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SF3B1-mutant myelodysplastic syndrome as a distinct disease subtype - A Proposal of the IWG for the Prognosis of MDS

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Abstract:
The 2016 revision of the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues is characterized by a closer integration of morphology and molecular genetics. Notwithstanding, the myelodysplastic syndrome (MDS) with isolated del(5q) remains so far the only MDS subtype defined by a genetic abnormality. About half of MDS patients carry somatic mutations in spliceosome genes, with *SF3B1* being the most commonly mutated one. *SF3B1* mutation identifies a condition characterized by ring sideroblasts, ineffective erythropoiesis, and indolent clinical course. A large body of evidence supports recognition of *SF3B1*-mutant MDS as a distinct nosologic entity. To further validate this notion, we interrogated the dataset of the International Working Group for the Prognosis of MDS (IWG-PM). Based on the findings of our analyses, we propose the following diagnostic criteria for *SF3B1*-mutant MDS: (i) cytopenia as defined by standard hematologic values; (ii) somatic *SF3B1* mutation; (iii) morphologic dysplasia (with or without ring sideroblasts); (iv) bone marrow blasts <5% and peripheral blood blasts <1%. Selected concomitant genetic lesions represent exclusion criteria for the proposed entity. In patients with clonal cytopenia of undetermined significance, *SF3B1* mutation is almost invariably associated with subsequent development of overt MDS with ring sideroblasts, suggesting that this genetic lesion provides presumptive evidence of MDS in the setting of persistent unexplained cytopenia. Diagnosis of *SF3B1*-mutant MDS has considerable clinical implications in terms of risk stratification and therapeutic decision making. In fact, this condition has a relatively good prognosis and may respond to luspatercept with abolishment of transfusion requirement.

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Special Report

SF3B1-mutant myelodysplastic syndrome as a distinct disease subtype - A Proposal of the International Working Group for the Prognosis of Myelodysplastic Syndromes (IWG-PM)

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Abstract

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condition has a relatively good prognosis and may respond to luspatercept with abolishment of transfusion requirement.

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Introduction

The World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues has been revised in 2016.[1,2] While several novel molecular findings with diagnostic and/or prognostic importance have been incorporated into this revision, a closer integration of morphology and molecular genetics is still needed for many hematologic malignancies.

According to the WHO classification of myeloid neoplasms, myelodysplastic syndromes (MDS) are a group of clonal disorders characterized by morphologic dysplasia in hematopoietic cells, ineffective hematopoiesis, and peripheral cytopenia(s).[3] In the last few years, the ascertainment of clonal nature has become feasible in clinical practice with the use of massive parallel sequencing for identification of somatic gene mutations.[4] Mutated driver genes include those of RNA splicing, DNA methylation, histone modification, transcription regulation, DNA repair, signal transduction, and cohesin complex.[5,6]

Defining the genetic basis is clinically relevant not only in the diagnostic approach to MDS, but also in the prognostication and therapeutic decision making.[4] This paradigm is represented by the MDS with isolated del(5q), the only MDS subtype currently defined by a genetic abnormality.[3] Deletion 5q is a disease-defining genetic lesion as haploinsufficiency of several genes mapping on the deleted chromosomal region, including *CSNK1A1* and *RPS14*, explains the molecular pathophysiology of the

disease.[7,8] It also predicts response to lenalidomide, which induces the ubiquitination and degradation of CSNK1A1, abolishing the selective advantage of hematopoietic cells carrying del(5q).[9,10]

About half of MDS patients carry somatic mutations in spliceosome genes, and of these, *SF3B1* is the most commonly mutated one. The *SF3B1* gene encodes the splicing factor 3b subunit 1, and is typically mutated in MDS with ring sideroblasts (MDS-RS).[11,12] The revised WHO classification specifically accounts for this genetic lesion, and a diagnosis of MDS-RS can now be made if ring sideroblasts comprise as few as 5% of nucleated red cells and a somatic mutation of *SF3B1* is present.[3] Several lines of evidence support recognition of somatic *SF3B1* mutation as a disease-defining genetic lesion. In fact, it (i) most often represents a founding genetic lesion; (ii) is a major determinant of disease phenotype; (iii) has an independent prognostic value on survival and risk of progression to acute myeloid leukemia (AML); (iv) may predict response to specific agents.[13-17]

In this report, we analyzed the available evidence supporting the recognition of *SF3B1*-mutant MDS as a distinct nosologic entity. To validate our proposal, we interrogated the dataset of the International Working Group for the Prognosis of Myelodysplastic Syndromes (IWG-PM), including 3479 patients with known *SF3B1* mutation status from 18 centers or networks.

Current principles of MDS classification

According to the WHO classification, MDS are currently categorized according to the number of cytopenia at presentation, the number of lineages manifesting dysplasia, the percentage of ring sideroblasts, and of blasts in the bone marrow and the peripheral blood (Table 1).[3] While only one genetic abnormality, del(5q), is used to define a specific MDS subtype, i.e. MDS with isolated del(5q), selected cytogenetic abnormalities are recognized as "MDS defining" in a cytopenic patient, as they provide presumptive evidence of MDS even in the absence of definitive morphologic features.

The MDS with ring sideroblasts (MDS-RS) is subdivided into a condition with single (erythroid) lineage dysplasia (MDS-RS-SLD), and a condition with multilineage dysplasia (MDS-RS-MLD).[3,18]

SF3B1 mutation is critical to the pathophysiology of myelodysplasia and ring sideroblasts

SF3B1 mutation is an initiating genetic lesion in MDS

Several lines of evidence are consistent with the notion that *SF3B1* mutation may be an initiating genetic event and that primitive lympho-myeloid hematopoietic stem cells represent the propagating cells in *SF3B1*-mutant MDS.[6,11-13,15,19,20]

Previous reports showed that *SF3B1* mutations are typically heterozygous and the overall median VAF is approximately 40%.[6,11-13] These data have been confirmed by the analysis of VAF reported in the IWG dataset, which showed median values for the observed variants ranging from 0.35 to 0.43.

Computational prediction in MDS-RS patients with one or more recurrent driver mutations based on targeted sequencing data, coupled with mutational analysis of the *SF3B1* gene in hematopoietic stem/progenitor cells, demonstrated that the *SF3B1* mutation may occur alone or as the first event in most cases, whereas it appears to be secondary to other oncogenic mutations in a minority of cases,[15,19,20] In these latter subjects, most frequently *SF3B1* mutations are occurring on the background of *TET2*-, *DNMT3A*- or *ASXL1*-mutated age-related hematopoietic clones (Figure 1).[14,15]

Phenotypic and functional evidence also indicated that the most primitive lympho-myeloid hematopoietic stem cells (Lin−CD34+CD38−CD90+CD45RA−) represent the origin of the mutated *SF3B1* clone in MDS with ring sideroblasts, and also represent the rare MDS propagating cells.[15,20] Mutations identified in the hematopoietic stem cell compartment were also present in downstream myeloid and erythroid progenitor cells.[15]

Relationship between *SF3B1* mutation, aberrant mRNA splicing, and ring sideroblasts

The strong association between *SF3B1* mutation and myelodysplasia with ring sideroblasts was evident since the first reports.[11,12] A subsequent study provided evidence that, when accounting for cases assigned to non-sideroblastic WHO categories, *SF3B1* mutation had a positive predictive value of 98% for disease phenotype with ring sideroblasts.[13] These data are consistent with a causal relationship between *SF3B1* mutation and bone marrow ring sideroblasts.

Following these genotype-phenotype correlation analyses, investigations were then performed to explore the abnormal biologic pathways and networks downstream of the mutation. Studies on cell lines and primary human cells showed that the mutant SF3B1 protein retains altered function, resulting in deregulated expression and splicing of key genes and pathways in myelodysplastic hematopoietic stem and progenitor cells.[21,22] Conditional knock-in mouse models of the most common *SF3B1* mutation, *Sf3b1*(K700E), confirmed that *Sf3b1*(K700E) mice develop macrocytic anemia, erythroid dysplasia, and long-term hematopoietic stem cell expansion.[23,24]

RNA sequencing studies in *SF3B1*-mutated cells provided evidence that most of the aberrant splicing events selectively observed in *SF3B1*-mutated samples are caused by misrecognition of 3' splice sites, resulting in a frameshift.[16,25] These studies also indicated that approximately 50% of the aberrant mRNAs induced by *SF3B1* mutations undergo degradation by a nonsense-mediated mRNA decay (NMD) pathway, resulting

in down-regulation of canonical transcripts and protein expression.[16,25] In addition, it is also possible that NMD-insensitive aberrant transcripts are translated into aberrant proteins with altered function.[16,25,26]

Two genes involved in mitochondrial iron metabolism synthesis, *PPOX* and *ABCB7*, were found to be significantly downregulated in *SF3B1*-mutated samples. As *PPOX* encodes protoporphyrinogen oxidase, which catalyzes the dehydrogenation of protoporphyrinogen IX to form protoporphyrin IX, it is likely that haploinsufficiency of this gene may induce defective heme synthesis and iron accumulation into the mitochondria. *ABCB7*, the causative gene of congenital sideroblastic anemia with cerebellar ataxia, uniformly showed reduced expression in *SF3B1*-mutated samples, consequent to abnormal splicing and NMD.[16,27] Forced ABCB7 expression was found to restore erythroid colony growth and decreased mitochondrial ferritin expression level in CD34+ cells from MDS with ring sideroblasts, supporting the hypothesis that *ABCB7* is implicated in the phenotype of this disorder.[28,29]

SF3B1 **mutation is a major determinant of disease phenotype in MDS**

SF3B1 mutation is associated with a highly homogeneous disease phenotype and distinctive demographic features

Patients with *SF3B1*-mutant MDS show a homogeneous disease phenotype characterized by erythroid dysplasia with ring sideroblasts, and ineffective

erythropoiesis.[13,14] Furthermore, cases with multilineage dysplasia according to current WHO morphological criteria have only very mild dysplasia in granulocytic or megakaryocytic lineage without significant effects on peripheral cytopenia (Figure 3).[14]

These observations are fully confirmed by interrogating the IWG registry. Patients reported in this dataset were originally classified according WHO criteria 2008. These analyses clearly show that *SF3B1* mutations are enriched in the RARS category, accounting for 82% of cases, as well as in the RCMD-RS category (75%) (Table 2). In addition, *SF3B1* mutations are also reported in 9% of patients with RCUD or RCMD. It must be noted that most of these patients harboring an *SF3B1* mutation and 5% or more RS are expectedly reclassified into the category of MDS-RS according to 2016 WHO criteria.[3,18] In addition, we took advantage from the large IWG dataset to explore the relationship between *SF3B1* mutation type, VAF and disease phenotype. No significant association was found between the most common *SF3B1* mutations or VAF and WHO categories (P=0.11 and P=0.08, respectively).

