bjh short report

Distinct bone marrow immunophenotypic features define the splicing factor 3B subunit 1 (*SF3B1*)-mutant myelodysplastic syndromes subtype

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Summary

Splicing factor 3B subunit 1 (*SF3B1*) mutations define a distinct myelodysplastic syndromes (MDS) patient group with a relatively favourable disease course and high response rates to luspatercept. Few data are available on bone marrow phenotype beyond ring sideroblasts in this subgroup of patients with MDS. In the present study, we identified immunophenotypic erythroid, myelomonocyte and progenitor features associated with *SF3B1* mutations. In addition, we illustrate that *SF3B1*-mutation type is associated with distinct immunophenotypic features, and show the impact of co-occurrence of a *SF3B1* mutation and a deletion of chromosome 5q on bone marrow immunophenotype. These genotype–phenotype associations and phenotypic subtypes within *SF3B1*-MDS provide leads that may further refine prognostication and therapeutic strategies for this particular MDS subgroup.

Keywords: myelodysplastic syndromes, *SF3B1*, mutational analysis, flow cytometry, diagnostic haematology.

Introduction

Mutations in splicing factor 3B subunit 1 (*SF3B1*) are amongst the most prevalent genetic lesions observed in patients with myelodysplastic syndromes (MDS).[1,2] Since patients with MDS harbouring a mutation in *SF3B1* show a relatively favourable disease course and may respond well to luspatercept,[3] Malcovati *et al.*[4] recently proposed to classify *SF3B1*-mutatant MDS as a separate MDS-disease subtype. Apart from the strong correlation between the presence of a *SF3B1* mutation and ring sideroblasts, few data are reported on cellular phenotype in patients with *SF3B1*-mutatant MDS.[5] Hence, we evaluated the immunophenotype of the bone marrow cells in patients with MDS who were classified according to the *SF3B1* disease subtype. To address potential factors associated with interpatient variability within the *SF3B1*-based disease subtype, we evaluated the association of additional mutations and type of the *SF3B1* mutation with the distinct immunophenotypic features of bone marrow cells present in the *SF3B1*-MDS subtype. In addition, we studied the impact of co-occurrence of a chromosome 5q deletion (del(5q)), the only other genetic lesion defining a MDS-disease subtype, on the cellular phenotype of the *SF3B1*-disease subtype.

Methods

To this end, we analysed bone marrow specimens of 162 patients with MDS and 48 age-matched healthy individuals (further referred to as controls) (Table SI) included between 2009 and 2020 at the Amsterdam UMC location VUmc. The patients with MDS were split in two groups: patients with

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MDS meeting the criteria for the *SF3B1*-MDS subtype defined by Malcovati *et al.*[4] (*SF3B1*-MDS subtype) ($n = 53$) (Table SII) and the remaining patients with MDS (other-MDS) ($n = 109$). All bone marrow samples were assessed for MDS-associated mutations by next generation sequencing and MDS-associated dysplastic cell surface marker expression and cell subset distribution by immunophenotyping (Table SIII, Data S1). The research was conducted in accordance with the Declaration of Helsinki.

Results and discussion

First, we identified immunophenotypic features that were significantly different between both *SF3B1*-MDS subtype and other-MDS, and *SF3B1*-MDS subtype and controls (Fig 1).

Erythroid progenitors and mast cells as a percentage of bone marrow-nucleated cells were significantly higher in the *SF3B1*-MDS subtype compared to other-MDS and controls (Fig 1A). Only 8% of *SF3B1*-MDS subtype cases had increased values of cluster of differentiation 34 (CD34)-positive myeloid progenitors (>2% $CD34^+$ cells of nucleated cells) compared to 30% of other-MDS cases (Fisher's exact, $P = 0{\cdot}002$), which can at least partially be explained by the exclusion of MDS with excess of blasts from the *SF3B1* subtype. In the *SF3B1*-MDS subtype, neutrophil percentages were decreased compared to controls but less compared to other-MDS, for lymphocyte percentages the opposite was observed (Fig 1A).

