SF3B1 mutation predicts unfavorable treatment-free survival in Chinese chronic lymphocytic leukemia patients

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Background: Splicing factor 3b subunit 1 (*SF3B1*), a splicing factor modulating RNA alternative splicing, is frequently mutated in multiple hematological malignancies including myelodysplastic syndromes and chronic lymphocytic leukemia (CLL). The clinical impact of *SF3B1* mutation on CLL remains controversial especially for patients of Asian descent.

Methods: We retrospectively analyzed the frequency of *SF3B1* mutation by Sanger sequencing in 399 newly diagnosed Chinese CLL patients.

Results: *SF3B1* mutation was detected in 5.5% (22/399) of the studied cohort with 59.1% of them being c.A2098G (p.K700E). *SF3B1* mutation was common in patients with unmutated immunoglobulin heavy chain variable region gene, positive CD38 and positive ZAP-70. Survival analysis showed that *SF3B1* mutation was associated with short treatment-free survival (TFS), but not overall survival (OS). We then developed 2 new risk models, named CLL-IPI-S and CLL-PI, according to the *SF3B1* mutation status and CLL-international prognostic index (CLL-IPI); CLL-PI showed greater power to predict TFS than CLL-IPI in Chinese CLL patients.

Conclusions: Our data suggest a low incidence and adverse clinical significance of *SF3B1* mutation in newly diagnosed Chinese CLL patients.

Keywords: Splicing factor 3b subunit 1 mutation (*SF3B1* mutation); chronic lymphocytic leukemia (CLL); prognosis; risk models

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Introduction

Chronic lymphocytic leukemia (CLL) is a common kind of mature lymphocytic proliferative disease with heterogeneous clinical course. Although some patients can be "watch and wait," a considerable number of patients need immediate treatment upon diagnosis and have diverse treatment responses and survival times. Multiple factors

contribute to this heterogeneity (1-5). Age, β2-microglobulin (β2-MG) concentration, stage, tumor protein 53 (*TP53*) status, and immunoglobulin heavy variable-region gene (*IGHV*) status, compose the CLL-international prognostic index (CLL-IPI), which can stratify patients into 4 groups: low [0–1], intermediate [2–3], high [4–6], and very high risk [7–10]. Although CLL-IPI is widely used to assess the prognosis, novel prognosis-related genetic aberrations are not included in this system (6).

Splicing factor 3b subunit 1 (*SF3B1*), located in 2q33.1, encodes the subunit 1 of splicing factor 3b, a component of the U2 small nuclear ribonucleoproteins complex, which is crucial to RNA alternative splicing (7). *SF3B1* mutation leads to the development and progression of multiple malignant diseases (8), including myelodysplastic syndromes (9), breast cancer (10), and CLL (11). In CLL, the most frequent aberration of *SF3B1* is a missense mutation with a hotspot of c.A2098G (p.K700E) (4,12). The *SF3B1* mutation occurs in 10–20% newly diagnosed CLL patients from western countries and suggests unfavorable prognosis (4,12). However, the incidence of *SF3B1* mutation is relatively low in Chinese CLL patients, and its prognostic value remains controversial (5,13). In this study, we retrospectively analyzed the incidence and clinical impact of *SF3B1* mutation in 399 newly diagnosed CLL patients. Additionally, new risk models based on *SF3B1* mutational status and CLL-IPI were developed in order to predict the survival of Chinese CLL patients better.

Methods

Patients

A total of 399 previously untreated CLL patients [including 201 patients reported by Xia *et al.* (5)] diagnosed from January 2000 to December 2017 in our hospital were enrolled in this single-center retrospective study. Diagnosis of CLL was based on the International Workshop on CLL-National Cancer Institute criteria. The hospital ethics committee approved this study, and all patients were provided and signed informed consent according to the Declaration of Helsinki.

SF3B1 mutation detection

Genomic DNA from CLL samples was extracted as previously reported (5). Briefly, PCR amplification was performed for exon 14-16 of *SF3B1*. Primers used were as follows: *SF3B1* exon 14 forward primer 5'-TGACTGTCCTTTCTTTGTTTAC-3' and reverse primer 5'-ATAGTAAGACCCTGTCTCCTA-3'; exon 15-16 forward primer 5'-TTGGCTGAATAGTTGATATATTGAGAG-3' and reverse primer 5'-AAACACTTTAAAATTCTGTTAGAACCA-3'. Sanger sequencing was performed in exon 14-16.

Data collection

Laboratory data such as absolute lymphocyte count (ALC), platelet count (PLT), hemoglobin (Hb), serum albumin (ALB) concentration, lactate dehydrogenase (LDH), thymidine kinase 1 (TK-1), and β2-MG were accessible from the hospital-based laboratory service within 24 h after the first admission.

Detection of *TP53*, *NOTCH1*, and *MYD88* mutation, and *IGHV* mutation status were performed as previously described (14). The cut-off of 98% homology to germline was used to dichotomize *IGHV* mutational status.

Karyotype analysis of CLL cells was performed after CpG-oligodeoxynucleotide and interleukin-2 stimulation. Fluorescence *in situ* hybridization (FISH) was carried out to detect 17p deletion, 11q deletion, 13q deletion, and trisomy 12 according to the procedures described previously (5). CD38 and ZAP-70 were detected via flow cytometry, and the cut-off values for positivity were 30% and 20% respectively.