In agreement with previous findings, when compared to *SF3B1*-unmutated MDS, *SF3B1*-mutated MDS show significantly lower hemoglobin values, consistent with a high degree of ineffective erythropoiesis, higher neutrophil and platelet counts, and lower bone marrow blasts (P<0.001) (Table 2). It is worth noting that 89% and 86% of patients with *SF3B1*-mutant MDS have normal or nearly normal neutrophil and platelet counts (i.e. absolute neutrophil count, ANC, >1.0 x 10^9/L, and PLT count >100 x 10^9/L) at the time of the registration into the IWG dataset.

Compared with the whole MDS population, *SF3B1*-mutated MDS display a significantly higher prevalence of females, resulting in a male to female ratio close to 1:1 (Table 2). Notably, a similar profile is also typically observed in the only genetically-defined MDS subtype, i.e. MDS with del(5q).[18] In addition, individuals with *SF3B1*-mutated MDS have a disease onset at a significantly older age than those with *SF3B1*-unmutated MDS (P<0.001) (Table 2).

WHO classification criteria fail to segregate distinct subsets within *SF3B1*-mutant MDS

Previous reports suggested that the current WHO classification criteria do not allow identification of distinct subsets within *SF3B1*-mutated MDS, supporting the notion that *SF3B1* mutation is a major determinant of disease phenotype in MDS.[14,30,31] In fact, the threshold of 15% for ring sideroblasts failed to stratify the prognosis of *SF3B1*-mutated patients.[14,31] In addition, single- or multi-lineage dysplasia did not show effect on survival or risk of disease progression within *SF3B1*-mutated patients.[14] This observation is fully confirmed by the analysis of IWG dataset that clearly show that the presence of a single or a multi-lineage dysplasia according to WHO morphological criteria does not have any impact on survival of patients with *SF3B1*-mutated MDS (P=0.4) (Figure 4A). Conversely, in agreement with previous reports,[14] the occurrence of an excess blasts significantly affects survival of patients with *SF3B1*-mutated MDS (P<0.001) (Figure 4B), suggesting that clonal evolution may overcome the prognostic advantage of *SF3B1* mutation.

Taken together, these results suggest that *SF3B1* mutation is the major determinant of disease phenotype, irrespective of current WHO classification criteria. In agreement with this conclusion, a previous study adopting unsupervised hierarchical clustering analyses showed that *SF3B1* mutation is recognized as a hierarchically high classification criterion identifying a highly homogeneous group of patients, and that, within the group of MDS with ring sideroblasts, two subsets were segregated according to *SF3B1* mutation status.[30]

SF3B1 **mutation is a favorable prognostic factor**

When analyzing the whole MDS study population, several studies suggested that *SF3B1* mutations had a positive prognostic value on overall survival and risk of disease progression. Some conflicting results were obtained when these analyses were adjusted for phenotypic covariates, mostly due to high collinearity of genotype- and phenotype-related variables.[11,13,14,30]

An analysis on the largest cohort of *SF3B1*-mutated MDS patients so far reported showed that the mutation retained an independent positive prognostic value in multivariable analyses including demographic and disease-related factors. The independent prognostic value of *SF3B1* mutations was confirmed when the analyses were focused on sideroblastic categories. By contrast, within MDS with excess blasts,

the mutation did not retain significant effect on survival and risk of disease progression.[14]

These findings are confirmed by the analysis of IWG dataset that shows that *SF3B1* mutation identifies a subgroup of MDS with favorable prognosis (P<0.001) (Figure 5A). A stratified analysis within IPSS-R categories[32] indicates that this positive prognostic value is significant within very low and low IPSS-R categories (P=0.002), whereas it is not retained within intermediate (P=0.66) and high- or very high-risk groups (P=0.11). Notably, the positive prognostic value of *SF3B1* mutation is also confirmed within the categories of RARS (P<0.001) and of RCMD-RS (P=0.003) (Figure 5B and 5C). In addition, in order to estimate the prognostic effect of the mutation in 2016 MDS-RS categories, we generated two groups of patients including RARS and *SF3B1*-mutated RCUD, and RCMD-RS and *SF3B1*-mutated RCMD, respectively. Compared with the respective 2016 categories, these groups comprised occasional patients with *SF3B1*-mutation and less than 5% RS. The positive prognostic value of *SF3B1* mutation was fully confirmed within the categories of single-lineage (P<0.001) and multi-lineage dysplasia (P=0.003) (Figure 5D and 5E). No significant effect of *SF3B1* mutation type and VAF was observed on survival. Taken together, these data suggest that within MDS-RS, *SF3B1* mutation represents a classification criterion stronger than single- or multi-lineage dysplasia, and concur to support the recognition of MDS with mutated *SF3B1* as a distinct disease entity.

Analysis of the IWG dataset confirmed that the mutation did not retain significant effect on survival and risk of disease progression within MDS with excess blasts (Figure 5F), suggesting that subclonal mutations driving clonal evolution may overcome the prognostic advantage of *SF3B1* mutation.

***SF3B1* mutation constrains the spectrum of genetic events driving clonal progression**

The available evidence suggests that progression to higher-risk MDS or AML occurs with a relatively low frequency in *SF3B1*-mutated MDS, and is driven by a restricted repertoire of cooperating genetic lesions.[6,14]

The IWG dataset enabled us to validate and expand these observations by testing the prognostic value of co-occurring cytogenetic abnormalities and somatic mutations in the largest cohort of *SF3B1*-mutant MDS reported so far. Overall, only 3% of patients with MDS and *SF3B1* mutation reported in the IWG dataset had a poor or very poor risk karyotype according to IPSS-R stratification (Table 2). This figure decreased to 1% in patients without excess blasts. Within these latter, a significant effect of IPSS-R poor or very poor cytogenetic risk compared to very low, low or intermediate risk groups was noticed on OS (P=.032, P=.007 and P=0.49, respectively). Within IPSS-R poor or very poor cytogenetic risk, the negative prognostic value of monosomy 7 was fully confirmed (n=7, P<0.001).

A recent comprehensive transcriptomic analysis showed that a high proportion of *SF3B1* mutated cases clustering in the category with high risk of leukemic transformation showed over-expression of *EVI1*, resulting from aberrant gene fusions, including *NRIP1-EVI1* and *RUNX1-EVI1,* or 3q26 abnormality.[33] Accordingly, in a recent study on genomic classification of AML, a clustering of *SF3B1*-mutated cases has been also reported in AML with inv(3) or t(3;3).[34] Thirteen *SF3B1*-mutated patients in the IWG dataset harbored an inv(3) or t(3;3). These subjects showed markedly lower OS (median, 27 vs 60 months) and higher risk of AML evolution (5-year cumulative incidence, 75% vs 40%) compared to *SF3B1*-mutated patients without chromosome 3q26 abnormalities, though these differences did not reach statistical significance (P=.13 and P=.11, respectively).

Overall, *SF3B1* mutation is associated with a restricted spectrum of subclonal mutations driving clonal progression (Figure 1). According to the available evidence, mutations in epigenetic regulators, including *TET2*, *DNMT3A* and *ASXL1*, did not affect survival of MDS with *SF3B1* mutation.[14] Conversely, *RUNX1* mutations have been reported to be significantly associated with increased risk of disease evolution.[6,14]

We tested the prognostic value of the number of mutations and the most frequent co-occurring or biologically relevant mutated genes in *SF3B1*-mutant MDS within the IWG dataset. When focusing the analysis on *SF3B1*-mutant MDS without excess blasts, the number of co-occurring mutations (i.e. isolated *SF3B1* mutation vs 1, 2 or 3 additional mutations) did not significantly affect OS (P values ranging from 0.90 to 0.07) (Figure 6A). The prognostic value of *RUNX1* mutations was confirmed highly significant on

both OS and cumulative incidence of AML evolution (P<.001) (Figure 6B and 6C). In addition, significant effects on OS were noticed for mutations in *EZH2* (P=.003) (Figure 6D), previously reported associated with increased risk of developing transfusion-dependency in *SF3B1*-mutated MDS,[14] and in *NF1* (P=.003), a functional target of mutant *SF3B1*-associated splicing.[16] The effect of *RUNX1* and *EZH2* mutations was confirmed in a multivariable analysis adjusted for IPSS-R risk categories (HR=2.66, P<0.001 and HR=2.25, P=0.001, respectively), whereas *NF1* mutations did not retain statistical significance (HR=1.43, P=0.50).

In addition, a significant co-occurrence has been reported between *SF3B1* mutations and JAK-STAT pathway activating mutations, including the classical *JAK2* (V617F) and less frequently *CALR* or *MPL* mutations.[13,14,35-38] This mutation pattern is typically associated with the MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), currently recognized by the WHO classification as a distinct disease entity.[39] The available evidence suggests that *SF3B1* mutations act as initiating lesions, responsible for myelodysplastic features, i.e. ineffective erythropoiesis and ring sideroblasts, whereas *JAK2*, *MPL* or *CALR* mutations drive the emergence of subclones conferring the myeloproliferative phenotype.[14,36] Within the IWG dataset, a significantly higher prevalence of *JAK2* and *MPL* mutations was observed in *SF3B1*-mutated compared to *SF3B1*-unmutated MDS (Figure 2A). Although these patients did not fulfil WHO criteria for a diagnosis of MDS/MPN-RS-T, a significantly higher platelet count was found in *SF3B1*-mutated patients carrying either *JAK2* or *MPL* mutation compared with those wild type for these co-mutations (P<0.001).

