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Research paper

Comparison of therapy-related myelodysplastic syndrome with ring sideroblasts and *de novo* myelodysplastic syndrome with ring sideroblasts

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ABSTRACT

Presence of RS is closely associated with *SF3B1* mutation in *de novo* MDS. RS is also present in a subset of therapy-related MDS (t-MDS), but data is not available in t-MDS with RS (t-MDS-RS). Using NGS gene panel, we assessed t-MDS-RS (n = 38) and compared the result with d-MDS-RS (n = 174). Commonly mutated genes were *TP53* (56.5%), *TET2* (39.1%), *SF3B1* (35.7%), *ASXL1* (30.4%), *DNMT3A* (17.4%), *RUNX1* (17.4%) and *SRSF2* (14.3%). Compared with d-MDS-RS, *TP53* mutation was more common but *SF3B1* mutation was less common in t-MDS-RS ($p < 0.05$). In t-MDS-RS, Mutations in 4 genes (*SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2*) involving the RNA splicing were found in about 50% of patients compared to ~90% in d-MDS-RS. Overall survival was by far worse in t-MDS-RS compared to d-MDS-RS (median overall survival: 10.9 months and 111.9 months in t-MDS-RS and d-MDS-RS, respectively, $p < 0.05$). Progression to acute myeloid leukemia was more common in t-MDS-RS (18.4% vs. 7.4% in t-MDS-RS and d-MDS-RS, respectively, $p < 0.05$). Unlike *de novo* MDS, t-MDS-RS did not have different outcome compared to t-MDS without RS (median OS: 10.9 months vs. 14.3 months, respectively, $p = 0.2341$). Our data demonstrate that presence of RS is not associated with superior outcome in t-MDS. Mutation profiles suggest RS in t-MDS might be a secondary event in at least 50% of the cases or not related to mutations in RNA splicing machinery unlike d-MDS where mutations in RNA splicing machinery occur early and as associated with ineffective erythropoiesis.

1. Introduction

Ring sideroblasts (RS) are defined as erythroid precursors in which there are ≥ 5 siderotic cytoplasmic granules covering ≥ 1/3 of the nuclear circumference [1]. Although reactive cases have been reported, the presence of RS is mostly regarded as a sign of dysplasia supporting the diagnosis of myelodysplastic syndrome (MDS) [2]. In the updated World Health Organization (WHO) classification, myelodysplastic syndrome with RS (MDS-RS) is a subset of MDS that is characterized by cytopenias, morphological dysplasia, < 5% blasts in bone marrow (BM) and < 1% blasts in peripheral blood (PB) and increased number of RS [3]. RS being ≥ 15% of bone marrow erythroid precursors is required for the diagnosis but the cutoff can be lowered to 5% when *SF3B1* mutation is present.

SF3B1 encodes a core component of the U2 small nuclear ribonucleoproteins (snRNP) that participate in RNA splicing and the 3′ processing of the precursor messenger RNA [4]. *SF3B1* mutation in patients with MDS is: 1) associated with the presence of RS; 2) has a distinct gene expression profile; and 3) is associated with a favorable outcome [5–8]. Patnaik and colleagues showed that the actual percentage of RS in cases of MDS is not associated with patient outcome. In part related to these data, in the WHO classification the required number of RS to establish a diagnosis of MDS-RS is now 5% when a *SF3B1* mutation is present [9].

MDS-RS is subdivided into cases with single lineage dysplasia and those with multilineage dysplasia in the current WHO classification system. The former was previously classified as refractory anemia with ring sideroblasts (RARS) in the 2008 WHO classification. The latter was not recognized as a subset of MDS-RS at that time and was classified as refractory cytopenia with multilineage dysplasia (RCMD).

Therapy-related MDS (t-MDS) is a subset of MDS which occurs in patients who have received cytotoxic therapies, usually for a prior malignancy [3]. Although morphologically similar to *de novo* MDS, t-MDS is enriched for very high and high risk groups as defined in the Revised International Prognostic Scoring System (IPSS-R), including patients with *TP53* mutation and a complex karyotype, and associated

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with a dismal outcome [10,11].