Statistical analyses

SPSS 23 (IBM Corporation, Armonk, NY, USA) and MedCalc Statistical Software version 15.2.2 (MedCalc Software bvba, Ostend, Belgium) were used to analyze data. Categorical variables were presented in percentage (%) and analyzed by the χ^2 test. Overall survival (OS) was defined as the time from diagnosis to death or last follow-up, and treatment-free survival (TFS) was calculated as the time between diagnosis and first-line treatment. Survival curves were constructed by the Kaplan-Meier method, and the log-rank test was used for statistic associations. The Cox proportional hazards model was established to evaluate different factors at diagnosis on survival by univariate and multivariate analyses. For the multivariate analysis, we included variables whose P value is less than 0.05 during the univariate analysis. Receiver-operator characteristic (ROC) curve and corresponding area under the curve (AUC) were constructed to assess the predictive accuracy of CLL-IPI

and new risk models, and the differences in AUCs were tested by a nonparametric approach developed by DeLong *et al.* (15). The Hosmer-Lemeshow goodness of fit test tested the calibration of risk models, and a calibration plot was drawn with observed events/total events and corresponding expected events/total events like the X axis and Y axis, respectively. P<0.05 was defined as a statistically significant value. Graphs were made by SPSS 23 and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Patient characteristics

A total of 399 newly diagnosed Chinese CLL patients (261 male patients and 138 female patients) were enrolled in this study. Median age was 60 years old (16–93 years). There were 355 patients in Rai I-IV or Binet B/C. *SF3B1* mutation was seen in 22 patients (5.5%) at sites of c.1866G>T, c.1877A>T, c.1996A>G, c.2098A>G, c.2219G>A, c.2221A>G, c.2223G>T, and c.2225G>A. The details of patients with *SF3B1* mutation are shown in *Tables S1*,*S2*. The median follow-up time was 60 months (2–230 months). As of the last follow-up, 293 patients have been treated, and their regimens included chemoimmunotherapy (278/293) and ibrutinib (7/293). Information about treatment regimens are not available in 8 patients. Detailed information is presented in *Table 1*.

Table 1 Baseline characteristics of 399 chronic lymphocytic leukemia patients

Variables (N=399)	N (%)
Age >65 years	128 (32.1)
Male	261 (65.4)
Rai I-IV or Binet B/C	355 (89.0)
Lymphocytes >50×10^9/L	86 (21.6)
Platelets <100×10^9/L	103 (25.8)
Hemoglobin <100 g/L	82 (20.6)
Albumin <40 g/L	138 (34.6)
LDH > ULN	100 (25.1)
TK-1 > ULN (N=346)	57 (16.5)
β2-MG >3.5 mg/L	179 (44.9)
TP53 disruption	94 (23.6)
ATM deletion (N=390)	52 (13.3)
Del (13q) (N=300)	108 (36.0)
Trisomy 12 (N=340)	74 (21.8)
IGHV unmutated (N=382)	161 (42.1)
SF3B1 mutated	22 (5.5)
NOTCH1 mutated (N=389)	30 (7.7)
MYD88 mutated (N=386)	31 (8.0)
CD38 (≥30%) (N=379)	109 (28.8)
ZAP-70 (≥20%) (N=379)	159 (42.0)

β2-MG, β2-microglobulin; *IGHV*, immunoglobulin heavy variable-region gene; LDH, lactate dehydrogenase; *SF3B1*, splicing factor 3b subunit 1; TK-1, thymidine kinase 1; *TP53*, tumor protein 53; ULN, upper limit of normal.

Clinical, cytogenetic and molecular associations

The relationships between *SF3B1* mutation and patients' clinical, cytogenetic, and molecular characteristics are shown in *Table 2*. *SF3B1* mutation was common in patients with unmutated *IGHV* (P=0.035), positive CD38 (P=0.049), and positive ZAP-70 (P=0.005). Also, *IGHV* usage was available in 378 patients, and we analyzed the association of *SF3B1* mutation with the 10 most used *IGHV* genes in Chinese CLL patients. A preferable *IGHV* 4-59 gene usage was observed in *SF3B1*-mutated subjects (*Table 3*).

Prognostic impact of SF3B1 mutation

Patients with mutated *SF3B1* had inferior TFS than those with unmutated *SF3B1* (P=0.012), while no significant difference was seen in OS between the two groups (*Figure 1*). The 1- and 3-year TFS were 53.6% and 36.9% respectively, for *SF3B1*-unmutated patients *vs.* 36.4% and 10.9% respectively, for *SF3B1*-mutated patients.

The results of Cox regression analysis are presented in *Table 4*. Univariate Cox regression analysis showed that Rai I-IV or Binet B/C (P<0.001), ALC >50×10^9/L (P<0.001), PLT <100×10^9/L (P<0.001), Hb <100 g/L (P<0.001), ALB <40 g/L (P=0.001), LDH > upper limit of normal (ULN) (P<0.001), β2-MG >3.5 mg/L (P<0.001), CD38 ≥30% (P=0.015), ZAP70 ≥20% (P=0.046), *TP53* disruptions (P<0.001), *ATM* deletion (P=0.014), unmutated *IGHV* (P<0.001), mutated *NOTCH1* (P=0.001) as well as mutated *SF3B1* (P=0.020) had adverse impacts on TFS, and age >65 years (P=0.001), Rai I-IV or Binet B/C (P=0.005), PLT <100×10^9/L (P=0.011), Hb <100 g/L (P<0.001), ALB

Table 2 The relationships between *SF3B1* status and patients' clinical, cytogenetic, and molecular characteristics

Variables	*SF3B1* UM	*SF3B1* M	P value
Gender			
Male	244	17	0.229
Female	133	5	
Age			
>65 years	123	5	0.334
≤65 years	254	17	
Stage			
Rai I-IV or Binet B/C	335	20	1.000
Rai 0 or Binet A	42	2	
Lymphocytes			
$>50\times10^9$/L	82	4	0.897
$\leq50\times10^9$/L	295	18	
Platelets			
$<100\times10^9$/L	99	4	0.400
$\geq100\times10^9$/L	278	18	
Hemoglobin			
<100 g/L	77	5	1.000
≥100 g/L	300	17	
Albumin			
<40 g/L	133	5	0.229
≥40 g/L	244	17	
LDH			
>ULN	94	6	0.806
Normal	283	16	
TK-1			
> ULN	55	2	0.622
Normal	271	18	
β2-MG			
>3.5 mg/L	168	11	0.618
≤3.5 mg/L	209	11	
TP53 disruption			
Positive	88	6	0.673
Negative	289	16	
ATM deletion			
Positive	47	5	0.312
Negative	321	17	
Del (13q)			
Positive	103	5	0.684
Negative	181	11	
Trisomy 12			
Positive	71	3	0.904
Negative	252	14	
IGHV status			
M	213	8	0.035
UM	147	14	
NOTCH1 status			
M	26	4	0.138
UM	341	18	
MYD88 status			
M	31	0	0.327
UM	334	21	
CD38 (≥30%)			
Positive	99	10	0.049
Negative	259	11	
ZAP-70 (≥20%)			
Positive	144	15	0.005
Negative	214	6	

β2-MG, β2-microglobulin; *IGHV*, immunoglobulin heavy variable-region gene; LDH, lactate dehydrogenase; M, mutated; *SF3B1*, splicing factor 3b subunit 1; TK-1, thymidine kinase 1; *TP53*, tumor protein 53; ULN, upper limit of normal; UM, unmutated.