Clinical features and outcomes of patients with MDS with ring sideroblasts without *SF3B1* mutation

About 20% of MDS-RS according to current WHO criteria do not harbor the *SF3B1* mutation.[5,6,11-14] The available evidence is suggesting that *SF3B1*-unmutated MDS-RS have clinical features and outcome significantly different from *SF3B1*-mutated MDS, with a significantly higher prevalence of myeloid and megakaryocyte dysplasia (Figure 3) and reduced survival.[14] These findings are fully confirmed by the interrogation of the IWG dataset that showed that *SF3B1*-negative MDS-RS have a significantly shorter survival compared to the *SF3B1*-mutated group (Figure 5B and 5C). While no specific mutation profile was identified in this subset, a significantly higher prevalence of mutations in *TP53* was reported.[14] Mutation patterns of *SF3B1*-unmutated MDS-RS within the IWG dataset are reported in Figure 1B and 2B.

Although the molecular basis of this subset remains to be clarified, at present it seems rational to confirm *SF3B1*-unmutated cases with ring sideroblasts within the distinct category of MDS-RS according to current WHO classification criteria.[18]

Relationship between *SF3B1* mutation and del(5q)

SF3B1 mutations have been reported in about 20% of patients classified with the category of MDS with isolated del(5q), associated with a variable proportion of ring sideroblasts.[5,6,13,14] These cases are classified within the category of MDS with isolated del(5q) according to current WHO criteria (Table 1).[18]

The reported co-occurrence of *SF3B1* and del(5q) is consistent with the prevalence of this genotype within the IWG dataset (Table 2). We analyze the clinical outcome of patients with MDS with isolated del(5q) according to *SF3B1* mutation status within the IWG-PM dataset, and no significant difference in overall survival was noticed (P=.57). In addition, no significant effect of the presence or absence of del(5q) on survival of *SF3B1*-mutated MDS without excess of blasts was found (P=.40).

A study combining single hematopoietic stem and progenitor cell and DNA mutational analysis by targeted sequencing and exome sequencing, provided evidence that del(5q) usually precedes recurrent driver mutations in isolated del(5q) MDS, whereas in cases of ring sideroblastic anemia del(5q) may be either preceded or be followed by *SF3B1* mutation.[19] Although genetic ontogeny of these myelodysplastic clones might inform the classification process and determine whether a case with concomitant del(5q) and *SF3B1* mutation should be more appropriately classified as MDS with isolated del(5q) or MDS with mutated *SF3B1*, in many cases clonal hierarchy cannot be easily and unequivocally solved in the everyday clinical practice. Therefore, at present it seems sensible that these cases should be classified according to current WHO criteria with

the category of MDS with isolated del(5q).[18] Additional information useful to the classification of these cases might derive from studies investigating the effect of this genotype and clonal hierarchy on response to lenalidomide and luspatercept.

Proposed diagnostic criteria for MDS with mutated *SF3B1*

According to the available evidence and the results of the IWG dataset analysis, the following classification criteria are proposed for the MDS with mutated *SF3B1*: (i) cytopenia defined by standard hematologic values;[40] (ii) somatic *SF3B1* mutation; (iii) isolated erythroid or multilineage dysplasia (ring sideroblasts are not required for the diagnosis); (iv) bone marrow blasts <5% and peripheral blood blasts <1%; (v) WHO criteria for MDS with isolated del(5q), MDS/MPN-RS-T or other MDS/MPN, and primary myelofibrosis or other MPN are not met. Due to their significant negative prognostic value and distinctive interaction with *SF3B1* mutations, the following genetic lesions represent robust exclusion criteria for the proposed entity (Table 3): (i) poor risk genetic features, including monosomy 7, inv(3) or abnormalities of chromosome 3q26, resulting in aberrant gene fusions and over-expression of *EVI1*, and complex karyotype (≥3 chromosomal abnormalities); (ii) co-occurring mutations in *RUNX1* and/or *EZH2*.

Clinical and hematological features and survival of patients classified according to the proposed criteria are reported in Table 4 and Figure 7.

Significance of *SF3B1* mutation in Clonal Hematopoiesis of Indeterminate Potential (CHIP) and Clonal Cytopenia of Undetermined Significance (CCUS)

SF3B1 have been reported as driver mutated genes in a fraction of individuals with CHIP.[41,42] In these subjects without any hematologic phenotype, median variant allele frequency of driver mutations was typically significantly lower than that observed in patients receiving a diagnosis of MDS.[43] Whether these studies intercepted a very early phase of the evolutionary trajectory of *SF3B1*-mutated clones preceding clinical expressivity or whether additional genetic events are required to promote their expansion, remains to be clarified.

In addition, *SF3B1* mutations were detected in a fraction of patients with idiopathic cytopenia of undetermined significance (ICUS) not fulfilling diagnostic criteria for MDS (CCUS).[44-46] Preliminary observations suggested that in these patients, *SF3B1* mutations were highly predictive of developing MDS with ring sideroblasts,[46] suggesting that this genetic lesion in cytopenic patients might provide presumptive evidence of MDS even in the absence of definitive morphological features, as previously acknowledged for selected cytogenetic abnormalities, including del(5q).[3,47,48] However, prospective studies are warranted to validate these observations and establish the value of *SF3B1*-mutated clones in the context of cytopenia of undetermined significance, and patients with these features should be carefully monitored and repeated tests, including bone marrow examination, should be performed to reach a conclusive diagnosis.

Functional consequences of *SF3B1* mutation are candidate therapeutic targets

Emerging experimental and clinical evidence suggests that *SF3B1* mutation and its functional consequences on erythropoiesis are candidate targets for therapeutic intervention.

SF3B1-mutant patients have high degree of ineffective hematopoiesis that results in elevated erythroferrone levels and inappropriately low serum hepcidin, as typically observed in congenital iron loading anemias due to ineffective erythropoiesis.[26,49] Transforming growth factor-β superfamily ligand traps have been found to reduce aberrant Smad2/3 signaling and enhance late-stage erythropoiesis in animal models of ineffective erythropoiesis.[50-52]

Luspatercept is a recombinant fusion protein that binds transforming growth factor-β superfamily ligands to reduce Smad2/3 signaling. In a phase 2 study, luspatercept was found to be effective for the treatment of anemia in lower-risk MDS.[53] In a subsequent phase 3, placebo-controlled study on transfusion-dependent patients with MDS-RS, luspatercept treatment abolished transfusion requirement in about 40% of cases.[54] The fact that more than 90% of these patients carried a somatic mutation of *SF3B1*, indicates that this drug can be particularly effective in *SF3B1*-mutant MDS-RS.

Several compounds can modulate RNA splicing by a direct interaction with the SF3b complex.[55,56] Emerging experimental evidence suggests that cancer cells bearing point mutations in the RNA splicing factor-encoding genes are dependent on wild-type spliceosome function, thus resulting in the preferential killing of spliceosome-mutant cells.[56] These data demonstrate the therapeutic potential of splicing modulation in spliceosome-mutant cancers and clinical studies are ongoing.

Conclusions and open questions

The available evidence and the findings of our analyses indicate that *SF3B1*-mutant MDS represents a distinct entity, mainly characterized by ineffective erythropoiesis, relatively good prognosis, and potential response of anemia to luspatercept treatment.

A limited number of concomitant genetic abnormalities are associated with poor outcome, and represent exclusion criteria for the proposed nosologic entity. Co-occurrence of JAK-STAT pathway activating mutations is typically associated with thrombocytosis, indicating the diagnosis of MDS/MPN-RS-T. A fraction of patients with *SF3B1* mutation have relative or absolute monocytosis, indicating a CMML, but the concurrent genetic lesions driving this phenotype remain to be clarified.

In patients with CCUS, *SF3B1* mutation is almost invariably associated with subsequent development of overt MDS with ring sideroblasts, suggesting that this mutation might

be included among the genetic lesions that provide presumptive evidence of MDS even in the absence of definitive morphological features.

Finally, *SF3B1*-unmutated MDS-RS appears to be a more heterogeneous group with less favorable prognosis and a largely obscure molecular basis, and additional efforts are warrant to fully elucidate the pathophysiology of these disorders.

Authorship

Contribution: LM and MC conceived this special report; KS and DN performed statistical analyses of IWG dataset; EP, PG, SO and EHL contributed to interpretation of the data and report design; RB., JB., DB., PJC, BLE, PF, TH; MH, JJ, RSK, JPM, MJW, MF, GGM, TAG, AK, MM, AP, DS, MRS, MAS, DS, ST, FT, PV collected clinical and molecular data; AvdL, DH, and HT were responsible for curation of IWG dataset. All Authors contributed to manuscript preparation and approved its content.

Conflict-of-interest disclosure

RB: consulting for AbbVie, Astex, Celgene, Daiichi-Sankyo, Forty Seven, and NeoGenomics; honoraria for serving on steering and data safety monitoring committees for Celgene; research funding from Celgene and Takeda. MH: honoraria from Novartis, Pfizer, PriME Oncology; consulting or advisory role for Abbvie, Bayer Pharma AG, Daiichi Sankyo, Novartis, Pfizer; research funding to institution from Astellas, Bayer Pharma AG, BergenBio, Daiichi Sankyo, Karyopharm, Novartis, Pfizer, Roche. DS: institutional research funding by H3 Biosciences; Consulting for Celgene. LM, KS, EP, DN, JB, TH, DB, PJC, BLE, PF, JJ, RSK, JPM, MJW, MF, GGM, TAG, AK, MM, AP, DS, MRS, MAS, ST, FT, PV, AvdL, DH, HT, PLG, SO, EHL, MC declare no competing financial interests.