Ring sideroblasts can be observed in a subset of cases of t-MDS. Whereas there is ample data regarding the presence of RS and pertinent molecular profiling in *de novo* MDS, data is not available for the t-MDS with RS (t-MDS-RS). DNA damage and disrupted DNA repair mechanisms or acquired somatic mutations in pretreatment samples are associated with higher risk in development of t-MDS, but these mechanisms do not readily explain presence of RS in t-MDS [12,13]. Therefore, in this study we assessed cases of t-MDS-RS for gene mutations using next generation sequencing methods and we compared these results to cases of *de novo* MDS-RS.

2. Material and methods

2.1. Patients

We reviewed all newly diagnosed cases of myelodysplastic syndrome (MDS) with increased (≥ 15% of erythroid precursors) RS, both *de novo* and therapy-related, from January 1, 2012 through December 31, 2018 at The University of Texas MD Anderson Cancer Center. Clinical, hematological and cytogenetic data were collected for all patients. We excluded patients with increased blasts (≥ 5% in bone marrow and ≥ 1% in peripheral blood). All cases were collected consecutively and classified according to the updated World Health Organization (WHO) classification system [3]. Overall risk for each patient was determined using the Revised International Prognostic Scoring System for MDS (IPSS-R) [14]. Informed consent was obtained from the patients or their guardians. This study was conducted in accord with the Declaration of Helsinki and was approved by the IRB at The University of Texas MD Anderson Cancer Center in Houston, Texas, USA.

2.2. Morphological assessment

In all cases, routine hematoxylin and eosin (H&E) histologic sections of BM trephine biopsy specimens and well-prepared Wright–Giemsa-stained bone marrow aspirate smears were reviewed. A 500-nucleated cell differential cell count was performed. Perls' reaction for iron was performed on BM aspirate smears.

2.3. Cytogenetic analysis

Conventional cytogenetic analysis was performed on metaphase cells prepared from BM aspirates cultured for 24 or 48 h without mitogens. Chromosomal analysis was performed using standard, published methods in the Clinical Cytogenetic Laboratory and the results were reported according to International System for Human Cytogenetic Nomenclature (ISCN) 2016 [15]. A minimum of 20 metaphase spreads were examined if available. Cytogenetic risk was determined using the Revised International Prognostic Scoring System for MDS (IPSS-R) [14].

2.4. Targeted next-generation sequencing

Targeted next-generation sequencing (NGS) studies using panels of genes commonly altered in myeloid neoplasia were performed as part of the clinical workup (28-gene or 81-gene panels) as described previously [16]. Of note, the spliceosome-related genes *SF3B1, SRSF2, U2AF1*, and *ZRSR2* were only included in the 81-gene panel. NGS data were analyzed using MiSeq Reporter (TruSeq) or SureCall (Haloplex). The Integrative Genomics Viewer (IGV, Broad Institute) was used to visualize read alignment and confirm variant calls. A custom-developed, in-house software package (OncoSeek) was used to annotate sequence variants and to interface the data with the IGV. For clinical reporting, the assay sensitivity was 1%. Nomenclature of genetic variants was designated following the Human Genome Variation Society recommendations.

Table 1
Patient characteristics.

Variables	t-MDS-RS (n = 38)	*De novo* MDS-RS (n = 174)	P value
Age (years)	69 (15-88)	71 (40-88)	0.3578
Men: women (men %)	26:12 (68.4%)	118: 59 (67.2%)	1
Median RS (%)	35.5 (15-90)	50 (15-95)	0.1376
Hemoglobin (g/dL)	9.1 (7-13.4)	9.2 (6.3-14.5)	0.6502
ANC (x 10^9/L)	1.75 (0.14-7.27)	2.39 (0.06-9.53)	0.0022
Platelets (x 10^9/L)	65 (17-428)	218 (8-507)	< 0.0001
BM blasts (%)	1 (0-4)	2 (0-4)	0.5025
Cytogenetic Risk			< 0.0001
Very good	0 (0%)	4 (2.3%)	
Good	11 (28.9%)	112 (64.4%)	
Intermediate	4 (10.5%)	36 (20.7%)	
Poor	4 (10.5%)	12 (6.9%)	
Very poor	19 (50%)	10 (5.7%)	
IPSS-R risk			< 0.0001
Very low	2 (5.7%)	28 (16.6%)	
Low	7 (20%)	100 (59.2%)	
Intermediate	5 (14.3%)	25 (14.8%)	
High	14 (40%)	13 (7.7%)	
Very high	7 (20%)	3 (1.8%)	

t-MDS-RS; therapy-related myelodysplastic syndrome with ring sideroblasts, RS; ring sideroblasts, ANC; absolute neutrophil count, BM; bone marrow, IPSS-R; The Revised International Prognostic Scoring System.