<40 g/L (P<0.001), LDH > ULN (P<0.001), β2-MG >3.5 mg/L (P<0.001), *TP53* disruptions (P<0.001), unmutated *IGHV* (P<0.001), and mutated *NOTCH1* (P<0.001) predicted shorter OS. Multivariate Cox regression analysis revealed that Rai I-IV or Binet B/C (P<0.001), ALC $>50\times10^9$/L (P=0.024), PLT $<100\times10^9$/L (P=0.028), β2-MG >3.5 mg/L (P=0.002), *TP53* disruptions (P=0.006), unmutated *IGHV* (P=0.012), and mutated *SF3B1*

Table 3 The association of *SF3B1* mutation and the 10 most used *IGHV* genes in Chinese chronic lymphocytic leukemia patients

IGHV gene	*SF3B1* M	*SF3B1* UM	P value
VH3-23			
Yes	0	38	0.211
No	22	318	
VH4-34			
Yes	2	34	1.000
No	20	322	
VH3-7			
Yes	0	30	0.311
No	22	326	
VH4-39			
Yes	2	23	0.968
No	20	333	
VH1-69			
Yes	2	21	0.882
No	20	335	
VH3-30			
Yes	2	27	1.000
No	20	329	
VH4-59			
Yes	4	12	0.010
No	18	344	
VH3-48			
Yes	2	11	0.171
No	20	345	
VH3-21			
Yes	0	9	1.000
No	22	347	
VH3-33			
Yes	0	13	1.000
No	22	343	

IGHV, immunoglobulin heavy variable-region gene; M, mutated; *SF3B1*, splicing factor 3b subunit 1; UM, unmutated.

(P=0.023) were independently associated with inferior TFS, and ALB <40 g/L (P=0.004), *TP53* disruptions (P=0.022), and unmutated *IGHV* (P=0.001) were independent prognostic factors for OS.

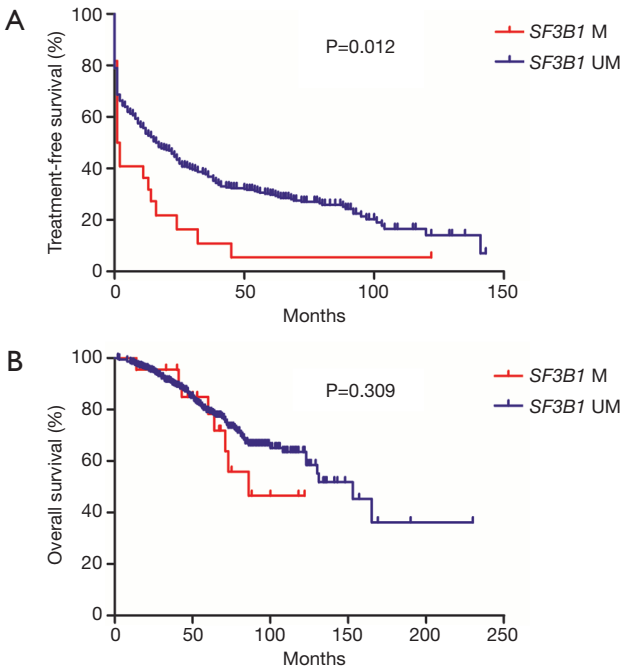


Figure 1 Kaplan-Meier curves of treatment-free survival (A) and overall survival (B) for different *SF3B1* mutation status. *SF3B1*, splicing factor 3b subunit 1; UM, unmutated.

Subgroup analysis of SF3B1 mutation

We divided patients into different subgroups according to age, *IGHV* mutation status, stage, *TP53* status, and β2-MG concentration, and analyzed the prognostic impact of *SF3B1* mutation in these subgroups (*Figure 2*). Worse TFS was observed in *SF3B1*-mutated patients in subgroups such as age ≤65 years old (P<0.001), *IGHV* unmutated (P=0.028), Rai I-IV or Binet B/C (P=0.002), *TP53* normal (P<0.001), and β2-MG ≤3.5 mg/L (P=0.019).

New risk models based on SF3B1 mutation and CLL-IPI

We developed two new risk models, CLL-IPI-S and CLL-PI, based on *SF3B1* mutational status and CLL-IPI (*Table 5*). CLL-IPI-S consisted of 6 adverse variables, including *TP53* disruptions, *IGHV* unmutated, β2-MG >3.5 mg/L, Rai I-IV or Binet B/C, age >65 years old, and *SF3B1* mutated. Because age was not associated with adverse TFS in Chinese CLL patients, in the second system, we replaced age with *SF3B1* mutation in CLL-IPI and named it CLL-PI. ROC

Table 4 Univariable and multivariate Cox regression analysis of treatment-free survival and overall survival

