The clinical impact of IKZF1 mutation in acute myeloid leukemia

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Article

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Abstract

The genetic heterogeneity generates great challenges in the understanding and treating acute myeloid leukemia (AML), while the knowledge about *IKZF1* mutation in AML was extremely limited. Previously, we described the distribution pattern of *IKZF1* mutation in AML, but its clinical impact remained undefined due to limited cases. Herein, we devoted to answer this question in one relatively large cohort. Among 530 newly diagnosed AML patients, we identified 28 *IKZF1* mutations in 22 patients (22/530, 4.15%). *IKZF1*-mutated patients presented one young median morbid age (P=0.027) and the high frequency of mixed lineage leukemia involvement (P=0.012). In AML, *IKZF1* mutation showed significant co-occurrences with *CEBPA* (P<0.001), *SF3B1* (P=0.001), or *CSF3R* (P=0.008) mutation. Though *IKZF1*-mutated AML was more classified into the intermediate-risky group (P=0.005), it showed one inferior complete remission rate (P=0.041). Furthermore, AML with high variant allele frequency of *IKZF1* mutation showed one relatively shorter overall survival duration (P=0.047), and it was one independent factor for the increased risk of death (hazard ratio, 3.14; 95% CI, 1.27-7.75; P=0.013). In subgroup analysis, our results showed that *IKZF1* mutation conferred one poor therapeutic response and prognosis for *SF3B1*-mutated AML (P=0.002). Therefore, *IKZF1* mutation exhibited one unique distribution mode and essential prognostic role in AML.

Introduction

Acute myeloid leukemia (AML) is an aggressive hematological malignancy, and it exhibits one great genetic heterogeneity. Though the initiation and maintenance has been demonstrated associated with the common mutations (*NPM1*, *FLT3-ITD*, *CEBPA* mutations, et al.) or rearrangements (*PML-RARA*, *RUNX1-RUNX1T1*, *CBFβ-MYH11*, et al.) in a portion of AML, its pathogenesis still remained not fully understood. It is certain that additional recurrent genetic alterations with one relatively lower frequency are also involved in this process, and we focus on *IKZF1* mutation.

IKZF1, encodes IKAROS, belongs to the transcription factor family of zinc finger DNA-binding proteins, which also contains *IKZF2* (HELIOS), *IKZF3* (AIOLOS), *IKZF4* (EOS), and *IKZF5* (PEGASUS)[1]. The locus of *IKZF1* is mapped on the short arm of chromosome 7 at 7p12.2, in which 8 exons are contained, and one protein with the length of 519 amino acids is encoded. IKZF1 contains four zinc fingers at the N-terminal that directly bind to DNA at the core motif A/GGAAA and additional two zinc fingers at the C-terminal required for forming homo- and hetero-dimerization between different IKZF proteins. Except of full-length IKZF1 (IK1), at least 16 isoforms have been identified due to alternative splicing or intragenic deletion. The same as IK1, IK2 and IK3 retain the function of DNA-binding, while IK4 to IK6 loss this property and play one dominant negative role on full length IKZF1[2]. Functionally, IKZF1 plays a critical role in almost every steps of normal lymphoid differentiation, and maintains the lymphoid lineage ontogeny and homeostasis[3, 4]. Alterations involving *IKZF1* mainly include the deletion and mutation[5]. Up to now, the relationship between *IKZF1* alterations and acute leukemia remained partially unmasked.

Consistent with its important role in lymphoid system, *IKZF1* alterations frequently affected acute lymphoblastic leukemia (ALL)[6, 7], in which *IKZF1* deletion rather than *IKZF1* mutation was predominate. Furthermore, *IKZF1* alterations were preferable to distribute in Philadelphia chromosome (Ph)-positive ALL and Ph-like ALL[8–10], and they conferred one poor prognosis for these ALL subtypes[11, 12]. In contrast, *IKZF1* alterations were less studied in AML and available studies were limited. It has been reported that *IKZF1* deletion, caused by monosomy 7 or deletion of 7p, was recurrent in pediatric AML[13]. In addition, *IKZF1* mutation as well as monosomy 7 preferred to appear in *EVI1*-rearranged AML[14]. In our previous study, we described the distribution pattern of *IKZF1* mutation in one relatively small cohort of AML patients, and found that *IKZF1* and *SF3B1* mutations were one pair of concomitant mutations in AML[15]. Though *IKZF1* alterations-related studies have been accumulated in AML, the distribution pattern of *IKZF1* mutation was incomplete due to limited cases, and its prognostic role remained uninvestigated. In this study, we addressed these questions and defined the clinical impact of *IKZF1* mutation in AML with one comprehensive view in one relatively large cohort.

Materials And Methods

Patients

From 01/01/2010 to 31/08/2020, 530 newly diagnosed AML patients, 265 from The First Affiliated Hospital to Zhejiang University School of Medicine while 265 patients from Changhai Hospital, were involved in this study, retrospectively. Among them, 500 were diagnosed as *de novo* AML, 22 were secondary/therapy-related AML, and 8 were mixed lineage leukemia (MLL). Acute promyelocytic leukemia (APL) was excluded from this study. The diagnosis criterion for AML was according to the 2016 revision World Health Organization classification of myeloid neoplasms and acute leukemia[16]. The genetic-based risky stratification was referred to the 2017 European Leukemia Net (ELN) recommendations for diagnosis and management of AML in adults[17]. For these patients, the treatment strategy was adapted as below: As to young AML patients, IA (idarubicin, cytarabine), DA (doxorubicin, cytarabine), or HAA (homoharringtonine, aclacinomycin, cytarabine)-based standard chemotherapy was adapted as induction therapy; three cycles of high-dose or intermediate-dose cytarabine combined with another four cycles of standard chemotherapy as consolidation therapy for low-risk and intermediate-risk patients; standard chemotherapy combined with hematopoietic stem cell transplantation (HSCT) as consolidation therapy for high-risk patients; HSCT was also applied for intermediate-risk patients when it was strongly requested for patients. As to elderly AML patients, AZA + HAG (azacitidine, G-CSF, low dose of homoharringtonine and cytarabine), AZA + VEN (azacitidine, venetoclax), GHAA (aclacinomycin, G-CSF, low dose of homoharringtonine and cytarabine), or CAG (G-CSF, low dose of cytarabine and aclacinomycin) regimen was adapted as induction and consolidation therapy. The standard of complete remission (CR) and relapse was referred to national comprehensive cancer network (NCCN) (2021.V3) guideline for AML. The last follow-up for these patients was conducted at 31/01/2021. Overall survival (OS) duration was calculated from the date of diagnosis to the date of death. Relapse free survival (RFS) duration was calculated from the date of CR to the date of relapse. Written consents were obtained from all

patients following the Declaration of Heisinki, and the study was approved by the ethics committee of The First Affiliated Hospital to Zhejiang University School of Medicine and Changhai Hospital.

