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Mutation status and burden can improve prognostic prediction of patients with lower-risk myelodysplastic syndromes

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Abstract

Patients with lower-risk myelodysplastic syndromes (LR-MDS) as defined by the International Prognostic Scoring System (IPSS) have more favorable prognosis in general, but significant inter-individual heterogeneity exists. In this study, we examined the molecular profile of 15 MDS-relevant genes in 159 patients with LR-MDS using next-generation sequencing. In univariate COX regression, shorter overall survival (OS) was associated with mutation status of *ASXL1* (P = .001), *RUNX1* (P = .031), *EZH2* (P = .049), *TP53* (P = .016), *SRSF2* (P = .046), *JAK2* (P = .040), and *IDH2* (P = .035). We also found significantly shorter OS in patients with an adjusted *TET2* variant allele frequency (VAF) ≥18% versus those with either an adjusted *TET2* VAF <18% or without *TET2* mutations (median: 20.4 vs 47.8 months; P = .020; HR = 2.183, 95%CI: 1.129-4.224). After adjustment for IPSS, shorter OS was associated with mutation status of *ASXL1* (P < .001; HR = 4.306, 95% CI: 2.144-8.650), *TP53* (P = .004; HR = 4.863, 95% CI: 1.662-14.230) and *JAK2* (P = .002; HR = 5.466, 95%CI: 1.848-16.169), as well as adjusted *TET2* VAF ≥18% (P = .008; HR = 2.492, 95% CI: 1.273-4.876). Also, OS was increasingly shorter as the number of mutational factors increased (P < .001). A novel prognostic scoring system incorporating the presence/absence of the four independent mutational factors into the IPSS further stratified LR-MDS patients into three prognostically different groups (P < .001). The newly developed scoring system redefined 10.1% (16/159) of patients as a higher-risk group, who could not be predicted by the currently prognostic models. In conclusion, integration of the IPSS with mutation status/burden of certain MDS-relevant genes may improve the prognostication of patients with LR-MDS and could help identify those with worse-than-expected prognosis for more aggressive treatment.

KEYWORDS

lower-risk myelodysplastic syndromes, mutation burden, mutation status, prognosis, variant allele frequency

Jiang and Luo contributed equally to this work.

1 | INTRODUCTION

Myelodysplastic syndromes (MDS) are a group of clonal hematological malignancies with significant clinical heterogeneity and genetic diversity.[1,2] The life expectancy of MDS patients ranges from several months to several years; thus, accurate survival prediction is critical for treatment decision-making.[3] The International Prognostic Scoring System (IPSS) is the most commonly used tool for prognostic assessment of patients with untreated primary MDS in clinical practice, trial eligibility and treatment recommendation.[4,5] Patients with low risk (IPSS score: 0) and intermediate 1 (Int-1) risk (IPSS score: 0.5-1.0) are grouped together as the lower-risk (LR) group, and generally tend to have an indolent course of disease progression. However, a significant subset of LR-MDS patients have a worse outcome and a much higher frequency of progression into secondary acute myeloid leukemia (sAML).[6-8]

Attempts have been made to identify patients with greater-than-expected risk in LR-MDS patients. The MD Anderson Lower-Risk Prognostic Scoring System (LR-PSS) could stratify LR-MDS into three prognostically distinct groups.[9] The recently revised IPSS (IPSS-R) reassigns MDS patients into a lower- versus a higher-risk group (IPSS-R score of ≤3.5 vs >3.5).[10] Despite these refinements, a significant proportion of patients with poor outcomes could not be identified.[8]

Recurrent mutations in MDS are associated with overall survival (OS), leukemic transformation, and response to hypomethylating agents (HMA).[11-15] In a pivotal study of LR-MDS, *ASXL1*, *EZH2*, *TP53*, and *RUNX1* mutations were shown to be independent prognostic predictors.[16] Also, combination of *EZH2* mutation status with LR-PSS could predict a subset of LR-MDS patients with poor prognosis not identified otherwise.[16]

Mutation burden has also been associated with prognosis in MDS patients.[3,5] In general, increasing number of mutations is associated with progressively poorer prognosis.[13,17-19] For mutations of a specific gene, higher *TP53* mutation allelic burden was negatively associated with OS in both unselected MDS and LR-MDS;[20-22] higher *TET2* clonal burden predicted an increased response to decitabine in MDS.[15] In addition to MDS, variant allele frequency (VAF) has been associated with prognosis in patients with AML.[23,24]

In the current study, we examined the mutation status and burden of 15 MDS-relevant genes in a group of patients with LR-MDS by next-generation sequencing (NGS). The results showed association between poor prognosis with *ASXL1* mutant type (MT), *TP53* MT, *JAK2* MT, and adjusted *TET2* VAF ≥18%. A scoring system that combines IPSS and mutation status/burden was further developed to identify LR-MDS patients with greater-than-predicted risk.

2 | SUBJECTS AND METHODS

2.1 | Patients

This study included all patients with LR-MDS (IPSS score ≤1.0) upon initial diagnosis who were treated at the First Affiliated Hospital, Zhejiang University School of Medicine during a period between February 2011 and January 2018. The diagnosis of MDS was based on the 2016 World Health Organization (WHO) classification.[25] Patients with IPSS score >1.0 were excluded from data analysis. Metaphase cytogenetic and mutational analysis was conducted prior to treatment in all subjects. Single nucleotide polymorphism array (SNP-A) analysis was further carried out on patients with *TET2* mutations confirmed by NGS. This study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. The study was conducted in compliance with the Helsinki Declaration. All patients provided informed consent for the use of samples for research purposes.

2.2 | Metaphase cytogenetic analysis

Unstimulated bone marrow cells were obtained upon initial diagnosis (prior to treatment). Cytogenetic slides were prepared using a standard protocol, and then R-banded. Twenty metaphases were analyzed and the karyotypes were described according to the current International System for Human Cytogenetic Nomenclature.[26]

2.3 | Mutational analysis

Genomic DNA was extracted from bone marrow mononuclear cells. Sample integrity was verified using standard NGS criteria (≥50 ng/μL, and OD 260/280:1.8-2.0). We used a custom targeted NGS approach that combined multiplex PCR-based target enrichment and library generation with ultra-deep high-throughput parallel sequencing using the Ion Proton Platform or MiSeq.[27,28] A total of 15-MDS-relevant genes, including *TET2*, *SF3B1*, *U2AF1*, *ASXL1*, *SRSF2*, *DNMT3A*, *RUNX1*, *EZH2*, *JAK2*, *NRAS*, *TP53*, *CBL*, *ETV6*, *IDH1*, and *IDH2*, were covered, and exons with coding regions known to be hotspots or related with MDS (average depth >800 X) were targeted. The raw sequence data are available on the Sequence Read Archive (SRA) (PRJNA550098).

