



EUROPEAN  
HEMATOLOGY  
ASSOCIATION



Ferrata Storti  
Foundation

**Haematologica** 2017  
Volume 102(9):1502-1510

# Molecular analysis of myelodysplastic syndrome with isolated deletion of the long arm of chromosome 5 reveals a specific spectrum of molecular mutations with prognostic impact: a study on 123 patients and 27 genes

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## ABSTRACT

The only cytogenetic aberration defining a myelodysplastic syndrome subtype is the deletion of the long arm of chromosome 5, which, along with morphological features, leads to the diagnosis of myelodysplastic syndrome with isolated deletion of the long arm of chromosome 5. These patients show a good prognosis and respond to treatment such as lenalidomide, but some cases progress to acute myeloid leukemia; however, the molecular mutation pattern is rarely characterized. Therefore, we investigated a large cohort of 123 myelodysplastic syndrome patients with isolated deletion of the long arm of chromosome 5, diagnosed following the World Health Organization classifications 2008 and 2016, by sequencing 27 genes. A great proportion of patients showed no or only one mutation. Only seven genes showed mutation frequencies >5% (*SF3B1*, *DNMT3A*, *TP53*, *TET2*, *CSNK1A1*, *ASXL1*, *JAK2*). However, the pattern of recurrently mutated genes was comparable to other myelodysplastic syndrome subtypes by comparison to a reference cohort, except that of *TP53* which was significantly more often mutated in myelodysplastic syndrome with isolated deletion of the long arm of chromosome 5. As expected, *SF3B1* was frequently mutated and correlated with ring sideroblasts, while *JAK2* mutations correlated with elevated platelet counts. Surprisingly, *SF3B1* mutations led to significantly worse prognosis with cases with isolated deletion of the long arm of chromosome 5, but showed a comparable outcome to other myelodysplastic syndrome subtypes with *SF3B1* mutation. However, addressing genetic stability in follow-up cases might suggest different genetic mechanisms for progression to secondary acute myeloid leukemia compared to overall myelodysplastic syndrome patients.

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Received: February 8, 2017.

Accepted: June 16, 2017.

Pre-published: June 22, 2017.

doi:10.3324/haematol.2017.166173

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: [www.haematologica.org/content/102/9/1502](http://www.haematologica.org/content/102/9/1502)

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## Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal bone marrow neoplasms characterized by ineffective hematopoiesis, morphologic dysplasia and peripheral cytopenias. While the degree of dysplasia and blast percentage are disease classifying, the specific types of cytopenias have a minor impact on MDS classification. The diagnosis of MDS is driven by the number of dysplastic cell lineages as well as blast counts <1% in the peripheral blood (PB) or <5% in the bone marrow (BM), or <19% in PB or BM for subtypes with excess blasts and at least one cytopenia.<sup>1</sup> The risk stratification for MDS patients is categorized according to the Revised International Prognostic Scoring System (IPSS-R), whereby, along with morphological features, cytogenetics also plays a crucial role. Some cytogenetic aberrations can also define an MDS even in the absence of morphologic dysplasia.

However, also according to the new World Health Organization (WHO) 2016 classification, the only cytogenetic aberration defining a MDS subtype is the deletion of the long arm of chromosome 5 (del(5q)), giving the diagnosis of MDS with isolated del(5q). Overall, less than 50% of MDS patients show an aberrant karyotype, of which del(5q) is the most common aberration presenting in 10–20% of MDS patients.<sup>2,3</sup> In about 55% of patients with del(5q) this aberration appears as a sole abnormality, in 17% it presents with one additional aberration, while in another 28% del(5q) appears with two or more additional cytogenetic lesions, leading by definition to a complex karyotype.<sup>4</sup> This is shifted towards sole del(5q) (82%) when addressing only low- and intermediate-1-risk MDS according to the IPSS.<sup>5</sup> However, the WHO classification emphasizes that the main impact of cytogenetics is the prognostic rather than the classifying information. It was demonstrated that MDS with isolated del(5q) shows a good prognosis, while MDS with del(5q) within a complex karyotype shows a poor prognosis.<sup>2,4</sup> In recent years the prognostic impact of additional aberrations was addressed, showing that the presence of only one aberration in addition to del(5) (excluding the abnormality -7 or del(7q)) had no adverse effect on prognosis.<sup>6</sup>

Therefore, in the new WHO classification 2016 a diagnosis of MDS with isolated del(5q) allows the presence of one additional aberration excluding aberrations affecting chromosome 7.<sup>1</sup> In addition to the good prognosis of these patients, a sensitivity to specific treatments, such as lenalidomide, was demonstrated.<sup>7,8</sup> Morphologically, MDS with isolated del(5q) is defined by blast counts <1% in the PB and <5% in the BM, severe macrocytic anemia and frequent thrombocytosis. Patients who have MDS with isolated del(5q) show a lower risk for progression to acute myeloid leukemia (AML) than those with other MDS. However, about 10% of these patients evolve to secondary (s)-AML.<sup>4,9,10</sup> The underlying pathobiological mechanisms are still being debated, while recent studies indicate that *TP53* mutation as well as karyotype evolution predict disease progression.<sup>11,12</sup>

Furthermore, the separation of MDS from reactive causes remains a challenge. In recent years large data sets became available showing that a limited number of genes were mutated in patients with MDS. Eighty to ninety percent of patients show at least one mutation in one of the >100 addressed genes, supporting the clonal hematopoiesis of the disease and with that the diagnosis.<sup>13,14</sup> Moreover, it was demonstrated that the increasing number of gene mutations correlates with the disease outcome in MDS patients; the addition of this data improves the existing risk stratifications.<sup>13,14</sup>

Mutations in *TP53* are generally associated with adverse outcome and an aggressive disease course in MDS. Furthermore, in MDS with isolated del(5q), a *TP53* mutation seems to predict a poorer response to lenalidomide and a higher risk of transformation to AML.<sup>11</sup> Thus, the respective mutation status should be addressed not only at the time of diagnosis, but also before treatment decisions are made, as recommended in the new WHO 2016 classification.<sup>1</sup>

Therefore, the aim of the study herein was to determine the frequency of mutations in a large cohort of 123 patients with MDS and isolated del(5q) with a 27 gene panel, and to combine clinical data and prognostic information.