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References

1. Swerdlow S.H. CE, Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Arber, D.A., Hasserjian R.P., Le Beau, M.M., Orazi, A., Siebert, R. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon: IARC; 2017.
2. Cazzola M. Introduction to a review series: the 2016 revision of the WHO classification of tumors of hematopoietic and lymphoid tissues. Blood. 2016;127(20):2361-4.
3. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.
4. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. Blood. 2013;122(25):4021-34.
5. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241-7.
6. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616-27.
7. Ebert BL, Pretz J, Bosco J, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature. 2008;451(7176):335-9.
8. Schneider RK, Adema V, Heckl D, et al. Role of casein kinase 1A1 in the biology and targeted therapy of del(5q) MDS. Cancer Cell. 2014;26(4):509-20.
9. List A, Dewald G, Bennett J, et al. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med. 2006;355(14):1456-65.
10. Kronke J, Fink EC, Hollenbach PW, et al. Lenalidomide induces ubiquitination and degradation of CK1alpha in del(5q) MDS. Nature. 2015;523(7559):183-8.
11. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med. 2011;365(15):1384-95.
12. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478(7367):64-9.
13. Malcovati L, Papaemmanuil E, Bowen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011;118(24):6239-46.
14. Malcovati L, Karimi M, Papaemmanuil E, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126(2):233-41.

15. Mortera-Blanco T, Dimitriou M, Woll PS, et al. SF3B1-initiating mutations in MDS-RSs target lymphomyeloid hematopoietic stem cells. Blood. 2017;130(7):881-90.
16. Shiozawa Y, Malcovati L, Galli A, et al. Aberrant splicing and defective mRNA production induced by somatic spliceosome mutations in myelodysplasia. Nat Commun. 2018;9(1):3649.
17. Fenaux P, Platzbecker U, Mufti GJ, et al. The Medalist trial: results of a phase 3, randomized, double-blind, placebo-controlled study of luspatercept to treat anemia in patients with very low-, low-, or intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts (RS) who require red blood cell (RBC) transfusions [abstract]. Blood. 2018;132(Suppl 1):1. Abstract 3.
18. Hasserjian RP, Orazi A, Brunning R, et al. Myelodysplastic syndromes: Overview. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. Lyon (France): IARC; 2017. p. 98-106.
19. Woll PS, Kjallquist U, Chowdhury O, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. Cancer Cell. 2014;25(6):794-808.
20. Mian SA, Rouault-Pierre K, Smith AE, et al. SF3B1 mutant MDS-initiating cells may arise from the haematopoietic stem cell compartment. Nat Commun. 2015;6:10004.
21. Dolatshad H, Pellagatti A, Fernandez-Mercado M, et al. Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndrome hematopoietic stem and progenitor cells. Leukemia. 2014;29:1798.
22. Pellagatti A, Armstrong RN, Steeples V, et al. Impact of spliceosome mutations on RNA splicing in myelodysplasia: dysregulated genes/pathways and clinical associations. Blood. 2018;132(12):1225-40.
23. Obeng EA, Chappell RJ, Seiler M, et al. Physiologic Expression of Sf3b1(K700E) Causes Impaired Erythropoiesis, Aberrant Splicing, and Sensitivity to Therapeutic Spliceosome Modulation. Cancer Cell. 2016;30(3):404-17.
24. Mupo A, Seiler M, Sathiaseelan V, et al. Hemopoietic-specific Sf3b1-K700E knock-in mice display the splicing defect seen in human MDS but develop anemia without ring sideroblasts. Leukemia. 2017;31(3):720-7.

25. Darman RB, Seiler M, Agrawal AA, et al. Cancer-Associated SF3B1 Hotspot Mutations Induce Cryptic 3? Splice Site Selection through Use of a Different Branch Point. Cell Rep. 2015;13(5):1033-45.
26. Bondu S, Alary AS, Lefevre C, et al. A variant erythroferrone disrupts iron homeostasis in SF3B1-mutated myelodysplastic syndrome. Sci Transl Med. 2019;11(500).
27. Dolatshad H, Pellagatti A, Liberante FG, et al. Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes. Leukemia. 2016;30(12):2322-31.
28. Boultwood J, Pellagatti A, Nikpour M, et al. The role of the iron transporter ABCB7 in refractory anemia with ring sideroblasts. PLoS ONE. 2008;3(4):e1970.
29. Nikpour M, Scharenberg C, Liu A, et al. The transporter ABCB7 is a mediator of the phenotype of acquired refractory anemia with ring sideroblasts. Leukemia. 2012;27:889-96.
30. Malcovati L, Papaemmanuil E, Ambaglio I, et al. Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. Blood. 2014;124(9):1513-21.
31. Patnaik MM, Hanson CA, Sulai NH, et al. Prognostic irrelevance of ring sideroblast percentage in World Health Organization-defined myelodysplastic syndromes without excess blasts. Blood. 2012;119(24):5674-7.
32. Greenberg PL, Tuechler H, Schanz J, et al. Revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes. Blood. 2012;120(12):2454-65.
33. Shiozawa Y, Malcovati L, Galli A, et al. Gene expression and risk of leukemic transformation in myelodysplasia. Blood. 2017;130(24):2642-53.
34. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med. 2016;374(23):2209-21.
35. Malcovati L, Della Porta MG, Pietra D, et al. Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Blood. 2009;114(17):3538-45.
36. Broseus J, Alpermann T, Wulfert M, et al. Age, JAK2(V617F) and SF3B1 mutations are the main predicting factors for survival in refractory anaemia with ring sideroblasts and marked thrombocytosis. Leukemia. 2013;27(9):1826-31.
37. Broseus J, Florensa L, Zipperer E, et al. Clinical features and course of refractory anemia with ring sideroblasts associated with marked thrombocytosis. Haematologica. 2012;97(7):1036-41.

38. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379-90.
39. Orazi A, Hasserjian RP, Cazzola M, Thiele J, Malcovati L. Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis. In: Swerdlow S.H. CE, Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Arber, D.A., Hasserjian R.P., Le Beau, M.M., orazi, A., Siebert, R., editor. WHO Classification of Tumors Of Haematopoietic and Lymphoid Tissues. Lyon (France): IARC; 2017. p. 93-4.
40. Greenberg PL, Tuechler H, Schanz J, et al. Cytopenia levels for aiding establishment of the diagnosis of myelodysplastic syndromes. Blood. 2016;128(16):2096-7.
41. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488-98.
42. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477-87.
43. Malcovati L, Cazzola M. The shadowlands of MDS: idiopathic cytopenias of undetermined significance (ICUS) and clonal hematopoiesis of indeterminate potential (CHIP). Hematology Am Soc Hematol Educ Program. 2015;2015(1):299-307.
44. Kwok B, Hall JM, Witte JS, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. 2015;126(21):2355-61.
45. Cargo CA, Rowbotham N, Evans PA, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. Blood. 2015;126(21):2362-5.
46. Malcovati L, Galli A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood. 2017;129(25):3371-8.
47. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292-302.
48. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-51.
49. Ambaglio I, Malcovati L, Papaemmanuil E, et al. Inappropriately low hepcidin levels in patients with myelodysplastic syndrome carrying a somatic mutation of SF3B1. Haematologica. 2013;98(3):420-3.

50. Suragani RN, Cadena SM, Cawley SM, et al. Transforming growth factor-beta superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. Nat Med. 2014;20(4):408-14.
51. Dussiot M, Maciel TT, Fricot A, et al. An activin receptor IIA ligand trap corrects ineffective erythropoiesis in beta-thalassemia. Nat Med. 2014;20(4):398-407.
52. Fenaux P, Kiladjian JJ, Platzbecker U. Luspatercept for the treatment of anemia in myelodysplastic syndromes and primary myelofibrosis. Blood. 2019;133(8):790-4.
53. Platzbecker U, Germing U, Gotze KS, et al. Luspatercept for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): a multicentre, open-label phase 2 dose-finding study with long-term extension study. Lancet Oncol. 2017;18(10):1338-47.
54. Fenaux P, Platzbecker U, Mufti GJ, et al. The Medalist trial: results of a phase 3, randomized, double-blind, placebo-controlled study of luspatercept to treat anemia in patients with very low-, low-, or intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts (RS) who require red blood cell (RBC) transfusions. Blood. 2018;132(Suppl 1):1. Abstract
55. Lee SC, Abdel-Wahab O. Therapeutic targeting of splicing in cancer. Nat Med. 2016;22(9):976-86.
56. Seiler M, Yoshimi A, Darman R, et al. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. Nat Med. 2018;24(4):497-504.

Table 1. Diagnostic criteria for MDS entities

Name	Dysplastic lineages	Cytopenias	Ring sideroblasts*	BM and PB blasts	Cytogenetics°
MDS with single lineage dysplasia (MDS-SLD)	1	1 or 2	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	2 or 3	1-3	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)					
MDS-RS-SLD	1	1 or 2	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS-MLD	2 or 3	1-3	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1-3	1-2	None or any	BM <5%, PB <1%, no Auer rods	del(5q) alone or with 1 additional abnormality except −7 or del(7q)
MDS with excess blasts (MDS-EB)					
MDS-EB-1	0-3	1-3	None or any	BM 5%-9% or PB 2%-4%, no Auer rods	Any
MDS-EB-2	0-3	1-3	None or any	BM 10%-19% or PB 5%-19% or Auer rods	Any
MDS, unclassifiable (MDS-U)					
1% blood blasts	1-3	1-3	None or any	BM <5%, PB = 1%,‡ no Auer rods	Any
SLD and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
defining cytogenetic abnormality	0	1-3	<15%§	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	1-3	1-3	None	BM <5%, PB <2%	Any

*Ring sideroblasts as % of marrow erythroid elements. °Cytogenetics by conventional karyotype analysis.
† If *SF3B1* mutation is present. ‡ One percent PB blasts must be recorded on at least 2 separate occasions. § Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD.