Characteristic	Treatment-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age >65 years	0.97 (0.76–1.24)	0.8			1.91 (1.29-2.82)	0.001	–	–
Rai I-IV or Binet B/C	6.46 (3.43–12.16)	<0.001	4.06 (1.96–8.38)	<0.001	7.52 (1.85–30.55)	0.005	–	–
Lymphocytes >50×10^9/L	1.76 (1.35–2.28)	<0.001	1.41 (1.05–1.90)	0.024	1.53 (0.98–2.39)	0.064		
Platelets <100×10^9/L	2.05 (1.60–2.63)	<0.001	1.41 (1.04–1.92)	0.028	1.70 (1.13–2.55)	0.011	–	–
Hemoglobin <100 g/L	1.84 (1.42–2.40)	<0.001	–	–	2.79 (1.86–4.20)	<0.001	–	–
Albumin <40 g/L	1.50 (1.19–1.90)	0.001	–	–	2.80 (1.89–4.14)	<0.001	1.95 (1.23–3.07)	0.004
LDH > ULN	1.91 (1.48–2.46)	<0.001	–	–	2.84 (1.92–4.22)	<0.001	–	–
TK-1 > ULN	1.29 (0.94–1.78)	0.112			1.37 (0.81–2.30)	0.239		
β2-MG >3.5 mg/L	2.45 (1.93–3.10)	<0.001	1.58 (1.19–2.11)	0.002	3.23 (2.11–4.93)	<0.001	–	–
CD38 ≥30%	1.37 (1.07–1.77)	0.015	–	–	1.34 (0.87–2.06)	0.185	–	–
ZAP70 ≥20%	1.27 (1.01–1.62)	0.046	–	–	1.31 (0.88–1.96)	0.184	–	–
TP53 disruption	1.71 (1.32–2.22)	<0.001	1.54 (1.13–2.09)	0.006	3.09 (2.08–4.60)	<0.001	1.72 (1.08–2.75)	0.022
ATM deletion	1.49 (1.08–2.04)	0.014	–	–	1.18 (0.67–2.07)	0.574	–	–
IGHV unmutated	2.04 (1.60–2.58)	<0.001	1.43 (1.08–1.90)	0.012	3.07 (2.04–4.62)	<0.001	2.24 (1.40–3.56)	0.001
NOTCH1 mutated	1.93 (1.30–2.86)	0.001	–	–	2.97 (1.77–4.96)	<0.001	–	–
MYD88 mutated	1.02 (0.67–1.54)	0.943	–	–	0.60 (0.25–1.49)	0.273	–	–
SF3B1 mutated	1.72 (1.09–2.72)	0.020	1.78 (1.08–2.93)	0.023	1.45 (0.70–3.00)	0.312	–	–

β2-MG, β2-microglobulin; CI, confidence interval; *IGHV*, immunoglobulin heavy variable-region gene; LDH, lactate dehydrogenase; HR, hazard ratio; *SF3B1*, Splicing factor 3b subunit 1; TK-1, thymidine kinase 1; *TP53*, tumor protein 53; ULN, upper limit of normal.

curve was conducted to analyze the power of CLL-IPI, CLL-IPI-S, and CLL-PI in predicting TFS of Chinese CLL patients. For 6-month TFS (*Figure 3A*), the AUC of CLL-IPI, CLL-IPI-S, and CLL-PI were 0.664, 0.669, and 0.676 (CLL-IPI *vs.* CLL-PI, P=0.055), respectively. For 12-month TFS (*Figure 3B*), the AUC of CLL-PI was significantly larger than CLL-IPI (0.717 *vs.* 0.702, P=0.011) and CLL-IPI-S (0.717 *vs.* 0.705, P=0.027). For overall TFS, CLL-PI was a superior predictor for TFS than CLL-IPI (AUC: 0.773 *vs.* 0.758, P=0.029) and showed better tendency when compared with CLL-IPI-S (AUC: 0.773 *vs.* 0.762, P=0.096) (*Figure 3C*).

We further validated the potency of CLL-IPI-S (low-risk 0–2; intermediate risk 3–5; high-risk 6–8; very high-risk 9–11) and CLL-PI (low-risk 0–1; intermediate risk 2–3; high-risk 4–7; very high-risk 8–10) for predicting TFS in Chinese CLL patients (*Table 5*). Both CLL-IPI-S and CLL-PI could significantly separate Chinese CLL patients into 4 groups as CLL-IPI (P<0.001). Median TFS of low, intermediate, high, and very high-risk patients were 69, 25, 11, and 1 month for CLL-IPI, 69, 12, 6, and 1 month for CLL-IPI-S, and 79, 25, 8, and 1 month for CLL-PI (*Figure 4*). P value of the Hosmer-Lemeshow test was 0.321, 0.630, and 0.243 for CLL-IPI, CLL-IPI-S, and CLL-PI, respectively.

Discussion

Mutations affecting alternative splicing pathway genes play important roles in the pathogenesis and progression of CLL (7). *SF3B1*, encoding an important splicing factor, is frequently mutated in 10–20% of newly diagnosed CLL patients according to previous reports (12). *SF3B1* mutation may lead to increased DNA damage, CLL-associated RNA alteration and Notch signaling activation through DVL2 alternative splicing, contributing to the poor prognosis of