## Methods

### Patients cohort

We investigated 123 patients (35 male, 88 female, ratio: 1:2.5) diagnosed as having MDS with isolated del(5q), strictly classified according to the WHO classification of 2008 and including the added guidelines of 2016 with respect to cytomorphology and cytogenetics (blast counts below 5% in the BM and below 1% in the PB, and isolated 5q deletion or one additional aberration that does not affect chromosome 7).<sup>1</sup> All patients' samples, taken between 2005 and 2013, were sent from different hematological centers to the MLL Munich leukemia laboratory for diagnostic purposes. The median age of the patients was 75 years (range: 35–93 years). One hundred and nineteen patients showed an isolated del(5q) while four cases appeared with only one additional cytogenetic aberration (two patients with del(13q), one with del(9q) and one with a trisomy 8). Follow-up data was available in 111/123 cases with a median follow up of 41 months. One hundred and twelve cases from the cohort presented herein were also included in the study by Heuser *et al.*<sup>15</sup> The mutation patterns and prognostic impact of MDS with del(5q) were compared to a MDS cohort of 944 patients representing all MDS subtypes.<sup>15</sup> Of the 123 patients diagnosed with MDS with isolated del(5q), 39 were already included in this recent study, resulting in a reference cohort of 905 patients after excluding these 39 cases.<sup>15</sup> This reference cohort contained the following MDS subtypes: 41 refractory anemia (RA), 81 refractory anemia with ringed sideroblasts (RARS), 27 RARS with thrombocytosis (RARS-T), 194 refractory cytopenia with multilineage dysplasia (RCMD), 183 refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS), 191 refractory anemia with excess blasts-1 (RAEB-1), and 188 refractory anemia with excess blasts-2 (RAEB-2), diagnosed according to the WHO 2008 classification guidelines.<sup>16</sup> The median age was 73 years (range: 23–91 years), the male to female ratio was 1:7. Follow-up data in the reference cohort was available in 869 cases with a median follow up of 62 months.

All patients gave their consent for genetic analyses and the use of laboratory results for research purposes. The study adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board of the laboratory.

### Cytomorphology

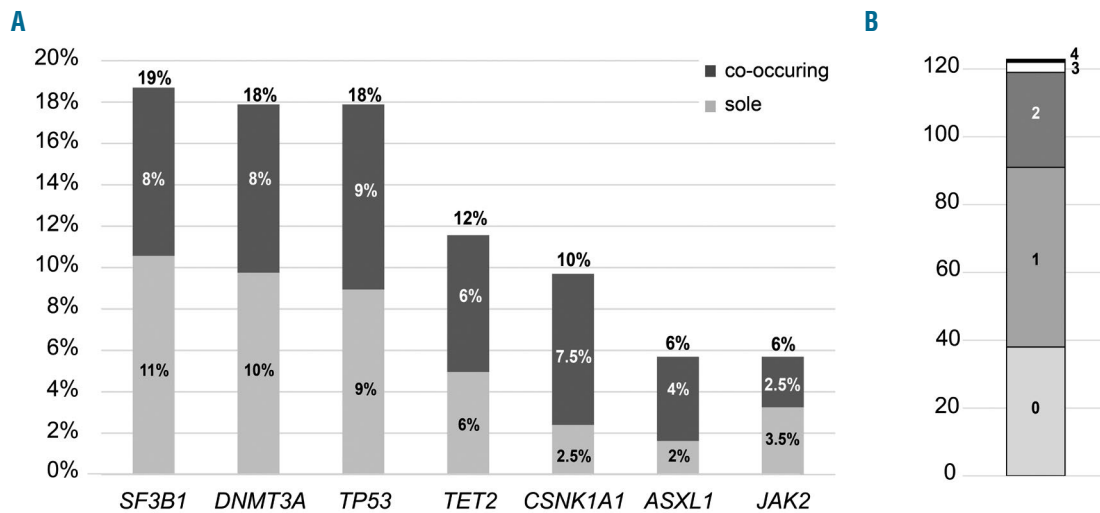
Cytomorphology of BM and/or PB samples was performed in all cases following May-Grünwald Giemsa staining, cytochemistry with myeloperoxidase (MPO), non-specific esterase (NSE) and iron staining (Fe).<sup>17</sup>

### Cytogenetics

Chromosome preparations and banding analysis of BM and/or PB samples were performed for all 123 cases as previously described according to standard methods.<sup>18</sup> For classification of abnormalities and karyotypes, the 2016 International System for Human Cytogenetic Nomenclature (ISCN) guidelines were used.<sup>19</sup>

### Next-generation sequencing (NGS)

All patients were analyzed *via* a myeloid gene panel containing *ASXL1*, *BCOR*, *BRAF*, *CSNK1A1*, *CBL*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3-TKD*, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *NRAS*, *KRAS*, *MPL*, *NPM1*, *PHF6*, *RUNX1*, *SF3B1*, *SRSF2*, *TET2*, *TP53*, *U2AF1* and *WT1*. The library of 26 genes was generated with the ThunderStorm (RainDance Technologies, Billerica, MA, USA), and *CSNK1A1* with the Access Array System (Fluidigm, San Francisco, CA, USA). Both libraries were sequenced and demultiplexed on a MiSeq instrument (Illumina, San Diego, CA, USA) as described previously.<sup>20</sup> The FASTQ files were further processed



**Figure 1. Mutation frequencies and distributions.** (A) The frequencies of the seven most often mutated genes are given, as well as their appearance as a sole abnormality. (B) Illustration of the distribution of the number of mutations per patient.

using the Sequence Pilot software version 4.1.1 Build 510 (JSI Medical Systems, Ettenheim, Germany) for alignment and variant calling. Analysis parameters were set according to the manufacturer's default recommendation. The validity of the somatic mutations was checked against the publicly accessible Catalogue Of Somatic Mutations In Cancer (COSMIC) v69 database, and functional interpretation was performed using SIFT 1.03, PolyPhen 2.0 and MutationTaster 1.0 algorithms.<sup>21</sup> Additionally, *TP53* variants were verified using the International Agency for Research on Cancer (IARC) repository.<sup>22</sup> Single-nucleotide polymorphisms (SNP) were annotated according to the National Center for Biotechnology Information Single Nucleotide Polymorphism Database (NCBI dbSNP). The detection limit for small nuclear variants was 3% variant allele frequency (VAF). Variants ( $n=9$ ) not yet described in any public database were excluded from statistical analyses.