2.5. Statistics

Fisher's exact test or the chi-square test was used for categorical variables and the Mann-Whitney test was applied for numerical variables. Survival probability was determined using the Kaplan–Meier method, with differences compared by the log-rank test. Patients undergoing stem cell transplant were censored in survival analysis. A P value (two-sided) of < 0.05 indicated statistical significance. Statistical analysis was performed using Prism software (version 7.0; GraphPad).

3. Results

3.1. Patient characteristics

The study cohort consisted of 38 patients with t-MDS-RS and 174 patients with *de novo* MDS-RS. The clinicopathologic and laboratory features of these patients are summarized in Table 1. In the t-MDS-RS group, there were 26 men and 12 women with a median age of 69 years (range, 15–88). Most patients (n = 34) showed multilineage dysplasia. All patients had neoplasms in the past including hematological malignancies (n = 23), carcinomas (n = 12), sarcoma (n = 1), thymoma (n = 1), and malignant mesothelioma (n = 1). Patients were treated for primary neoplasms with combined chemotherapy only (n = 24), chemotherapy and radiation therapy (n = 11), or radiation only (n = 3). In the *de novo* MDS-RS group, there were 118 men and 59 women with a median age of 71 years (range, 40–88). In this group, 58 patients had MDS-RS with single lineage dysplasia and 116 patients had MDS-RS with multilineage dysplasia.

Comparing the t-MDS-RS and *de novo* MDS-RS, patients with t-MDS-RS had significantly lower absolute neutrophil counts (median: 2.39 × 10^9/L and 1.75 × 10^9/L, respectively, p = 0.0022) and platelet counts (median: 218 × 10^9/L and 65 × 10^9/L, p < 0.0001) (Table 1). Other variables including median age, gender, hemoglobin, bone marrow blasts, and median percentage of RS showed no significant differences. In t-MDS-RS group, patients were treated with hypomethylating agent (HMA)-based therapy, chemotherapy and supportive care/no treatment in 26 (68.4%), 3 (7.8%), and 9 (23.6%) patients, respectively. In de novo MDS-RS group, patients were treated with HMA-based therapy, chemotherapy and supportive care/not treatment in 110 (63.2%), 10 (5.7%) and 54 (31%), respectively. The trend was similar in two groups (p = 0.6271).

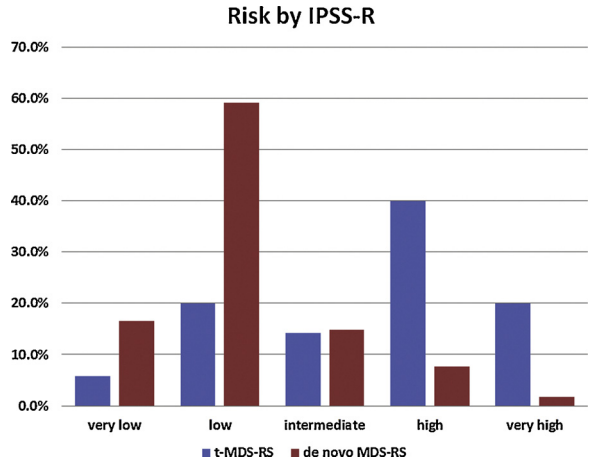

Fig. 1. Distribution of risk by The Revised International Prognostic Scoring System (IPSS-R) in therapy-related myelodysplastic syndrome with ring sideroblasts and de novo myelodysplastic syndrome with ring sideroblasts.
IPSS-R; The Revised International Prognostic Scoring System, t-MDS-RS; therapy-related myelodysplastic syndrome with ring sideroblasts.

3.2. Cytogenetics and IPSS-R risk

In the t-MDS-RS group, 11 (28.9%), 4 (10.5%), 4 (10.5%), and 19 (50%) patients had good, intermediate, poor and very poor cytogenetic risk, respectively. There were no patients with very good cytogenetic risk in the t-MDS-RS group. In the *de novo* MDS-RS group, 4 (2.3%), 112 (64.4%), 36 (20.7%), 12 (6.9%) and 10 (5.7%) patients had very good, good, intermediate, poor and very poor cytogenetic risk, respectively.

