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Original Article

Double *CEBPA* mutations are prognostically favorable in non-M3 acute myeloid leukemia patients with wild-type *NPM1* and *FLT3-ITD*

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Abstract: This study is aimed to investigate the pattern of *CEBPA* mutations and its clinical significance in Chinese non-M3 acute myeloid leukemia (AML) patients. The entire coding region of *CEBPA* gene was amplified by PCR and then sequenced in samples from 233 non-M3 AML patients. Fifty mutations were identified in 37 (15.8%) patients with eleven (4.7%) double mutated *CEBPA* (*dmCEBPA*) and twenty-six (11.1%) single mutated *CEBPA* (*smCEBPA*). *dmCEBPA* was exclusively observed in M1 and M2 subtypes of FAB classification ($P = 0.008$), whereas *smCEBPA* occurred in almost all subtypes ($P = 0.401$). Patients with *dmCEBPA* had significantly younger age and higher WBC counts than those with *wtCEBPA* ($P = 0.016$ and 0.043, respectively). Both *dmCEBPA* and *smCEBPA* were mainly present in cytogenetically normal patients. Patients with *dmCEBPA* achieved higher rate of complete (CR) than *wtCEBPA* patients (88% vs. 51%, $P = 0.037$), whereas *smCEBPA* and *wtCEBPA* groups are similar (47% vs. 51%, $P = 0.810$). Patients with dm*CEBPA* had a superior overall survival (OS) compared with patients with wt*CEBPA* ($P = 0.033$), whereas patients with sm*CEBPA* had a similar OS as patients with wt*CEBPA* ($P = 0.976$). *dmCEBPA* but not *smCEBPA* was also associated with favorable outcome in patients with wild-type *NPM1* and *FLT3-ITD* (*NPM1*^{wt}*FLT3-ITD*^{wt}). Our data confirm that *dmCEBPA* but not *smCEBPA* is prognostically favorable in *NPM1*^{wt}*FLT3-ITD*^{wt} AML, and suggest that the entity AML with mutated *CEBPA* should be definitely designated as AML with *dmCEBPA* in WHO classification and *smCEBPA* should be excluded from the favorable risk of molecular abnormalities.

Keywords: *CEBPA*, mutation, prognosis, acute myeloid leukemia

Introduction

The hallmark of acute myeloid leukemia (AML) is the differentiation arrest and neoplastic accumulation of myeloid precursor cells in the bone marrow. Researches on the pathogenesis of AML have identified the molecular changes caused by acquired cytogenetic abnormalities in leukemic patients [1, 2]. Inappropriately activated transcription factors involved in these structural rearrangements disturb normal programs of myeloid cell proliferation, differentiation, and survival [1-3]. Other genetic and epigenetic lesions accumulate and act in concert with aberrant transcription factors in multiple pathways of leukemogenesis [4-6]. Recurrent cytogenetic abnormalities have been demonstrated as powerful predictors of the outcome in AML patients [7-9]. Aberrant molecular events have further refined the prognosis in AML [10].

CCAAT/enhancer binding protein alpha (*CEBPA*) gene, located on chromosome 19q13.1, encodes a protein of 358 amino acids which consists of a N-terminal transcriptional activation domain (TAD) and a C-terminal basic leucine zipper (bZIP) domain. The *CEBPA* transcription factor is widely expressed during the differentiation of myelopoiesis and is involved in cell cycle block, proliferation inhibition, and repression of self-renewal [11-13]. *CEBPA* mutations have been reported in approximately 5~14% of all AML patients among which the

Table 1. Patient characteristics according to *CEBPA* mutation status in non-M3 AML