### Statistical analysis

Dichotomous variables were compared between different groups using the  $\chi^2$ -test and continuous variables by the Student's *t*-test; results were considered significant at  $P<0.05$ . Adjustment for multiple testing was not done. Statistical analyses were performed using SPSS version 19.0 (IBM Corporation, Armonk, NY, USA); the reported *P*-values are two-sided. Survival curves were calculated for overall survival (OS) according to Kaplan-Meier and compared using the two-sided log-rank test. OS was considered as being the time from diagnosis to death or last follow up.

## Results

### Incidence of MDS with isolated del(5q) following the new WHO classification

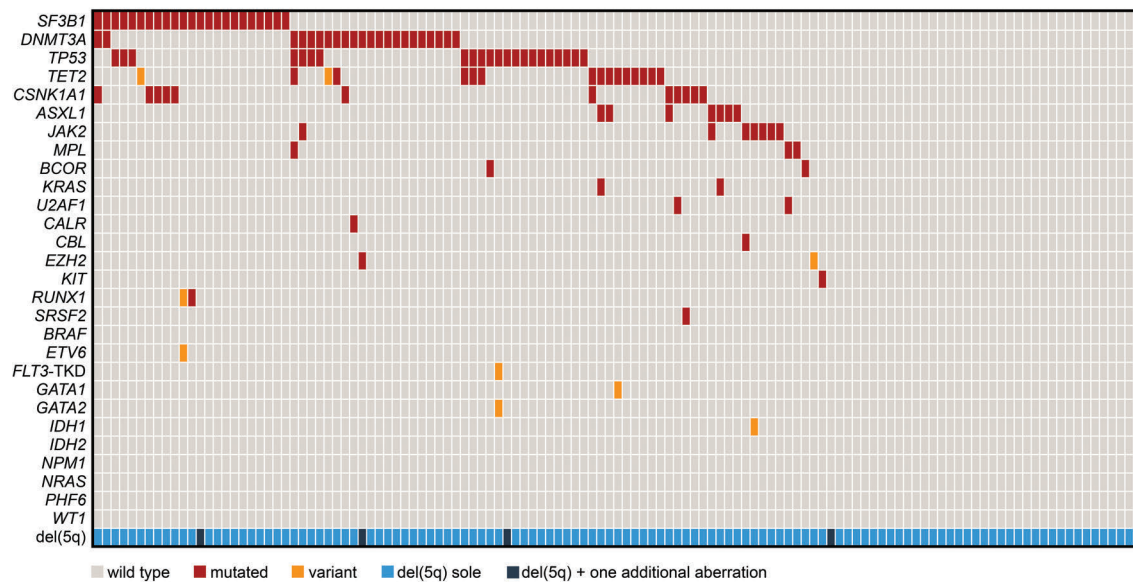
Following the recently revised 2016 version of the WHO classification for myeloid neoplasms, one additional chromosomal aberration (other than aberration of chromosome 7), in addition to the deletion of the long arm of chromosome 5, allows for the diagnosis of MDS with isolated del(5q).<sup>1</sup> Therefore, we analyzed a well characterized MDS cohort for incidences of del(5q) and other diagnostic

criteria leading to a diagnosis of MDS with isolated del(5q).<sup>13</sup> Of 944 MDS patients, 84 (9%) carried a del(5q) either as a sole abnormality or co-occurring with other lesions. Of 84 cases, 53 (63%) showed a sole del(5q), while only five cases (6%) appeared with one additional aberration and 26 (31%) showed a complex karyotype. Of the 53 patients with isolated del(5q), 44 fulfilled the diagnostic criteria of <1% blasts in the PB and <5% in the BM. Furthermore, 4/5 cases with one additional lesion were also diagnosed as MDS with isolated del(5q), while the fifth case harbored a -7 as an additional aberration. Therefore, 48 cases were diagnosed as MDS with isolated del(5q), whereof only 4/48 cases (8%) were grouped to these patients based on the new WHO classification and its additional criteria, while the main group was diagnosed using the criteria of the WHO 2008 classification, without allowing for additional lesions.<sup>1</sup>

### Molecular genetic characterization of MDS with isolated del(5q)

For a comprehensive molecular characterization we assembled a cohort of 123 patients diagnosed with MDS with isolated del(5q) and investigated 27 genes by mutation analyses. The most frequently mutated gene was *SF3B1* (23/123; 19%), followed by *DNMT3A* (22/123; 18%), *TP53* (22/123; 18%), *TET2* (14/121; 12%), *CSNK1A1* (12/123; 10%), *ASXL1* and *JAK2* (both 7/123; 6%) (Figure 1A). All other analyzed genes showed mutation frequencies below 5%, with *BRAF*, *ETV6*, *FLT3-TKD*, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *NPM1*, *NRAS*, *PHF6* and *WT1* showing no mutations at all. Nearly all mutations were heterozygous with a median VAF for six of the most frequently mutated genes of 15-30% (range: 2-50% VAF). Solely *CSNK1A1* showed VAF of 3-78%, mimicking a homozygous mutation status, caused by the location of *CSNK1A1* on chromosome 5q in the commonly deleted region. Addressing the number of mutations per patient revealed a large number of patients with no mutation (38/123; 31%) or one mutation (53/123; 43%) in any of





**Figure 2. Molecular and cytogenetic characterization of patients with MDS with isolated del(5q).** Illustration of all 123 samples, each column represents one patient. All 27 analyzed genes as well as the occurrence of del(5q) as a sole aberration or with one additional cytogenetic aberration are given for each patient. Light gray: wild-type; red: mutated; orange: variant; light blue: del(5q) sole; dark blue: del(5q) and one additional lesion.

the 27 analyzed genes. Two genes were mutated in 23% (28/123) of patients, while three and four mutations were detected in only 2% (3/123) and 1% (1/123) of patients, respectively, (Figure 1B). Looking at the co-occurrence of gene mutations resulted in a single association of *CSNK1A1* and *SF3B1* mutations. Only 16% of *CSNK1A1* wild-type (wt) cases showed a *SF3B1* mutation (18/111), while 42% of *CSNK1A1*-mutated patients displayed a co-occurring *SF3B1* mutation (5/12;  $P=0.047$ ). Albeit the most frequent gene mutations rarely overlapped and occurred frequently as sole mutations, they were not completely mutually exclusive (Figure 1A and Figure 2).