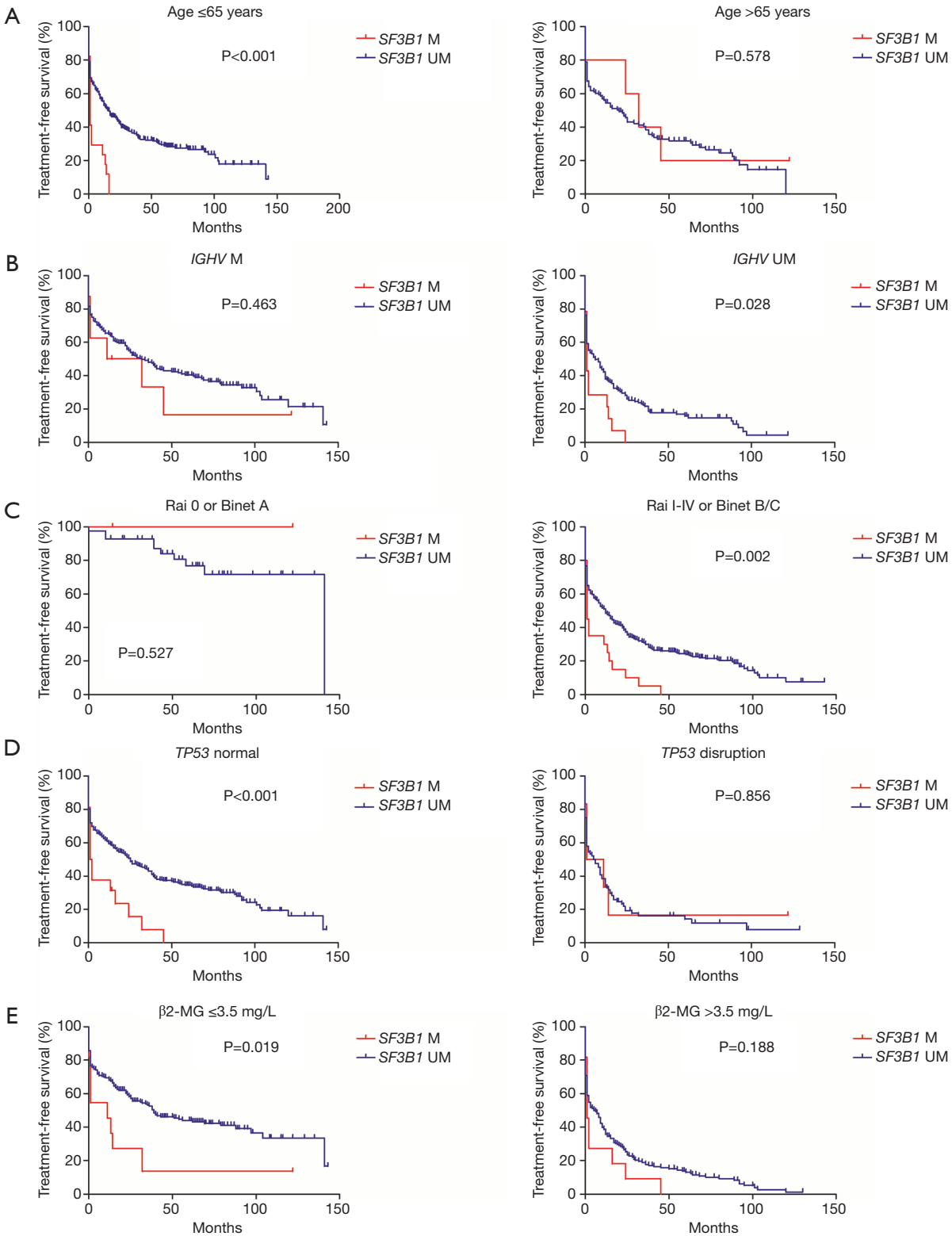


Figure 2 Kaplan-Meier curves of treatment-free survival for different *SF3B1* mutation status stratified by age (A), *IGHV* mutation status (B), stage (C), *TP53* status (D), and serum β2-MG concentration (E). β2-MG, β2-microglobulin; *IGHV*, immunoglobulin heavy variable region gene; M, mutated; *SF3B1*, splicing factor 3b subunit 1; *TP53*, tumor protein 53; UM, unmutated.

Table 5 Details of risk models for treatment-free survival

Model	Variables	Score	Risk stratification	Total score
CLL-IPI	*TP53* disruption	4	Low	0–1
	IGHV unmutated	2	Intermediate	2–3
	β2-MG >3.5 mg/L	2	High	4–6
	Rai I-IV or Binet B/C	1	Very high	7–10
	Age >65 years	1	–	–
CLL-IPI-S	*TP53* disruption	4	Low	0–2
	IGHV unmutated	2	Intermediate	3–5
	β2-MG >3.5 mg/L	2	High	6–8
	Rai I-IV or Binet B/C	1	Very high	9–11
	Age >65 years	1	–	–
	SF3B1 mutated	1	–	–
CLL-PI	*TP53* disruption	4	Low	0–1
	IGHV unmutated	2	Intermediate	2–3
	β2-MG >3.5 mg/L	2	High	4–7
	Rai I-IV or Binet B/C	1	Very high	8–10
	SF3B1 mutated	1	–	–

β2-MG, β2-microglobulin; CLL-IPI, chronic lymphocytic leukemia-international prognostic index; *IGHV*, immunoglobulin heavy variable-region gene; *SF3B1*, splicing factor 3b subunit 1; *TP53*, tumor protein 53.

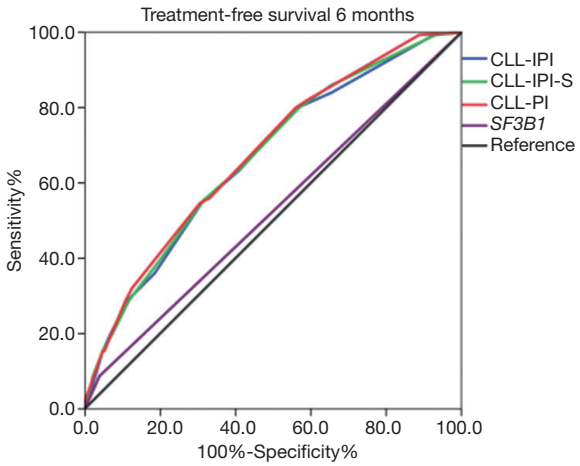
CLL patients harboring this mutation (11,16,17). In this study, we retrospectively analyzed the clinical, cytogenetic and molecular characteristics of 399 newly diagnosed Chinese CLL patients with different *SF3B1* mutational status, explored the effects of *SF3B1* mutation on their survival, and developed new risk models on the basis of CLL-IPI and *SF3B1* mutational status to better predict the TFS of newly diagnosed CLL patients.

The incidence of *SF3B1* mutation in Chinese CLL patients from our study was 5.5%, which was lower than that in patients from western countries. All mutations were missense, and the most common mutation was c.A2098G (p.K700E). Consistent with previous reports, *SF3B1* mutation mainly occurred in patients with unmutated *IGHV*, positive CD38, and positive ZAP-70. Strefford *et al.* (18), Rossi *et al.* (19), and Jeromin *et al.* (20) reported that *SF3B1* mutation showed a higher frequency in stereotyped *IGHV3-21*, *IGHV3-48*, and *IGHV1-69* users. However, *SF3B1* mutation in Chinese CLL patients was common in patients with *IGHV4-59*. Furthermore, in contrast to the results of western countries, *SF3B1* mutation could only predict adverse TFS but failed to predict unfavorable OS in our cohort. Through subgroup analysis, we found that *SF3B1* mutation could only represent adverse prognosis in subgroups such as age ≤65 years old, *IGHV* unmutated, Rai I-IV or Binet B/C, *TP53* normal and β2-MG ≤3.5 mg/L.