Table 2. Characteristics for 3479 patients with known *SF3B1* mutation status within the IWG dataset

Variable	*SF3B1* WT	*SF3B1* Mutated	p-value
N	2684	795	
Sex			
Female	978 (36)	349 (44)	<0.001
Male	1706 (64)	446 (56)	
Age (yrs.) at Sample, median (range)	69 (11, 99)	72 (34, 94)	<0.001
< 40	61 (2)	3 (<1)	<0.001
40 – 49 yrs.	131 (5)	22 (3)	
50 – 59 yrs.	326 (12)	78 (10)	
60 – 69 yrs.	822 (31)	205 (26)	
70 – 79 yrs.	959 (36)	348 (44)	
80 – 89 yrs.	313 (12)	125 (16)	
≥ 90 yrs.	15 (1)	6 (1)	
Unknown	57 (2)	8 (1)	
WHO 2008			
del(5q)	91 (3)	20 (3)	<0.001
RARS	60 (2)	273 (34)	
RA/RCUD	238 (9)	21 (3)	
RCMD	520 (19)	18 (2)	
RCMD-RS	56 (2)	171 (22)	
RAEB-1	412 (15)	49 (6)	
RAEB-2	426 (16)	28 (4)	
Unknown	735 (27)	206 (26)	
FAB			
RA	611 (23)	61 (8)	<0.001
RARS	103 (4)	352 (44)	
RAEB	763 (28)	86 (11)	
RAEB-T	48 (2)	5 (1)	
CMML	61 (2)	4 (1)	
Unknown	1098 (41)	287 (36)	
Blast %, median (IQR)	4.0 (1, 9.0)	2.0 (1.0, 4.0)	<0.001
< 5 %	1347 (50)	635 (80)	<0.001
5 – 10 %	649 (24)	94 (12)	
11 – 20 %	486 (18)	33 (4)	
21 – 30 %	23 (1)	2 (<1)	
Unknown	179 (7)	31 (4)	
Hemoglobin (g/dl), median (IQR)	9.9 (8.7, 11.3)	9.5 (8.6, 10.5)	<0.001
< 8.0	307 (11)	102 (13)	<0.001
8.0 – 9.99	1000 (37)	353 (44)	
10.0 – 11.99	774 (29)	249 (31)	
≥12.0	447 (17)	34 (4)	
Unknown	156 (6)	57 (7)	
Absolute Neutrophil Count (ANC)	1603 (8000, 3300)	2730 (1700, 4241)	<0.001
< 0.5	262 (10)	20 (3)	<0.001
0-5 – 0.99	393 (15)	43 (5)	
1.0 – 1.8	415 (15)	96 (12)	
≥ 1.8	940 (35)	410 (52)	
Unknown	674 (25)	226 (28)	
Platelets ($x10^9$/l), median (IQR)	93 (48, 171)	261 (150, 378)	<0.001
< 50	639 (24)	41 (5)	<0.001
50 – 100	668 (25)	60 (8)	
100 – 149	410 (15)	76 (10)	
150 – 449	662 (25)	422 (53)	
≥ 450	74 (3)	118 (15)	
Unknown	231 (9)	78 (10)	
IPSS-R			
Very Low	263 (10)	152 (19)	<0.001
Low	610 (23)	352 (44)	
Intermediate	531 (20)	86 (11)	
High	391 (14)	45 (6)	
Very High	320 (12)	9 (1)	
Unknown	569 (21)	151 (19)	
IPSS-R Cytogenetic Risk			
Very Good	79 (3)	38 (5)	<0.001
Good	1681 (63)	608 (76)	
Intermediate	322 (12)	93 (12)	
Poor	154 (6)	14 (2)	
Very Poor	271 (10)	7 (1)	
Unknown	177 (7)	35 (4)	

Table 3. Proposed diagnostic criteria for the MDS with mutated *SF3B1*.

Cytopenia defined by standard hematologic values
Somatic *SF3B1* mutation
Isolated erythroid or multilineage dysplasia°
Bone marrow blasts <5% and peripheral blood blasts<1%
WHO criteria for MDS with isolated del(5q), myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis or other myelodysplastic/myeloproliferative neoplasms, and primary myelofibrosis or other myeloproliferative neoplasms are not met
Normal karyotype or any cytogenetic abnormality other than del(5q); monosomy 7; inv(3) or abn. 3q26, complex (≥3)
Any additional somatically mutated gene other than *RUNX1* and/or *EZH2**

°Ring sideroblasts are not required for the diagnosis.

*Additional *JAK2V617F*, *CALR*, or *MPL* mutations strongly support the diagnosis of myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis.

Table 4. Clinical and hematological features of 495 patients within the IWG cohort classified according to the proposed entity of MDS with mutated SF3B1

Sex	
Female	212 (43)
Male	283 (57)
Age (yrs.) at Sample, median (range)	70 (11, 99)
< 40	0 (0)
40 – 49 yrs.	10 (2)
50 – 59 yrs.	50 (10)
60 – 69 yrs.	115 (23)
70 – 79 yrs.	236 (48)
80 – 89 yrs.	79 (16)
≥ 90 yrs.	2 (<1)
Unknown	3 (1)
WHO 2008	
RARS	238 (48)
RA/RCUD	15 (3)
RCMD-RS	156 (32)
RCMD	17 (3)
Unknown	68 (14)
IPSS-R	
Very Low	130 (26)
Low	269 (54)
Intermediate	21 (4)
High	3 (1)
Very High	0 (0)
Unknown	72 (15)
IPSS-R Cytogenetic Risk Group	
Very Good	26 (5)
Good	415 (84)
Intermediate	54 (11)
Poor	0 (0)
Very Poor	0 (0)
Hemoglobin (g/dl), median (IQR)	9.8 (8.7, 11.1)
< 8.0 (g/dl)	51 (10)
8.0 – 9.99 (g/dl)	216 (44)
10.0 – 11.99 (g/dl)	174 (35)
≥12.0 (g/dl)	19 (4)
Unknown	35 (7)
Absolute Neutrophil Count (ANC) ($x10^9$/l), median (IQR)	1887 (900, 3600)
< 0.5	4 (1)
0.5 – 0.99	14 (3)
1.0 – 1.8	49 (10)
≥ 1.8	271 (55)
Unknown	157 (32)
Platelets ($x10^9$/l), median (IQR)	115 (56, 238)
< 50	12 (2)
50 – 100	12 (2)
100 – 149	49 (10)
150 – 449	290 (59)
≥ 450	89 (18)
Unknown	43 (9)

Figure 1. Patterns of the mutations observed in MDS patients reported to the dataset of the International Working Group for MDS. A) Distribution of somatic lesions in the analyzed genes according to the WHO category. Each column represents an individual patient sample. B) Distribution of somatic lesions in the analyzed genes in patients with MDS-RS with or without *SF3B1* mutation.

Figure 2. Frequency of co-occurring or mutually exclusive mutated genes in *SF3B1*-mutated or -unmutated MDS in the IWG Dataset. A) Most frequent co-occurring or mutually exclusive mutated genes in *SF3B1*-mutant MDS in the IWG Dataset. Maroon and navy bars represent relative frequencies (percentage) of mutated genes in *SF3B1*-mutated and *SF3B1*-wild type MDS, respectively. Red or blue gene labels indicate significantly higher frequencies of the co-mutated gene in *SF3B1*-mutated or *SF3B1*-wild type MDS, respectively (P values ranging from .019 to <.001). B) Most frequent co-occurring or mutually exclusive mutated genes in *SF3B1*-wild type versus *SF3B1*-mutant MDS-RS in the IWG Dataset. Navy and maroon bars represent relative frequencies (percentage) of mutated genes in *SF3B1*-wild type and *SF3B1*-mutant MDS-RS, respectively. Blue or red gene labels indicate significantly higher frequencies of the co-mutated gene in *SF3B1*-wild type or *SF3B1*-mutant MDS, respectively (P values ranging from .047 to .002).

Figure 3. Tridimensional scatter plot of *SF3B1*-mutated and unmutated MDS with ring sideroblasts according to bone marrow dysplastic features. Red dots identify MDS associated with *SF3B1* mutation, whereas blue dots identify MDS unmutated for

SF3B1. The degree of dysmyelopoiesis and dysmegakaryopoiesis are measured as percentage of lineage dysplastic cells.[14]

Figure 4. Effect of current WHO classification criteria on overall survival of patients with *SF3B1*-mutated MDS. Plot A reports overall survival of patients with *SF3B1*-mutated MDS according to the presence of single-lineage (black curve, n=267) or multi-lineage (red curve, n=171) dysplasia (P=0.4). Plot B reports overall survival of patients with *SF3B1*-mutated MDS according to bone marrow blasts lower (black curve, n=341) or equal/higher (red curve, n=85) than 5% (P<0.001).

Figure 5. Overall survival of patients with MDS classified according to *SF3B1* mutation status. Plot A reports overall survival of the whole MDS population according to *SF3B1* mutation status: *SF3B1*-mutated MDS (red curve, n=769) have a significantly longer survival compared with *SF3B1*-unmutated MDS (black curve, n=2555) (P<0.001). Plots B reports overall survival of *SF3B1*-mutated (red curve, n=267) and –unmutated (black curve, n=54) patients with RARS (P<.001). Plots C reports overall survival of *SF3B1*-mutated (red curve, n=171) and –unmutated (black curve, n=56) patients with RCMD-RS (P=.003). Plot D reports overall survival of patients with RARS or *SF3B1*-mutated RCUD according to *SF3B1* mutation status (mutated *SF3B1*, red curve, n=287; unmutated, black curve, n=54) (P<.001). This group is overlapping the category of MDS-RS-SLD according to 2016 WHO criteria, except for comprising occasional patients with *SF3B1*-mutation and less than 5% RS. Plot E reports overall survival of patients with RCMD-RS or *SF3B1*-mutated RCMD according to *SF3B1*

