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ARTICLE

Cytogenetics and molecular genetics

The mutational burden of therapy-related myeloid neoplasms is similar to primary myelodysplastic syndrome but has a distinctive distribution

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Abstract

Therapy-related myeloid neoplasms (T-MN) are poorly characterized secondary hematological malignancies following chemotherapy/radiotherapy exposure. We compared the clinical and mutational characteristics of T-MN ($n = 129$) and primary myelodysplastic syndrome (P-MDS, $n = 108$) patients. Although the somatic mutation frequency was similar between T-MN and P-MDS patients (93% in both groups), the pattern was distinct. *TP53* mutations were more frequent in T-MN (29.5 vs. 7%), while spliceosomal complex mutations were more common in P-MDS (56.5 vs. 25.6%). In contrast to P-MDS, the ring sideroblasts (RS) phenotype was not associated with better survival in T-MN, most probably due to genetic association with *TP53* mutations. *SF3B1* was mutated in 96% of P-MDS with ≥15% RS, but in only 32% T-MN. *TP53* mutations were detected in 92% T-MN with ≥15% RS and *SF3B1* wild-type cases. Interestingly, T-MN and P-MDS patients with "Very low" or "Low" Revised International Prognostic Scoring System (IPSS-R) showed similar biological and clinical characteristics. In a Cox regression analysis, *TP53* mutation was a poor prognostic factor in T-MN, independent of IPSS-R cytogenetics, disease-modifying therapy, and *NRAS* mutation. Our data have direct implications for T-MN management and provide evidence that, in addition to conventional disease parameters, mutational analysis should be incorporated in T-MN risk stratification.

Introduction

Therapy-related myeloid neoplasms (T-MNs) are secondary malignancies that occur following chemotherapy (CT) and/or radiotherapy (RT) exposure for the treatment of malignant or non-malignant diseases [1]. They account for 5–10% of patients newly diagnosed with MNs. The World Health Organization (WHO) included therapy-related myelodysplastic syndrome (T-MDS), myelodysplastic/myeloproliferative neoplasm (T-MDS/MPN), and acute myeloid leukemia (T-AML) within the single clinical group of T-MN, due to the perceived similarity between their biological characteristics and outcome. The incidence of these clinically aggressive malignancies varies from <1 to 24% [2] depending on the type of primary disease (non-malignant vs. malignant), the type and intensity of genotoxic exposure, and the length of follow-up.

The prognosis of T-MN is generally poor, with a median survival <12 months, which is attributed to high-risk cytogenetic changes. Unlike primary MDS (P-MDS), which has validated prognostic classifications (e.g., Revised International Prognostic Scoring System [IPSS-R]), there is no universal standard to prognosticate T-MN. Recently, a

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modified version of IPSS-R was applied to segregate T-MDS and T-AML (<30% bone marrow [BM] blasts) into different prognostic groups [3]. Intriguingly, the overall survival (OS) between T-MN and P-MDS was different, even when matched for IPSS-R. This indicates a possible contribution of sub-chromosomal genetic lesions to the pathogenesis and dismal outcome of this disease.

Although comprehensive mutation profiling of P-MDS has been reported on large patient cohorts (157–2250 patients) [4–7], limited data are available for T-MN patients. Mutational frequency in T-MN was reported as highly variable and lower than in P-MDS (33–59 vs. 78–90%, respectively) [8, 9]. This is likely due to relatively small sample size and limited repertoire of genes analyzed. Importantly, very few studies have compared the mutation frequency of T-MN and P-MDS using a uniform bioinformatics pipeline. This study analyzes the somatic mutation profile of T-MN and P-MDS patients registered in the South Australian-MDS Registry.

Materials and methods

Patient samples

The South Australian-MDS (SA-MDS) Registry is a statewide registry enrolling patient diagnosed with P-MDS, AML with ≤30% BM blasts, MDS/MPN overlap, or T-MN. The demographic, clinical, laboratory, treatment, and follow-up data of enrolled patients were analyzed.

The study was conducted in accordance with the Declaration of Helsinki and was approved by ethics committees of participating institutions. Diagnosis was from peripheral blood/BM smear examination by expert hematopathologists. Perl's stain was used to characterize BM iron stores and ring sideroblasts (RS). The WHO classification [1] was employed to further classify the MNs. Where applicable, the IPSS-R classification [10] was used for prognostic purposes in both P-MDS and T-MN. Patients were categorized as receiving "best supportive care only" or "disease-modifying therapy" (DMT). DMT was further specified as hypomethylating agent, intensive CT, allogeneic hematopoietic stem cell transplant (allo-HSCT), investigational therapy, or any combination of these.

Mutational profiling

Genomic DNA collected at diagnosis from 108 P-MDS and 129 T-MN patients was subjected to sequencing and mutational analysis. Patients with samples obtained only at time-points other than diagnosis were excluded from both cohorts ($n = 37$ and 15, respectively). Paired germline samples (hair follicle or BM mesenchymal stromal cell DNA) were available for 31/108 (29%) P-MDS and 58/129 (45%) T-MN samples. The genomic profile of T-MN and P-MDS samples (BM or germline) were analyzed using either custom-designed 217-gene NimbleGen Capture Platform (Roche NimbleGen Inc., Madison, WI, USA), Ion AmpliSeq (Thermo Fisher Scientific Inc., Waltham, MA, USA), or Fluidigm Access Array (Fluidigm Inc., San Francisco, CA, USA) platforms (see Supplementary Information for details). Sequence reads were aligned to the GRCh37 human reference genome (Supplementary method).

Forty-three genes known to be recurrently mutated in myeloid malignancies were selected for further analysis (Table S1). Only variants at sites with a total read depth >100, supported by more than five alternate variant reads and a variant allele frequency (VAF) ≥3%, were retained for further analysis. Exceptions to this were *ASXL1* (c.1934dupG) and *SRSF2* (P95) variants (see Supplementary Information). Figure S1 summarizes the criteria used for selecting somatic mutations. Comparison was performed with paired germline samples when available. All selected variants were manually curated for functional deleteriousness.