Variables	Total (n = 233)	*wtCEBPA* (n = 196)	*smCEBPA* (n = 26)	*P*	*dmCEBPA* (n = 11)	*P*
Sex, male/female	135/98	114/82	16/10	0.834	5/6	0.534
Median age, years (range)	53 (15-93)	54 (15-93)	52 (17-85)	0.489	35 (15-61)	0.016
Median WBC, × 10^9/L (range)	16.1 (0.6-528.0)	14.5 (0.8-528.0)	35.5 (1.3-528.0)	0.072	44.7 (2.4-507.0)	0.043
Median hemoglobin, g/L (range)	73 (32-147)	72 (32-142)	74 (45-147)	0.220	85 (56-108)	0.168
Median platelets, × 10^9/L (range)	40 (3-447)	37 (3-447)	53 (4-203)	0.137	21 (3-76)	0.084
FAB				0.158		0.022
M0	1	1	0		0	
M1	27	18	6		3	
M2	115	95	12		8	
M4	51	49	2		0	
M5	28	24	4		0	
M6	11	9	2		0	
WHO				0.005		0.007
AML with t (8; 21)	37	37	0		0	
AML with inv (16)	1	1	0		0	
AML with 11q23	4	3	0		1	
AML without maturation	23	15	6		2	
AML with maturation	82	62	12		8	
Acute myelomonocytic leukemia	50	48	2		0	
Acute monoblastic and monocytic leukemia	25	21	4		0	
Acute erythroid leukemia	11	9	2		0	
Karyotype classification				0.005		0.099
Favorable	39	39	0		0	
Intermediate	153	121	23		9	
Poor	29	25	2		2	
No data	12	11	1		0	
Karyotype				0.021		0.180
normal	119	91	20		8	
t (8;21)	38	38	0		0	
inv (16)	1	1	0		0	
11q23	4	3	0		1	
complex	23	20	2		1	
others	36	32	3		1	
No data	12	11	1		0	
Gene Mutation						
NPM1 (+/-)	26/203	23/169	3/23	1.000	0/11	0.618
FLT3 ITD (+/-)	22/207	19/173	2/24	1.000	1/10	1.000
C-KIT (+/-)	15/214	15/177	0/26	0.226	0/11	1.000
IDH1/2	14/207	13/171	1/25	1.000	0/11	1.000
DNMT3A	17/204	14/170	2/24	1.000	1/10	0.595
N/K-RAS	20/201	15/169	4/22	0.266	1/10	1.000
CR (+/-)	83/74	66/63	9/10	0.810	8/1	0.037

majority are cytogenetically normal (CN) [14-17]. Although *CEBPA* mutations can occur across the whole coding region, two types of mutations are predominant: (1) N-terminal frame-shift mutations that lead to loss of translation of the full-length 42-kDa CEBPA protein (p42 CEBPA) and to the overexpression of a truncated, dominant negative 30-kDa CEBPA isoform (p30 CEBPA); (2) C-terminal in-frame insertions/deletions that prevent homodimerization or hetero-dimerization of CEBPA [15-18]. These *CEBPA* mutation types can occur as mono- or bi-allelic mutations. More than half of *CEBPA*-mutated patients harbor bi-allelic mutations with a N-terminal frame-shift mutation on one allele and a C-terminal in-frame mutation on the other allele [19]. The clinical significance of *CEBPA* mutations has been extensively