2.4 | SNP-A-based karyotyping

DNA was extracted from bone marrow. Sample quantity and purity were assessed by spectrophotometry (Nanodrop 2000, Thermo Fisher Scientific). CytoScan 750K arrays and reagents (Thermo Fisher Scientific) were used for SNP-A testing. The array analysis and interpretation were carried out using Chromosome Analysis Suite (ChAS; Thermo Fisher Scientific) software version 4.0. Copy number variations (CNV) called by the ChAS software algorithm are denoted as true aberrations, with the exception of those known to be normal genomic variants based on a publicly available database (http://dgv.tcag.ca/dgv/app/home). We used SNP-A to screen for microdeletions and uniparental disomy (UPD) at chromosome 4q/24 involving *TET2* locus.

2.5 | Mutation VAF

Variant allele frequency (referred to as raw VAF) is defined as the number of variant reads divided by the number of total reads and reported as a percentage. Variants with a VAF of <1% were excluded from analysis. Mutations were annotated using multiple databases, including 1000 genomes, COSMIC, PolyPhen-2, and dbSNP.

Adjusted VAF was acquired with the adjustment of raw VAF based on copy number and zygosity confirmed by SNP-A. VAF of homozygous mutation was reduced to as half the value of raw VAF. Hemizygous mutation VAF was adjusted based on the formula "adjusted VAF = a/1 + a (a = raw VAF value)." There is no adjustment for heterozygous or compound heterozygous mutations.

The R language-based web tool Cutoff Finder (http://molpath.charite.de/cutoff/) was used to determine the optimal VAF cutoff in a given gene for survival stratification.[29] In cases with multiple mutations of a certain gene, we chose the higher/highest VAF for calculation. For each cutoff, survival was examined in the two separated groups using the function survfit from the R package survival. Finally, the optimal cutoff for differences in survival was selected (lowest *P* value under log-rank test).[29]

2.6 | Follow up and response assessment

Overall survival was defined as the period from the date of initial diagnosis to the date of death regardless of the cause. Data were censored at the last follow up. Response to decitabine was assessed using the modified International Working Group (IWG) criteria.[30] Patients with complete remission (CR), partial remission (PR), marrow CR (mCR), or hematological improvement (HI) were regarded as responders. Patients with stable disease, failure, or disease progression were regarded as non-responders.

2.7 | Statistical analysis

Survival analysis was carried out using the Kaplan-Meier method followed by the log-rank test. Univariate COX regression was used to select mutational variables for entry into multivariate COX regression with stepwise backward selection ($P < .05$). For the genes of which $P \geq .05$ in the univariate analysis, the R language-based web tool Cutoff Finder was then used to find the optimal VAF cutoff for differences in survival. Independent variables in the multivariate regression also included: age (≥ vs <60 years), gender, and currently common prognostic scoring systems (IPSS, IPSS-R or LR-PSS excluding age). Associations between mutation status and leukemic conversion were evaluated by Chi-squared test or Fisher's exact test. Bonferroni correction was applied for multiple testing. All statistical analyses were carried out using SPSS 23.0 software. $P < .05$ (2-sided) was considered statistically significant. Venn diagram was generated by BioVenn.[31]

3 | RESULTS

3.1 | Clinical characteristics of the study population

A total of 159 LR-MDS patients (median age: 58 years, range: 14-89 years; 95 men and 64 women) were included in data analysis (Table S1). Based on the IPSS score, 29 (18.2%) patients had low risk and the remaining 130 (81.8%) had Int-1 risk (Table 1). The 2016 WHO MDS types included: MDS with single lineage dysplasia (MDS-SLD;

TABLE 1 Demographic and baseline characteristics of the study population

Variable	Baseline distribution in cohort
Demographics	
Age (y), median (range)	58 (14-89)
Male gender, N (%)	95 (59.7)
2016 WHO classification, N (%)	
MDS-SLD	25 (15.7)
MDS-RS	16 (10.1)
MDS-MLD	76 (47.8)
MDS-EB-1	33 (20.8)
MDS-EB-2	3 (1.9)
MDS-U	5 (3.1)
MDS with isolated del(5q)	1 (0.6)
Blood counts at time of mutation analysis	
Hemoglobin level (g/dL), mean ± SD	8.1 ± 2.9
Neutrophil count ($\times 10^9$/L), mean ± SD	1.9 ± 2.9
Platelet count ($\times 10^9$/L), mean ± SD	113.5 ± 155.2
Bone marrow at time of mutation analysis	
Bone marrow blasts (%), mean ± SD	3.1 ± 2.6
Abnormal karyotype, N (%)	45 (28.3)
IPSS, N (%)	
Low	29 (18.2)
Int-1	130 (81.8)
Treatment, N (%)	
Best supportive care	99 (62.3)
HSCT	14 (8.8)
Decitabine	42 (26.4)
Chemotherapy	4 (2.5)
Outcome, N (%)	
Leukemic transformation	25 (15.7)
Death	58 (36.5)

Abbreviations: HSCT, hematopoietic stem cell transplantation; Int-1, intermediate 1; IPSS, International Prognostic Scoring System; MDS-EB-1, myelodysplastic syndromes with excess blasts-1; MDS-EB-2, MDS with excess blasts-2; MDS-MLD, MDS with multilineage dysplasia; MDS-RS, MDS with ring sideroblasts; MDS-SLD, MDS with single lineage dysplasia; MDS-U, MDS unclassifiable; WHO, World Health Organization.

n = 25, 15.7%), MDS with ring sideroblasts (MDS-RS; n = 16, 10.1%), MDS with multilineage dysplasia (MDS-MLD; n = 76, 47.8%), MDS with excess blast-1 (MDS-EB-1; n = 33, 20.8%), MDS-EB-2 (n = 3, 1.9%), MDS unclassifiable (MDS-U; n = 5, 3.1%), and MDS with isolated del (5q) (n = 1, 0.6%). Forty-five (28.3%) patients had abnormal karyotypes. The four most frequent abnormal karyotypes were trisomy 8 (13.2%), del (20q) (4.4%), del (5q) (3.7%), and del (7) (2.5%) (Figure 1A).

Median follow up was 21.2 months (IQR: 13.6-36.6 months). A total of 58 (36.5%) patients died during the follow up. Median OS was 47.8 months (95% CI: 38.0-57.6 months). Leukemic conversion occurred in 25 (15.7%) patients during the follow up.

3.2 | Gene mutational profile

Next-generation sequencing revealed mutations in all 15-target genes (Figure 1B; Table S2). The five most frequent mutated genes were *TET2* (14.5%), *SF3B1* (13.8%), *U2AF1* (10.7%), *ASXL1* (10.1%), and *DNMT3A* (7.5%). Genes less frequently mutated were *RUNX1* (6.9%), *EZH2* (6.3%), *TP53* (5.0%), *CBL* (4.4%), *SRSF2* (4.4%), *IDH1* (3.8%), *NRAS* (3.1%), *JAK2* (2.5%), *IDH2* (1.9%), and *ETV6* (1.9%). One hundred and one (63.5%) patients had at least one mutated gene. Fifty-nine (37.1%) had one mutated gene only, 31 (19.5%) had two mutated genes, and 11 (6.9%) had at least three mutated genes.