### Comparison of mutation frequencies in MDS with isolated del(5q) and other MDS subtypes

In order to analyze the differences in the mutation patterns of MDS with isolated del(5q) and all other MDS subtypes, we investigated the most frequently mutated genes in comparison to a reference cohort, represented by the previously published MDS cohort after excluding the cases diagnosed as MDS with isolated del(5q).<sup>13</sup> Comparing the mutation frequencies of the seven most frequently mutated genes in MDS with isolated del(5q) with all other MDS subtypes revealed that the respective six mutated genes, *SF3B1*, *DNMT3A*, *TP53*, *TET2*, *ASXL1* and *JAK2*, were also ranked within the 15 most frequently mutated genes in all MDS subtypes (Figure 3; *CSNK1A1* was not addressed due to missing data in the MDS reference cohort). Of note, *TP53* mutations were found significantly more often in MDS with isolated del(5q) in comparison to all other MDS subtypes, with a mutation frequency of 18% (22/123) compared to 6% in the MDS reference cohort (52/905;  $P<0.001$ ).

### Clinical and morphological correlations

Upon dividing the patients into groups defined by a BM blast count of <2% and 2-5%, as described in the IPSS-R,

we could not detect any correlation with the number of mutations per patient. However, patients who had no mutation ( $n=38$ ) were younger compared to patients with at least one mutation ( $n=85$ ; 70 vs. 76 years,  $P=0.018$ ). There was no difference between these two patient groups as regards to sex, white blood cell count, hemoglobin level or platelet count. Taking single gene mutations into account revealed that *TP53* mutations correlated with older age (78 vs. 73 years,  $P=0.038$ ). All 22 patients with a *TP53* mutation were  $\geq 67$  years of age, with only two patients under 70; 11 patients were between 70 and 79 years of age, eight patients were between 80 and 89 years of age, and one patient in the group was  $\geq 90$  years of age. Addressing the correlation between percentages of ring sideroblasts (RS) and *SF3B1* mutations also showed that in MDS with isolated del(5q) these two parameters significantly correlated with a mean of 17% RS (range: 0-80%) in *SF3B1*-mutated patients and only 1% RS in *SF3B1* wt patients (range: 0-12%,  $P=0.004$ ). Furthermore, *JAK2* mutations correlated with a platelet count of  $>450,000/\mu\text{l}$  (80% in *JAK2* mutated vs. 12% in *JAK2* wt,  $P=0.002$ ).

### Prognostic impact of gene mutations

Looking at the prognostic relevance of gene mutations showed, surprisingly, that *SF3B1*-mutated patients ( $n=20$  with follow-up data) had a significantly inferior outcome than that of *SF3B1* wt patients ( $n=91$ ; median OS: 50 months vs. not reached,  $P=0.010$ ; Figure 4A). Furthermore, we analyzed the OS compared to the MDS reference cohort representing all other than isolated del(5q) MDS subtypes ( $n=869$  with follow-up data). Overall the median OS was significantly favorable for patients with MDS with isolated del(5q) (62 months vs. not reached,  $P<0.001$ ; Figure 4B). To better characterize the unexpected worse impact of *SF3B1* mutations we analyzed both cohorts with respect to their *SF3B1* mutation status as well as

cytogenetics in terms of normal or aberrant karyotypes (Figure 4C). *SF3B1* mutations within a normal karyotype showed favorable outcomes in MDS patients (median OS: 93 months), however, this effect was reduced by an additional aberrant karyotype (median OS: 64 months). Therefore, *SF3B1*-mutated patients possessing aberrant karyotypes showed comparable outcomes (median OS: 50 vs. 64 months), irrespective of diagnoses of MDS with isolated del(5q) or any other subtype of MDS, while *SF3B1* wt patients with isolated del(5q) had a favorable outcome compared to all other *SF3B1* wt MDS patients (median OS: not reached vs. 49 months). However, we did not find a prognostic impact of *TP53* mutations in our cohort (Online Supplementary Figure S1; median OS was not reached in *TP53* wt (n=92) and *TP53* mutated (n=19) patients;  $P=0.094$ ).

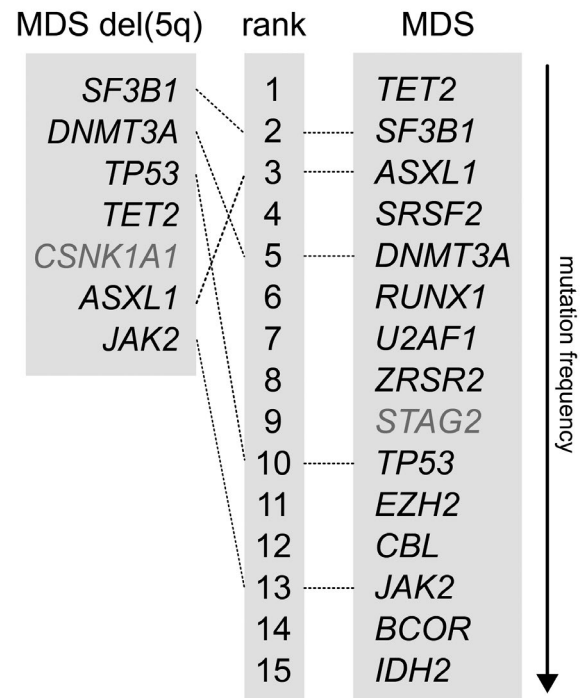
### Molecular genetics during follow up and progression to AML

Follow-up samples were available in 13 patients with a median time between two investigations of 22 months (range: 5-47 months, cases with less than four months between two investigations were excluded). In a further 6/123 (5%) cases we could follow a progression to AML with a median transformation time to s-AML of 24 months (range: 10-53 months).