These differences could be due to the following reasons. First, the differences of the clinical, cytogenetic and molecular background between CLL patients from Asian and western countries. Second, Nadeu *et al.* (12) reported that the incidence of *SF3B1* mutation was 13% and nearly half the mutations were subclones with variant allele frequency <12%, which could not be detected by Sanger sequencing. Therefore, in our study, we missed some subclonal mutation due to Sanger sequencing, accounting for the lower prevalence of *SF3B1* mutation in our cohort.

ROC curve was conducted to compare the power of TFS prediction of three risk models. Although the improvement was minor, CLL-PI had larger AUC than CLL-IPI and CLL-IPI-S with a significant statistical difference. Moreover, the calibration of CLL-PI was fairly satisfactory. However, *SF3B1* mutation just represented adverse

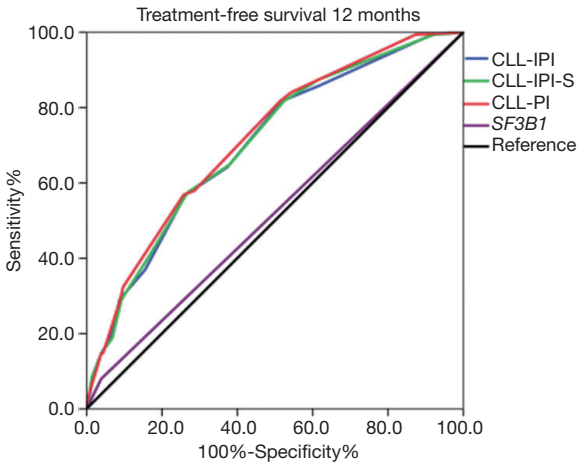
A



	AUC	SE	95%CI
CLL-IPI	0.664	0.029	0.614–0.711
CLL-IPI-S	0.669	0.028	0.619–0.716
CLL-PI	0.676	0.027	0.627–0.723
SF3B1	0.524	0.013	0.473–0.575

CLL-IPI *vs.* CLL-PI; P=0.055; CLL-IPI-S *vs.* CLL-PI; P=0.163.

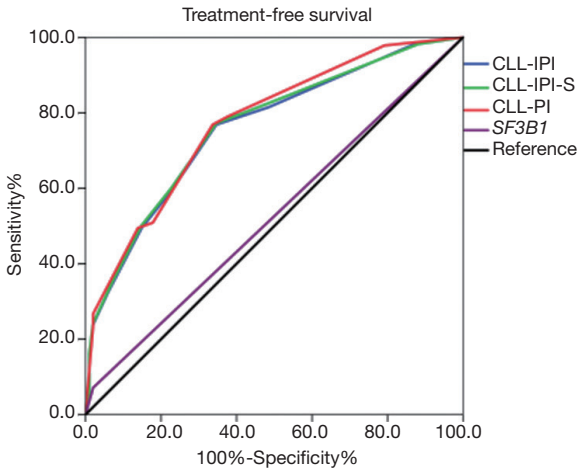
B



	AUC	SE	95%CI
CLL-IPI	0.702	0.0262	0.653–0.747
CLL-IPI-S	0.705	0.0261	0.657–0.751
CLL-PI	0.717	0.0253	0.669–0.761
SF3B1	0.520	0.0123	0.469–0.571

CLL-IPI *vs.* CLL-PI; P=0.011; CLL-IPI-S *vs.* CLL-PI; P=0.027.