3.3 | Mutation status versus OS

Mutation status associated with OS was examined by univariate COX regression analysis: *ASXL1* (P = .001), *RUNX1* (P = .031), *EZH2* (P = .049), *TP53* (P = .016), *SRSF2* (P = .046), *JAK2* (P = .040), and *IDH2* (P = .035) mutations were significantly associated with shorter OS (Table 2). Furthermore, mutation status of *IDH2* was associated with conversion to sAML (P = .045 after Bonferroni correction; Table S3).

3.4 | Mutation VAF versus OS

Using R language-based web tool Cutoff Finder, a raw *TET2* VAF threshold of 17.6% was the optimal cutoff for outcome prediction (P = .017; Figure S1A). By contrast, there was no significantly optimal raw VAF value for any other genes in the context of patients' survival (Figure S1B-H).

As raw VAF could not completely represent mutation burden which was also influenced by allelic status, we next carried out SNP-A analysis on *TET2*-mutated patients. NGS identified 23 patients with *TET2* mutations, but DNA was available for SNP-A analysis in 20 of these cases (Table 3; Table S4). Heterozygous *TET2* mutations were found in 17 cases without 4q/24 aberration, hemizygous *TET2* mutations were identified in two patients with 4q24 microdeletions, and compound heterozygous *TET2* mutations were discovered in one case. VAF of *TET2* mutations were then adjusted. For the three patients without SNP-A analysis, whether the *TET2* mutation was heterozygous, hemizygous, or homozygous, adjusted *TET2* VAF was always <18% in one of the cases, whereas adjusted VAF was always ≥18% in the other two cases according to the adjustment method of VAF. After excluding three *TET2*-mutated cases without SNP-A analysis, we found an adjusted *TET2* VAF threshold of 17.6% (rounded to 18%) remained the best cutoff for prognostic stratification by Cutoff Finder (P = .029; Figure S2).

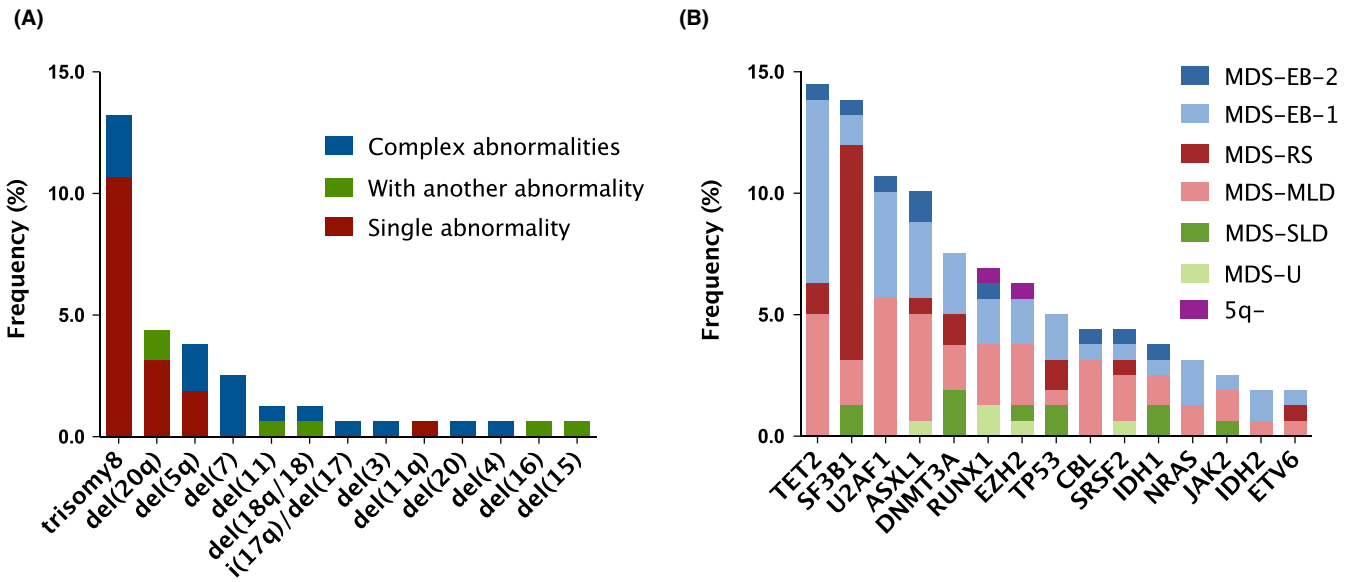

FIGURE 1 Cytogenetic and genomic spectrum in lower-risk myelodysplastic syndromes (LR-MDS). A, Frequency of abnormal karyotypes in 159 LR-MDS patients. B, Frequency of 15 mutated genes in 159 LR-MDS patients with different 2016 WHO subtypes. LR-MDS, lower-risk myelodysplastic syndromes; WHO, World Health Organization

TABLE 2 Univariate analyses of mutation status for overall survival

Mutational variable	Mutation status	N	Median OS (months)	*P* value
TET2	MT	23	20.9	.128
	WT	136	47.8	
SF3B1	MT	22	39.6	.322
	WT	137	47.8	
U2AF1	MT	17	49.0	.323
	WT	142	47.8	
ASXL1	MT	16	20.4	.001
	WT	143	50.4	
DNMT3A	MT	12	NR	.841
	WT	147	46.5	
RUNX1	MT	11	24.3	.031
	WT	148	49.0	
EZH2	MT	10	17.0	.049
	WT	149	49.0	
TP53	MT	8	7.8	.016
	WT	151	47.8	
CBL	MT	7	NR	.321
	WT	152	47.8	
SRSF2	MT	7	25.6	.046
	WT	152	49.0	
IDH1	MT	6	NR	.522
	WT	153	46.5	
NRAS	MT	5	56.8	.824
	WT	154	46.5	
JAK2	MT	4	16.8	.040
	WT	155	47.8	
IDH2	MT	3	24.9	.035
	WT	156	47.8	
ETV6	MT	3	16.6	.388
	WT	156	47.8	

Abbreviations: MT, mutant type; NR, not reached; OS, overall survival; WT, wild type.

3.5 | *TET2* VAF versus OS

Overall survival was not associated with *TET2* mutation status per se (median: 20.9 vs 47.8 months; *P* = .128; Figure 2A), but with *TET2* mutation burden (low, as defined by wild type plus adjusted *TET2* VAF at <18% vs high, defined as adjusted *TET2* VAF at ≥18%). Out of the 159 study subjects, *TET2* mutation was identified in 23 patients, among which 19 (82.6%) had adjusted *TET2* VAF at ≥18%. OS was significantly shorter in subjects with high *TET2* mutation burden (adjusted VAF ≥18%) versus those with low *TET2* mutation burden (adjusted VAF <18% plus *TET2* wild type) (median: 20.4 vs 47.8 months; *P* = .020; HR = 2.183, 95% CI: 1.129-4.224; Figure 2B). When compared with patients with no *TET2* mutations alone, patients with an adjusted *TET2* VAF ≥18% also had significantly shorter OS (median: 20.4 vs 47.8 months; *P* = .023; HR = 1.465, 95% CI: 1.053-2.040; Figure S3).