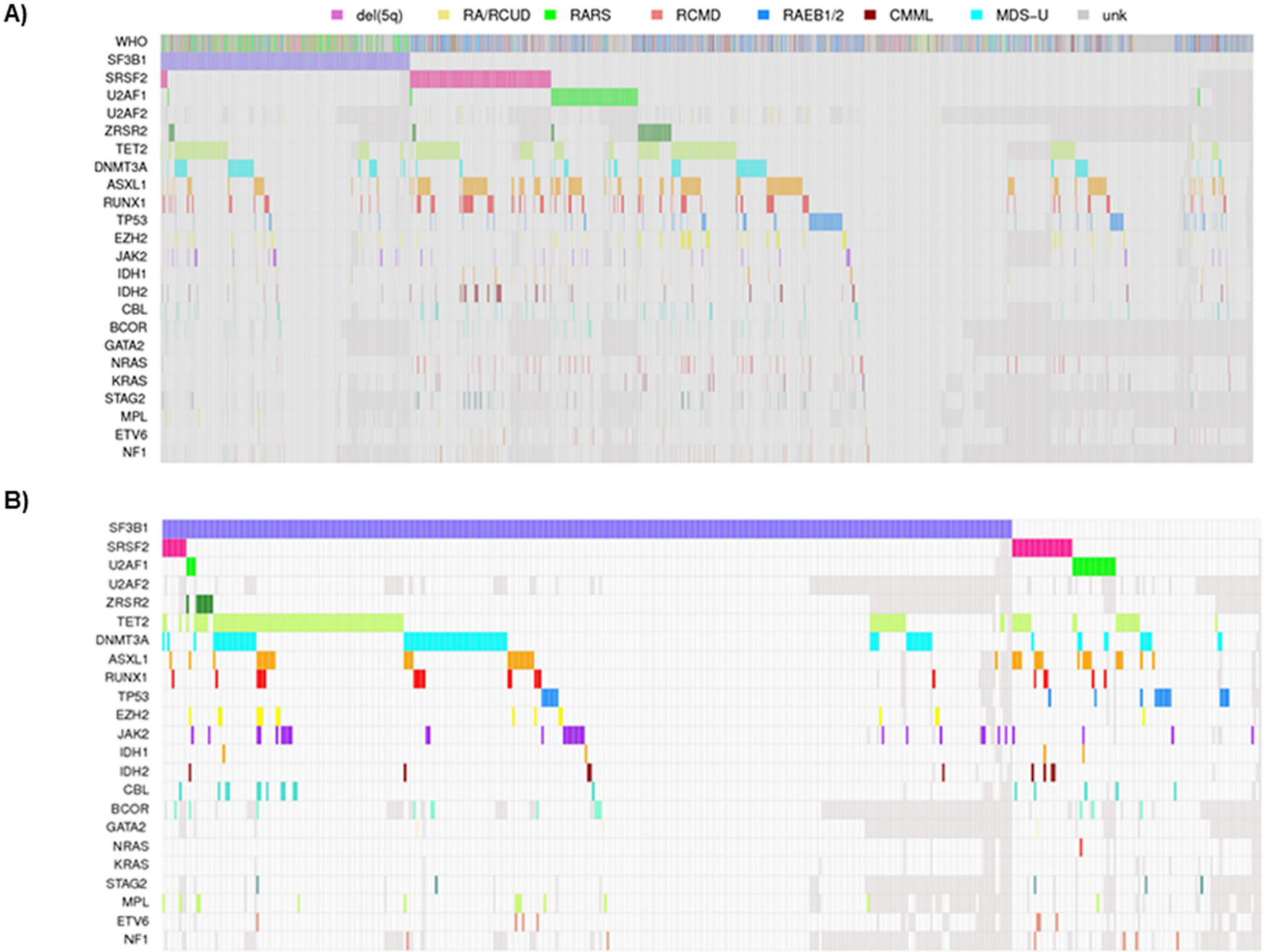
mutation status (mutated *SF3B1*, red curve, n=189; unmutated, black curve, n=56) (P=.003). This group is overlapping the category of MDS-RS-MLD according to 2016 WHO criteria, except for comprising occasional patients with *SF3B1*-mutation and less than 5% RS. Plot F reports overall survival of *SF3B1*-mutated (red curve, n=77) and – unmutated patients (black curve, n=823) with MDS-EB (P=0.34).

Figure 6. Overall survival of patients with *SF3B1* mutant MDS according to additional somatic mutations. Plot A reports overall survival by isolated *SF3B1* mutation (n=201, black curve) versus *SF3B1* mutation associated with additional somatic mutations within *SF3B1*-mutated MDS without excess blasts (*SF3B1* plus one additional mutation, n=192, red curve; two additional mutations, n=66, green curve; three or more additional mutations, n=23, blue curve) (including patients sequenced for all of the following 15 genes: *SF3B1, TET2, DNMT3A, SRSF2, ASXL1, RUNX1, TP53, EZH2, JAK2, U2AF1, IDH1, IDH2, CBL, NRAS, ETV6*). Plot B and C report overall survival and cumulative incidence of AML evolution of *SF3B1*-mutated MDS without excess blasts according to *RUNX1* mutation status (mutated, n=21, red curve; unmutated, n=505, black curve) (P<.001). Cumulative incidence of AML evolution was estimated with a competing risk approach, considering death for any cause as a competing event. Plot D overall survival of *SF3B1*-mutated MDS without excess blasts according to *EZH2* mutation status (mutated, n=20, red curve; unmutated, n=499, black curve) (P=.003).

Figure 7. Survival and risk of leukemic evolution of patients classified within the proposed entity of MDS with mutated *SF3B1.* Plot A reports overall survival of

patients classified within the proposed entity of MDS with mutated *SF3B1* (n=486). Plot B reports cumulative incidence of AML evolution of evaluable patients (n=52) classified within the proposed entity of MDS with mutated *SF3B1.* Cumulative incidence of AML evolution was estimated with a competing risk approach, considering death for any cause as a competing event.

Figure 1


A)
del(5q)
RA/RCUD
RARS
RCMD
RAEB1/2
CMML
MDS-U
unk
WHO
SF3B1
SRSF2
U2AF1
U2AF2
ZRSR2
TET2
DNMT3A
ASXL1
RUNX1
TP53
EZH2
JAK2
IDH1
IDH2
CBL
BCOR
GATA2
NRAS
KRAS
STAG2
MPL
ETV6
NF1
B)
SF3B1
SRSF2
U2AF1
U2AF2
ZRSR2
TET2
DNMT3A
ASXL1
RUNX1
TP53
EZH2
JAK2
IDH1
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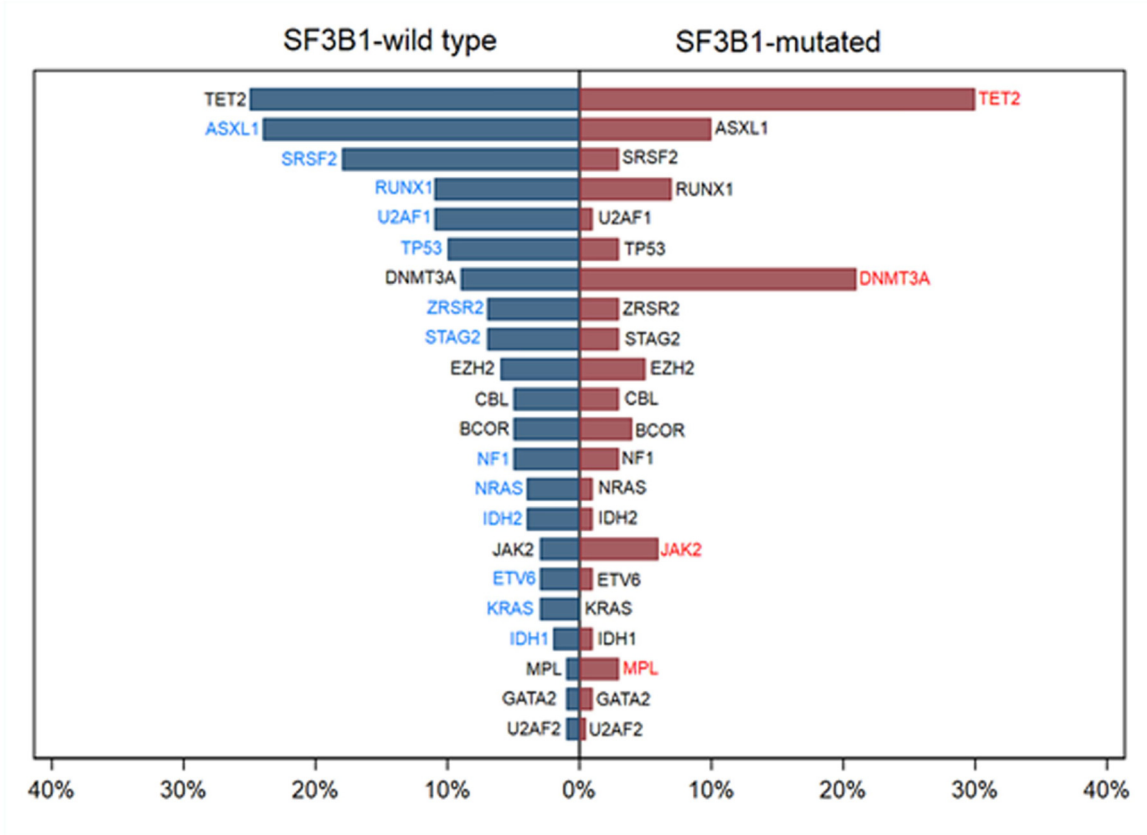
CBL
BCOR
GATA2
NRAS
KRAS
STAG2
MPL
ETV6
NF1