Targeted Exome Sequencing

Genomic DNA was isolated from bone marrow diagnostic samples. A targeted sequencing gene panel, including 185 genes, was specifically used for next-generation sequencing at Acornmed Biotechnology Co. Ltd (Tianjin, China). Multiplex libraries were sequenced using NovaSeq instrument (Illumina). To filter raw variant results, the following criteria were used: average effective sequencing depth on target per sample ≥ 1,000x; mapping quality ≥ 30; and base quality ≥ 30; variant allele frequency (VAF) ≥ 1% for single nucleotide variations (SNVs), insertions, or deletions (InDels). Alignment of the trimmed reads was performed using Burrows-Wheeler alignment (BWA, version 0.7.12). PCR duplicates were marked using the MarkDuplicates tool from Picard. IndelRealigner and BaseRecalibrator from Genome Analysis Toolkit (GATK; version 3.8) were applied for realignment and recalibration of the BWA data, respectively. Variant calling, including SNVs and InDels, was performed in Mutect2. ANNOVAR software was used to annotate all the variants including 1000G projects, COSMIC, SIFT, and PolyPhen.

Statistical analysis

Statistical analyses were carried out using R (version 3.5.1). Mann-Whitney U test was used for continuous variables. Chi-square test or Fisher's exact test was used for categorical variables with adjustment for multiple testing using the Benjamini-Hochberg method. For analyzing the associations between different fusions and mutations, the false discovery rate correction was applied. Survival analysis was performed by the Kaplan-Meier method and differences assessed by the log-rank test. Cutoff point of the VAF of *IKZF1* mutation was determined by maximally selected log-rank statistics. Variables with $P < 0.2$ by univariate analysis were entered into a multivariate analysis using a Cox proportional hazards model to identify the statistically integrate known clinical and genetic risk factors and potential confounders. A two-sided $P < 0.05$ was considered statistically significant.

Results

The clinical features of IKZF1-mutated AML

In total, 530 newly diagnosed AML patients were involved in our study, and the mutational study was displayed for all of them **(Figure S1)**. *IKZF1* mutation was identified in 22 patients (4.15%, 22/530) **(**Table 1**)**. Though no bias of distribution in gender was found in *IKZF1*-mutated ($IKZF1^{MUT}$) and *IKZF1*-wild type ($IKZF1^{WT}$) group, AML patients with $IKZF1^{MUT}$ showed one relatively young median age compared to those with $IKZF1^{WT}$ (42.5 years of $IKZF1^{MUT}$ vs. 50 years of $IKZF1^{WT}$, P = 0.027). *IKZF1* mutation preferred to affect *de novo* AML (19/22, 86.4%), while the frequency of MLL in $IKZF1^{MUT}$ group (2/22, 9.1%) was significantly higher than it in $IKZF1^{WT}$ group (6/508, 1.2%) (P = 0.012). Though AML with *IKZF1* mutation mostly presented the morphologic subtype of AML-M2 (7/22, 31.8%), M4 (5/22, 22.7%), or M5 (5/22, 22.7%), no imbalanced distribution was found according to French-American-British (FAB) classification between two groups. In addition, there were no significant differences in the level of white blood cell (WBC) (P = 0.171), hemoglobin (HB) (P = 0.656), platelet (PLT) (P = 0.887) as well as the percentage of BM blast (P = 0.339) between $IKZF1^{MUT}$ and $IKZF1^{WT}$ groups **(**Table 2**)**. Therefore, besides of younger morbid age and high frequency of MLL involvement, the basic clinical presentation of $IKZF1^{MUT}$ AML patients was similar to $IKZF1^{WT}$ patients.

Table 1
IKZF1-mutated AML patients in our cohort

UPN	Age/Sex	Diagnosis	Karyotype	*IKZF1* mutation			Other genetic mutation
				MutFreq	cHGVS	pHGVS	
1	60/M	*de novo* AML	46,XY	0.01	c.1233delC	p.L411fs	*MPL, CEBPA, CCDC168*
				0.20	c.1054dupA	p.H351fs	
2	52/F	*de novo* AML	46,XX,t(8;21)	0.02	550C > T	p.R184W	*NRAS, KRAS, ASXL2*
3	45/M	*de novo* AML	46,XY	0.45	c.49_50insGC	p.S17fs	*NOTCH1, DNMT3A, CSF3R*
4	30/F	*de novo* AML	46,XX	0.03	c.1505_1511del	p.R502fs	*CEBPA*
				0.02	637C > T	p.R213X	
5	23/M	*de novo* AML	46,XY,9q-	0.44	c.663delG	p.E221fs	*SRCAP, FLT3-ITD, CEBPA*
6	52/F	*de novo* AML	45–47,XX,der(1),der(5),7p-,+8,r(12),-13,13q-,-15,18q-,-22,+20q-[cp18]/46,XX[2]	0.23	476A > G	p.N159S	*TP53*
7	55/F	*de novo* AML	46,XX	0.40	c.909_910del	p.N303fs	*NRAS, KIT, CEBPA*
8	34/F	*de novo* AML	NA	0.04	c.253_260del	p.L85fs	*KRAS, KIT, FLT3-ITD, CSF3R, CEBPA*
9	40/M	*de novo* AML	46,XY,t(11;19)	0.46	573C > A	p.H191Q	*WT1, TET2, NRAS, KIT, FAT1, CEBPA*
10	23/M	*de novo* AML	46,XY	0.06	815C > T	p.A272V	*STAG2, NRAS, NF1*
11	28/F	*de novo* AML	45,X,-X	0.11	484C > T	p.R162W	*NRAS, KIT, EZH2, CSF3R, CEBPA*
12	60/F	MLL	46,XX[20]	0.70	c.331C > T	p.R111X	*NRAS, IDH2, DNMT3A*
13	52/F	*de novo* AML	46,XX[20]	0.43	476A > G	p.N159S	*SF3B1, PTPN11, ETNK1*
14	61/M	*de novo* AML	47,XY,+3(q21)[20]	0.38	c.214G > T	p.E72X	*SF3B1, PTPN11, FLT3, BCOR*
				0.42	c.1150delT	p.S384fs	
15	45/M	*de novo* AML	46,XY,der(3)(q27)[10]	0.45	c.184_185insAA	p.Q62fs	*SF3B1, PTPN11*
				0.47	550C > T	p.R184W	
16	24/M	*de novo* AML	46,XY,del(8)(q22){5}/46,XY{5}	0.02	427C > T	p.R143W	*WT1, CEBPA*
				0.03	637C > T	p.R213X	
17	34/F	*de novo* AML	46,XX[20]	0.20	c.336delinsGCCCG	p.L112fs	*WT1, CTCF, CSF3R, CEBPA*

M, male; **F**, female; **AML**, acute myeloid leukemia; **MLL**, mix lineage leukemia; **s/t-AML**, secondary/therapy-related AML; **NA**, not available.