In these 19 cases with a follow-up sample mutation analyses were performed, in 18/19 cases cytogenetics were also available. Overall, in 5/13 (38%) patients showing no transformation to AML no clonal evolution was detectable, neither in karyotype nor in the mutation pattern, leaving 8/13 (62%) patients with clonal evolution. In 4/13 cases (31%) a change in cytogenetics occurred and in 7/13 patients (54%) additional gene mutations appeared; in 3/13 patients clonal evolution occurred in both instances. Studying patients progressing to AML after MDS revealed a similar pattern regarding stability in genetics with 3/5 cases (60%) showing no genetic evolution, 1/5 (20%) a clonal evolution in cytogenetics and 2/6 (33%) exhibiting a change in the mutation pattern. Of note, none of the patients gained a mutation in *TP53* or *FLT3* during progression to AML. Addressing the gains of genetic lesions in the disease course showed no specific pattern. Cytogenetic gains included -Y, del(11q), del(13q) and inversion 3 (inv(3)), and gained mutations affected *ASXL1*, *BCOR*, *CSNK1A1*, *DNMT3A*, *IDH1*, *JAK2*, *RUNX1* and *TET2*. However, recurrent gains were limited to *JAK2* (n=2) and *RUNX1* (n=3), with two cases showing a gain of *RUNX1* mutation progressing to AML and one progressing to MDS RAEB-1. Furthermore, the VAF of all gained mutations were <10% (median 4%, range: 3-7%), except for those of *RUNX1* during transformation to AML, which showed VAF of 14% and 25%, respectively. An illustration of the different types of clonal evolution is given in Figure 5.

### Discussion

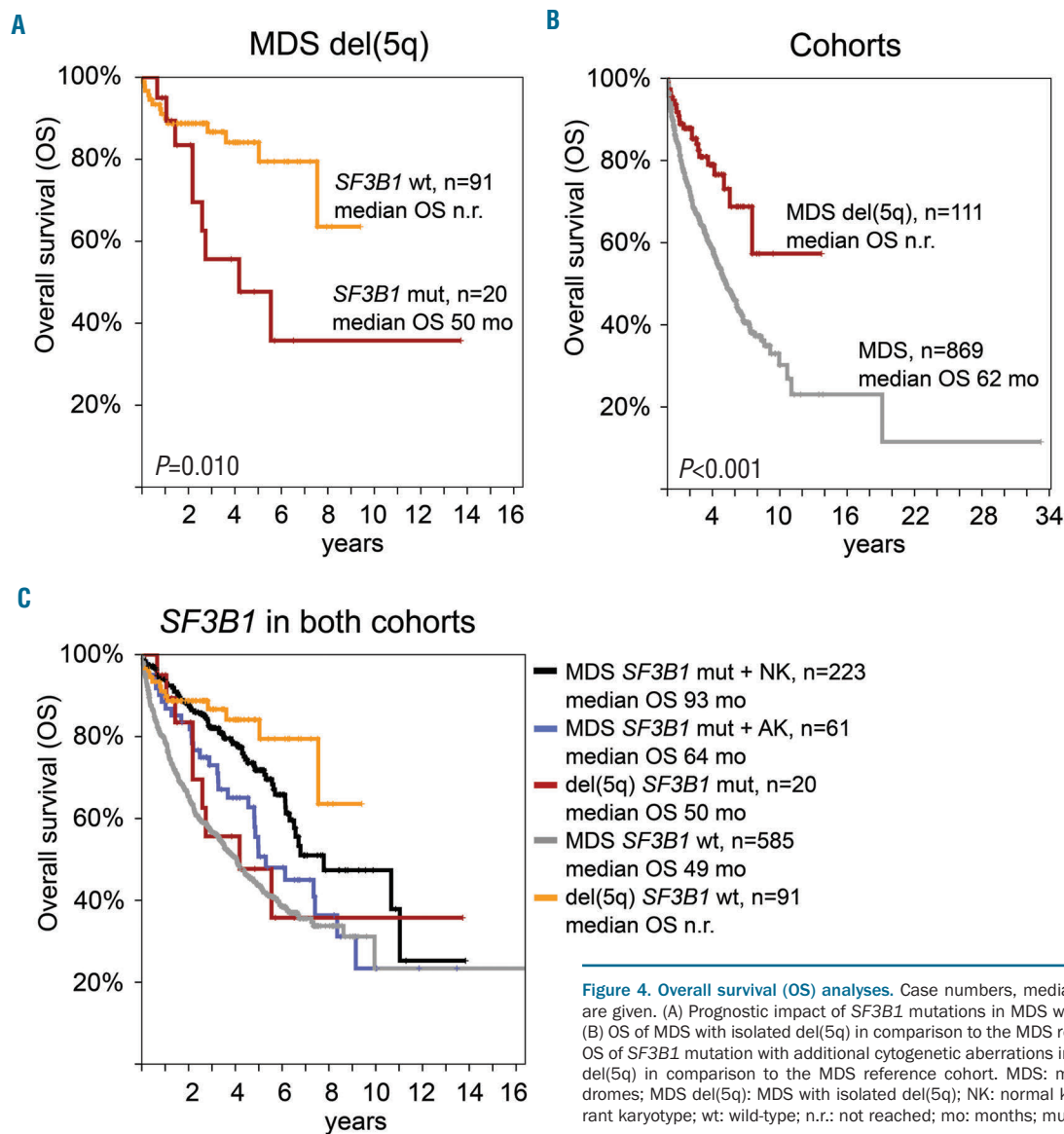
In the study herein, we investigated the molecular mutation pattern of a large cohort of 123 MDS patients with isolated del(5q) classified according to the WHO classification of 2008 and including the added guidelines of 2016. A large proportion of patients showed no or only one mutation in 27 analyzed genes, with only seven genes