In the *SF3B1*-MDS subtype, immunophenotype of erythroid progenitor cells was aberrant.[6,7] The coefficient of variation (CV) of CD71 was significantly higher compared to both other-MDS and controls, and the mean expression of CD71 was significantly lower (Fig 1B). Moreover, the percentage of erythroid cells with diminished (dim) CD71 expression was increased in *SF3B1*-MDS subtype (Fig 1B). The abnormal CD71 expression in *SF3B1*-MDS subtype suggests that the process of CD71 shedding is more severely disturbed in these patients compared to other-MDS.[8] In addition, erythrocyte progenitors displayed a higher sideward-light-scatter (further referred to as side scatter, SSC) than other-MDS and controls (Fig 1B). In *SF3B1*-MDS subtype, erythroid SSC correlated significantly with the percentage of ring sideroblasts (Spearman rank $\rho = 0{\cdot}35$, $P = 0{\cdot}03$), and the CV of CD71 negatively correlated with haemoglobin levels (Spearman rank $\rho = -0{\cdot}28$, $P = 0{\cdot}03$). Both the presence of ring sideroblasts and severe macrocytic anaemia are frequently observed in patients with the *SF3B1*-MDS subtype.[5]

Myeloid progenitors in the *SF3B1*-MDS subtype had a higher SSC compared to other-MDS and controls (Fig 1C). Neutrophils and monocytes ($CD45^{high}$, SSC^{dim}) in the *SF3B1*-MDS subtype displayed decreased CD11b expression (Fig 1C). In the *SF3B1*-MDS subtype, CD11b expression on monocytes and neutrophils correlated significantly ($\rho = 0{\cdot}8$, $P < 0{\cdot}001$), suggesting a similar mechanism is responsible for the decreased CD11b expression in both cell subsets. Besides decreased CD11b expression, monocytes in the *SF3B1*-MDS subtype expressed significantly lower levels of CD36 and CD64 (Fig 1C). CD11b, CD36 and CD64 are involved in many immunological processes including cell migration, the complement system and cell adhesion.[9,10] The reduced expression levels in *SF3B1*-MDS most likely impact cell function[11] and may be caused directly by mutations in *SF3B1* (e.g. altered splicing of the surface proteins) or induced by *SF3B1*-associated microenvironment factors (e.g. presence of specific inflammatory cytokines).[12] Future studies are warranted to clarify how *SF3B1* mutations influence (mature) myeloid cell functions and the potential consequences for MDS pathogenesis and therapeutic strategies.

Subsequently, we studied the *SF3B1*-associated features in the context of two potential factors associated with remaining interpatient variability within the *SF3B1*-MDS subtype: the presence of additional mutations and the type of *SF3B1* mutation. Patients within the *SF3B1*-MDS subtype harbouring only a mutation in *SF3B1* ($n = 20$) displayed lower CD64 expression on monocytes compared to patients with additional mutations ($n = 33$) ($P = 0{\cdot}04$). Monocyte CD64 expression in *SF3B1*-mutant cases with additional mutations was more comparable to controls. The other immunophenotypic features did not differ significantly between these patient groups, which accords with the prominent role of *SF3B1* in disease phenotype and the similar clinical characteristics of *SF3B1*-MDS with or without additional mutations.[4]

Distinct mutations in *SF3B1* occur in MDS and may impact phenotype.[1,13] The patients within the *SF3B1*-MDS subtype harbouring the most prevalent *SF3B1* mutation type, K700E ($n = 27$), displayed significantly lower expression of CD11b on monocytes ($P < 0{\cdot}001$) and neutrophils ($P < 0{\cdot}001$), higher percentages of mast cells ($P < 0{\cdot}001$), and lower CD36 expression on monocytes ($P = 0{\cdot}03$) compared to patients with the *SF3B1*-MDS subtype with other *SF3B1* mutations ($n = 26$) (Figure S1 and Table SIV). The observation that the K700E *SF3B1* mutation is associated with distinct immunophenotypic features compared to other *SF3B1*-mutation types, emphasises the need to take mutation type into account for future clinical (e.g. response to luspatercept) and translational studies. For the latter, the use of, e.g. mouse models or cell lines comprising one *SF3B1*-mutation type may not accurately reflect *SF3B1*-mutant MDS in general, and the evaluation of multiple *SF3B1*-mutation types is preferable. A recent study into a different mutation type in *SF3B1*, K666N, complements our findings, as K666N *SF3B1*-mutatant MDS had a distinct transcriptome and a relatively poor prognosis compared to MDS with other *SF3B1*-mutation types.[13]

A del(5q) is the only other genetic lesion defining a distinct MDS subgroup and in ~20% of this subgroup a mutation in *SF3B1* co-occurs.[1] Since del(5q) MDS display specific immunophenotypic features,[14] we explored whether patients