UPN	Age/Sex	Diagnosis	Karyotype	*IKZF1* mutation			Other genetic mutation
				MutFreq	cHGVS	pHGVS	
18	50/M	s/t-AML	46,XY[20]	0.04	c.637C > T	p.R213X	*WT1, TET2, SF3B1, GATA2, DNMT3A*
19	21/M	MLL	46,XY[20]	0.23	c.472G > A	p.G158S	*GATA2, CEBPA, CCND3*
20	23/M	*de novo* AML	46,XY[20]	0.49	472G > A	p.G158S	*RUNX1, GATA2, CEBPA*
21	15/F	*de novo* AML	46,XX,-2,-6,-11,del(13)(q13q22), +3mar[6]/46,XX[4]	0.03	c.520A > C	p.K174Q	*CCND3*
22	66/F	*de novo* AML	46,XX[20]	0.01	c.472G > A	p.G158S	/
				0.14	c.482T > G	p.L161R	

M, male; **F**, female; **AML**, acute myeloid leukemia; **MLL**, mix lineage leukemia; **s/t-AML**, secondary/therapy-related AML; **NA**, not available.

Table 2
The baseline characteristics of our AML cohort.

Characteristic	*IKZF1*^{WT} group	*IKZF1*^{MUT} group	P
N, % of total	508, 95.8%	22, 4.2%	
Age (years)	50 (11–82)	42.5 (15–66)	0.027
Gender			
Male (N)	284 (55.9%)	11 (50%)	0.585
Female (N)	224 (44.1%)	11 (50%)	
Peripheral blood			
White blood cells (10^9/L)	11.35 (0.4-484.8)	17.72 (1.67-428.01)	0.171
Hemoglobin (g/L)	83 (2-204)	87.5 (57–148)	0.656
Platelets (10^9/L)	51 (2-565)	59 (7-917)	0.887
Bone marrow blasts (%)	59 (11.5–98)	61.9(20–96)	0.339
Diagnosis (N)			
De novo AML	481 (94.7%)	19 (86.4%)	0.012
Mixed lineage leukemia	6 (1.2%)	2 (9.1%)	
Secondary/therapy-related AML	21 (4.1%)	1 (4.5%)	
French-American-British (N)			
M0	21 (4.1%)	2 (9.1%)	0.728
M1	27 (5.3%)	1 (4.5%)	
M2	170 (33.5%)	7 (22.7%)	
M4	101 (19.9%)	5 (22.7%)	
M5	161 (31.7%)	5 (22.7%)	
M6	11 (2.2%)	0 (0%)	
M7	1 (0.2%)	0 (0%)	
Undefine	16 (3.1%)	2 (9.1%)	
Cytogenetics (N)			
Normal karyotype	250 (49.2%)	12 (54.5%)	0.886
Complex karyotype	44 (8.7%)	2 (9.1%)	0.736
Monosomal karyotype	16 (3.1%)	0 (0%)	0.793
-5/5q-/monosomy 5	21 (4.1%)	0 (0%)	0.637
-7/monosomy 7	17 (3.3%)	0 (0%)	0.758
-17/17p abnormalities	11 (2.2%)	0 (0%)	0.989
Chromosome 3 abnormalities	16 (3.1%)	2 (9.1%)	0.417
Gene fusions (N)			
RUNX1-RUNX1T1	65 (12.8%)	1 (4.5%)	0.376
CBFB-MYH11	36 (7.1%)	0 (0%)	0.365
BCR-ABL1	10 (2.0%)	0 (0%)	0.489
KMT2A rearrangements	19 (3.7%)	0 (0%)	0.712
European Leukemia Net 2017 (N)			
Low	151 (29.7%)	3 (13.6%)	0.005
Intermediate	214 (42.1%)	17 (77.3%)	
High	143 (28.2%)	2 (9.1%)	

Characteristic	*IKZF1*^{WT} group	*IKZF1*^{MUT} group	P
Complete remission (N)	400 (84.6%)	15 (68.2%)	0.041
No complete remission (N)	73 (15.4%)	7 (31.8%)	

The genetic features of IKZF1-mutated AML

In our cohort, 28 *IKZF1* mutations were identified in 22 patients. In detail, 25 mutations were identified in 19 *de novo* AML patients (19/22, 86.4%), 2 mutations were in 2 MLL patients (2/22, 9.1%), and 1 mutation was in 1 secondary/therapy-related AML patient (1/22, 4.5%). Among those mutations, 13 were missense mutation, 5 were nonsense mutation, and 10 were frame-shift mutation. Missense mutation preferred to localize at the exon 5 (92.31%, 12/13), which influenced the DNA-binding of IKZF1, while only one affected the exon 7 (7.69%, 1/13). 40% (6/15) of nonsense mutation and frame-shift mutation disrupted the DNA-binding domain and lost the dimerization domain, while 60% (9/15) of them only disrupted the dimerization domain. Both of the DNA binding domain and the dimerization domain were required for the functional integrity of IKZF1, so all of above mutations possibly impaired its transcription activity (Fig. 1A **and** Table 1).