**Figure 3. Comparison of mutation frequencies between MDS with isolated del(5q) and all MDS subtypes.** Mutation frequencies for MDS were taken from the previously published reference cohort.<sup>13</sup> The six most frequently mutated genes in MDS with isolated del(5q) are compared to the 14 most frequently mutated genes in the MDS reference cohort. *CSNK1A1* and *STAG2* (typed in gray) were not analyzed in the MDS reference cohort or MDS del(5q) cohort, respectively. MDS: myelodysplastic syndromes; MDS del(5q): MDS with isolated del(5q).

showing mutation frequencies >5% (*SF3B1*, *DNMT3A*, *TP53*, *TET2*, *CSNK1A1*, *ASXL1*, *JAK2*). Albeit in other studies more genes were investigated for mutation analyses, in overall MDS the median number of mutations is higher with three mutations per patient<sup>13</sup> or nearly half of the patients showing two or three mutations<sup>14</sup> in contrast to MDS with isolated del(5q). This indicates the specific and narrow spectrum of genetic lesions in this specific MDS subtype. However, the most frequently mutated genes were comparable to all other MDS subtypes investigated in a large MDS reference cohort.<sup>13</sup> The 15 most frequently mutated genes have also been identified by another large MDS study and by meta-analysis showing consistent data for the MDS mutation landscape.<sup>14,23</sup> Furthermore, *SF3B1* was frequently mutated and correlated with the presence of RS, which is in line with previously published data on other MDS entities.<sup>24-26</sup>

In contrast, *TP53* was mutated significantly more often in MDS with isolated del(5q). In a number of studies mutations of *TP53* were shown to occur in high-risk or therapy-related MDS, MDS-derived leukemia or within a complex karyotype.<sup>27-29</sup> Incidences of *TP53* mutations in MDS with isolated del(5q) were found to be 5%, much lower than in other MDS subtypes.<sup>12</sup> However, in another previous study the incidence of *TP53* mutations, investigated by back tracking *TP53*-mutated samples in low-risk MDS with del(5q), was 18%, suggesting a previous underestimation of *TP53* subclonal mutations.<sup>11</sup> It was shown that the *TP53* mutation was already present in the early



**Figure 4. Overall survival (OS) analyses.** Case numbers, median OS and  $P$ -values are given. (A) Prognostic impact of  $SF3B1$  mutations in MDS with isolated del(5q). (B) OS of MDS with isolated del(5q) in comparison to the MDS reference cohort. (C) OS of  $SF3B1$  mutation with additional cytogenetic aberrations in MDS with isolated del(5q) in comparison to the MDS reference cohort. MDS: myelodysplastic syndromes; MDS del(5q): MDS with isolated del(5q); NK: normal karyotype; AK: aberrant karyotype; wt: wild-type; n.r.: not reached; mo: months; mut: mutated.

stages of the disease and increased during the course of the disease.<sup>11</sup> This is in line with the present data showing a  $TP53$  mutation incidence of 18%, with 7/22 cases having VAF of <10%.

Surprisingly,  $SF3B1$  mutations led to significantly worse OS, but ultimately showed a comparable outcome, still favorable, to all other MDS subtypes with a  $SF3B1$  mutation and an aberrant karyotype. Since a high frequency of MDS patients show a normal karyotype, the need to analyze the impact of karyotype information on  $SF3B1$  mutations in overall MDS in our reference cohort seemed the next logical step. This demonstrated that an accompanying altered karyotype also reduces the favorable impact of  $SF3B1$  mutations. Therefore, the highly favorable impact of  $SF3B1$  mutation was limited to sole  $SF3B1$  mutations without any additional lesions, and might be an explanation as to why the favorable impact is reduced and the overall good prognosis is not driven by  $SF3B1$  mutations alone in MDS with isolated del(5q). However, this finding is in contrast to previous studies which demonstrate that

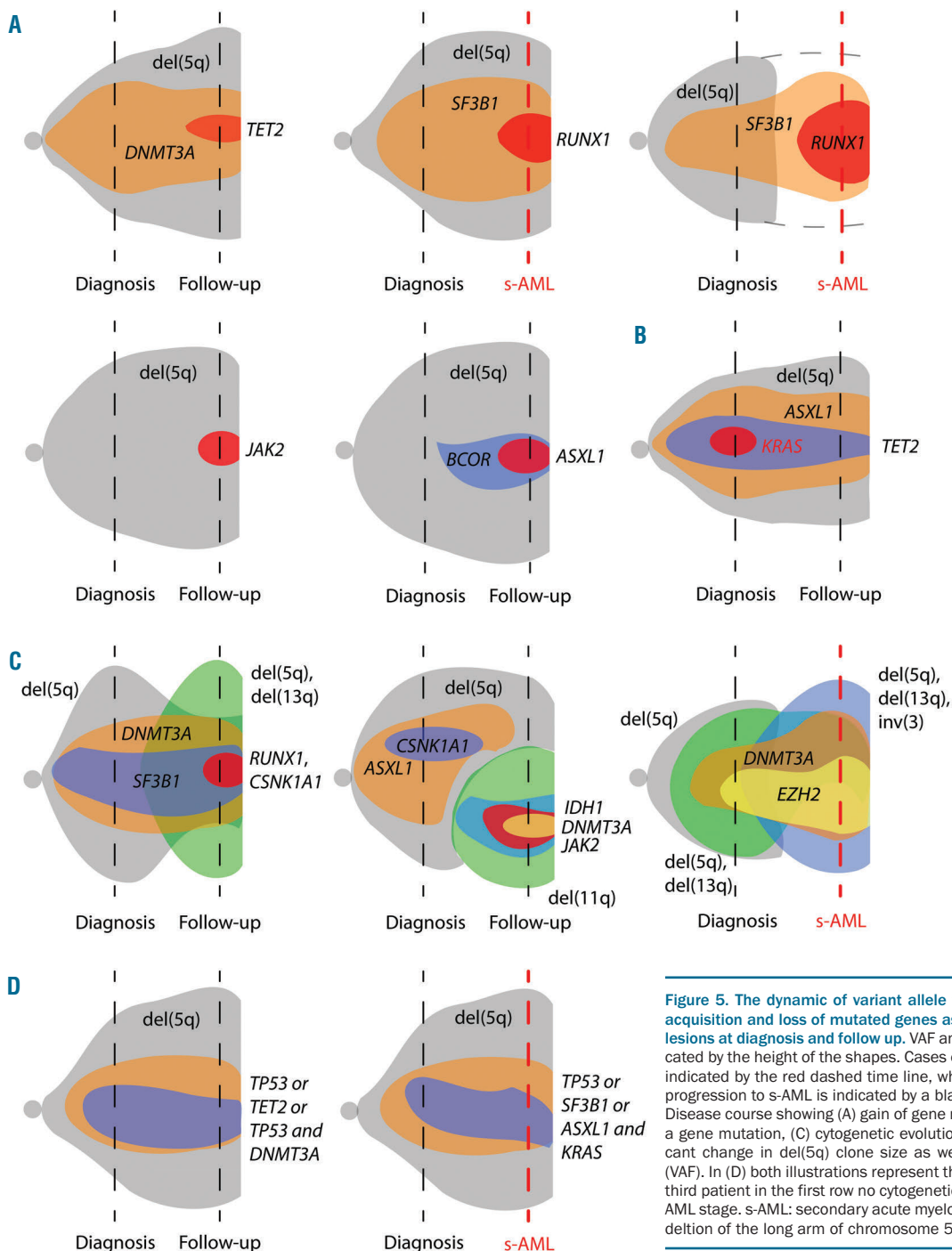
the prognostic effect of  $SF3B1$  mutations is independent of variables that could coexist, such as age, sex and cytogenetics.<sup>24,26</sup> In other studies, however,  $SF3B1$  mutations did not keep independent prognostic significance in multivariate analyses or showed no prognostic impact whatsoever, indicating that perhaps MDS represents a heterogeneous disease and as such should be analyzed in respective subgroups.<sup>30-33</sup>