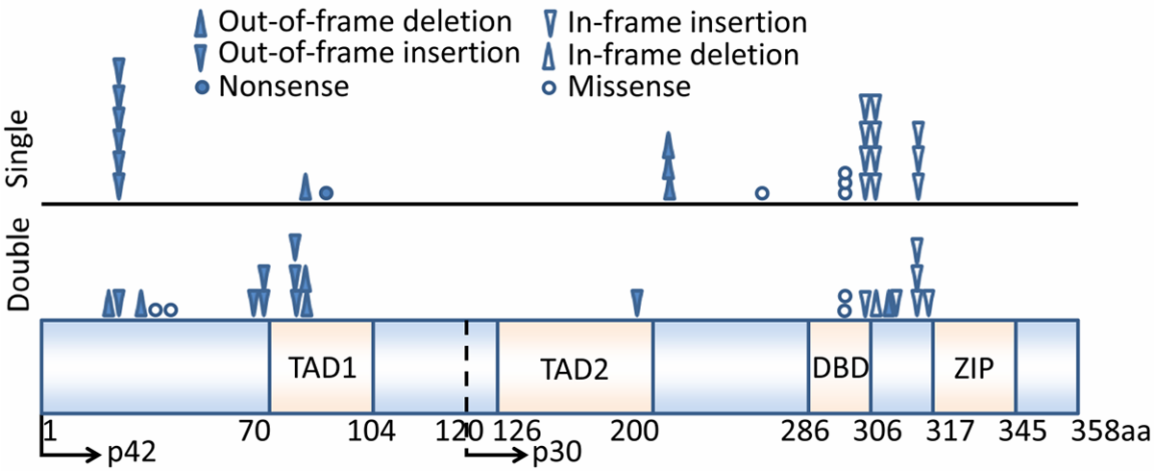

Figure 1. Schematic representation and location of the 50 *CEBPA* mutations. Functional regions of two transactivation domains (TAD1 and TAD2), DNA-binding domain (DBD), and the bZIP region are depicted. Amino acid (aa) numbering and the alternative translation start site at position 120aa are also depicted.

explored. AML with mutated *CEBPA* has recently been included in the current WHO classification as a provisional entity due to its prognostically favorable influence in normal karyotype [20]. However, more recent studies have identified that the favorable impact on AML outcome is predicted by double mutated *CEBPA* (*dmCEBPA*) rather than single mutated *CEBPA* (*smCEBPA*) [21-24]. In the present study, we evaluated the frequency, the main associated features, and the prognostic significance of *CEBPA* mutations in a cohort of Chinese de novo non-M3 AML patients.

Materials and methods

Patients and samples

This study was approved by the Ethics Committee Board of the Affiliated People's Hospital of Jiangsu University. Bone marrow aspirates of 233 non-M3 AML patients were collected after informed consent written by patients or their guardians. The diagnosis and classification were conducted according to the French-American-British Cooperative Group Criteria and the 2008 World Health Organization (WHO) proposal [20, 25]. Treatment protocol was described as reported previously [26]. The main clinical and laboratory features of the patient cohort were summarized in **Table 1**.

Cytogenetic analysis

Conventional cytogenetic analysis was performed in the cytogenetics laboratory of our hospital. Chromosomes were prepared routinely by the direct method or 24h short-term culture of bone marrow cells. Karyotypes were analyzed on R-banded metaphases. The definition of a cytogenetic clone and descriptions of karyotypes followed the International System for Human Cytogenetic Nomenclature [27]. Karyotypes were classified according to the revised MRC prognostic classification [9].

Cell separation and DNA isolation

The mononuclear cells were separated by density-gradient centrifugation using Ficoll. Subsequently, genomic DNA was extracted using the Genomic DNA Purification Kit (Gentra, Minneapolis, MN, USA) according to the manufacturer's instructions.

Gene mutation detection

Mutation of the *CEBPA* gene was detected in genomic DNA by PCR and direct sequencing. Two overlapping primer pairs were used to amplify the entire coding region of *CEBPA* (NM_004364.3): P1 (477 bp) 5'-TGCCGGGAGAACTCTAACT-3' (sense) and 5'-CCCGACGCGCTCGTACA-3' (antisense), P2 (688 bp) 5'-CTGGACGGCAGGC TGGA-3' (sense) and 5'-CAGGGCGGTCCCACAGC-3' (antisense). The total PCR reaction volume of 25 μL contained 1× PCR buffer for KOD FX, 0.4 μM of primer set 1 or 0.2 μM of primer set 2, 0.4 μM of each dNTP, and 0.5U KOD FX polymerase (Toyobo, Osaka, Japan). The following conditions were performed: 98°C for 2 minutes; 35 cycles of 98°C for 10 seconds, 60°C (P1) or 68°C (P2) for 30