Cytogenetic aberrations were common in AML, and then we analyzed the relationship between *IKZF1* mutation and them. Our results showed that normal karyotype (P = 0.886), complex karyotype (P = 0.736), monosomal karyotype (P = 0.793), -5/5q-/monosomy 5 (P = 0.637), and − 17/17p abnormalities (P = 0.989) did not preferred to co-occur with *IKZF1* mutation significantly. -7/monosomy 7 led to *IKZF1* haploid-insufficiency, and no *IKZF1* mutation affected those patients, indicating that *IKZF1* mutation possibly impaired the function of IKZF1^{WT}, and did not need to disrupt the WT allele by loss of chromosome 7. *IKZF1* mutation preferred to locate in AML with chromosome 3 abnormalities, but only two *IKZF1*^{MUT} patients were found in this sub-group and no significant distribution preference of *IKZF1* mutation was exhibited (P = 0.417). At the same time, *IKZF1* mutation also showed rare co-occurrence with common gene fusion in AML, such as *RUNX1-RUNX1T1*, *CBFβ-MYH11*, *BCR-ABL1*, or *KMT2A* rearrangement, while only one *IKZF1*^{MUT} and *RUNX1-RUNX1T1*-positive patient was identified in our cohort (Table 2). Therefore, the partner of *IKZF1* mutation, which contributed to leukemogenesis, was not such cytogenetic aberrations.

Then, we analyzed the relationship of *IKZF1* mutation with other gene mutations. *IKZF1*^{MUT} significantly exhibited more preferable to co-occur with *CEBPA* (P < 0.000), *SF3B1* (P = 0.001), and *CSF3R* (P = 0.008) mutation than *IKZF1*^{WT}. Besides, *NRAS* (P = 0.645), *WT1* (P = 0.260), *GATA2* (P = 0.093), *KIT* (P = 0.638), *EZH2* (P = 0.333), *PTPN11* (P = 0.648), *DNMT3A* (P = 0.670), and *TET2* (P = 0.987) mutations were exhibited to co-occur with *IKZF1* mutation, but no preferable bias was found between *IKZF1*^{MUT} and *IKZF1*^{WT} groups. In contrast, *IKZF1* mutation seemingly exhibited mutually exclusion with *NPM1* (P = 0.063), *ASXL1* (P = 0.171), *IDH1* (P = 0.232), *IDH2* (P = 0.576), *TP53* (P = 0.730) *SRSF2* (P = 1) or *U2AF1* (P = 0.616) mutation though without significance (Fig. 1B and 1C). So, our results strongly indicated that the possible cooperation of *IKZF1* mutation with *CEBPA*, *SF3B1* or *CSF3R* mutation existed in AML pathogenesis.

The prognostic role of IKZF1 **mutation in AML**

ELN 2017 prognostic stratification well predicted the clinical outcome of AML patients[17]. Though compared to *IKZF1*^{WT} group, *IKZF1*^{MUT} group showed one high frequency of patients belonging to ELN-intermediate-risk group (77.3% of *IKZF1*^{MUT} vs. 42.1% of *IKZF1*^{WT}), and low frequency of ELN-low-risk (13.6% of *IKZF1*^{MUT} vs. 29.7% of *IKZF1*^{WT}) and ELN-high-risk group (9.09% of *IKZF1*^{MUT} vs. 28.1% of *IKZF1*^{WT}) (P = 0.005), the rate of CR in *IKZF1*^{MUT} group was significantly more inferior to it in *IKZF1*^{WT} group (68.2% of *IKZF1*^{MUT} vs. 84.6% of *IKZF1*^{WT}, P = 0.041) under our treatment strategy (Table 2). To further determine the prognostic role of *IKZF1* mutation in AML, we compared the OS as well as RFS for *IKZF1*^{MUT} and *IKZF1*^{WT} group. Our results indicated that *IKZF1*^{MUT} patients showed similar OS (P = 0.76) and RFS (P = 0.64) with *IKZF1*^{WT} patients (Fig. 2A and 2B). Though *IKZF1* mutation conferred one disadvantaged therapeutic response for AML patients in our cohort, overall, it finally did not influence their survival duration.

To interpret the contrast phenomena and define the prognostic role of *IKZF1* mutation further, we displayed the subgroup analysis for *IKZF1*^{MUT} patients according to the VAF, the type of mutation, or the mutational count in single patient. In VAF-based stratification, *IKZF1*^{MUT} patients with high VAF (> 0.230) exhibited one lower CR rate than *IKZF1*^{MUT} patients with low VAF (≤ 0.230), though without significance **(Table S1)**. Notably, *IKZF1*^{MUT} patients with high VAF showed one significantly inferior OS compared to those with low VAF or *IKZF1*^{WT} (P = 0.047), but their RFS did not exhibit the statistic difference (P = 0.4) (Fig. 2C and 2D). However, the type of mutations, missense mutation or frame-shift mutation as well as nonsense mutation, did not influence the OS (P = 0.95) and RFS (P = 0.9) of *IKZF1*^{MUT} patients **(Figure S1C and S1D)**, while the mutational count in single patient, 1 or 2, also exhibited no significance in OS (P = 0.93) and RFS (P = 0.28) **(Figure S1E and S1F)**. Therefore, high burden of *IKZF1* mutation predicated one poor prognostic role in AML.

Univariate And Multivariate Analysis For Overall Survival Duration

To assess the true contribution of high burden of *IKZF1* mutation to the poor prognosis of AML, we displayed univariate and multivariate analysis for OS, in which the baseline characteristics and genetic alterations were included. In univariate analysis, we identified 17 factors influencing OS of our AML cohort significantly, including *IKZF1* mutation with high VAF. When they were submitted to multivariate analysis, our results strongly indicated that *IKZF1* mutation with high VAF was one independent risky factor for the death of AML (hazard ratio [HR], 3.14; 95% CI, 1.27–7.75; P = 0.013). In the baseline characteristics, advanced age and high WBC count predicted increased risk of death, while bone marrow transplantation predicted the decreased risk. In the genetic alterations, core-binding factor rearrangement, *IDH2* mutation, and *bi-allele CEBPA* mutation predicated one relatively good prognosis, while

DNMT3A mutation, *TP53* mutation, and aberrant karyotype exhibited the inverse effect on the OS of AML (Table 3). Thus, our results supported that the independent role of high burden of *IKZF1* mutation on the poor OS of AML.