Statistical analysis

Comparison of patient characteristics was performed using Mann–Whitney, Fisher's exact, or χ^2 tests, as appropriate. Overall survival (OS) was calculated from the date of diagnosis to that of last contact or death. Approximately 21% P-MDS and 39% T-MN patients in our cohort received DMT reflecting current MDS management. Hence, we adjusted the OS using a time-varying covariate to account for DMT exposure. Patients alive at the last contact date or lost to follow-up were censored. Survival analyses were performed using the Kaplan–Meier method and curves were compared using the log-rank test in univariate analysis. These factors were further analyzed in a multivariate Cox regression model with time-varying covariate. The statistical analyses were performed using GraphPad Prism (version 6.07 for Windows, GraphPad Software) and R (https://www.r-project.org). All tests were two sided, unless specified, and differences were considered significant if $p < 0.05$.

Results

Clinical characteristics

The demographic, clinical, and laboratory characteristics of 108 P-MDS and 129 T-MN patients in our cohort are shown in Table 1. T-MN patients were younger than P-MDS (71.1 vs. 75.3 years, $p < 0.001$) at diagnosis of their MN.

Table 1 Demographic and clinical characteristics of P-MDS and T-MN patients subjected to mutational analysis

Variable	P-MDS ($n = 108$)	T-MN ($n = 129$)	p Value
Median age at diagnosis (range)	75.3 years (20.3–97.3)	71.1 years (20.7–89.9)	0.0012
Gender (male:female)	71:37	73:56	n.s.
WHO morphological subtype			
MDS-SLD/MDS-RS-SLD	12 (11.1%)	13 (10.1%)	n.s.
MDS-MLD/MDS-RS-MLD	38 (35.2%)	40 (31%)	n.s.
MDS-EB-1	22 (20.4%)	17 (13.2%)	n.s.
MDS-EB-2	9 (8.3%)	10 (7.8%)	n.s.
MDS with isolated del (5q)	1 (0.9%)	0	n.s.
CMML	11 (10.2%)	7 (5.4%)	n.s.
Others	11 (10.2%)	7 (5.4%)	n.s.
Hypoplastic MDS	2 (1.9%)	1 (0.8%)	
MDS with fibrosis	0	1 (0.8%)	
MDS/MPN-RS-T	5 (4.6%)	0	
aCML	0	3 (2.3%)	
MDS/MPN-U	3 (2.8%)	1 (0.8%)	
MDS-U	1 (0.9%)	1 (0.8%)	
AML (≤30% blasts)	4 (3.7%)	10 (7.8%)	n.s.
AML-NOS	3 (2.8%)	1 (0.8%)	
AML-MRC	1 (0.9%)	7 (5.4%)	
APML	0	2 (1.6%)	
AML (>30% blasts)	–	21 (16.3%)	
AML with t(9;11)		2 (1.6%)	
AML-NOS		7 (5.4%)	
AML-MRC		12 (9.3%)	
AML – unknown blast count	0	3 (2.3%)	
WHO subtype unknown	0	1 (0.8%)	
Specific cytogenetic groups[a]			
del5/5q ± other abnormalities[b]	1 (0.9%)	5 (4%)	n.s.
del7/7q ± other abnormalities[b]	7 (6.5%)	23 (18.4%)	0.0099
Ch.3 abnormalities[c]	1 (0.9%)	2 (1.6%)	n.s.
11q23 translocation[c]	0	4 (3.2%)	n.s.
Marker chromosome[d]	2 (1.9%)	26 (20.8%)	<0.0001
Monosomal karyotype[d]	5 (4.6%)	33 (26.4%)	<0.0001
Any chromosomal translocation[d]	11 (10.2%)	49 (39.2%)	<0.0001
Complex karyotype (≥3 abnormalities)	9 (8.3%)	36 (28.8%)	<0.0001
IPSS-R score			<0.0001
Very low	13 (12%)	8 (6.2%)	
Low	39 (36.1%)	14 (10.9%)	
Intermediate	25 (23.1%)	23 (17.8%)	
High	13 (12%)	24 (18.6%)	
Very high	9 (8.3%)	27 (20.9%)	
Not applicable	9 (8.3%)	27 (20.9%)	
AML >30% blasts	–	21 (16.3%)	
APML	0	2 (1.6%)	
MDS/MPN overlap	9 (8.3%)	4 (3.2%)	
Unknown	0	6 (4.7%)	
Treatment[e]			0.0018
Supportive care	85 (78.7%)	79 (61.2%)	
Azacitidine/others including clinical trials	14 (13%)	25 (19.4%)	
Induction chemotherapy	8 (7.4%)	23 (17.8%)	
Allo-HSCT	2 (1.9%)	13 (10.1%)	
Median overall survival (range)	31.4 months (26.8–43.4)	10.6 months (9.1–13.6)	<0.0001

P-MDS primary MDS, *T-MN* therapy-related myeloid neoplasm, *RS* ring sideroblasts, *MDS-SLD* MDS with single lineage dysplasia, *MDS-RS-SLD* MDS with ring sideroblasts with single lineage dysplasia, *MDS-MLD* MDS with multilineage dysplasia, *MDS-RS-MLD* MDS with ring sideroblasts with multilineage dysplasia, *MDS-EB-1* MDS with excess blasts-1, *MDS-EB-2* MDS with excess blasts-2, *CMML* chronic myelomonocytic leukemia, *MDS/MPN-RS-T* myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, *aCML* atypical chronic myeloid leukemia, *MDS/MPN-U* myelodysplastic/myeloproliferative neoplasm, unclassifiable, *MDS-U* MDS, unclassifiable, *AML* acute myeloid leukemia, *AML-NOS* AML, not otherwise specified, *AML-MRC* AML with myelodysplasia related changes, *APML* acute promyelocytic leukemia, *IPSS-R* Revised International Prognostic Scoring System, *Allo-HSCT* allogeneic hematopoietic stem cell transplant, *n.s.* not significant

[a] $n = 125$ for T-MN group, as cytogenetics were unknown in four patients

[b] Includes the presence of additional chromosomal abnormalities but not amounting to complex karyotype

[c] Chr3 abnormalities and 11q23 translocation, occurring either alone or in combination with other chromosomal abnormalities excluding del5/5q, del7/7q, or complex karyotype

[d] With or without the presence of other cytogenetic abnormalities

[e] Patients who received more than one type of therapy

The most common primary disease in T-MN patients requiring cytotoxic therapy was lymphoproliferative disease (36.4%), followed by breast (14.7%) and prostate (11.6%) cancers (Table S2). Approximately 10% T-MN received cytotoxic therapy for autoimmune diseases. Patients received CT (45.7%), RT (21.7%), or both (combined modality treatment [CMT], 32.6%) to treat their primary disease. Notably, 36.8% of lymphoproliferative

disease and myeloma patients developing T-MN had autologous HSCT.