Using the IPSS-R system, in the t-MDS-RS group 2 (5.7%), 7 (20%), 5 (14.3%), 14 (40%) and 7 (20%) patients had very low, low, intermediate, high and very high risk, respectively. In the *de novo* MDS-RS group, 28 (16.6%), 100 (59.2%), 25 (14.8%), 13 (7.7%), and 3 (1.8%) patients had very low, low, intermediate, high and very high risk, respectively, (Fig. 1).

3.3. Comparison of mutation profiles in t-MDS-RS and de novo MDS-RS

A total of 23 and 117 t-MDS-RS and *de novo* MDS-RS patients had available NGS data. In t-MDS-RS, 14 and 9 patients underwent 81-gene and 28-gene panels, respectively. In *de novo* MDS-RS, 81 and 36 patients underwent 81-gene and 28-gene panels, respectively. Twenty-one of 23 (91.3%) t-MDS-RS patients had gene mutations, with a median of 2 mutations per case (range, 0–8). In order of decreasing frequency, commonly (> 10%) mutated genes included *TP53* (13/23, 56.5%), *TET2* (9/23, 39.1%), *SF3B1* (5/14, 35.7%), *ASXL1* (7/23, 30.4%), *DNMT3A* (4/23, 17.4%), *RUNX1* (4/23, 17.4%) and *SRSF2* (2/14, 14.3%) (Fig. 2). The median mutant allelic frequency of *SF3B1* mutation was 36.9% (range, 18.7% to 48.5%). Mutations in 4 genes (*SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2*) involved in RNA splicing were found in 50% of patients.

One hundred eight of 117 (92.3%) patients in the *de novo* MDS-RS group had gene mutations, with a median of 2 mutations per case (range, 0–10). In order of decreasing frequency, commonly mutated genes included *SF3B1* (58/81, 71.6%), *TET2* (48/117, 41%), *DNMT3A* (26/117, 22.2%), *ASXL1* (24/117, 20.5%), *SRSF2* (9/81, 11.1%), *RUNX1* (12/117, 10.3%), U2AF1 (8/81, 9.9%) and *TP53* (11/117, 9.4%) (Fig. 2). The median mutant allelic frequency of *SF3B1* mutation was 38.6% (range: 9.1% to 48.4%). Mutations in one of the 4 RNA splicing genes (*SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2*) were observed in 88.9% of patients.

As expected, *TP53* mutation was more frequent in the t-MDS-RS patients (56.5% vs. 9.4%, $p < 0.0001$). In comparison, *SF3B1* mutation was more common in *de novo* MDS-RS patients (71.6% vs. 35.7%, $p = 0.0136$). No differences were observed in the mutant allelic frequency *SF3B1* mutation in either group ($p = 0.6548$, Mann-Whitney test). There were no significant differences in the frequency of other gene mutations between the groups.

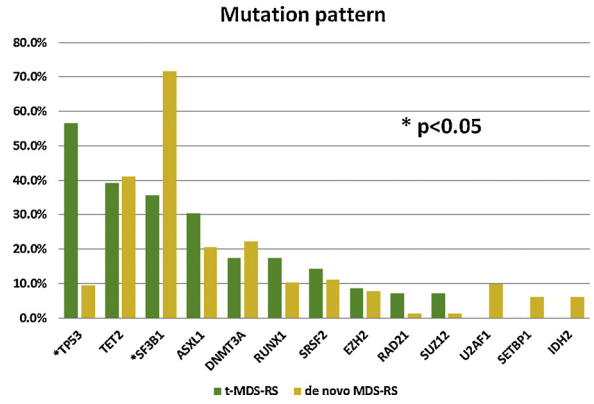

Fig. 2. Common mutations in therapy-related myelodysplastic syndrome with ring sideroblasts and de novo myelodysplastic syndrome with ring sideroblasts. Asterisk (*) denotes $p < 0.05$.
t-MDS-RS; therapy-related myelodysplastic syndrome with ring sideroblasts.