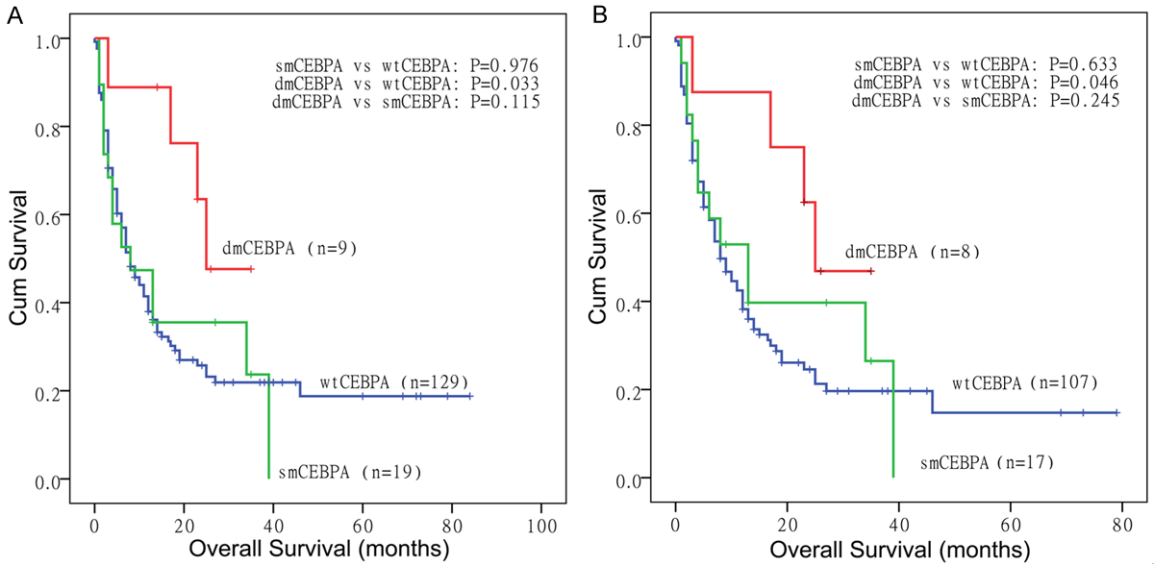

Figure 2. Kaplan-Meier survival curves for OS in non-M3 AML patients. A: All patients; B: *NPM1*^{wt}*FLT3-ITD*^{wt} patients.

seconds, and 68°C for 3 minutes; then 72°C for 7 minutes. PCR products were electrophoresed on 2% agarose gels, purified using Axygen AP-GS500 kit (Axygen, CA, USA) and then sequenced in both directions with PCR primers on an ABI 3730 Prism Sequencer (Applied Biosystems, CA, USA). In samples with a *CEBPA* sequence variation, the entire coding region was amplified with primers P1F and P2R under the previously described PCR conditions except for annealing at 65°C and was cloned into the pMD19-T vector (TaKaRa, Japan). 5 to 10 clones were sequenced in each patient with the primers used to amplify the entire coding region of *CEBPA*.

NPM1, FLT3 internal tandem duplication (ITD), *C-KIT*, *DNMT3A*, *IDH1/IDH2*, and *N/K-RAS* mutations were detected as described previously [28-30]. Briefly, genomic DNA was amplified using gene-specific primers. Mutation scanning was performed for PCR products of all genes except for *FLT3-ITD* using HRMA with the LightScanner™ platform (Idaho, Utah, USA). All positive samples were directly DNA sequenced to confirm the results of HRMA. *FLT3* internal tandem duplication (ITD) was detected using direct DNA sequencing.

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 software package (SPSS, Chicago, IL). Statistical significance of the difference between groups for continuous variables was determined by the Mann-Whitney test. Statistical significance of the difference between groups for categoric variables was determined by Fisher exact test or Chi-square analysis. Overall survival (OS) was estimated using the Kaplan-Meier method and were compared using the log-rank test. Multivariate analyses were performed using Cox proportional hazards regression. The significance of results was defined as a level of $P<0.05$ at both tails.