Most T-MN patients presented in MDS phase (74%) and fewer with AML (≥20% blasts, 26%). Two patients had therapy-related acute promyelocytic leukemia at diagnosis. Therapy-related chronic myelomonocytic leukemia (T-CMML) and T-MDS/MPN were infrequently seen in our cohort (7 and 4 cases, respectively). The morphological MDS subtypes were not different between P-MDS and T-MN groups (Table 1).

Comparing T-MN patients with or without leukemic presentation (T-AML vs. T-MDS), T-AML showed a higher frequency of 11q23 translocations ($p = 0.004$). The OS of T-AML was poorer than that of T-MDS (3.5 vs. 13.2 months, $p < 0.0001$), despite a higher proportion of T-AML patients being treated with induction CT (41.2 vs. 9.5%) and allo-HSCT (17.7 vs. 7.4%; Table S3).

Poor risk cytogenetic abnormalities and high IPSS-R scores are common in T-MN

A higher proportion of T-MN patients had IPSS-R Poor and Very poor risk cytogenetic abnormalities. Conversely, Very low and Low risk cytogenetics were more common in P-MDS ($p < 0.0001$; Table 1). Consequently, the IPSS-R distribution was skewed towards High and Very high categories in T-MN and Very low and Low in P-MDS groups (Table 1).

Cytogenetic abnormalities, including del7/7q (18.4 vs. 6.5%, $p = 0.009$), complex karyotype (≥3 abnormalities, 28.8 vs. 8.3%; $p < 0.0001$), chromosomal translocations (39.2 vs. 10.2%; $p < 0.0001$), monosomal karyotype (26.4 vs. 4.6%; $p < 0.0001$), and marker chromosomes (20.8 vs. 1.9%; $p < 0.0001$), were more common in T-MN compared to P-MDS (Table 1). The 11q23 translocations (*MLL* or *KMT2A* fusions) were only seen in T-MN patients and were not associated with topoisomerase exposure.

T-MN and P-MDS have a similar overall mutation frequency, but the mutation pattern is distinct

The mutational spectrum of T-MN is depicted in Fig. 1a, b and Tables S4 and S5. In P-MDS, 323 nonsynonymous somatic mutations were detected in 100/108 (92.6%) patients in 35 of the 43 genes tested (Fig. S2 and Tables S4 and S5). Similarly, 347 nonsynonymous mutations were detected in 34 genes in 120/129 (93.0%) T-MN patients. Mutations in >1 gene were detected in 73 and 66% of P-MDS and T-MN, respectively (Fig. 1 and Fig. S2). The mutation burden was similar between P-MDS and T-MN (median, 3 mutations). Of the 43 genes analyzed, no mutations were seen in *MYD88, PDGFRA*, and *SRP72* in either P-MDS or T-MN (Table S4).

Although the overall mutation frequency was similar, the mutation pattern was different between P-MDS and T-MN. *TP53* mutations were more frequent in T-MN than in P-MDS (29.5 vs. 7.0%; $p < 0.0001$) (Fig. 1 and Table S4). A total of 56 *TP53* mutations (41 missense, 7 nonsense, 5 splice-site, and 3 frameshift) were detected in 38 T-MN patients (Fig. 1c). Multiple *TP53* mutations were detected in a higher proportion of T-MN (10.8%) than P-MDS (3.0%; $p = 0.03$). Two *TP53* mutations were detected in 11 T-MN patients, while 3 and 4 *TP53* mutations were detected in 2 and 1 T-MN patients, respectively. Considering the highest VAF in patients with multiple mutations, the median VAF of *TP53* mutations was high (39% in 38 patients).

The presence of *TP53* mutation was strongly associated with complex karyotype ($p < 0.0001$; Fig. S3A). Additionally, patients with *TP53* VAF ≥20% (25/30; 83%) had a higher frequency of complex karyotype compared to patients with VAF <20% (3/7, 43%; $p = 0.04$). The median number of mutations in genes other than *TP53* in patients with mutated *TP53* (*TP53*^{mut}) was much lower compared to that in patients with wild-type *TP53* (*TP53*^{wt}; 0 vs. 3 mutations/sample, $p < 0.0001$; Fig. 1b). The BM blast count was <5% in half of the patients at the time of T-MN diagnosis. Conspicuously, no *TP53*^{mut} patient had BM morphology consistent with T-CMML. The frequency of primary hematological malignancy (55.3 vs. 40.6%) or CT exposure (81.6 vs. 76.9%) was not different between T-MN with *TP53*^{mut} and *TP53*^{wt}.

We also assessed the impact of *TP53* VAF on the OS of T-MN patients. There was no difference in this parameter when T-MN were segregated by a *TP53* VAF median value of 39%. However, patients with a VAF of <20% showed longer OS than patients with ≥20% (10.4 vs. 7.7 months, $p = 0.03$; Fig. S3B). There was no difference in the proportion of patients with High or Very high IPSS-R scores or AML between these two groups.

Spliceosome mutations were more frequent in P-MDS compared to T-MN (56.5 vs. 25.6%; $p < 0.0001$; Table S4 and Fig. 1a). Within the spliceosomal complex, *SF3B1* (25.9 vs. 6.2%; $p < 0.0001$) and *U2AF1* mutations (7.4 vs. 1.6%; $p = 0.04$) were detected at higher frequency in P-MDS compared to T-MN, while there was no difference in the frequency of *SRSF2* or *ZRSR2* mutations. As expected, most of the spliceosome mutations were mutually exclusive, except in one patient (PID 1355) harboring two *SF3B1* variants (K666M; VAF 27.5% and K666N; VAF 10.4%) with each likely being in separate sub-clones.