Figure 2

A)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SF3B1-mutated
TET2
ASXL1
SRSF2
RUNX1
U2AF1
TP53
DNMT3A
ZRSR2
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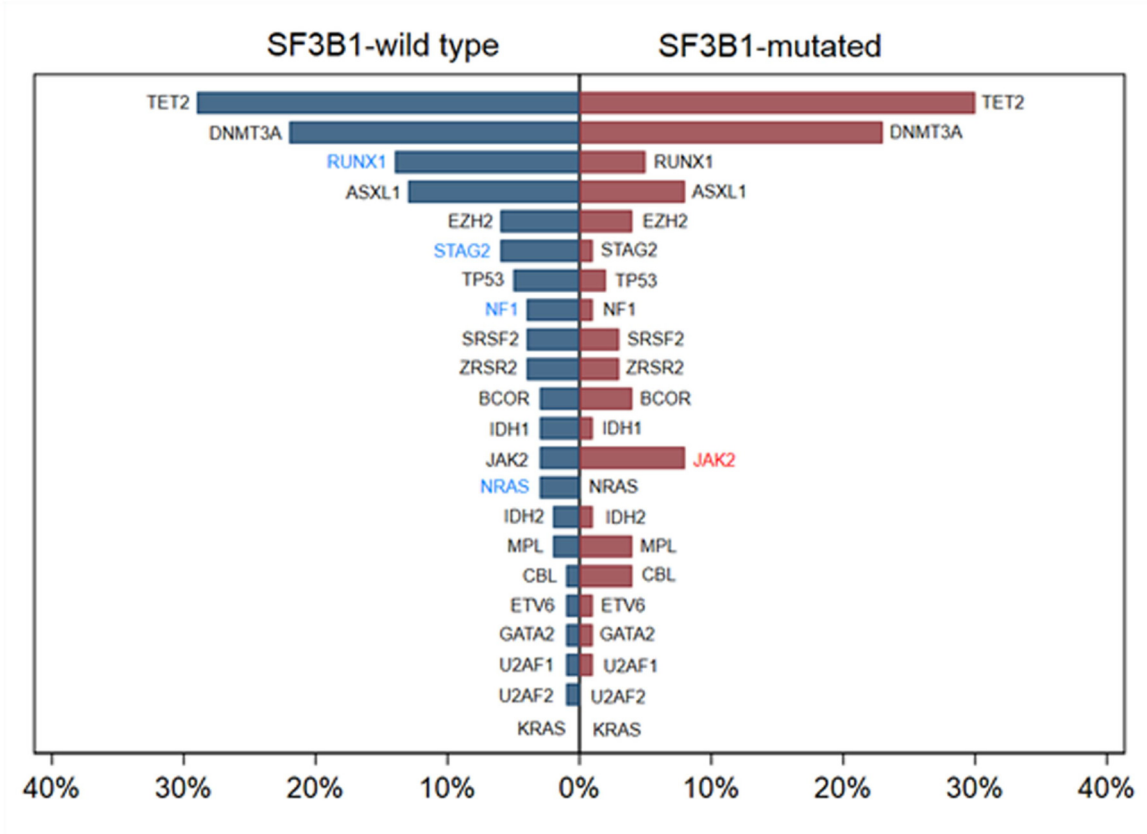
EZH2
CBL
BCOR
NF1
NRAS
IDH2
JAK2
ETV6
KRAS
IDH1
MPL
GATA2
U2AF2
40%
30%
20%
10%
0%
10%
20%
30%
40%

B)


SF3B1-wild type
SF3B1-mutated
TET2
DNMT3A
RUNX1
ASXL1
EZH2
STAG2
TP53
NF1
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ZRSR2
BCOR
IDH1
JAK2
NRAS
IDH2
MPL
CBL
ETV6
GATA2
U2AF1
U2AF2
KRAS
40%
30%
20%
10%
0%
10%
20%
30%
40%

Figure 3

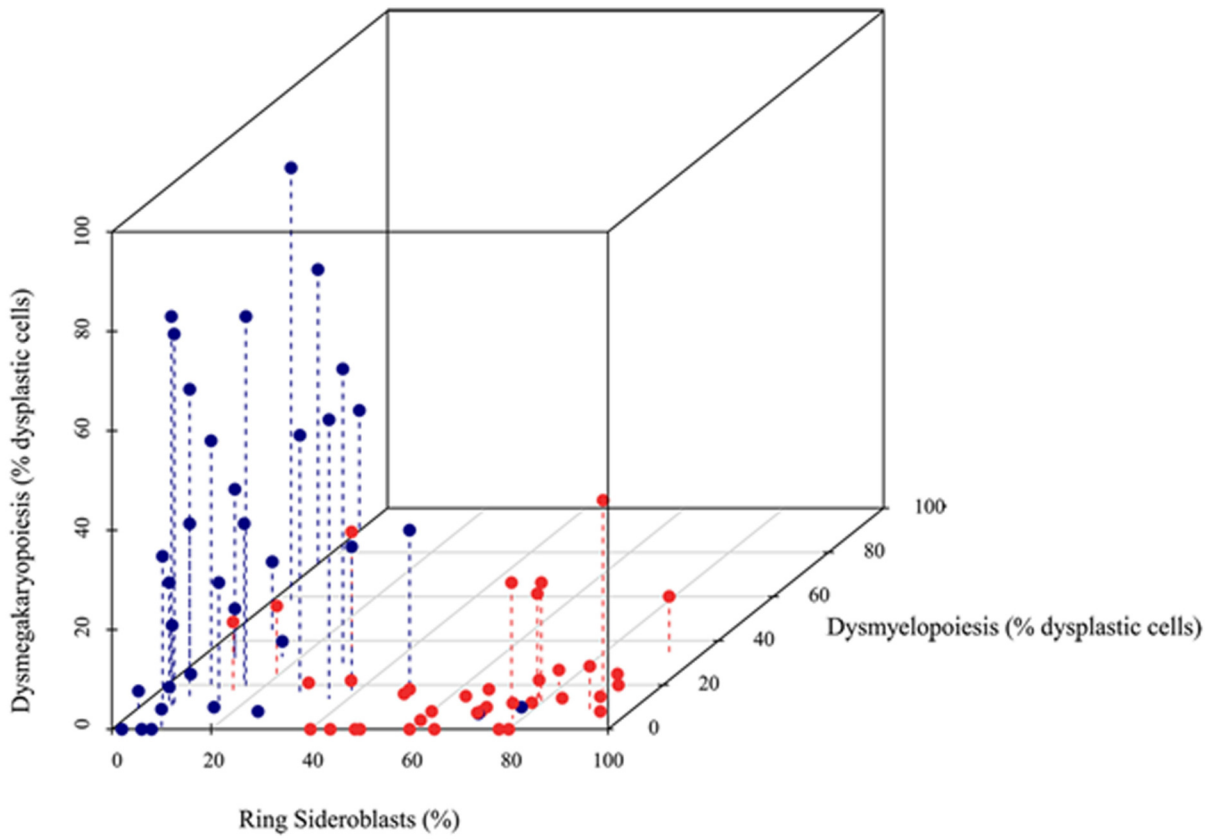
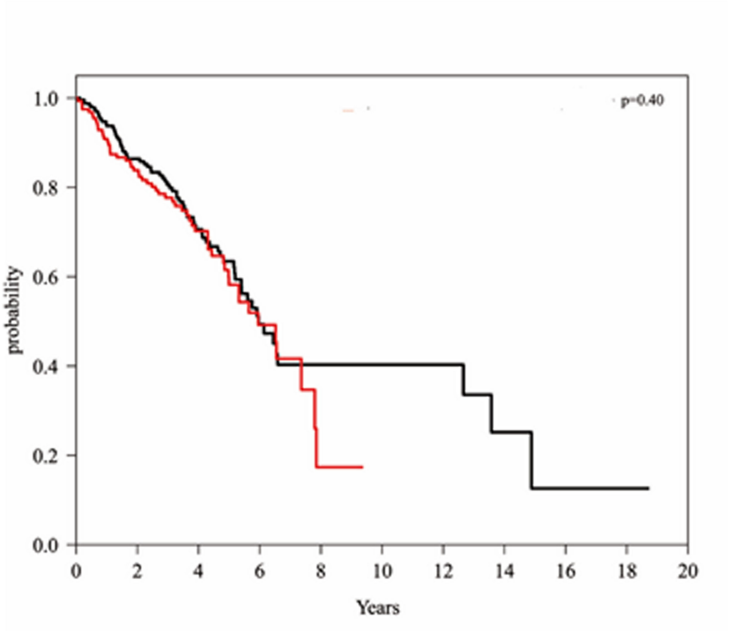

Dysmegakaryopoiesis (% dysplastic cells)
0
20
40
60
80
100
Ring Sideroblasts (%)
0
20
40
60
80
100
Dysmyelopoiesis (% dysplastic cells)
0
20
40
60
80
100

Figure 4

A)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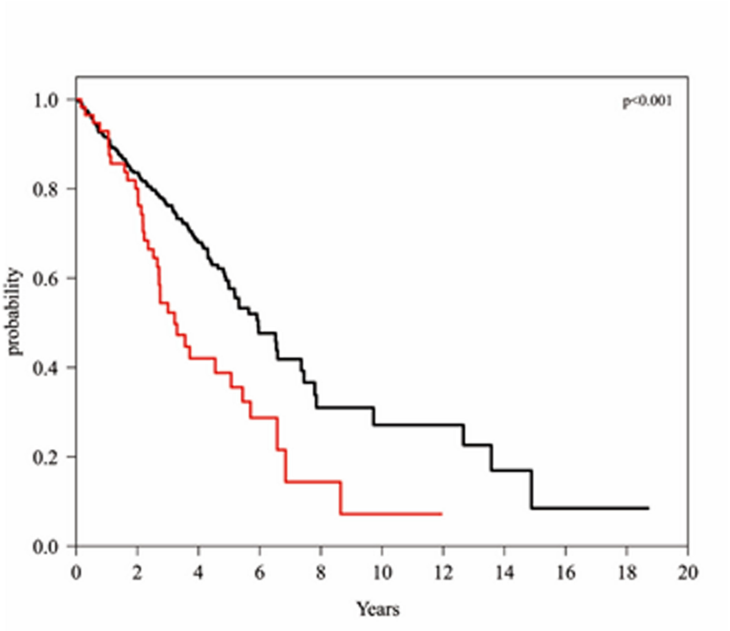
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
Years

B)


p<0.001
probability
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0.8
0.6
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4
6
8
10
12
14
16
18
20
Years