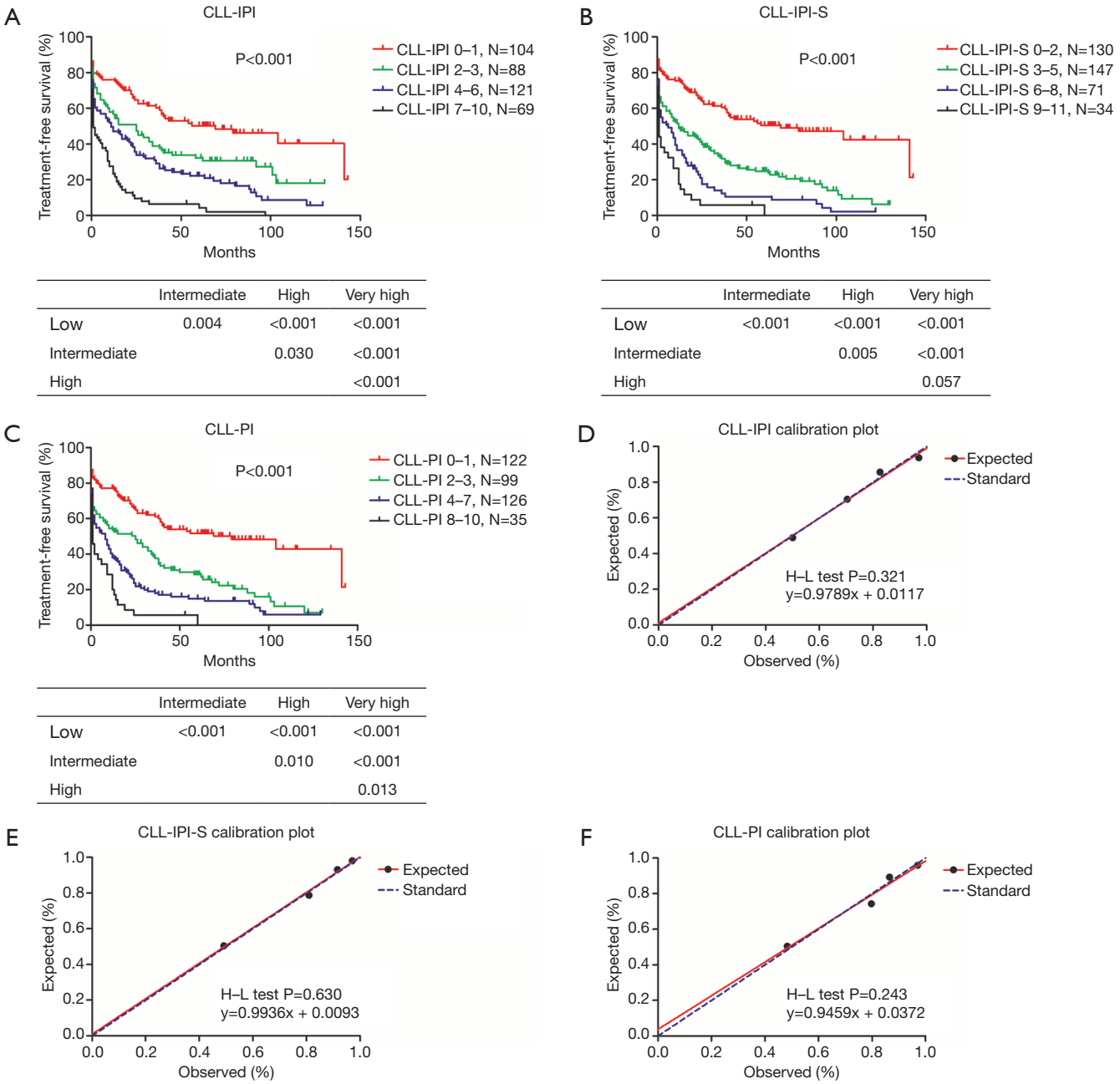
C



	AUC	SE	95%CI
CLL-IPI	0.758	0.0264	0.712–0.800
CLL-IPI-S	0.762	0.0262	0.716–0.804
CLL-PI	0.773	0.0257	0.728–0.814
SF3B1	0.526	0.0104	0.474–0.577

CLL-IPI *vs.* CLL-PI; P=0.029; CLL-IPI-S *vs.* CLL-PI; P=0.096.

Figure 3 Comparison of AUC among CLL-IPI, CLL-IPI-S, and CLL-PI in 6-month TFS (A), 12-month TFS (B), and overall TFS (C). AUC, area under the curve; CI, confidence interval; CLL-IPI, chronic lymphocytic leukemia-international prognostic index; SE, standard error; *SF3B1*, splicing factor 3b subunit 1; TFS, treatment-free survival.



	Intermediate	High	Very high
Low	0.004	<0.001	<0.001
Intermediate		0.030	<0.001
High			<0.001

	Intermediate	High	Very high
Low	<0.001	<0.001	<0.001
Intermediate		0.005	<0.001
High			0.057

	Intermediate	High	Very high
Low	<0.001	<0.001	<0.001
Intermediate		0.010	<0.001
High			0.013

Figure 4 Kaplan-Meier curves of treatment-free survival stratified by four CLL-IPI (A), CLL-IPI-S (B), and CLL-PI (C) risk grades. Calibration plot of CLL-IPI (D), CLL-IPI-S (E), and CLL-PI (F). CLL-IP, chronic lymphocytic leukemia-international prognostic index.

prognosis for TFS. Thus CLL-PI could only stratify Chinese patients in terms of TFS and had limited scope of application. Also, these risk models were developed based on a small Chinese cohort, and confirmation was needed in a larger cohort to estimate the repeatability of these models and judge the feasibility in patients from western countries.

So far, more than 13 studies have investigated the prognostic value of *SF3B1* mutation in CLL (21). Most of them were from western countries, and only two studies investigated Asian populations. Our study focused on the effects of *SF3B1* mutation on Chinese CLL patients, and the sample size was the largest among these Asian studies.

Furthermore, our study was the first to combine *SF3B1* mutation with CLL-IPI, and eventually improve the potency of CLL-IPI for predicting TFS in Chinese CLL patients.

In summary, we analyzed *SF3B1* mutation in 399 newly diagnosed Chinese CLL patients. The incidence of *SF3B1* mutation was 5.5% and was lower than that in previous studies from western countries. Also, *SF3B1* mutation could only predict shorter TFS. New risk models were established according to the CLL-IPI and *SF3B1* mutational status in order to better stratify Chinese patients in terms of TFS. Admittedly, there were some limitations in our study, such as small sample size, low-sensitivity sequencing, and short follow-up time. A larger study cohort and advanced next-generation sequencing technology are needed in order to identify the role of *SF3B1* mutation further and confirm the potency of CLL-PI in Chinese CLL patients.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the hospital ethics committee (2018-SRFA-087) of the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital.

References

1. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 2011;475:101-5.
2. Quijada-Alamo M, Hernandez-Sanchez M, Robledo C, et al. Next-generation sequencing and FISH studies reveal the appearance of gene mutations and chromosomal abnormalities in hematopoietic progenitors in chronic lymphocytic leukemia. J Hematol Oncol 2017;10:83.
3. Quijano S, Lopez A, Rasillo A, et al. Impact of trisomy 12, del(13q), del(17p), and del(11q) on the immunophenotype, DNA ploidy status, and proliferative rate of leukemic B-cells in chronic lymphocytic leukemia. Cytometry B Clin Cytom 2008;74:139-49.
4. Rasi S, Khiabanian H, Ciardullo C, et al. Clinical impact of small subclones harboring NOTCH1, SF3B1 or BIRC3 mutations in chronic lymphocytic leukemia. Haematologica 2016;101:e135-8.
5. Xia Y, Fan L, Wang L, et al. Frequencies of SF3B1, NOTCH1, MYD88, BIRC3 and IGHV mutations and TP53 disruptions in Chinese with chronic lymphocytic leukemia: disparities with Europeans. Oncotarget 2015;6:5426-34.
6. International CLL-IPI working group. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol 2016;17:779-90.
7. Fabbri G, Dalla-Favera R. The molecular pathogenesis of chronic lymphocytic leukaemia. Nat Rev Cancer 2016;16:145-62.
8. Obeng EA, Chappell RJ, Seiler M, et al. Physiologic Expression of Sf3b1(K700E) Causes Impaired Erythropoiesis, Aberrant Splicing, and Sensitivity to Therapeutic Spliceosome Modulation. Cancer Cell 2016;30:404-17.
9. Malcovati L, Papaemmanuil E, Bowen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood 2011;118:6239-46.
10. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61-70.
11. Wang L, Brooks AN, Fan J, et al. Transcriptomic Characterization of SF3B1 Mutation Reveals Its Pleiotropic Effects in Chronic Lymphocytic Leukemia. Cancer Cell 2016;30:750-63.
12. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. Blood 2016;127:2122-30.
13. Wu SJ, Lin CT, Agathangelidis A, et al. Distinct molecular genetics of chronic lymphocytic leukemia in Taiwan: clinical and pathogenetic implications. Haematologica 2017;102:1085-90.
14. Wang WT, Zhu HY, Wu YJ, et al. Elevated absolute NK