Table 3
Univariate and multivariate analysis for overall survival duration

Variable	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
ELN adverse risk	1.72	1.19–2.47	0.00000001	-	-	-
TP53 mutation	2.70	1.39–5.21	0.000004	1.98	1.20–3.28	0.008
CBF-AML	0.37	0.26–0.52	0.00002	0.40	0.23–0.68	0.00065
Advanced age	2.01	1.32–3.04	0.000046	1.64	1.13–2.39	0.010
Aberrant karyotype	1.94	1.18–3.18	0.0007	2.11	1.34–3.32	0.0013
High WBC count	1.65	1.20–2.28	0.0019	1.97	1.38–2.80	0.00017
DNMT3A mutation	1.73	1.11–2.70	0.0032	1.59	1.05–2.41	0.030
BMT	0.60	0.42–0.84	0.0078	0.56	0.36–0.85	0.0068
MLL	2.20	0.88–5.50	0.013	1.85	0.94–3.65	0.078
KRAS mutation	1.75	0.99–3.10	0.016	1.60	0.95–2.68	0.076
ASXL2 mutation	0.49	0.31–0.78	0.020	0.55	0.27–1.13	0.103
BCOR mutation	1.73	0.92–3.26	0.030	1.19	0.69–2.04	0.539
IKZF1 mutation with high VAF	2.38	0.70–8.17	0.031	3.14	1.27–7.75	0.013
Bi-allele CEBPA mutation	0.53	0.34–0.85	0.034	0.46	0.24–0.87	0.017
SF3B1 mutation	2.03	0.65–6.35	0.082	1.73	0.68–4.41	0.248
SRCAP mutation	0.55	0.32–0.95	0.095	0.56	0.27–1.17	0.124
IDH2 mutation	0.65	0.38–1.12	0.191	0.39	0.20–0.79	0.009

ELN, European Leukemia Net; **CBF**, core-binding factor; **WBC**, white blood cells; **BMT**, bone marrow transplantation; **MLL**, mixed lineage leukemia.

The prognostic role of IKZF1 mutation in specific genetic AML subtypes

To analyze the prognostic role of *IKZF1* mutation in some genetic subtype of AML, we further compared the clinical outcome between *CSF3R*, *CEBPA* or *SF3B1*-mutated patients with or without *IKZF1* mutation due to their recurrent co-existences. In our cohort, *CSF3R* mutation did not confer one significant difference in the OS (P = 0.95) or RFS (P = 0.21) of AML **(Figure S2A and S2B)**, and *IKZF1* mutation also did not influence the OS (P = 0.92) or RFS (P = 0.35) of $CSF3R^{WT}$ or $CSF3R^{MUT}$ patients (Fig. 3A and 3B, **Table S2)**. *Bi-allele CEBPA* mutation conferred one good prognostic role for AML patients[18–21]. Consistent with it, AML patients in our cohort with *bi-allele CEBPA* mutation showed one extremely good OS compared to those without it (P = 0.034), though RFS was similar between two subgroups (P = 0.23) **(Figure S2C and S2D)**. In $IKZF1^{MUT}$ patients, *bi-allele CEBPA* mutation (11/13, 84.6%) was far more than *single-allele CEBPA* mutation (2/13, 15.4%). Interestingly, *IKZF1* mutation conferred one relatively lower CR rate in $CEBPA^{WT}$/*single-allele* $CEBPA^{MUT}$ group rather than *bi-allele* $CEBPA^{MUT}$ group **(Table S3)**. However, both of $CEBPA^{WT}$/*single-allele* $CEBPA^{MUT}$ and *bi-allele* $CEBPA^{MUT}$ AML showed similar OS (P = 0.052) and RFS (P = 0.65) between with or without *IKZF1* mutation in survival analysis (Fig. 3C and 3D). Besides, *SF3B1* mutation also showed no influence on the OS (P = 0.082) or RFS (P = 0.65) of our patients **(Figure S2E and S2F)**. Strikingly, $IKZF1^{WT}$/ $SF3B1^{MUT}$ AML patients exhibited one low CR (50%, P = 0.33), while the therapeutic response was even worse in $IKZF1^{MUT}$/ $SF3B1^{MUT}$ AML and none of them achieved CR all the course (0%, P < 0.001) **(Table S4)**. Consistently, *IKZF1* mutation combined with *SF3B1* mutation conferred on extremely poor OS on AML (P = 0.002), but the RFS of $IKZF1^{MUT}$/ $SF3B1^{MUT}$ AML patients was unavailable due to no CR achievement (P = 0.846) (Fig. 3E and 3F). Thus, *IKZF1* mutation conferred one poor prognosis when combined with *SF3B1* mutation.

Discussion

IKZF1 mutation was one rare mutation in AML, and its frequency was 4.15% (22/530) in this newly diagnosed AML cohort. In previous report, our results showed that the frequency of *IKZF1* mutation was 2.59% (5/193) in one small AML cohort[15], while it was in 1.35% (8/593), 0.5% (1/200), or 4.21% (4/95) in OHSU[22], TCGA[23], or TARGET[24] study, respectively. Interestingly, our study showed one relatively high frequency of *IKZF1* mutation compared to reports from foreign countries, especially in this cohort. This phenomenon was possibly attributed to two reasons: First, the difference existed in the genetic background of AML from different races; second, the depth of sequencing has been advanced in recent years, which was revealed that many *IKZF1*

mutations with low VAF (≤ 5%) were also identified in our cohort. *IKZF1* deletion, including monosomy 7 and focal deletion of 7p, also impaired the function of IKZF1, which was similar to *IKZF1* mutation[13]. In one published report, the frequency of *IKZF1* deletion was 3.75% (11/293) in AML[13], while the frequency was not obviously higher than it of *IKZF1* mutation, which was also revealed by the low frequency of -7/monosomy 7 in our cohort (3.20%, 17/530). Though the frequency of *IKZF1* mutation in ALL was comparable to it in AML, the frequency of *IKZF1* deletion in ALL was much higher than it in AML[8]. As these results indicated, *IKZF1* deletion was the dominate alteration of *IKZF1* in ALL, in contrast, *IKZF1* mutation as well as *IKZF1* deletion was equally dominate in AML. Therefore, *IKZF1* mutation was one main genetic alteration involving *IKZF1*, and it was also one non-negligible gene mutation in AML.