3.4. Survival analysis

Although the presence of RS was observed in both groups, overall survival was much worse for patients with t-MDS-RS versus patients with *de novo* MDS-RS (median overall survival, 10.9 months and 111.9 months, respectively, $p < 0.0001$) (Fig. 3-A). We further investigated if the dismal outcome in t-MDS-RS could be attributed to mutations in *ASXL1, RUNX1, SRSF2* or *TP53* (high risk mutations), which are known to be associated with poor outcome in MDS. Selecting cases with high risk mutations, the dismal outcome in t-MDS-RS was retained compared to *de novo* MDS-RS ($p < 0.0001$). Unlike *de novo* MDS-RS, where high risk mutations were associated with a poorer outcome ($p = 0.0289$), the high risk mutations were not associated with a poorer outcome in t-MDS-RS ($p = 0.4802$).

Progression to acute myeloid leukemia (≥20% blasts in either bone marrow or peripheral blood) was more common in patients with t-MDS-RS versus patients with *de novo* MDS-RS (18.4% versus 7.4%, respectively, $p = 0.0364$). We also compared the outcome of patients with t-MDS-RS to patients with t-MDS using a previously published t-MDS cohort (n = 105) [10]. Patients with t-MDS-RS had a similar outcome to patients with t-MDS without RS (median OS, 10.9 months vs. 14.3 months, respectively, $p = 0.2341$) (Fig. 3-B). Similar outcomes between t-MDS-RS and t-MDS without RS were observed when cases were analyzed based in IPSS-R very high/high risk, intermediate and low/very low risks ($p > 0.05$).

4. Discussion

Using an NGS gene panel, we assessed a series of cases of t-MDS-RS and compared the results with a group of *de novo* MDS-RS cases. Demographic factors (age and gender) showed no difference between the two groups. Significant differences were observed in the absolute neutrophil and platelet counts, which were much lower in patients with t-MDS-RS. There were also pronounced differences in cytogenetic risk. The poor and very poor cytogenetic risk groups constituted ˜60% of t-MDS-RS cases but only 12.6% in of *de novo* MDS-RS. In comparison, the good and very good cytogenetic risk groups represented about two thirds of *de novo* MDS-RS patients. In accord with the cytogenetic risk score, the IPSS-R showed a similarly skew to high risk pattern in patients with t-MDS-RS (Table 1). In *de novo* MDS-RS, very low and low risk by IPSS-R was significantly more common (75.8%) than in *de novo*

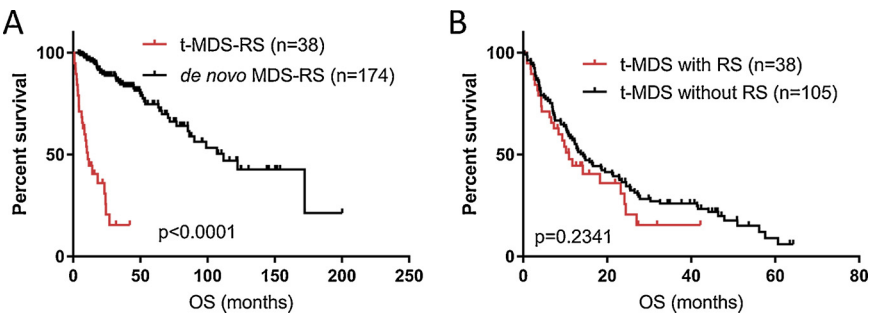

Fig. 3. (A) Comparison of overall survival between therapy-related myelodysplastic syndrome with ring sideroblasts and de novo myelodysplastic syndrome with ring sideroblasts. (B) Comparison of overall survival between therapy-related myelodysplastic syndrome with ring sideroblasts and therapy-related myelodysplastic syndrome without ring sideroblasts.
t-MDS-RS; therapy-related myelodysplastic syndrome with ring sideroblasts.

MDS in general (56˜59%) [10,14]. Interestingly, distribution of cytogenetic risk and IPSS-R risk in t-MDS-RS were similar to that in t-MDS in general [10].

In both t-MDS-RS and *de novo* MDS-RS, most (> 90%) patients had at least one mutation. The median number of mutations per case was 2 in each group, however, the pattern of mutations was different, particularly *TP53* and *SF3B1* (Fig. 2). In the t-MDS-RS group, the frequency of *TP53* mutation, 56.5%, was higher than in a previously published cohort of t-MDS patients (29.5˜39.3%) [11,17]. This difference may be attributed to dramatic improvements in NGS technology, such as target enrichment method and optical technology in NGS sequencer, thereby improving sensitivity. Furthermore, a mutant allelic frequency (MAF) of 5% and 3% were used as a cutoff in the previous studies compared with a 1% cutoff in the current study. Indeed, there were 3 cases of t-MDS-RS with *TP53* mutation in which the MAFs were < 5%. Excluding these 3 cases, the frequency of *TP53* mutation in this cohort of t-MDS-RS patients, 43.4%, is much closer to the frequency reported previously.