Figure 5

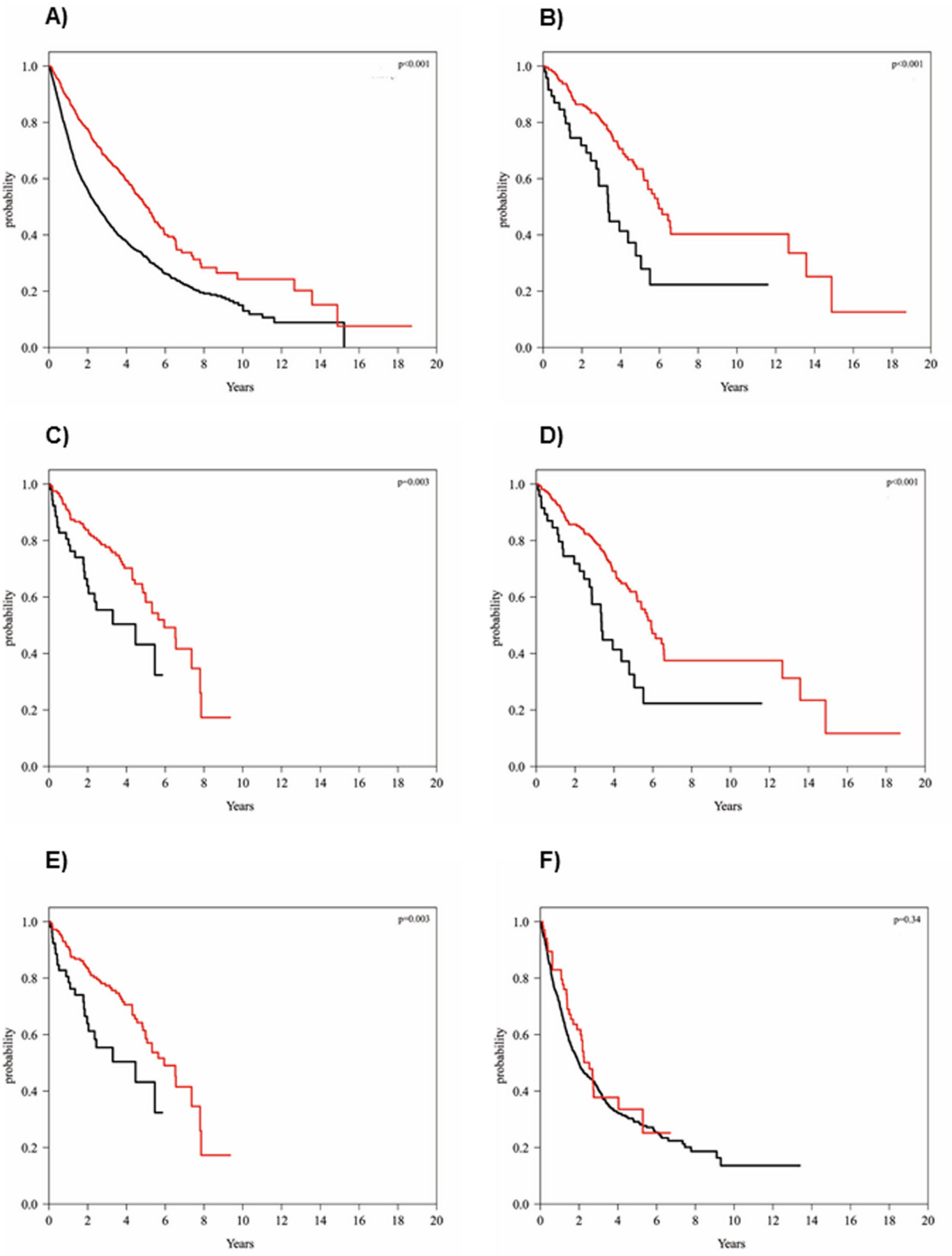
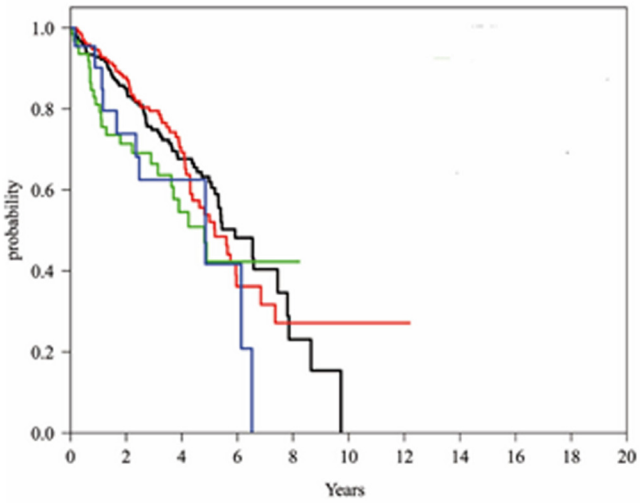
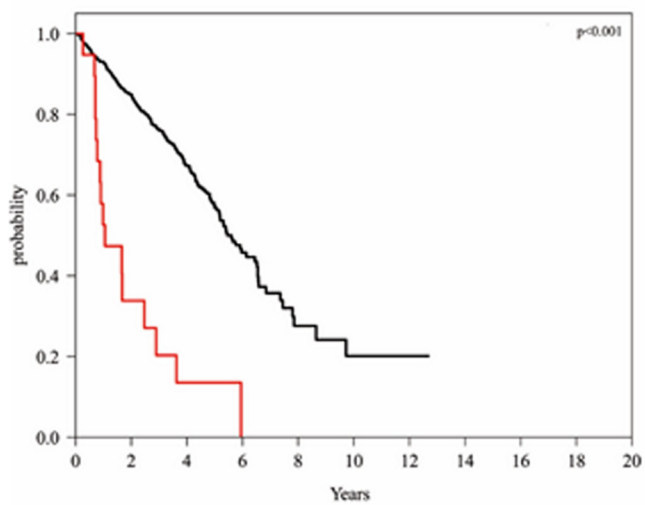

A)
p<0.001
probability
Years
B)
p<0.001
probability
Years
C)
p=0.003
probability
Years
D)
p<0.001
probability
Years
E)
p=0.003
probability
Years
F)
p=0.34
probability
Years

Figure 6

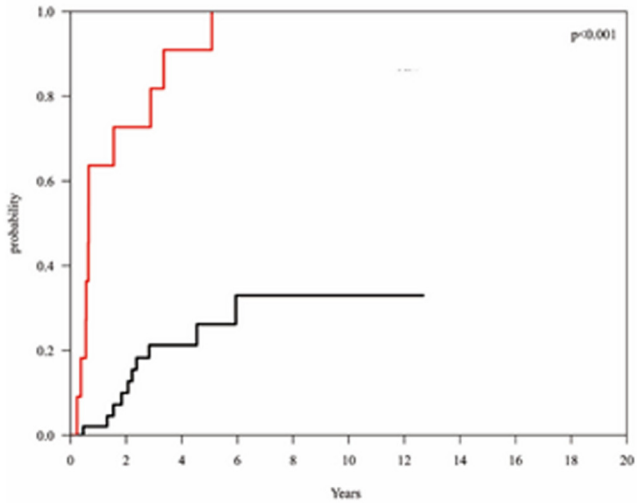
A)



B)



C)



D)

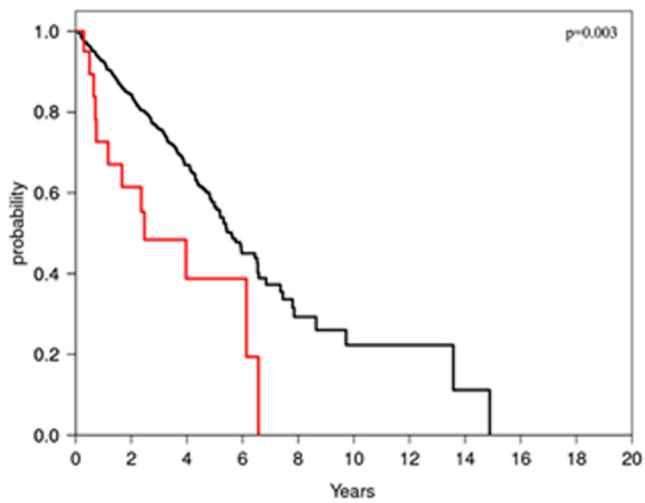
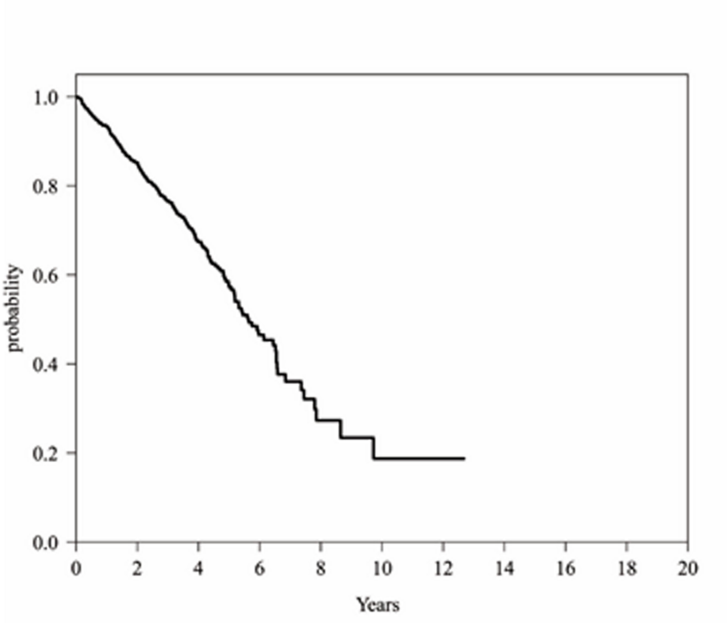


Figure 7

A)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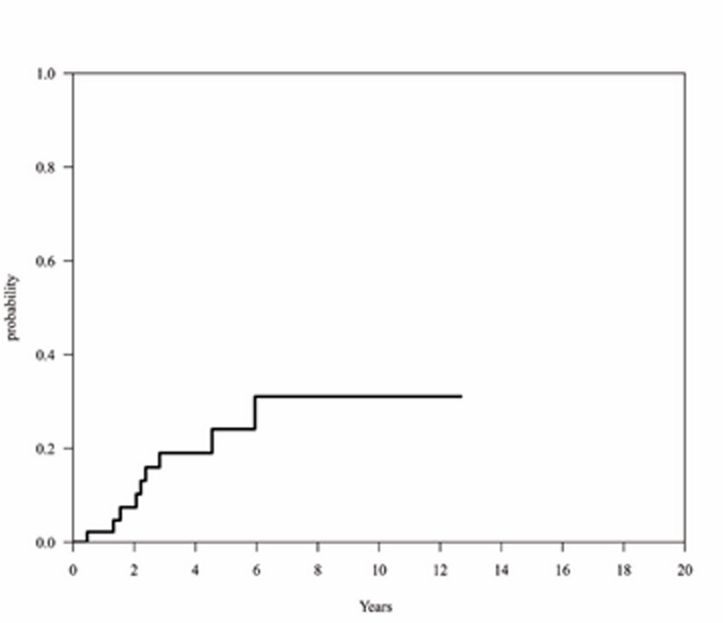
0.6
0.4
0.2
0.0
probability
0
2
4
6
8
10
12
14
16
18
20
Years

B)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probability
0
2
4
6
8
10
12
14
16
18
20
Years