In a subgroup analysis that included 99 subjects only receiving best supportive care without HMA, OS was also significantly shorter in patients with an adjusted *TET2* VAF ≥18% (n = 11) (median: 20.4 vs 55.9 months; *P* = .036; HR = 2.484, 95% CI: 1.063-5.805; Figure 2C). Response to decitabine also seemed to be associated with *TET2* burden: the adjusted *TET2* VAF value was 50.79%, 40.80%, and 41.50% in the three *TET2*-mutated patients who responded to decitabine treatment, and <18%, 16.20%, and 20.22% in three *TET2*-mutated patients who did not respond to decitabine (Table S5).

3.6 | Predictors of OS

The following variables were entered as independent variables in the multivariate COX regression analysis: the IPSS risk group, age (≥ vs <60 years), gender, mutation status of *ASXL1*, *RUNX1*, *EZH2*, *TP53*, *SRSF2*, *JAK2*, and *IDH2*, and adjusted *TET2* VAF (≥ vs <18%) (Table 4). The analysis showed that OS was independently associated with the following factors: IPSS Int-1 risk (*P* = .014; HR = 3.626, 95% CI: 1.292-10.174), *ASXL1* mutations (*P* < .001; HR = 4.306, 95% CI: 2.144-8.650), *TP53* mutations (*P* = .004; HR = 4.863, 95% CI: 1.662-14.230), *JAK2* mutations (*P* = .002; HR = 5.466, 95% CI: 1.848-16.169), and adjusted *TET2* VAF ≥18% (*P* = .008; HR = 2.492, 95% CI: 1.273-4.876).

In another similar model considering the LR-PSS risk category (Table S6), mutational variables including *ASXL1* mutations, *TP53* mutations, *JAK2* mutations, and adjusted *TET2* VAF ≥18% remained as independent predictors of worse outcome. Similar results were also detected in the IPSS-R risk group (Table S7).

Within the 43 subjects (27.0%) having at least one poor-risk mutational factor (*ASXL1* MT, *TP53* MT, *JAK2* MT, and adjusted *TET2* VAF ≥18%), OS was increasingly shorter as the number of poor-risk mutational factors increased (*P* < .001; Figure 3). OS was also significantly shorter in those with poor-risk mutational factors in subgroup analysis that included patients with IPSS low risk only (*P* = .029; Figure 4A), or IPSS Int-1 risk only (*P* < .001; Figure 4B). Similarly, in subgroup analysis that divided subjects using IPSS-R (≤ vs >3.5), the presence of poor-risk mutational factors was associated with a significantly shorter OS (*P* = .018 for IPSS-R at ≤3.5; Figure 4C; *P* < .001 for IPSS-R at >3.5; Figure 4D).

3.7 | Development of a mutational factors-based prognostic scoring system

Patients with IPSS Int-1 risk had a significantly worse median OS versus patients with IPSS low risk (44.3 months vs median OS not reached, *P* = .029; Figure 5A). Furthermore, LR-PSS category three patients had a significantly shorter median OS versus LR-PSS category two patients (23.7 months vs 47.8 months; *P* = .001) or LR-PSS category one patients (median OS not reached; *P* < .001) (Figure 5B). Moreover, patients with IPSS-R >3.5 had a shorter median OS

TABLE 3 Characteristics of *TET2*-mutated patients

			TET2 mutations			
Number	Cytogenetics	LOH4q24	Consequence	State	Raw VAF	Adjusted VAF
21	Normal	NA	c.3393_3314insT	Unknown	12.70	<18[a]
92	Normal	Absence	c.3409 + 1G>A	Heterozygous	44.50	44.50
109	Normal	Absence	c.3315_3316insA	Heterozygous	45.15	45.15
140	Normal	Absence	c.2604T > G	Heterozygous	54.20	54.20
156	Normal	NA	c.2068C > T	Unknown	52.20	≥18[b]
157	Normal	Absence	c.5543C > G	Heterozygous	16.20	16.20
177	Normal	Absence	c.2153delT	Heterozygous	45.60	45.60
192	46,XX,-11,+mar[10]	Absence	c.2440C > T	Heterozygous	50.79	50.79
306	Normal	NA	c.5476G > T	Unknown	38.70	≥18[b]
310	Normal	del4q24	c.3626T > C	Hemizygous	19.00	15.97
346	Normal	Absence	c.5618T > C	Heterozygous	42.37	42.37
352	Normal	Absence	c.4393C > T	Heterozygous	51.83	51.83
355	46,XX,del(20)(q11)[3]/46,XX[7]	Absence	c.2604T > G	Heterozygous	51.45	51.45
361	Normal	Absence	c.3646C > T	Heterozygous	20.22	20.22
366	47,XY,+mar[1]/45,XY,-15[3]/46,XY[16]	Absence	c.4793delA	Compound	32.20	32.20
			c.1664C > T	Heterozygous	46.60	46.60
380	46,XY,der(20)(q11)[9]/46,XY[1]	Absence	c.2604T > G	Heterozygous	48.10	48.10
382	Normal	Absence	c.3955-2A > G	Heterozygous	4.70	4.70
384	47,XY,+8[9]/46,XY[1]	Absence	c.5298delC	Heterozygous	40.40	40.40
386	Normal	del4q24	c.3626T > C	Hemizygous	89.90	47.34
404	46,XX,der(5)(q32)[16]/46,XX[4]	Absence	c.2230C > T	Heterozygous	40.80	40.80
412	47,XY,+8[10]	Absence	c.4546C > T	Heterozygous	1.40	1.40
430	Normal	Absence	c.5178delT	Heterozygous	41.50	41.50
446	Normal	Absence	c.5618T > C	Heterozygous	42.40	42.40

Abbreviations: LOH: loss of heterozygosity; NA, not available; VAF, variant allele frequency.

[a]Adjusted VAF was always <18% according to the adjustment method of VAF, whether the *TET2* mutation was heterozygous, hemizygous, or homozygous.

[b]Adjusted VAF was always ≥18% according to the adjustment method of VAF, whether the *TET2* mutation was heterozygous, hemizygous, or homozygous.

versus those with IPSS-R ≤3.5 (37.1 months vs 55.9 months; $P = .011$; Figure 5C).

We developed a prognostic scoring system, referred to as Lower-Risk Molecular Prognostic Scoring System (LR-M-PSS below), using the five risk factors identified in the current study (IPSS Int-1 risk, *ASXL1* MT, *TP53* MT, *JAK2* MT, and adjusted *TET2* VAF ≥18%). A score of 1 was assigned to each factor if present; otherwise, a score of 0 was assigned. The maximum possible overall score was 5.