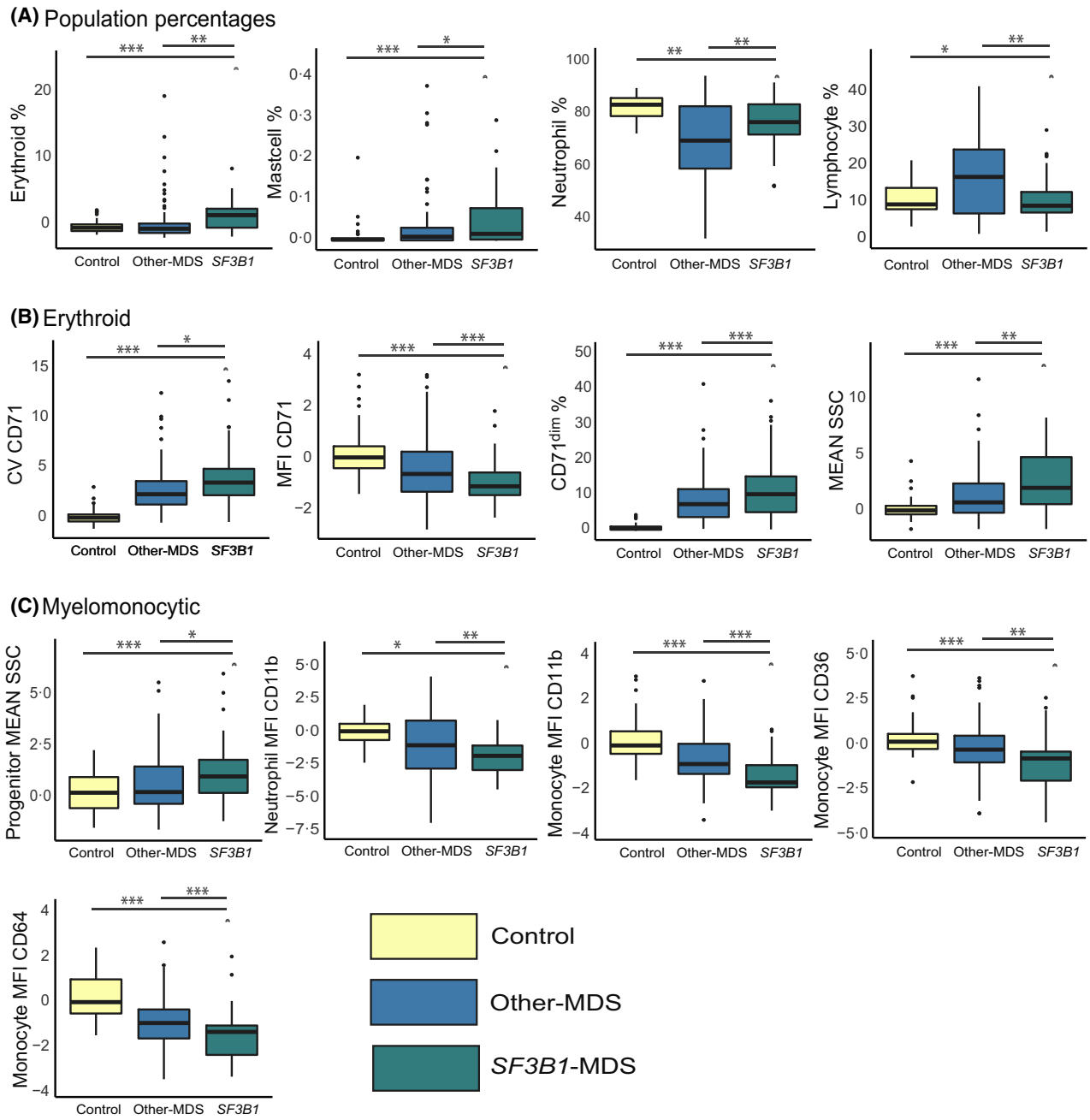

Fig 1. Immunophenotypic features associated with splicing factor 3B subunit 1-myelodysplastic syndromes (*SF3B1*-MDS) subtype. Patients within the *SF3B1*-MDS subtype (SF3B1-MDS) displayed immunophenotypic features that differed significantly from *SF3B1* wild-type MDS (Other-MDS) and controls (non-cytopenic and age-matched). Multiple bone marrow cell subsets (**A**), erythroid features (**B**) and myelomonocytic features (**C**) were associated with the *SF3B1*-MDS subtype. Erythroid progenitors were defined as CD36 and CD71 positive, myeloid progenitors as CD34 and CD117 positive, neutrophils as $CD45^{dim}$, side scatter (SSC) medium-high and CD34 negative, monocytes as CD45 high, SSC medium and CD11b, CD14 and CD36 positivity. Cell population percentages were calculated relative to total bone marrow white blood cell, with the exception of erythroid progenitors that were calculated relative to total nucleated cells. Antigen expression levels [both CVs and MFIs (on the *y*-axis)] were expressed relative to expression of controls.[15] The Mann–Whitney *U*-test was applied for the comparisons. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$. CV, coefficient of variation; dim, diminished; MFI, mean fluorescent intensity; SSC, sideward light scatter. [Colour figure can be viewed at wileyonlinelibrary.com]