Results

Frequency and types of CEBPA mutations in Chinese AML patients

Fifty mutations were identified in 37 (15.8%) patient samples (**Figure 1**; Table S1). Eleven patients (4.7%) had *dmCEBPA*, whereas twenty-six (11.1%) had *smCEBPA*. We observed four *CEBPA* mutations in one patient. In all 11 patients with two *CEBPA* mutations, we identified one patient with two *CEBPA* mutations in one allele, while the distribution of the mutations was biallelic in other patients as determined by cloning analysis. 10 patients with double mutations had both a N-terminal frameshift mutation resulting in premature truncation and a C-terminal mutation resulting in in-frame insertion/duplication/substitution/deletion or out-of-frame duplication/deletion. Among patients with a single *CEBPA* mutation, eight mutations were located in the N-terminal

Table 2. Multivariate analyses of prognostic factors for OS in non-M3 AML

	All patients		*NPM1*^{wt}*FLT3-ITD*^{wt} patients	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Sex	0.922 (0.56-1.497)	0.743	0.891 (0.560-1.418)	0.628
Age	1.938 (1.270-2.958)	0.002	2.073 (1.326-3.242)	0.001
WBC	1.555 (1.026-2.354)	0.037	1.140 (0.723-1.797)	0.573
Karyotype risk	1.979 (1.467-2.669)	< 0.001	1.953 (1.422-2.682)	<0.001
dmCEBPA vs *wtCEBPA*	0.596 (0.358-0.992)	0.047	0.650 (0.431-0.980)	0.040
smCEBPA vs *wtCEBPA*	0.869 (0.458-1.650)	0.669	0.763 (0.387-1.503)	0.434
FLT3	0.606 (0.304-1.208)	0.155	-	-
NPM1	0.852 (0.338-2.145)	0.734	-	-
C-KIT	0.906 (0.319-2.576)	0.853	0.842 (0.296-2.398)	0.748
N/K-RAS	1.695 (0.825-3.483)	0.151	1.576 (0.797-3.116)	0.191
IDH1/2	1.354 (0.643-2.850)	0.425	2.321 (1.037-5.197)	0.041
DNMT3A	1.061 (0.417-2.698)	0.901	1.848 (0.443-7.703)	0.399

HR: hazard ratio; CI: confidence interval; -: not included.

leading to premature truncation, the majority of mutations (15/26, 58%) were located in the C-terminal resulting in in-frame duplication/insertion/substitution, while three mutations were located in between TAD2 and DNA binding domain (DBD) regions resulting in frame-shift premature truncation.

Patient characteristics related to CEBPA mutation status

Because double *CEBAP* mutations were considered as a prognostic factor according to previous studies, we divided our patient cohort into the following three groups: patients with *dmCEBPA* (n = 11), patients with *smCEBPA* (n = 26), and patients with wild-type *CEBPA* (*wtCEBPA*, n = 196). The comparison of the clinical and laboratory features between three groups is summarized in **Table 1**.

smCEBPA was distributed in almost all subtypes of FAB classification (**Table 1**). Although the majority of patients with *smCEBPA* had M1 or M2 subtypes (18/26, 69%), there was no difference in the distribution of *smCEBPA* in the whole cohort [18/142 (13%) patients with M1 or M2 versus 8/91 (9%) patients with other subtypes, *P* = 0.401]. There was a trend that the patients with *smCEBPA* had higher WBCs than those with *wtCEBPA* (*P* = 0.072). *smCEBPA* was predominantly present in cytogenetically normal patients (*P* = 0.005). Concurrent other molecular mutations including *NPM1*, *FLT3*, *IDH1*, *N/K-RAS*, and *DNMT3A* occurred in patients with *wtCEBPA*, but no correlation was observed (*P* > 0.05).