The overall score was 0 in 21 subjects (13.2%), 1 in 102 subjects (64.2%), and ≥2 in the remaining 36 subjects (22.6%). The median OS was not reached in subjects with an overall score of 0, 49.0 months (95% CI: 36.8-61.2 months) in those with an overall score of 1, and 19.8 months (95% CI: 13.0-26.6 months) in those with an overall score of 2 or above ($P < .001$; Figure 5D).

Comparing the identification power of the higher-risk patients, 10.3% (3/29) of patients were redefined as higher-risk group by LR-M-PSS (overall score of ≥2) within the group of low IPSS scores, with a median OS of 12.9 months; whereas none could be upgraded to higher-risk group by either the LR-PSS or the IPSS-R (LR-PSS category 3 or IPSS-R at >3.5). In the group of Int-1 IPSS scores, 25.4% (33/130) of patients were identified as having poor survival by the LR-M-PSS, whereas 24.6% (32/130) were identified by LR-PSS and 50.8% (66/130) by IPSS-R.

Considering the overlap of the higher-risk group recognized by these models (Figure 6), IPSS-R obviously identified the largest number of the patients (41.5%, 66/159), covering most of the subjects identified by LR-PSS (81.3%, 26/32) and half of the patients by LR-M-PSS (50%, 18/36). However, our new model could identify an additional 10.1% (16/159) of patients who neither identified

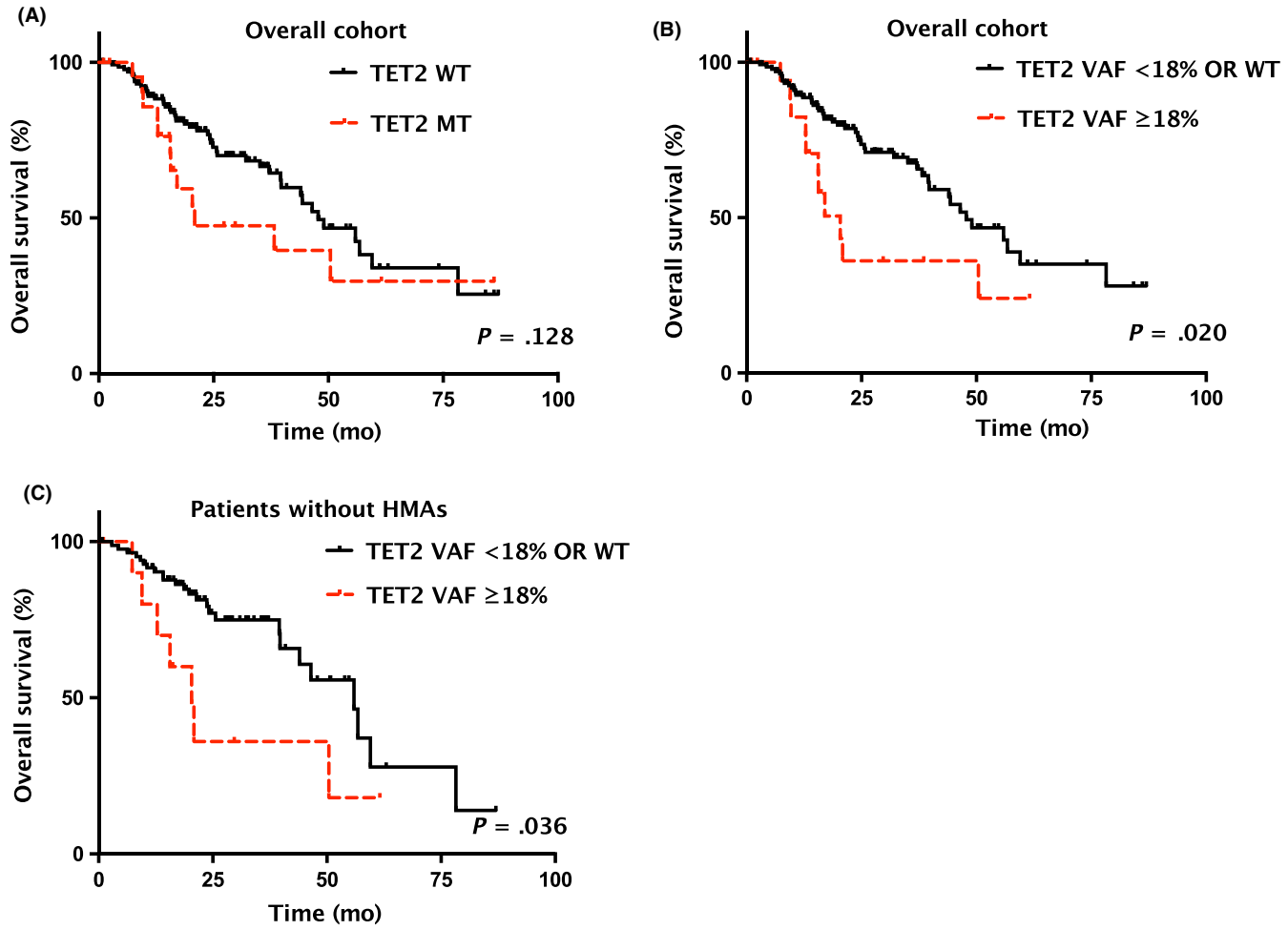

FIGURE 2 Overall survival (OS) by *TET2* mutation status and *TET2* variant allele frequency (VAF) in lower-risk myelodysplastic syndromes (LR-MDS). A, OS stratified by *TET2* mutation status in the overall cohort. B, OS stratified by adjusted *TET2* MT VAF <18% or WT vs adjusted *TET2* MT VAF ≥18% in the overall cohort. C, OS stratified by adjusted *TET2* MT VAF <18% or WT vs adjusted *TET2* MT VAF ≥18% in 99 patients without hypomethylating agents (HMA). MT, mutant type; OS, overall survival; WT, wild type

by IPSS-R nor by LR-PSS, with a median OS of 20.4 months (95% CI: 11.2-29.5 months). By contrast, the higher-risk patients identified by IPSS-R or LR-PSS but not LR-M-PSS had a median OS of 49.0 months (95% CI: 31.7-66.3 months) or 44.0 months (95% CI: 11.3-76.7 months).

4 | DISCUSSION

In the present study, *ASXL1* MT, *TP53* MT, *JAK2* MT, and high *TET2* mutation burden were found to be significantly associated with poorer prognosis independent of IPSS. A scoring system that combines these mutational factors with the IPSS could stratify LR-MDS patients into three prognostically distinct groups and help identify patients with greater-than-predicted risk for early intervention.