cell counts in peripheral blood predict good prognosis in chronic lymphocytic leukemia. J Cancer Res Clin Oncol 2018;144:449-57.
15. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837-45.
16. Agrawal AA, Seiler M, Brinton LT, et al. Novel SF3B1 in-frame deletions result in aberrant RNA splicing in CLL patients. Blood Adv 2017;1:995-1000.
17. Te Raa GD, Derks IA, Navrkalova V, et al. The impact of SF3B1 mutations in CLL on the DNA-damage response. Leukemia 2015;29:1133-42.
18. Strefford JC, Sutton LA, Baliakas P, et al. Distinct patterns of novel gene mutations in poor-prognostic stereotyped subsets of chronic lymphocytic leukemia: the case of SF3B1 and subset #2. Leukemia 2013;27:2196-9.
19. Rossi D, Bruscaggin A, Spina V, et al. Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. Blood 2011;118:6904-8.
20. Jeromin S, Weissmann S, Haferlach C, et al. SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. Leukemia 2014;28:108-17.
21. Zhang Z, Chen S, Chen S, et al. SF3B1 mutation is a prognostic factor in chronic lymphocytic leukemia: a meta-analysis. Oncotarget 2017;8:69916-23.

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Supplementary

Table S1 *SF3B1* mutation identified by Sanger sequencing in 399 Chinese CLL cases

Patient ID	Nucleotide change	Amino acid change	RefSeq
9	c.2221A>G	p.K741E	NM_001005526.2
20	c.2225G>A	p.G742D	NM_001005526.2
26	c.2098A>G	p.K700E	NM_001005526.2
32	c.2098A>G	p.K700E	NM_001005526.2
45	c.2225G>A	p.G742D	NM_001005526.2
56	c.2098A>G	p.K700E	NM_001005526.2
115	c.2223G>T	p.K741N	NM_001005526.2
121	c.1877A>T	p.N626I	NM_001005526.2
122	c.1877A>T	p.N626I	NM_001005526.2
127	c.2098A>G	p.K700E	NM_001005526.2
144	c.2098A>G	p.K700E	NM_001005526.2
171	c.2098A>G	p.K700E	NM_001005526.2
192	c.2098A>G	p.K700E	NM_001005526.2
219	c.1866G>T	p.E622D	NM_001005526.2
266	c.2098A>G	p.K700E	NM_001005526.2
277	c.2098A>G	p.K700E	NM_001005526.2
305	c.2098A>G	p.K700E	NM_001005526.2
326	c.2098A>G	p.K700E	NM_001005526.2
364	c.2219G>A	p.G740E	NM_001005526.2
375	c.2098A>G	p.K700E	NM_001005526.2
390	c.1996A>G	p.K666E	NM_001005526.2
392	c.2098A>G	p.K700E	NM_001005526.2

Table S2 Characteristics of patients with *SF3B1* mutation

Patient ID	Gender	Age	Concomitant alterations (11q/+12/ TP53/*NOTCH1*)*	*IGHV* mutational status	CD38 (%)	ZAP-70 (%)	Chromosome karyotype	Binet stage	TFS (mo)	OS (mo)
9	M	69	+12, *NOTCH1* M	UM	48	20.6	47,XY,+12[9]/46,XY[1]	C	24	41
20	M	78	11q-	M	0	22.3	46,XY,t(4;12)(q26;q23),del(11)(q22)[5]/46,XY[15]	A	32	75+
26	M	56	Normal	UM	0	17	Normal	B	2	43
32	M	47	*TP53* disruption	UM	0	30.2	NA	B	14	14
45	M	62	Normal	UM	56.3	29.3	43-45,XY,der(2)t(1;2),3q+,-11, -13,-14,+mar[5cp]	C	1	86
56	M	70	*NOTCH1* M	M	74.6	68.6	46,XY[3]	C	45	60
115	M	53	Normal	UM	0	0	46,XY,9p+[8]/46,XY[2]	C	1	47+
121	M	62	Normal	UM	32.1	32.1	46,XY,der(9)[2]/46,XY[8]	B	13	71
122	F	32	*TP53* disruption	M	32.1	32.1	NA	A	11	50+
127	F	55	11q-, +12, *TP53* disruption	UM	52	17	46,XX,der(10),der(11), -17,+mar[10cp]	C	1	73
144	M	55	Normal	UM	0	20.3	46,XY,17p-[7]/46,X, Yq-,2q+[1]/46,XY[2]	B	2	53+
171	F	55	Normal	M	78	0	46,XX,del(13)(q31[8])/46,XX[2]	A	14+	14+
192	M	70	Normal	UM	9	0	NA	B	0	40+
219	M	52	*TP53* disruption, *NOTCH1* M	UM	0	34	46,XY,11q-[2]/46,XY[18]	C	1	64
266	M	56	Normal	UM	62	63	Normal	A	1	68+
277	M	41	Normal	M	0	0	Normal	A	0	86+
305	M	54	11q-	M	12	10.4	Normal	B	1	88+
326	M	59	11q-	M	76	42	46,XY,del(11)(q21)[10]	B	1	118+
364	M	65	Normal	UM	85	20	46,XY,7p+[3]/46,XY,7p+, 11q-,13q-[5]/46,XY,4p+,7p+,11q-[1]/46,XY[1]	B	0	33+
375	M	78	*TP53* disruption	M	NA	NA	Normal	A	122+	122+
390	F	59	+12, *TP53* disruption, *NOTCH1* M	UM	0	33	47,XX,+12,-17,+mar[6]	B	0	100+
392	F	54	11q-	UM	17	NA	46,XX,del(6)(q10),+der(6)del(6)(q11),-19[5]/46,XX[5]	C	16	67+

*, genetic alterations detected by fluorescence *in situ* hybridization and/or sequencing. F, female; *IGHV*, immunoglobulin heavy variable region gene; M, male (gender); M, mutated; mo, months; NA, not available; OS, overall survival; *SF3B1*, splicing factor 3b subunit 1; TFS, treatment-free survival; *TP53*, tumor protein 53; UM, unmutated.