S.C. collected clinical data; and E.T., R.I., and E.B. performed blinded revision of diagnostic specimens.

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