A previous report showed that *ASXL1* and *EZH2* mutations were more common in LR-MDS than higher-risk MDS.[11] A subsequent study from the same group of investigators showed that *ASXL1*,

TABLE 4 Multivariate COX regression analysis for overall survival for IPSS

Variable	*P* value	Hazard ratio (95% CI)
IPSS (Int-1 vs low)	0.014	3.626 (1.292-10.174)
Age (≥ vs <60 y)	0.161	1.579 (0.833-2.991)
Gender (male vs female)	0.240	1.443 (0.783-2.662)
ASXL1 (MT vs WT)	< 0.001	4.306 (2.144-8.650)
RUNX1 (MT vs WT)	0.798	1.151 (0.391-3.390)
EZH2 (MT vs WT)	0.303	1.630 (0.643-4.131)
TP53 (MT vs WT)	0.004	4.863 (1.662-14.230)
SRSF2 (MT vs WT)	0.774	1.235 (0.293-5.193)
JAK2 (MT vs WT)	0.002	5.466 (1.848-16.169)
IDH2 (MT vs WT)	0.255	2.090 (0.587-7.441)
Adjusted *TET2* VAF (≥ vs <18%)	0.008	2.492 (1.273-4.876)

Abbreviations: CI, confidence interval; Int-1, intermediate 1; IPSS, International Prognostic Scoring System; MT, mutant type; VAF, variant allele frequency; WT, wild type.

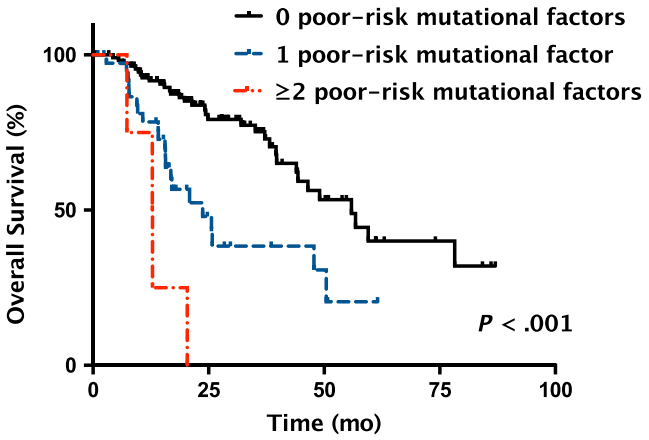

FIGURE 3 Overall survival (OS) by the number of poor-risk mutational factors in lower-risk myelodysplastic syndromes (LR-MDS). OS stratified by the number (0, 1, and ≥2, respectively) of poor-risk mutational factors of independent prognostic significance, including *ASXL1* MT, *TP53* MT, *JAK2* MT, and adjusted *TET2* VAF ≥18%. MT, mutant type

EZH2, and *NRAS* mutations were associated with poorer prognosis in LR-MDS independent of IPSS; mutations of *ASXL1*, *EZH2*, *TP53*, and *RUNX1* remained independently significant after adjusting for LR-PSS.[16] In the current study, we confirmed the association of *ASXL1*, *EZH2*, *TP53*, and *RUNX1* with worse outcome in the univariate analysis and the independent prognostic significance of *ASXL1* and *TP53* mutations in the multivariate analysis. We failed to show an association of *NRAS* mutation with patient survival, probably due to the small number of patients with *NRAS* mutation in our cohort. *JAK2* mutations have previously been reported to be associated with poor patient survival in MDS receiving hematopoietic stem cell transplantation (HSCT),[32] but not in LR-MDS.[16] The current study extended such an association to LR-MDS.

We also observed that LR-MDS patients with *IDH2* mutations were more likely to develop sAML, as previous studies of unselected MDS reported.[14,33] Mutations of genes involved in DNA methylation including *IDH2* might represent early events in MDS and play an indirect role in disease progression through a multistep evolutionary process.[34,35] *NARS* mutations were reported to be associated