In the other major myeloid genes, *TET2*, *ASXL1*, *DNMT3A*, *RUNX1*, and *EZH2*, the mutation frequency was similar between P-MDS and T-MN (Fig. 1a). *SETBP1* mutations were seen in 6.2% of T-MN patients, of which 6 (75%) had del7/7q abnormalities. The primary disease type and its treatment did not influence the mutation pattern in T-MN.

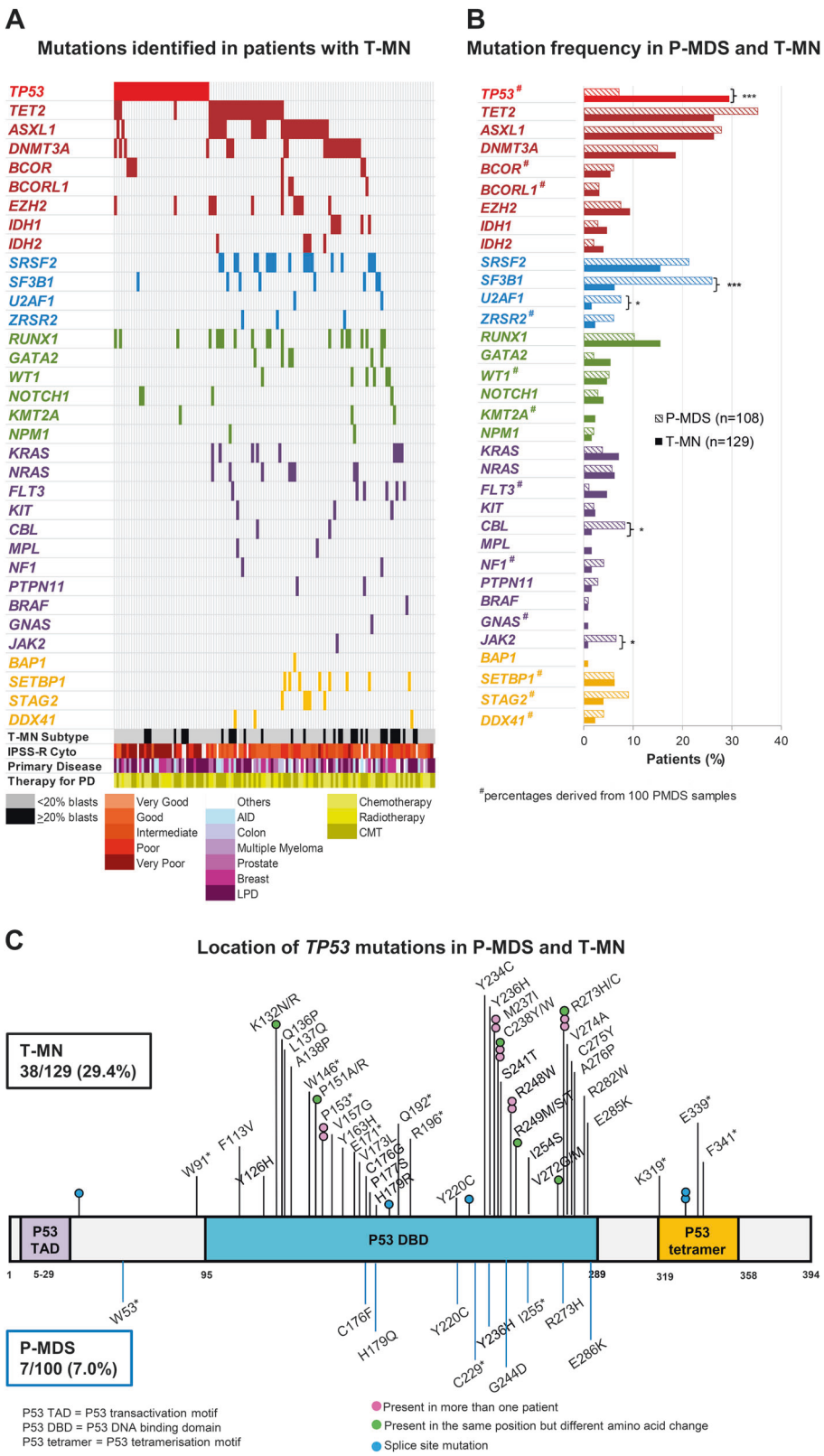

Fig. 1 Frequency and distribution of mutations in therapy-related myeloid neoplasms (T-MN) and primary MDS (P-MDS) cases: **a** Distribution of mutations in 129 T-MN patients. **b** Frequency of mutations in T-MN (solid fill) and P-MDS (hashed fill). *TP53* is the most commonly mutated gene in T-MN, while *TET2* followed by *SF3B1* are the most commonly mutated genes in P-MDS. In *TP53*-mutated cases, mutations in other genes are less frequent compared to non-*TP53*-mutated cases ($p < 0.0001$). The antecedent condition leading to T-MN and the therapy received are listed on the bottom rows. **c** Distribution of mutations in *TP53* according to key functional domains in P-MDS and T-MN. PD – primary disease; Others – other cancers; AID – autoimmune disease; Ca Colon – colon cancer; MM – multiple myeloma; Ca Prostate – prostate cancer; Ca Breast – breast cancer; LPD – lymphoproliferative disorders; CT – chemotherapy; RT – radiotherapy; CMT – combined modality treatment (CT and RT)

Additionally, the frequency and median number of mutations were not different between T-MDS and T-AML. *SETBP1*, *SF3B1*, and *ZRSR2* mutations were exclusively seen in T-MDS, while the mutation pattern in other genes was not different between the two groups (Table S6).