dmCEBPA was exclusively observed in M1 and M2 subtypes (**Table 1**). Overall, 11 (8%) out of 142 patients with M1 or M2 subtypes harbored *dmCEBPA*, while none of 91 patients with other subtypes who did so (*P* = 0.008). Patients with *dmCEBPA* had significantly younger age and higher WBC counts at diagnosis than those with *wtCEBPA* (*P = 0.016 and 0.043, respectively*). There was a trend that *dmCEBPA* group had lower platelet counts than *wtCEBPA* group (*P* = 0.084). *dmCEBPA* was mainly present in cytogenetically normal patients except for two patients harboring poor-risk karyotypes. Concurrent *FLT3-ITD*, *N-RAS*, and *DNMT3A* mutations occurred in two patients with *dmCEBPA*.

Prognostic impact of CEBPA mutation status

Survival data were obtained for 157 AML patients with mean follow-up time of 14 months (range, 1-84 months). There was no significant difference in the rate of complete remission (CR) after induction chemotherapy between *smCEBPA* and *wtCEBPA* groups (47% vs 51%, *P* = 0.810), however, more patients with *dmCEBPA* achieved CR than *wtCEBPA* patients (88% vs 51%, *P* = 0.037, **Table 1**). Patients with *dmCEBPA* had a superior overall survival (OS) compared with patients with *wtCEBPA* (estimated median 25 *vs* 8 months, respectively, *P* = 0.033; **Figure 2A**), whereas *smCEBPA* patients had a similar OS as *wtCEBPA* patients (estimated median 8 *vs* 8 months, respectively, *P* = 0.976; **Figure 2A**). Furthermore, in multivariate analysis that included sex, age (≤60 yrs vs >60 yrs), WBC count (≤30 vs >30 × 10^9/L), karyotype risk group, and mutational status of

seven genes as covariates, the presence of a *dmCEBPA* mutation remained an independent favorable prognostic factor for OS (**Table 2**). Because of the small size of *CEBPA* mutations, patients with *dmCEBPA*, compared with patients with s*mCEBPA*, showed the trend towards longer OS ($P = 0.115$, **Figure 2A**).

The impact of *CEBPA* mutations was also evaluated in patients with wild-type *NPM1* and *FLT3-ITD* (*NPM1*^{wt}*FLT3-ITD*^{wt}), *dmCEBPA* but not s*mCEBPA* was associated with favorable outcome (**Figure 2B**). Estimated median OS in *dmCEBPA*, s*mCEBPA, and wtCEBPA* groups was 25, 13 and 8 months, respectively. Multivariate analysis also confirmed *dmCEBPA* only as an independent favorable factor in *NPM1*^{wt}*FLT3-ITD*^{wt} patients (**Table 2**).

Discussion

A total of 37 (15.8%) out of 233 Chinese non-3 AML patients were identified to carry 50 *CEBPA* mutations after excluding all known *CEBPA* polymorphisms (data not shown). The frequency of *CEBPA* mutations detected in our study is in good accordance with previous studies [24, 31]. Three mutational hot spots were identified, one 5' of the TAD1 region, the other in bZIP and the third in the DBD of the *CEBPA* gene, which was comparable with previous studies [22-24, 32]. The proportion of s*mCEBPA* and *dmCEBPA* was different from previous studies in which the percentage of *dmCEBPA* was more than that of s*mCEBPA* [22-24]. However, a recent large-scale study has identified *dmCEBPA* in 104 (42.6%) out 2296 AML patient [33].

The observations are highly consistent across all studies: double *CEBPA* mutations occur mainly in M1/M2 subtypes and are highly associated with normal karyotypes. However, there is a discrepancy about clinical characteristics of AML with *CEBPA* mutations. Although the majority of studies did not find the correlation of *CEBPA* mutations with peripheral leukocytes in AML [22, 23, 32], two groups observed that *CEBPA* mutated patients had higher peripheral leukocytes than wild-type patients [34, 35], which was consistent with our results. Moreover, similar to two previous reports [24, 36], the present study found the association of double *CEBPA* mutation with younger age, whereas other studies did no find this correlation. However, a recent study on *CEBPA* mutations, the largest size of AML patients (a total of 2296 cases) till now, confirmed the association of *dmCEBPA* with younger age [33].