In MDS it was evidenced that transformation to s-AML is frequently accompanied by mutations in the  $FLT3$  and  $RAS$  genes and karyotype evolution.<sup>34-38</sup> In addition,  $RUNX1$ ,  $GATA2$  and  $CEBPA$  were identified by progression to s-AML, abrogating normal differentiation.<sup>39</sup> Furthermore, some genes have been identified as being affected by mutations occurring as late events in MDS patients, thus giving rise to a potential progression to AML, such as mutations in  $ASXL1$ ,  $RUNX1$ ,  $SRSF2$ ,  $IDH2$  or  $NRAS$ .<sup>34,35,38</sup> However, most of these genes were not identified to be affected in MDS with isolated del(5q), arguing for an alternative progression mechanism to that



found in all other MDS patients. Nevertheless, the recurrent gain of *RUNX1* mutations in the disease course might indicate transformation to AML. In a comprehensive study of the mutation patterns in AML, it became obvious that some genes were specific for s-AML, covering the spliceosomal genes as well as chromatin modifiers.<sup>38,59</sup> The high incidence of *SF3B1* mutations in MDS with isolated del(5q) would therefore reflect the MDS origin of the disease rather than a high *SF3B1* mutation frequency indicating disease progression. In previous studies, mutations in *TP53* and gain of cytogenetic aberrations were speculated

to indicate disease progression and transformation to AML.<sup>11,12</sup> However, the clonal evolution was comparable in MDS with isolated del(5q) to that described for overall MDS, with a karyotype evolution in 34% and mutation pattern in 53% of MDS cases transforming to AML.<sup>34</sup> Moreover, the clonal evolution appeared in the same manner in cases without progression to AML. Additionally, *TP53* mutations were never gained during the disease course, but the mutation burden increased (from 11% to 75%) in the one case which transformed to AML, while in follow-up cases without progression (n=2) the burden did



**Figure 5.** The dynamic of variant allele frequency (VAF) and acquisition and loss of mutated genes as well as cytogenetic lesions at diagnosis and follow up. VAF and clone size are indicated by the height of the shapes. Cases evolving to s-AML are indicated by the red dashed time line, while follow up without progression to s-AML is indicated by a black dashed time line. Disease course showing (A) gain of gene mutations, (B) loss of a gene mutation, (C) cytogenetic evolution, and (D) no significant change in del(5q) clone size as well as gene mutation (VAF). In (D) both illustrations represent three patients. For the third patient in the first row no cytogenetics was available at s-AML stage. s-AML: secondary acute myeloid leukemia; del(5q): deltion of the long arm of chromosome 5.

not change. Therefore, one might suppose that in patients with clonal evolution clinical progress will be encountered, while stable patients will also remain clinically stable. It was shown that clinical stability accompanies mutational stability, while developing new mutations resulted in AML progression in patients with MDS with isolated del(5q).<sup>40</sup> The previously published worse impact prognosis of *TP53* mutations was not observed in the present cohort. Our patients were unselected and the median follow up was 41 months. Thus, our cohort may include a larger proportion of patients earlier in their clinical course compared to cohorts enrolled in treatment studies. Therefore, the negative impact of *TP53* mutations may become obvious in later stages of the disease course or even subsequent to the need for starting treatment, a phenomenon that is not represented by the collection of patients presented herein. Of note, 60% (61/101) of our patients were under observation alone, or only received red blood cells or erythropoietin. However, the presence, in advance, of *TP53* mutations in the early stages of the disease would therefore be in line with our data, supported by the observed increase of clone size rather than the gain of *TP53* mutation during the disease course.<sup>11</sup> However, this needs to be further proven in larger cohorts as we had a small number of cases for follow up.

In summary, MDS with isolated del(5q) shows a very

limited mutation spectrum of the recurrently mutated genes in comparison to all other MDS subtypes. However, the seven frequently mutated genes resemble the MDS mutation landscape, with the exception of *TP53* mutations, which are more often found in MDS with isolated del(5q). Therefore, progression to s-AML is driven by the worse prognostic impact of *TP53* mutation in combination with del(5q) and potentially the gain of *RUNX1* mutations, rather than the otherwise identified accompanying mutations during MDS progression. The diagnostic and prognostic approach of MDS with isolated del(5q) should therefore include the mutation status of the seven most frequently mutated genes, including *SF3B1*, and *TP53* and *RUNX1* as potential progression markers, investigated with deep sequencing to catch all appearing mutations including those of a subclonal nature with low VAF.