The frequency of RS is similar in T-MN and P-MDS, but the genetic association is distinct

RS were seen upon Perl's stain in P-MDS (29.6%) and T-MN (24.8%). In 21.3% P-MDS and 14.7% T-MN, RS were present in ≥15% of erythroid cells. Spliceosomal complex mutations were seen in all P-MDS, but only in 37% T-MN with ≥15% RS ($p < 0.0001$), with *SF3B1* mutations being the most frequent (96 vs. 32%; $p < 0.0001$; Fig. 2a and Table S7). Interestingly, *TP53* mutations were detected in 92% T-MN with ≥15% RS and wild-type *SF3B1* (*SF3B1*^{wt}), compared to 29.5% in the entire T-MN cohort. Notably, the only P-MDS sample with ≥15% RS and *SF3B1*^{wt} lacked *TP53* mutation. A similar correlation was seen for *SF3B1* mutation frequency with any number of RS (Table S7, footnote and Fig. S4A).

Survival of P-MDS with ≥15% RS was better than those with <15% RS (37.5 vs. 27.7 months, $p = 0.008$), most probably due to the strong association of RS with favorable *SF3B1* mutations in P-MDS. The OS of P-MDS patients with *SF3B1* mutations was better than that of wild-type patients (38.2 vs. 26.8 months, $p = 0.004$). However, the OS in T-MN with ≥15% RS was not different from that of those patients with <15% RS (12 vs. 11 months; $p = 0.33$). In addition, the OS of T-MN with ≥15% RS was worse than that of P-MDS with ≥15% RS (12 vs. 38 months, $p < 0.0001$; Fig. 2b), likely due to higher prevalence of *TP53* mutations (Fig. 2c) in these T-MN cases. Similarly, when RS ≥1% were considered, the OS of T-MN was worse than that of P-MDS (Fig. S4B).

Overall, although the frequency of ≥15% RS was similar in P-MDS and T-MN, it was not associated with better survival in T-MN, most likely due to poor correlation with *SF3B1* mutation and stronger association with *TP53* mutations in *SF3B1*^{wt} cases.

T-MN and P-MDS with Very low or Low IPSS-R score share clinical and biological characteristics and have similar outcomes

T-MN patients with Very low IPSS-R score had a median survival of 106 months, which worsened with an increase in the score, with median survival in Very high IPSS-R group being only 7.8 months ($p < 0.0001$). The survival of T-MN cohort was significantly poor as compared to P-MDS cohort (10.6 vs. 31.4 months, $p < 0.0001$; Fig. 3a) and the difference between the two groups was maintained when the analysis was restricted to patients with ≤30% blasts (12.8 vs. 32.4 months; $p < 0.0001$; Fig. S5). T-MN with Intermediate IPSS-R score had poorer OS compared to Intermediate score P-MDS (14 vs. 35 months, $p = 0.01$; Table S8), while it was equally poor in T-MN and P-MDS with High or Very high IPSS-R scores (8.8 vs. 13 months, $p = 0.09$).

Interestingly, survival of T-MN with Very low or Low risk was not different from P-MDS (64 vs. 45 months, $p = 0.9$; Fig. 3b). Hence, we compared the clinical and genetic

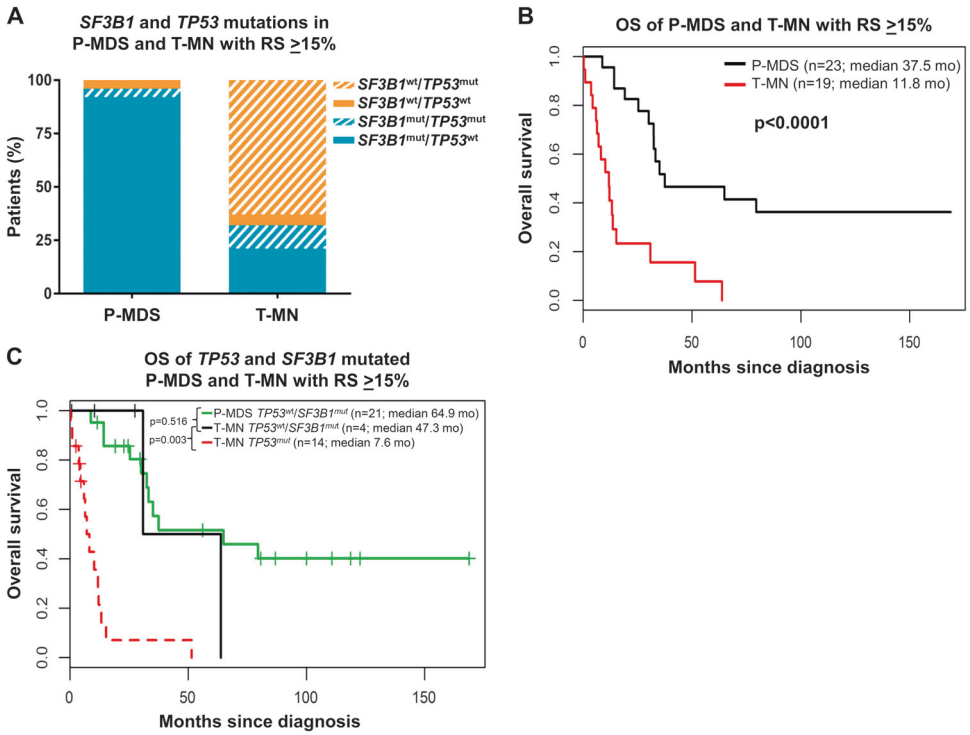

Fig. 2 Comparison of the genetic association of ring sideroblasts (RS) and survival in therapy-related myeloid neoplasms (T-MN) and primary MDS (P-MDS): **a** Distribution of *SF3B1* and *TP53* mutations in patients with ≥15% or more RS on bone marrow morphology. *SF3B1* mutation is most common in P-MDS, while *TP53* is most common in T-MN. **b** The overall survival of T-MN patients with ≥15% RS is poorer than P-MDS patients. **c** Poor OS in T-MN with ≥15% RS is most probably due to association with *TP53* mutation in T-MN cases