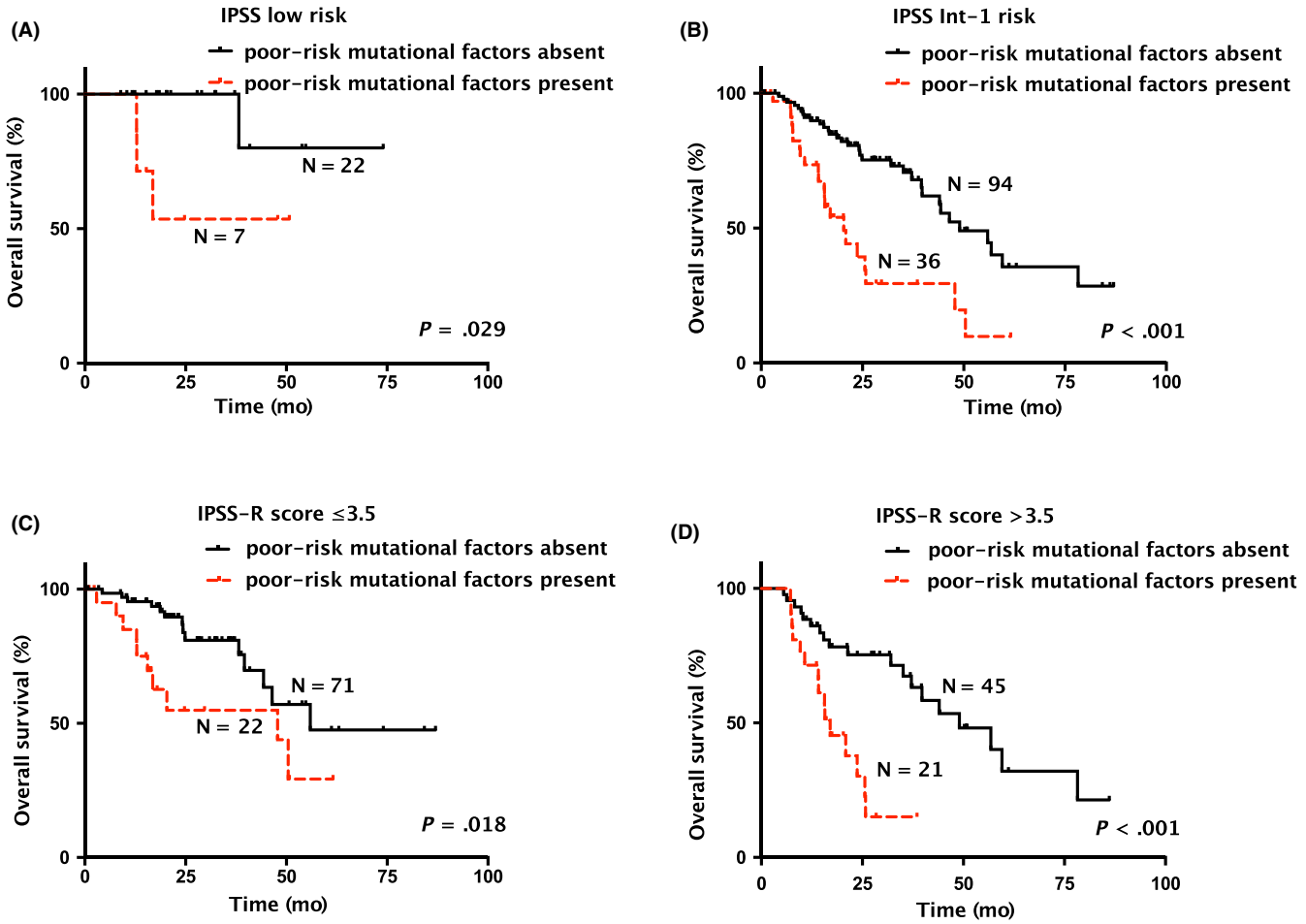

FIGURE 4 Overall survival (OS) by presence or absence of any poor-risk mutational factors in lower-risk myelodysplastic syndromes (LR-MDS) with each risk category of the International Prognostic Scoring System (IPSS) or the revised IPSS (IPSS-R). OS by presence or absence of any poor-risk mutational factors of independent prognostic significance, including *ASXL1* MT, *TP53* MT, *JAK2* MT, and adjusted *TET2* VAF ≥18%, in LR-MDS with (A) IPSS low risk, (B) IPSS Int-1 risk, (C) an IPSS-R score ≤3.5, or (D) an IPSS-R score >3.5. Int-1, intermediate 1; MT, mutant type

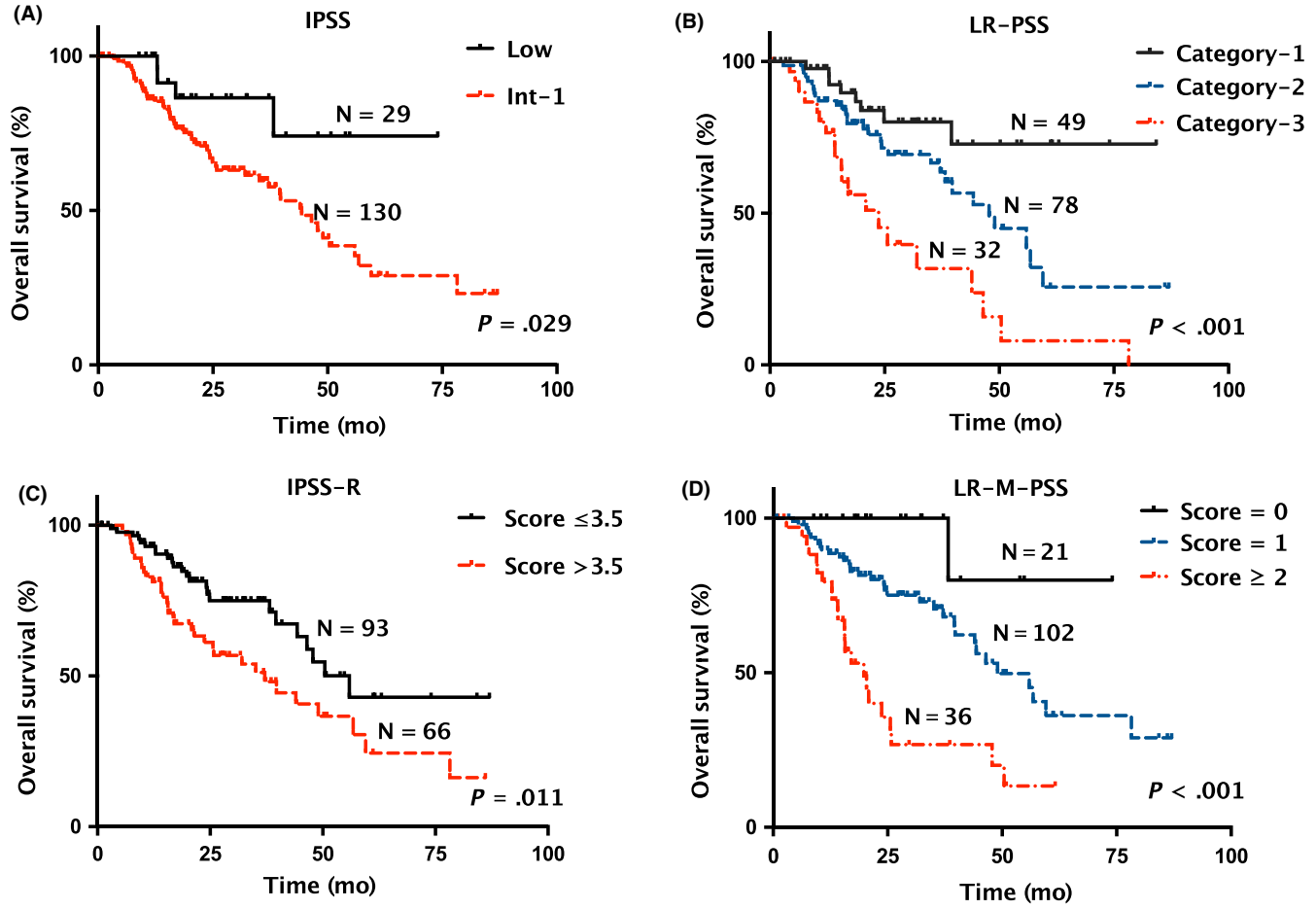

FIGURE 5 Overall survival (OS) by prognostic models in lower-risk myelodysplastic syndromes (LR-MDS). (A) OS stratified by IPSS low risk vs Int-1 risk, (B) LR-PSS categories, (C) IPSS-R score ≤3.5 vs >3.5, and (D) LR-M-PSS groups, respectively. Int-1, intermediate 1; IPSS, International Prognostic Scoring System; IPSS-R, revised IPSS; LR-M-PSS, Lower-Risk Molecular Prognostic Scoring System; LR-PSS, MD Anderson Lower-Risk Prognostic Scoring System