with MDS harbouring both a *SF3B1* mutation and a del(5q) have either an immunophenotype more similar to *SF3B1*-MDS subtype or to del(5q) MDS without a *SF3B1* mutation. We first identified which features were significantly different between del(5q) MDS without a mutation in *SF3B1* ($n = 12$) and the *SF3B1*-MDS subtype ($n = 53$) (Fig 2, Table SV), and

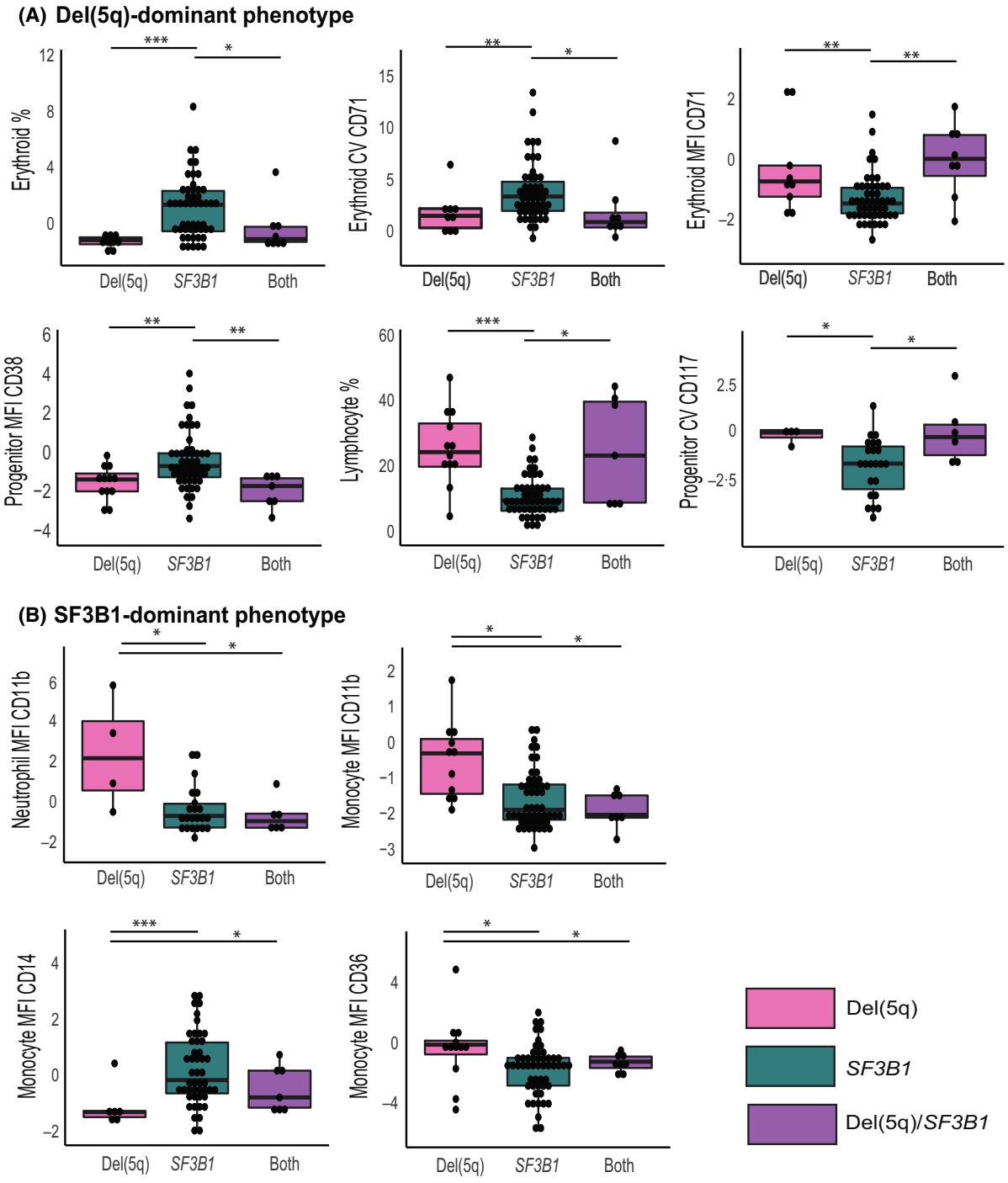

Fig 2. Immunophenotypic features in patients with del(5q), splicing factor 3B subunit 1-myelodysplastic syndromes (*SF3B1*-MDS) subtype, and patients with both genetic lesions. Immunophenotypic features significantly different between patients with the *SF3B1*-MDS subtype and patients with del(5q) were identified. Panel **A**, shows that of those features, six – concerning erythroid cells, myeloid progenitors and lymphocytes – were significantly different between patients with an isolated *SF3B1*-MDS subtype and patients with both a del(5q) and *SF3B1*-MDS. Panel **B**, shows that four other features – concerning maturing monocytes and neutrophils – were significantly different between del(5q) and patients with both a del(5q) and *SF3B1*-MDS subtype. Erythroid progenitors were defined as CD36 and CD71 positive, myeloid progenitors as CD34 and CD117 positive, neutrophils as $CD45^{dim}$, side scatter (SSC) medium-high and CD34 negative, monocytes as CD45 high, SSC medium and CD11b, CD14 and CD36 positivity. Population sizes were calculated relative to total bone marrow white blood cell, with the exception of erythroid progenitors that were calculated relative to total nucleated cells. Expression levels both CVs and MFIs (on the *y*-axis) were shown relative to expression of controls.[15] The Mann–Whitney *U*-test was applied for the comparisons. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$. CV, coefficient of variation; dim, diminished; MFI, mean fluorescent intensity; SSC, sideward light scatter. [Colour figure can be viewed at wileyonlinelibrary.com]

subsequently, studied these features in cases with both a *SF3B1* mutation and a del(5q) ($n = 8$). In patients with both genetic lesions, several features were more similar to del(5q) MDS, including the percentage of erythroid cells and expression of CD71 [CV and mean fluorescent intensity (MFI)] on erythrocyte progenitors, CD38 (MFI) and CD117 (CV) levels on progenitors and percentages of lymphocytes (Fig 2A). On the other hand, CD11b, CD14 and CD36 levels on monocytes and CD11b levels on neutrophils in patients with both a *SF3B1* mutation and a del(5q) were more similar to those in the *SF3B1*-MDS subtype (Fig 2B). In the proposed *SF3B1*-based MDS classification, patients with both a del(5q) and a *SF3B1* mutation are excluded from the *SF3B1*-based disease subtype and grouped with del(5q) patients as there were no clinical arguments to change the status quo.[3]