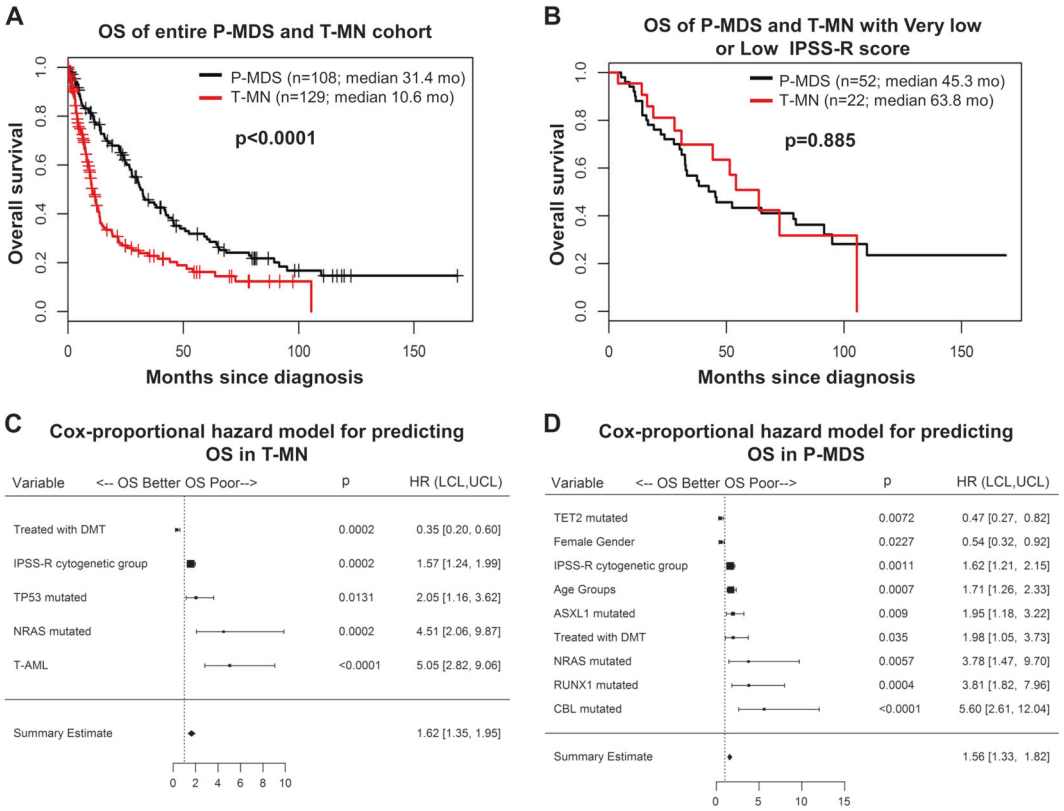

Fig. 3 Survival in Revised International Prognostic Scoring System (IPSS-R) Very low and Low risk therapy-related myeloid neoplasms (T-MN) is not different from the primary MDS (P-MDS) counterpart: **a** The overall survival (OS) of T-MN patients is poorer than P-MDS. **b** In contrast, the OS of patients with Low or Very low IPSS-R is similar in T-MN and P-MDS. **c** Cox proportional hazard model for OS in T-MN. **d** Cox proportional hazard model for OS in P-MDS. UCL – upper confidence limit; LCL – lower confidence limit

characteristics of P-MDS ($n = 52$) and T-MN ($n = 22$) patients with Very low or Low IPSS-R score (Table 2). There was no difference between their demographics and MDS subtypes. The majority of patients had normal karyotype (79 vs. 82%), and no poor-risk chromosomal abnormalities were seen in the remainder. The median number of mutations was not different (2 vs. 3 mutations). Importantly, the mutation profile of both groups was also remarkably similar. We did not find any difference in the frequency of mutations in either individual genes or pathways (Table 2).

Predictors of survival in T-MN

The OS (adjusted for DMT) was poor in T-MN patients compared to P-MDS patients (10.6 vs. 31.4 months, $p < 0.0001$), despite a higher proportion of T-MN patients receiving intensive CT (17.8 vs. 7.4%) and allo-HSCT (10.1 vs. 1.9%). The OS was compared between P-MDS and T-MN patients with respect to the mutational status of genes that were mutated in five or more patients. In univariate analysis, *TET2* and *SF3B1* mutations were associated with better OS, while *ASXL1*, *CBL*, *U2AF1*, *TP53*, and *NRAS* mutations were associated with poorer outcome in P-MDS. In T-MN, *TP53*, *NRAS*, *WT1*, and *BCOR* mutations were associated with a poorer OS, while *ASXL1* and *IDH2* mutations were favorable. There was no relationship between OS and the number of mutations.

In a multivariate Cox regression analysis (Fig. 3c), the presence of ≥20% BM blasts at diagnosis (hazard ratio (HR) 5.05, 95% confidence interval (CI) 2.82–9.06, $p < 0.0001$), IPSS-R cytogenetic category (HR 1.57, 95% CI 1.24–1.99, $p = 0.0002$), DMT (HR 0.35, 95% CI 0.20–0.60, $p = 0.0002$), and *NRAS* (HR 4.51, 95% CI 2.06–9.87, $p = 0.0002$) and *TP53* (HR 2.05, 95% CI 1.16–3.62, $p = 0.0131$) mutations were independent predictors of OS in the T-MN cohort. In P-MDS, in addition to IPSS-R cytogenetic and DMT categories, age, male gender, and mutations in *ASXL1*, *NRAS*, *RUNX1*, and *CBL* were independent factors associated with poor survival. Conversely, *TET2* mutations were associated with improved OS in P-MDS (Fig. 3d).

Discussion

T-MN represents an aggressive malignancy with extremely poor long-term outlook compared to de novo MDS/AML. Here, we present the clinical, laboratory, and genetic profiles of 129 well-characterized T-MN patients, compared with 108 P-MDS patients. Our results show that mutation

Table 2 Comparison of clinical and genetic characteristics of primary MDS and T-MN patients with IPSS-R Very low and Low score