In this study, we identified 28 *IKZF1* mutations in total. According to the consequences of encoded IKZF1 protein, we divided these mutations into three main mutational subtypes: The first subtype was nonsense or frame-shift mutations leading to loss of DNA-binding domain and dimerization domain, such as $IKZF1^{S17fs}$, $IKZF1^{E72X}$, $IKZF1^{L85fs}$, et al; the second subtype was nonsense or frame-shift mutations leading to only loss of dimerization domain, such as $IKZF1^{N303fs}$, $IKZF1^{S384fs}$, $IKZF1^{R502fs}$, et al; the third one was missense mutations involving DNA-binding domain, such as $IKZF1^{G158S}$, $IKZF1^{N159S}$, $IKZF1^{R184W}$, et al. Both DNA-binding domain and dimerization domain of IKZF1 were required for its action as one transcriptional factor, so the functional consequences of subtype one and two mutations were relatively clear, and it could be attributed to direct loss of functionally important domains. However, the impact of subtype three mutation on IKZF1 function was mostly unrevealed and highly various. In this study, we identified 13 missense mutations of *IKZF1*. Interestingly, most of these mutations involved DNA-binding domain localization on the exon 5 of *IKZF1* (92.30%, 12/13), while no one involved the dimerization domain, and one involved the connected region between DNA-binding domain and dimerization domain on the exon 7. Consistent with the distribution pattern, only one missense mutation on the dimerization domain was identified in our previous study. However, it was not the same in ALL, in which the frequencies of DNA-binding domain and dimerization domain involvements by *IKZF1* missense mutation were relatively balanced[8]. Therefore, *IKZF1* missense mutation in AML seemingly preferred to affect the DNA-binding domain, which was quite in contrast to it in ALL. It has been reported that $IKZF1^{G158S}$ and $IKZF1^{N159Y}$ were the hotspot mutation in ALL[8], while $IKZF1^{N159S}$ was in AML[15]. In our study, recurrent $IKZF1^{G158S}$ and $IKZF1^{N159S}$ were also identified in 3 and 2 patients, respectively. Besides, recurrent $IKZF1^{R184W}$ was identified in 2 patients from our study, while $IKZF1^{R184Q}$ has been reported in ALL. Notably, several *IKZF1* missense mutations on DNA-binding domain, including $IKZF1^{G158S}$, $IKZF1^{N159}$, $IKZF1^{R184Q}$ as well as $IKZF1^{Q156H}$, $IKZF1^{D186A}$, $IKZF1^{D186Y}$, have been demonstrated to exert one aberrant subcellular localization, which strongly confirmed the perturbation of IKZF1 function[8]. As these results indicated, IKZF1 aberration could also be caused by subtype three mutation, so consistent with *IKZF1* deletion, most of *IKZF1* mutations exhibited functional impacts on IKZF1.

It has been widely demonstrated that *IKZF1* alteration, including deletion and mutation, defined one unique aggressive B-cell ALL subtype with high relapse rate and poor survival duration[7, 25, 26]. Due to high frequency of co-occurrence with *BCR-ABL1* in ALL, the prognostic role of *IKZF1* alteration was also analyzed in the *BCR-ABL1*-positive ALL, and the same conclusion could be also drawn[11, 12]. Compared to ALL, the prognostic role of *IKZF1* alteration in AML remained largely unknown. Unlike AML with − 7/monosomy 7 showing one poor clinical outcome[17], *IKZF1* deletion, caused by loss of chromosome 7 as well as focal deletion, did not showed one significant impact on the relapsed frequency and survival duration[13]. In contrast to *IKZF1* deletion, the prognostic role of *IKZF1* mutation in AML was still not defined. In this study, AML patients with *IKZF1* mutation showed one relatively low CR rate when compared to those without it, but we did not find its significant impact on their OS and RFS. Interestingly, our results showed that $IKZF1^{MUT}$ patients with high VAF showed one significant inferior OS but not RFS compared to those with low VAF or $IKZF1^{WT}$, so the negative prognostic impact of *IKZF1* mutation in the whole cohort was possibly attributed to various VAFs of these patients. Consistent with it, the frequency of CR in $IKZF1^{MUT}$ patients was lower than it in $IKZF1^{WT}$ patients though without statistical significance. In detail, there were 7 $IKZF1^{MUT}$ patients with VAF below 10% (7/22), so *IKZF1* mutation possibly existed in sub-clones but not dominate-clones of AML, and less contributed to the development of AML in these patients. As our results indicated, when VAF was greater than 0.230, AML with *IKZF1* mutation showed one relatively poor prognosis and these patients accounted nearly half of $IKZF1^{MUT}$ patients (10/22). Therefore, AML with *IKZF1* mutation in dominate-clones still showed one more aggressive clinical course, and *IKZF1* mutation was also valuable for the prognostic stratification of these AML patients.

In AML, *IKZF1* mutation has been found to prefer to co-occur with *EVI1* rearrangement[14], and our previous study showed *SF3B1* mutation was also one important partner for this mutation[15]. Consistent with our previous report, *SF3B1* mutation was also preferable to co-occurred with *IKZF1* mutation in this study. Though alternative splicing factor mutations were more likely to affect myelodysplastic syndrome, they also played one important role in the biology of AML, and *SF3B1*, *SRSF2* as well as *U2AF1* were most commonly mutated[27]. Interestingly, *SF3B1* but not *SRSF2* or *U2AF1* mutation co-occurred with *IKZF1* mutation, so it strongly indicated that their specific cooperation in the pathogenesis of AML. Furthermore, we found that all of $IKZF1^{MUT}$/$SF3B1^{MUT}$ patients showed resistant to available therapies without CR achievement, so combination of these two mutations possibly defined one extremely aggressive AML subtype. In addition to *SF3B1* mutation, we also identified *CEBPA* mutation as the cooperator of *IKZF1* mutation in AML, and it was the most common gene mutation in $IKZF1^{MUT}$ AML, while more than half of $IKZF1^{MUT}$ patients accompanied with it (N = 13) in our study. It has been reported that *bi-allele* $CEBPA^{MUT}$ exhibited one extremely distinct pattern in the distribution of cooperated gene mutations with *single-allele* $CEBPA^{MUT}$, especially their distribution pattern with *NPM1* mutation and *FLT3-ITD*[28], but no reports has revealed the relationship with *IKZF1* mutation and our study first showed that *IKZF1* mutation both affected *bi-allele* $CEBPA^{MUT}$ and *single-allele* $CEBPA^{MUT}$ AML patients. Furthermore, *IKZF1* mutation more commonly appeared in *bi-allele* $CEBPA^{MUT}$ rather than *single-allele* $CEBPA^{MUT}$ AML patients, but it did not make the prognosis of *bi-allele* $CEBPA^{MUT}$ AML worse. Therefore, *IKZF1* mutation exhibited one specific distribution pattern in AML and played one significant prognostic role in some genetic subtypes.

In conclusion, our study showed that *IKZF1* mutation was one essential genetic alteration in AML and further provided the global view on the clinical, genetic, and prognostic features of $IKZF1^{MUT}$ AML. Compared to our previous study, this study was involved with one relatively larger cohort and provided information about its prognostic role in AML. Even so, there were two more questions needed to be answered in the future: First, though the functional consequences of *IKZF1* deletion was similar to *IKZF1* mutation, whether they shared the same pattern of distribution and prognosis in AML was required for further investigations; Second, accumulated evidences indicated *IKZF1* disruption also impaired the myeloid hierarchy[1, 4, 29], so what was the role of *IKZF1* mutation in the initiation and maintenance of AML needed to be studied.