In MDS with both genetic lesions, erythroid and progenitor immunophenotypic features are consistent with the proposed classification as they resemble isolated del(5q) MDS. These observations suggest that patients with MDS harbouring an *SF3B1* mutation and a del(5q) may benefit from lenalidomide instead of luspatercept as a first-line of therapy.[3]

Conclusions

To improve treatment outcome in MDS, identification of patient characteristics that correlate with response and the further development of compounds that target downstream effects of the pathogenic genetic mutations is highly warranted. *SF3B1*-mutant MDS represents a clinically important subtype, but individual patients still show variation in response to therapy. The genotype–phenotype associations and phenotypic subtypes within *SF3B1*-mutant cases, described in the present study, may help further refine prognostic estimation for individual patients, both in the context of current and experimental therapies.

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Author contributions

Carolien Duetz designed the study, performed the analysis and wrote the manuscript, supported by Theresia M. Westers and Arjan A. van de Loosdrecht. Florentien E. M. in 't Hout, Eline M. P. Cremers, Canan Alhan, Bianca Venniker-Punt, Heleen A. Visser-Wisselaar, Dana A. Chitu, Aniek O. de Graaf, Linda Smit, Joop H. Jansen provided data and critically reviewed the methodology and manuscript.

Conflict of interest

The authors declare no competing financial interests.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Patients' characteristics.

Table SII. Criteria *SF3B1* subtype.

Table SIII. Flow cytometry panels.

Table SIV. Frequency of *SF3B1* mutation type.

Table SV. Patient characteristics of del(5q) and *SF3B1*^{mut} patients.

Data S1. Mutations in ASXL1, CBL, CEBPA, DNMT3A, ETV6/TEL, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, NRAS, RUNX1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2 were determined by multiple sequencing methods.

Fig S1. *SF3B1*-associated parameters that were significantly different between *SF3B1*-mutation type K700E (yellow) and other mutation types (blue) are depicted. Expression levels (on the Y-axis) are shown relative to expression of controls.[15] The Mann Whitney *U* test was applied for the comparisons. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

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