Characteristic	P-MDS ($n = 52$)	T-MN ($n = 22$)	p Value
Median age at diagnosis (range)	76.2 (48.6–89.3)	74.6 (57.1–85.4)	0.60
Gender (male:female)	34:18	13:9	0.60
WHO morphological subtype			
MDS-SLD/MDS-RS-SLD	11 (21.2%)	8 (36.4%)	
MDS-MLD/MDS-RS-MLD	27 (51.9%)	10 (45.5%)	
MDS-EB-1	3 (5.7%)	1 (4.5%)	
MDS with isolated del(5q)	1 (1.9%)	0	
CMML-0	2 (3.8%)	3 (13.6%)	
MDS/MPN-RS-T	4 (7.7%)	0	
Others (hypoplastic MDS, MDS/MPN-U, MDS-U)	4 (7.7%)	0	
Patients with ring sideroblasts ≥15%	19 (38.8%)	4 (19.1%)	0.17
Bone marrow karyotype at diagnosis (%)			
Normal	41 (78.8%)	18 (81.8%)	0.78
delY	4 (7.7%)	1 (4.5%)	
del5q	1 (1.9%)	0	
del20q	1 (1.9%)	2 (9.1%)	
Other abnormalities	5 (9.6%)	1 (4.5%)	
Median number of mutations (range)	2 (0–9)	3(0–7)	0.67
Patients with mutations in (pathway, %)			
p53	3 (6.0%)	1 (4.6%)	1.00
Epigenetic	36 (70.6%)	17 (77.3%)	0.78
Splicing	35 (68.6%)	10 (45.5%)	0.07
Transcription	10 (20.0%)	4 (18.2%)	1.00
Signaling	9 (18.0%)	5 (22.7%)	0.75
Transformation to AML	7 (13.5)	5 (22.7)	0.32
Median time to AML transformation (months)	37.4 (11.9–88)	16.8 (4.4–70)	0.61
Treatment with disease-modifying therapy	3 (5.8)	3 (13.6)	0.35

P-MDS Primary MDS, *T-MN* therapy-related myeloid neoplasm, *IPSS-R* Revised International Prognostic Scoring System, *MDS-SLD* MDS with single lineage dysplasia, *MDS-RS-SLD* MDS with ring sideroblasts with single lineage dysplasia, *MDS-MLD* MDS with multilineage dysplasia, *MDS-RS-MLD* MDS with ring sideroblasts with multilineage dysplasia, *MDS-EB-1* MDS with excess blasts-1, *CMML* chronic myelomonocytic leukemia, *MDS/MPN-RS-T* myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, *MDS/MPN-U* myelodysplastic/myeloproliferative neoplasm, unclassifiable, *MDS-U* MDS, unclassifiable

burden in T-MN is as high as P-MDS but the pattern is different. Further, the strong genetic association of *SF3B1* mutation with RS is absent in T-MN. Also, the survival outcome between T-MN and P-MDS with Low or Very low IPSS-R score was not significantly different.

Unlike in P-MDS and AML, the cytogenetic categories are still not well defined in T-MN. Previous studies on T-MN (T-MDS and T-AML) have used various classification systems, including European LeukaemiaNet (ELN) [11], UK Medical Research Council (UK-MRC) [12], IPSS [13], and others [14]. Interestingly, the IPSS-R is one of the best predictive tools in T-MDS [15]; hence, we used the IPSS-R to stratify cytogenetics in our patients with ≤30% blasts. Of 21 T-AML patients with >30% blasts, 17 did not have complex, del7 or other chromosomal abnormalities, and would be categorized similarly by the IPSS-R, ELN, or UK-MRC classifications. The only difference would be for four patients with 11q23 abnormality, which is considered of intermediate risk in IPSS-R, but adverse in ELN or UK-MRC. Consistent with previous studies, our T-MN cohort showed earlier age of onset, higher frequency of poor-risk cytogenetic abnormalities, higher IPSS-R scores, and poorer OS in comparison to P-MDS [3, 8, 14, 16].

Frequency and pattern of mutations in our P-MDS cohort were similar to published literature [4–6]. We detected mutations in 93% of P-MDS patients, most commonly in *TET2*, *ASXL1*, *SF3B1*, *SRSF2*, and *DNMT3A*. Spliceosomal complex mutations were mutually exclusive, with *SF3B1*-mutated status being associated with RS and good prognosis. Notably, our T-MN cohort showed a high mutation frequency (93% of all T-MN) similar to P-MDS. Hence, on a gene level, T-MN is as heterogeneous as P-MDS [5, 6] or AML [17, 18], and this heterogeneity is not offset by an increased frequency of karyotypic abnormalities, a key feature of T-MN.

The frequency of mutations in our T-MN cohort was substantially different from previously published studies [8, 9, 19–22] for a number of reasons. Firstly, analyses employing low-sensitivity sequencing methods (e.g., Sanger [19, 20, 22]) reported generally lower mutation frequency than next-generation sequencing (NGS)-based studies. Secondly, most reports have focused only on mutational hotspots for some genes [8, 19, 23] such that mutations elsewhere in the gene have been overlooked. For example, in a large T-MN cohort ($n = 77$) that screened only for the *DNMT3A* hotspot by Sanger sequencing [19], 5.2% of cases were mutated, which was much lower than the 26% reported in an separate T-AML cohort sequenced on an NGS platform [23]. In closer agreement with the latter study, we found *DNMT3A* mutations in 27% T-AML and 16% T-MDS. Additionally, genes of considerable importance in the pathogenesis of MNs have been excluded in most studies. For example, spliceosomal complex genes, *ASXL1*, *SETBP1*, *WT1*, and *TET2*, were not included in panels selected by Ok et al. [8] or the "Copenhagen Series" [20]. Similarly, important genes such as *SF3B1*, *SRSF2*, *JAK2*, *DDX41*, *MPL*, *GNAS*, *PTEN*, and *SETBP1* were not included in another study on T-AML [9]. Lastly, studies performing deep sequencing on the most myeloid-relevant

genes are exclusively on T-AML [9, 23]. These factors have led to inconsistencies in reporting and understanding the mutational profile of the spectrum of T-MN. Notably, in comparisons between different T-AML cohorts, the mutation frequency ranged from 33 to 97% of patients [8, 9, 21, 23]. Our cohort is a spectrum of T-MDS and T-AML sequenced using a panel of genes covering most of the important genes/pathways. Importantly, our results are in line with comparable P-MDS and AML studies, which also show a much higher and comparable mutation frequency, with 80–90% or more showing at least one mutation [5, 17, 18].