SF3B1 mutation was present in almost three quarters of *de novo* MDS-RS cases in this cohort, similar to published data [5,6]. In contrast, *SF3B1* mutation was present in approximately one third of t-MDS-RS patients in this study. Although less frequent, the *SF3B1* mutation was a dominant clone in t-MDS-RS given that the MAFs were similar in both groups. Mutations in at least one of the spliceosome-related genes *SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2* were identified in approximately 50% and 90% of t-MDS-RS and *de novo* MDS-RS, respectively. Available data shows that spliceosome-related gene mutations occur early in *de novo* MDS, which induces RS and ineffective erythropoiesis [18,19]. The 50% frequency of t-MDS-RS patients that harbor mutations in spliceosome-related genes suggests that unidentified mechanisms resulting in RS might exist in t-MDS cases, or possibly RS is a secondary phenomenon in t-MDS.

The outcome of patients with t-MDS-RS was significantly worse compared with patients with *de novo* MDS-RS (Fig. 3-A). In Progression to AML within a relatively short follow-up interval was also more frequent in the t-MDS-RS group, 18.4% versus 7.4% in *de novo* MDS-RS patients. Therefore, patients with t-MDS-RS have more aggressive disease than patients with *de novo* MDS-RS. In addition, patients with t-MDS-RS had an outcome similar to other patients with t-MDS without RS (Fig. 3-B). Therefore, unlike the situation in *de novo* MDS, the presence of RS does not justify separating these cases from other cases of t-MDS.

Our result is somewhat similar to a recent study by Singhal and colleagues [17]. Although comparison between t-MDS-RS and *de novo* MDS-RS was not the main focus of their study, their study cohort included 19 and 23 patients with t-MDS-RS and *de novo* MDS-RS. *SF3B1* mutation was less common (32%) in t-MDS with ≥15% RS compared to *de novo* MDS-RS with ≥15% RS (96%). Most importantly, t-MDS-RS had significantly worse overall survival compared to *de novo* MDS-RS, corroborating our result. They concluded that poor outcome in the t-MDS-RS group was attributed to enriched *TP53* mutation (92%) because patients with wild-type *TP53* in this group had outcome as favorable as *de novo* MDS-RS with wild-type *TP53* and *SF3B1* mutation. Although *TP53* mutations were quite common (55.6%) in *SF3B1* wild-type patients in t-MDS-RS, however, we did not recapitulate the result (data not shown). In our cohort, *TP53* mutation alone or high risk mutations (*ASXL1*, *RUNX1*, *SRSF2* or *TP53* mutations) were not associated with poorer outcome in t-MDS-RS. Instead, intermediate/high/very high risk by IPSS-R was associated with poorer outcome, similar to t-MDS without RS. Our data suggest that RS might be irrelevant in t-MDS because 1) t-MDS-RS shares similar cytogenetic and IPSS-R risk distribution to t-MDS in general, 2) significant risk stratification of t-MDS-RS by IPSS-R, and 3) presence of RS is not associated with poorer outcome.

Our data have its limitation. Firstly, although the largest study so far, the number of patients with t-MDS-RS is marginally large. Secondly, two different types of NGS were conducted causing that spliceosome-related genes are not covered in all patients. A larger scale study cohort with a uniform NGS platform is needed to support our data.

In summary, t-MDS-RS patients generally fall in a higher risk group by cytogenetic risk score and the IPSS-R and frequently carry *TP53* mutations. *SF3B1* mutations and mutations in spliceosome-related genes are less common than *de novo* MDS-RS patients, but still occur in about 50% of cases suggesting that other mechanisms lead to RS in this context. The outcome of patients with t-MDS-RS is significantly worse than that of patients with *de novo* MDS-RS and is similar to that of patients with t-MDS without RS.

Declaration of Competing Interest

All authors have nothing to disclose.

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None.

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