Declarations

Competing Interests statement

The authors declare that they have no conflict of interest.

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Author contributions

Xiang Zhang designed this study. Min Yang, Yinjun Lou, Lei Wang, Ni Xiong, Xiaoxia Hu, Gusheng Tang, Liping Mao, Jiejin Qian, Weilai Xu, Juying Wei, Gaixiang Xu, Haitao Meng, Wenyuan Mai, Chunmei Yang and Wenjuan Yu collected and integrated the clinical materials. Lixia Liu, Mengmeng Zhang and Shanbo Cao conducted the sequencing experiments and mutational analysis. Xiang Zhang, Aijie Huang, Lixia Liu, and Chengcheng Wang displayed the data analysis. Xiang Zhang wrote the manuscript. Honghu Zhu, Hongyan Tong, Jianmin Yang and Jiayue Qin provided advices for this work. Jianmin Wang and Jie Jin revised the manuscript. All authors approved the manuscript.

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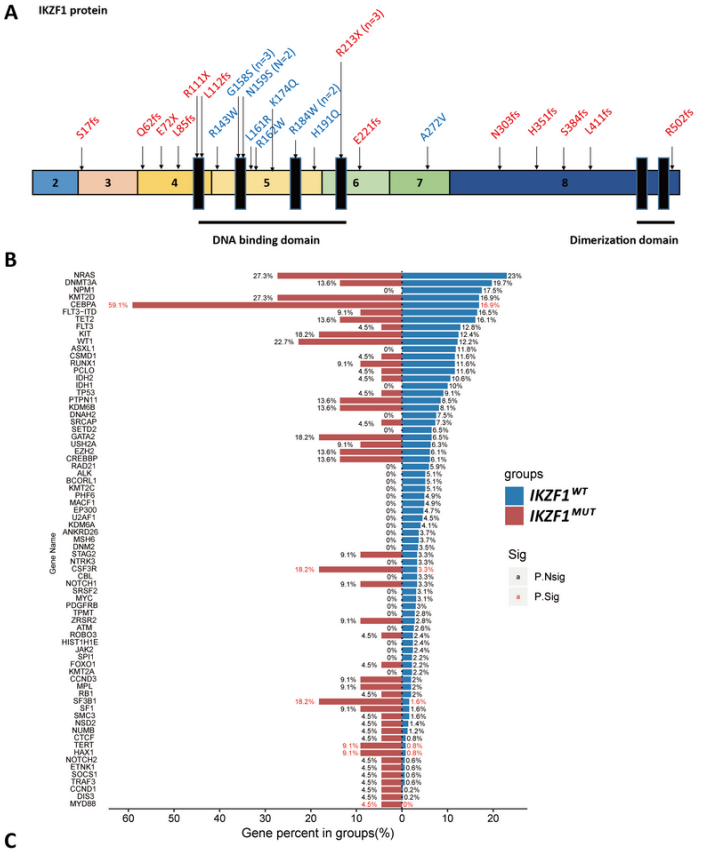
Figures



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Gene	*IKZF1*^{WT}	*IKZF1*^{MUT}	P	Gene	*IKZF1*^{WT}	*IKZF1*^{MUT}	P
NPM1	89 (17.5%)	0 (0%)	0.063	*TET2*	82 (11.6%)	3 (13.6%)	0.987
FLT3-ITD	84 (16.5%)	2 (9.1%)	0.527	*IDH1*	51 (10.0%)	0 (0%)	0.232
CEBPA	**86 (16.9%)**	**13 (59.1%)**	**<0.001**	*IDH2*	54 (10.6%)	1 (4.5%)	0.576
DNMT3A	100 (19.7%)	3 (13.6%)	0.670	*EZH2*	31 (6.1%)	3 (13.6%)	0.333
NRAS	117 (23%)	6 (27.3%)	0.645	***SF3B1***	**8 (1.6%)**	**4 (18.2%)**	**0.001**
ASXL1	60 (11.8%)	0 (0%)	0.171	*PTPN11*	43 (8.5%)	3 (13.6%)	0.645
RUNX1	59 (11.6%)	2 (9.1%)	0.983	***CSF3R***	**17 (3.3%)**	**4 (18.2%)**	**0.008**
TP53	46 (9.1%)	1 (4.5%)	0.730	*KIT*	63 (12.4%)	4 (18.2%)	0.638

Figure 1

***IKZF1* mutation in AML.** (**A**) The distribution of *IKZF1* mutations, which were identified in our cohort, on the protein. The nonsense or frameshift mutation was marked as red, while the missense mutation was marked as blue. (**B**) The difference of additional mutations distribution in *IKZF1*^{WT} and *IKZF1*^{MUT} groups, and the percentage of each gene mutation was exhibited. (**C**) The distribution of frequent AML-associated gene mutations in *IKZF1*^{WT} and *IKZF1*^{MUT} groups, and the count as well as percentage of each gene mutation were showed.