#### Acknowledgments

We thank all clinicians for sending samples to our laboratory for diagnostic purposes and for providing clinical information and follow-up data. In addition, we would like to thank all our co-workers at the MLL Munich Leukemia Laboratory for jointly approaching many aspects in the field of leukemia diagnostics and research. We also thank Karolína Perglerová for analyzing *FLT3-ITD* in the follow-up samples at MLL2 s.r.o., Prague, Czech Republic.

#### References

- Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
- Haase D, Germing U, Schanz J, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood*. 2007;110(13):4385-4395.
- Bernasconi P, Klersy C, Boni M, et al. World Health Organization classification in combination with cytogenetic markers improves the prognostic stratification of patients with de novo primary myelodysplastic syndromes. *Br J Haematol*. 2007;137(3):193-205.
- Mallo M, Cervera J, Schanz J, et al. Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q. *Leukemia*. 2011;25(1):110-120.
- Germing U, Lauseker M, Hildebrandt B, et al. Survival, prognostic factors and rates of leukemic transformation in 381 untreated patients with MDS and del(5q): A multicenter study. *Leukemia*. 2012;26(6):1286-1292.
- Schanz J, Tuchler H, Sole F, et al. New Comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. *J Clin Oncol*. 2012;30(8):820-829.
- Mallo M, del Rey M, Ibanez M, et al. Response to lenalidomide in myelodysplastic syndromes with del(5q): influence of cytogenetics and mutations. *Br J Haematol*. 2013;162(1):74-86.
- List AF, Dewald GW, Bennett J, et al. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. *N Engl J Med*. 2006;355(14):1456-1465.
- Giagounidis AA, Germing U, Haase S, et al. Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. *Leukemia*. 2004;18(1):113-119.
- Side LE, Curtiss NP, Teel K, et al. RAS, FLT3, and TP53 mutations in therapy-related myeloid malignancies with abnormalities of chromosomes 5 and 7. *Genes Chromosomes Cancer*. 2004;39(3):217-223.
- Jadersten M, Saft L, Smith A, et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. *J Clin Oncol*. 2011;29(15):1971-1979.
- Fernandez-Mercado M, Burns A, Pellagatti A, et al. Targeted re-sequencing analysis of 25 genes commonly mutated in myeloid disorders in del(5q) myelodysplastic syndromes. *Haematologica*. 2013;98(12):1856-1864.
- Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2014;28(2):241-247.
- Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616-3627.
- Heuser M, Meggendorfer M, Cruz MM, et al. Frequency and prognostic impact of casein kinase 1A1 mutations in MDS patients with deletion of chromosome 5q. *Leukemia*. 2015;29(9):1942-1945.
- Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al. WHO classification of tumours of haematopoietic and lymphoid tissues, 4<sup>th</sup> ed. Lyon: International Agency for Research on Cancer (IARC), 2008.
- Löffler H, Rastetter J, Haferlach T. Atlas of clinical hematology, 6<sup>th</sup> ed. Heidelberg: Springer, 2010.
- Schoch C, Schnittger S, Bursch S, et al. Comparison of chromosome banding analysis, interphase- and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. *Leukemia*. 2002;16(1):53-59.
- McGowan-Jordan J, Simons A, Schmid M. *ISCN 2016: An International System for Human Cytogenomic Nomenclature*. Basel, New York: Karger, 2016.
- Delic S, Rose D, Kern W, et al. Application of an NGS-based 28-gene panel in myeloproliferative neoplasms reveals distinct mutation patterns in essential thrombocythaemia, primary myelofibrosis and polycythaemia vera. *Br J Haematol*. 2016;175(3):419-426.
- Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;7(8):575-576.
- Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat*. 2007;28(6):622-629.
- Rose D, Kohlmann A, Nagata Y, et al. A robust molecular pattern for myelodysplastic syndromes in two independent cohorts investigated by next-generation sequencing can be revealed by comparative bioinformatic analyses. *Br J Haematol*. 2014;167(2):278-281.



24. Papaemmanuil E, Cazzola M, Boultonwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med.* 2011;365(15):1384-1395.
25. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature.* 2011;478(7367):64-69.
26. Malcovati L, Papaemmanuil E, Bowen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood.* 2011;118(24):6239-6246.
27. Haferlach C, Dicker F, Herholz H, et al. Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype. *Leukemia.* 2008;1539-1541.
28. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature.* 2015;518(7540):552-555.
29. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med.* 2011;364(26):2496-2506.
30. Patnaik MM, Lasho TL, Hodnefield JM, et al. SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. *Blood.* 2012;119(2):569-572.
31. Damm F, Thol F, Kosmider O, et al. SF3B1 mutations in myelodysplastic syndromes: clinical associations and prognostic implications. *Leukemia.* 2012;26(5):1137-1140.
32. Thol F, Kade S, Schlarman C, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. *Blood.* 2012;119(15):3578-3584.
33. Cazzola M, Rossi M, Malcovati L. Biologic and clinical significance of somatic mutations of SF3B1 in myeloid and lymphoid neoplasms. *Blood.* 2013;121(2):260-269.
34. Meggendorfer M, de AA, Nadarajah N, et al. Karyotype evolution and acquisition of FLT3 or RAS pathway alterations drive progression of myelodysplastic syndrome to acute myeloid leukemia. *Haematologica.* 2015;100(12):e487-e490.
35. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med.* 2016;374(23):2209-2221.
36. Takahashi K, Jabbour E, Wang X, et al. Dynamic acquisition of FLT3 or RAS alterations drive a subset of patients with lower risk MDS to secondary AML. *Leukemia.* 2013;27(10):2081-2083.
37. Murphy DM, Bejar R, Stevenson K, et al. NRAS mutations with low allele burden have independent prognostic significance for patients with lower risk myelodysplastic syndromes. *Leukemia.* 2013;27(10):2077-2081.
38. Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. *Nat Rev Cancer.* 2017;17(1):5-19.
39. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood.* 2015;125(9):1367-1376.
40. 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.