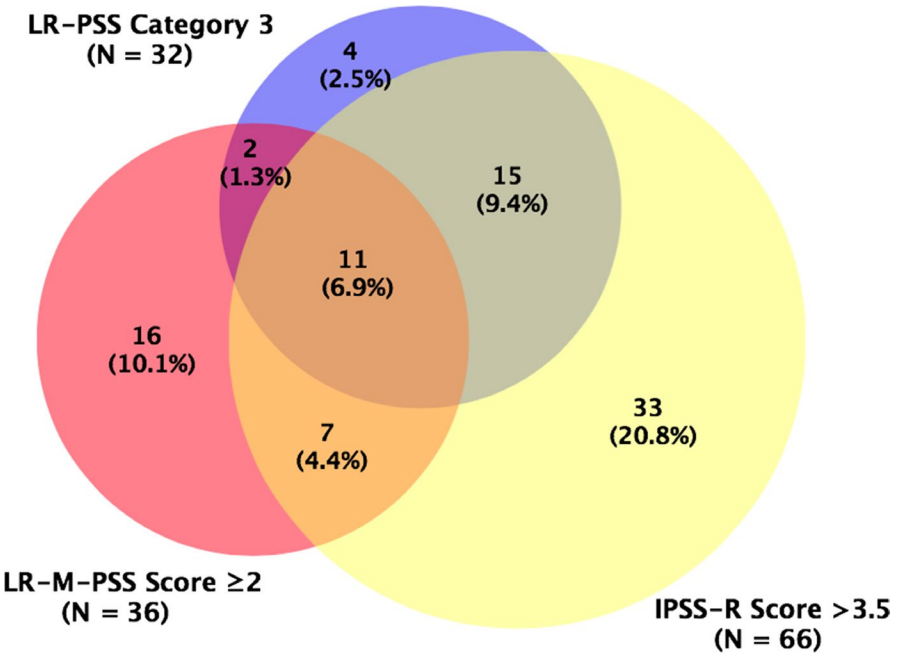

FIGURE 6 Venn diagram showing the overlap of lower-risk myelodysplastic syndromes (LR-MDS) patients with a poor prognosis identified by IPSS-R (IPSS-R at >3.5), LR-PSS (category 3), and LR-M-PSS (overall score of ≥2), respectively. Created using BioVenn.[31] IPSS-R, revised International Prognostic Scoring System; LR-M-PSS, Lower-Risk Molecular Prognostic Scoring System; LR-PSS, MD Anderson Lower-Risk Prognostic Scoring System

with leukemic transformation of MDS in several studies of serial sequences.[34-36] However, we failed to statistically find their relation in our study probably because the association was not obvious in the early stage of MDS.

A major finding in the current study is that the independent association of *TET2* mutation burden with prognosis in LR-MDS. *TET2* is one of the most commonly mutated genes in MDS,[11,13,16] and often found at the initiation and early progression of MDS.[37-39] *TET2* mutations had been reported to be associated with poorer survival[32,40,41] and higher response to HMA[15,42] by some, but not all, studies[11,12,16,17,43,44] in either unselected MDS or LR-MDS. In the current study, we found that *TET2* mutation status had no association with OS per se, but with *TET2* mutation burden. Specifically, the patients with an adjusted *TET2* VAF ≥18% had a significantly worse survival, compared with patients with an adjusted *TET2* VAF <18% or without *TET2* mutations. Such an association remained statistically significant even after adjustment for HMA. Our finding is in line with a previous study that indicated higher *TET2* VAF leading to worse survival in myeloid neoplasms.[32] Another interesting finding is that all three *TET2*-mutated subjects who responded to decitabine had >40% adjusted *TET2* VAF, whereas all three *TET2*-mutated subjects who did not respond to decitabine had adjusted *TET2* VAF of approximately less than 20%. This preliminary finding is consistent with a previous study that suggested higher *TET2* VAF in decitabine responders.[15]

Another major finding in the current study is that mutational factors (eg, *ASXL1* MT, *TP53* MT, *JAK2* MT, and adjusted *TET2* VAF ≥18%) could help to identify LR-MDS patients with poor survival within each risk group defined by either IPSS or IPSS-R. Patients with mutational factors had shorter OS in comparison with those without such mutational factors within the same IPSS or IPSS-R risk group. Also, survival was increasingly worse as the number of these mutational factors increased.

LR-PSS could stratify LR-MDS into three categories with distinct outcomes, and thus represents an advance in more accurate risk prediction.[9] Median OS in patients of LR-PSS category 3 has been shown to be equivalent to that of patients of the IPSS intermediate 2 (Int-2) risk group.[16] IPSS-R represents another attempt at more accurate risk prediction: it could reassign patients into a lower- or higher-risk group based on a cutoff score of 3.5 points.[10] The prognostic value of LR-PSS and IPSS-R had already been elaborately examined before,[8,16,45] which was also confirmed by our lower-risk MDS cohort. About 25% and 50% of patients were reassessed as higher-risk group by LR-PSS (category 3) or IPSS-R (IPSS-R at >3.5) respectively, within the IPSS Int-1 risk group in the current study. However, neither the LR-PSS nor the IPSS-R could be used to recognize patients with poor OS within the IPSS low-risk group, as reported previously.[45] It is expected that additional molecular predictors will contribute to better risk stratification of LR-MDS.

Combination of molecular data with the existing risk-prediction systems has already been explored. For example, by combining *EZH2* mutation status and LR-PSS, 29% of LR-MDS patients with either *EZH2* mutation or LR-PSS category 3 risk were identified as having a shorter-than-predicted OS.[16] In the present study, we developed a novel prediction model (abbreviated as LR-M-PSS) by combing the four mutational factors, including *ASXL1* MT, *TP53* MT, *JAK2* MT, and adjusted *TET2* VAF ≥18%, with the IPSS risk group. LR-M-PSS stratified LR-MDS patients into three groups with distinct prognosis (overall score of 0, 1, or ≥2). Approximately one-fourth of the study subjects fell into the high-risk group (overall LR-M-PSS score ≥2).

Comparing the identification power of the higher-risk patients, IPSS-R definitely had the advantage of identifying the largest number of patients as previously reported,[8,45] covering most of the patients identified by LR-PSS and half of the patients by LR-M-PSS. However, our model made a more refined risk prediction by subdividing LR-MDS patients into three prognostically distinct groups. LR-M-PSS could additionally identify more than 10% of patients who were failed to be recognized by both IPSS-R and LR-PSS. LR-M-PSS also could recognize these patients from the IPSS low-risk group, whereas the other two models could not.[45] Moreover, the higher-risk patients identified by LR-M-PSS only had a definitely poor survival, whereas patients identified by IPSS-R or LR-PSS but not LR-M-PSS seemed to have a longer-than-predicted survival. These preliminary findings indicated that mutation status and burden of certain genes could improve prognostic stratification of LR-MDS, and would be an important complement to the current prognostic models.

We acknowledged several limitations in this study. First, the optimal VAF cutoff was primarily determined based on the raw VAF data, which may lack biological significance to some extent. Thus, we further explored the prognostic significance of *TET2* mutation burden with the adjustment of VAF for copy number and zygosity. Additional limitations included the relatively small sample size and the lack of an independent validation cohort. More studies are needed to confirm our findings, and to improve prognostic prediction of LR-MDS. Notwithstanding the above limitations, our study allows for a new approach on the application of molecular data for prognostication of LR-MDS.

In summary, this study shows that *ASXL1* MT, *TP53* MT, *JAK2* MT, and high *TET2* mutation burden are important predictors for poor survival in LR-MDS. Our study highlights that integrating mutation status and burden of certain MDS-relevant genes into IPSS may improve risk stratification of patients with LR-MDS and help identify those with worse-than-expected prognosis for more aggressive treatment.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

HT and LJ conceived and designed the study. YL, SZ and LW analyzed and arranged the data. LM, HZ, CS, and WY carried out the

mutation analysis. YR, XZ, CM, WX, LY, HY, and CL provided patient samples and data. JJ guided the research with valuable comments. HT provided critical revision and suggestions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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