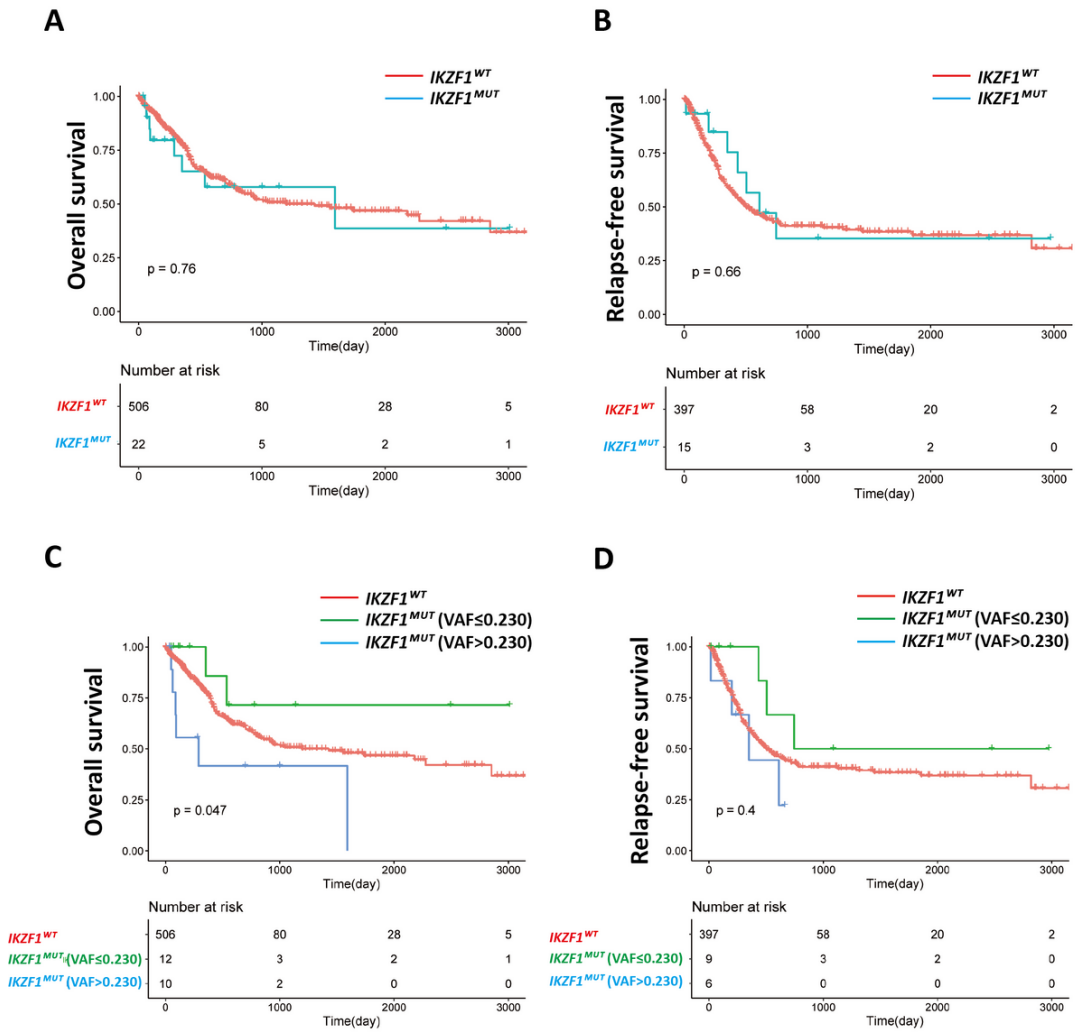

Figure 2

The prognostic role of *IKZF1* mutation in AML. (**A-B**) The OS (**A**) and RFS (**B**) of $IKZF1^{WT}$ and $IKZF1^{MUT}$ groups in our AML cohort. (**C-D**) The influence of *IKZF1* mutation burden on the prognosis of AML was studied, and the OS (**C**) as well as RFS (**D**) of $IKZF1^{WT}$, $IKZF1^{MUT}$ with VAF≥0.230, and $IKZF1^{MUT}$ with VAF<0.230 groups were showed.

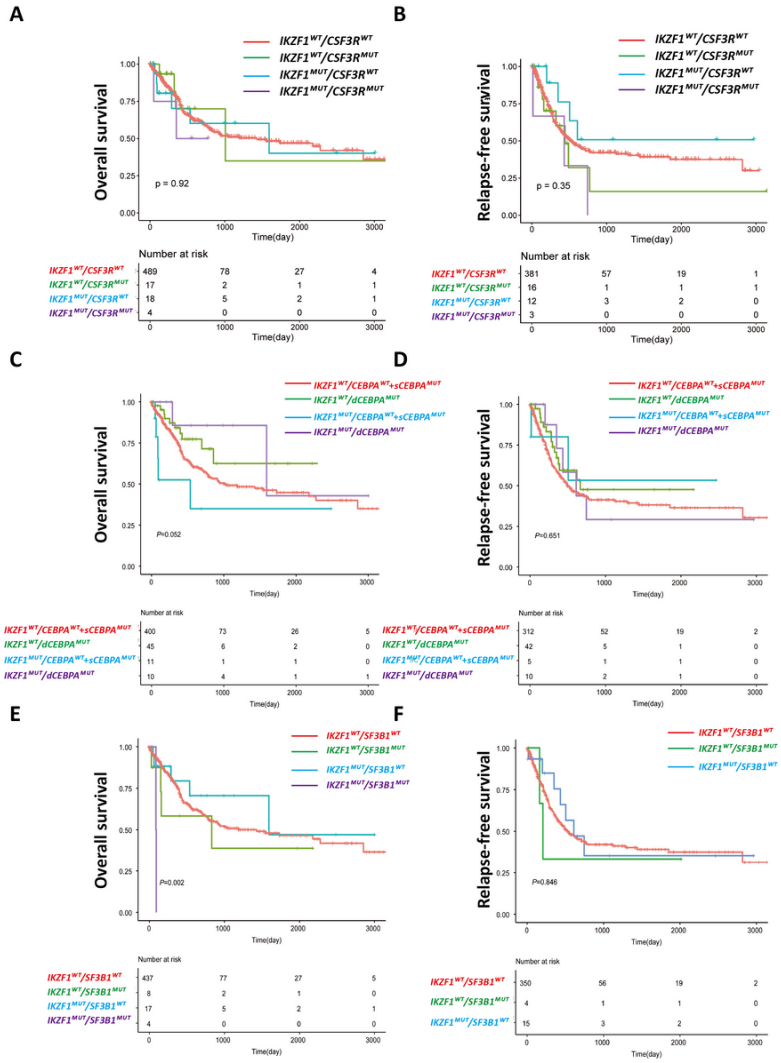

Figure 3

The prognostic role of *IKZF1* mutation in the specific genetic AML subtype. (**A-B**) The influence of *IKZF1* mutation on the OS (**A**) and PFS (**B**) of *CSF3R*-mutated AML. (**C-D**) The influence of *IKZF1* mutation on the prognosis of the *CEBPA*-mutated AML was studied, and the OS (**A**) as well as RFS (**B**) of $CEBPA^{WT}$ plus *single-allele* $CEBPA^{MUT}$ ($sCEBPA^{MUT}$) and *bi-allele* $CEBPA^{MUT}$ ($dCEBPA^{MUT}$) groups with or without $IKZF1^{MUT}$ were showed. (**E-F**) The prognostic role of combined *IKZF1* and *SF3B1* mutations on AML was investigated, and the OS (**E**) as well as RFS (**F**) of AML with different *IKZF1* or *SF3B1* mutated status were exhibited.

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