TP53 was the most commonly mutated gene in T-MN, followed by the spliceosomal complex. Other groups have also reported a similar high prevalence of *TP53* mutations in T-MN patients [8]. One study on a smaller pediatric T-MDS cohort found that *TP53* mutations mostly occur as an isolated genetic event, similarly to our findings [24]. The observed high frequency of *TP53* mutations in T-MN could be due to preferential selection and expansion of rare hematopoietic progenitor cells (HSPC) carrying *TP53* mutations following CT exposure, possibly due to their innate chemo-resistance nature. *TP53* mutations are known to be associated with complex cytogenetics. This was recapitulated in our T-MN cohort, with the association being stronger than that seen in P-MDS. Despite its strong association with complex cytogenetics, the *TP53* mutational status was independently associated with poor OS in a multivariate model. The OS of $TP53^{mut}$ T-MN further decreased as the mutated *TP53* allele load increased above 20%, as reported in P-MDS [25].

A detailed spectrum of spliceosomal complex mutations has not been previously reported in T-MN. These mutations occurred in up to a quarter of T-MN patients and were mutually exclusive. In contrast to P-MDS, however, we found that *SRSF2* mutations were the dominant spliceosomal mutation in T-MN (60%) instead of *SF3B1*. Both *SF3B1* and *SRSF2* are heterozygous mutations limited to specific codons resembling oncogenes and seen in normal individuals (clonal hematopoiesis) [26, 27]. It has been recently shown in a pre-clinical mouse model that HSPC carrying *SRSF2* mutations are able to survive CT-induced pro-mutagenic genomic insults and tolerate DNA damage accumulation [28]. It is possible that some age-related mutations (e.g., *SF3B1*) are not as resistant to such insults and are wiped out as hematopoiesis continues under the stress of mutagen exposure. This may explain the higher frequency of *SRSF2* in T-MN.

Interestingly, we find that the frequency of patients with RS is similar between P-MDS and T-MN. As published previously [29], 96% of P-MDS with ≥15% RS harbored *SF3B1* mutations, but only 32% of T-MN with RS had *SF3B1* mutations. Furthermore, >90% of $SF3B1^{wt}$ T-MN cases with RS had *TP53* mutation. This finding has not been previously reported in T-MN, although such an observation has been seen in P-MDS with RS, where 10% of $SF3B1^{wt}$ P-MDS cases harbored *TP53* mutations [29]. The exact biological mechanism behind this is not clear. However, *TP53* is known to play an important role in mitochondrial homeostasis via multiple mechanisms, which ultimately impact respiratory and oxidative phosphorylative functions [30]. It is possible that *TP53* mutations occurring in undifferentiated $CD34^{+}$ HSPC may downregulate genes involved in the mitochondrial ribosome and in the electron transport chain, leading to later appearance of RS [31]. Further studies are warranted to examine this phenomenon.

In agreement with recent findings [15], the OS, clinical and genetic characteristics of IPSS-R Very low and Low risk T-MN and P-MDS were not significantly different. However, study was underpowered for subgroup analysis and validation in a larger independent cohort is required.

Despite almost seven decades since T-MN were first described [32, 33], prognostic factors still remain to be firmly ascertained. Multiple studies show that karyotype and previous CT/RT exposure (i.e., being therapy related) are important prognostic factors [11, 12, 34–36]. With the BM blasts, the prognostic value was found to be more impactful at a cut-off level of 20% [12, 37]. Overall, the discriminatory power of traditional MDS prognostic classifications like IPSS, IPSS-R, WPSS (WHO classification-based Prognostic Scoring System), and others does not seem to be as robust in T-MN as for P-MDS [3, 15], and requires modification from the original schema [3]. This suggests that factors beyond the conventional parameters are operative in T-MN prognosis. In multivariable analysis, we found that patients with T-AML had the highest risk of death, followed by patients carrying *TP53* and *NRAS* mutations. Our findings are supported by studies showing poor prognosis of *TP53* and the RAS mutations in MDS patients treated with DMT or/and allo-HSCT [24, 38–41]. Interestingly, *TP53* VAF also predicted poor survival; T-MN patients with high *TP53* VAF (>20%) had poorer survival. This is in agreement with findings in P-MDS, albeit at a different clone size cut-off [25]. In our cohort, treatment with DMT was associated with improved OS, but the study is underpowered to draw a definite conclusion.

In conclusion, we show similarities and differences in mutational signatures and clinical outcomes between T-MN and P-MDS. The mutation frequency is similar in T-MN and P-MDS, but the mutation pattern is different. The T-MN mutational landscape is dominated by *TP53* and *SRSF2*, probably due to selection and expansion of HSPC harboring these mutations under genotoxic stress. The frequency of RS is similar in P-MDS and T-MN, but is not

associated with better survival in T-MN, most likely due to fewer *SF3B1* mutations and the presence of *TP53* mutations in *SF3B1* wild-type cases. Our results suggest that, in addition to conventional disease parameters, mutational analysis provides added prognostic markers and should be incorporated in T-MN risk stratification.

Data deposition

The sequence data have been deposited at the European Genome-Phenome Archive, which is hosted by the European Bioinformatics Institute, under accession #EGAS00001003547.

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Author contributions DS: planned and performed mutational analysis, analyzed data, and wrote the manuscript. LYAW: processed samples, analyzed data, and edited the manuscript. MMK and RC: analyzed data and edited the manuscript. JG, AWS, JF, and PP-SW: analyzed bioinformatics data and provided critical comments. MB, WTP, and SH: processed samples and analyzed data. SE: provided statistical advice. SM and PA: analyzed data and provided critical comments. SB and ALB: provided critical comments and edited the manuscript. NS and RG: provided clinical information and critical comments. TK, LBT, PGB, IDL, RJD: provided critical comments. JPM: provided critical comments and edited the manuscript. HSS: developed the project, analyzed data, and edited the manuscript. CNH: developed the project, analyzed data, and edited the manuscript. DKH: developed the project, analyzed data, and edited the manuscript.

Compliance with ethical standards

Conflict of interest SB received research funding and honoraria and served on advisory committees from Novartis and Bristol-Myers Squibb, and consultancy fees and honoraria from Qiagen and Cepheid. HSS received honoraria from Celgene. The other authors declare that they have no conflict of interest.

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