The impact of *CEBPA* mutations on outcome has been extensively evaluated. Earlier studies have led to the introduction of 'AML with mutated *CEBPA'*, which includes both s*mCEBPA* and s*mCEBPA*, into the current WHO classification as a provisional entity among 'AML with recurrent genetic abnormalities' [20]. However, later studies have shown only *dmCEBPA* but not s*mCEBPA* is a favorable prognostic factor. A recent study has confirmed that survival of AML with *dmCEBPA* is similar with those with *PML-RARA* [37]. Although in the present study patients with *dmCEPA* harbored higher peripheral leukocytes which also affected patients' outcome, multivariate COX analysis verified *dmCEBPA* as an independent favorable predictor after adjusting for other covariates including peripheral leukocytes. Because of limited case numbers the difference in outcome was not significant between *dmCEBPA* and s*mCEBPA* groups in this study. Initial study revealed that the presence of additional *FLT3-ITD* significantly worsen overall survival in the *CEBPA*-mutated group [38]. However, a subsequent study did not find the influence of *FLT3-ITD* on survival in the *CEBPA*-mutated group [34]. The aforementioned studies did not differentiate *dmCEBPA* from s*mCEBPA*. Concurrent *FLT3-ITD* or *NPM1* mutations are significantly less frequent in patients with *dmCEBPA* compared with those with s*mCEBPA*, therefore, the impact of *FLT3-ITD* in AML with *dmCEBP* still needs to be determined. Further endeavor has been made to investigate the clinically relevant aspect of whether the favorable prognosis of *dmCEBPA* is influenced by other additional molecular markers. Concurrent *TET2* mutations were adversely prognostic for OS [33, 39], whereas *GATA-2* mutations improved OS [39, 40].

Although it has been shown that AML patients carrying single *CEBPA* mutation have similar outcome as those with wild-type *CEBPA*, further risk stratification has been tried in AML with single *CEBPA* mutation. Two studies revealed that *FLT3-ITD* significantly impaired the survival of AML patients with s*mCEBPA* [24, 33]. Furthermore, Fasan et al revealed that s*mCEBPA* further decreased survival compared with *wtCEBPA* in patients with *FLT3-ITD* [33]. Moreover, two groups also observed that

NPM1^{mt}/*CEBPA*^{sm} genotype showed a trend toward favorable outcome compared with *NPM1*^{wt}/*CEBPA*^{sm} [24, 33]. Although *smCEBPA* was considered beneficial for outcome of AML with *NPM1* mutation by Dufour et al [41], the later results from the same group did not confirm this observation [33]. As for the role of *smCEBPA* in *NPM1*^{wt}*FLT-ITD*^{wt} AML, it is also controversial. Park et al found *smCEBPA* subgroup had longer survival than *wtCEBPA* subgroup in *NPM1*^{wt}*FLT-ITD*^{wt} AML [42], however, similar with the results of the large-scale study of Fasan et al [33], we did not identify the difference.

In conclusion, the results of our present study confirm that AML with *dmCEBPA* but not *smCEBPA* is associated with a favorable outcome in *NPM1*^{wt}*FLT3-ITD*^{wt} non-M3 AML. Our data suggest that the entity AML with mutated *CEBPA* should be definitely designated as AML with *dmCEBPA* in WHO classification and *smCEBPA* should be excluded from the favorable risk of molecular abnormalities [10].

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Disclosure of conflict of interest

None.

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Table S1. Description of *CEBPA* mutations in 37 AML patients

Patient no.	Age (years)/ Sex	FAB Subtype	Nucleotide change	AA change	Description of mutation	Karyotype	Other gene mutations
1	M/50	M2	c.62delG	S21fs*139	Frameshift in N-terminal and stop in TAD2	N	-
			c.912_913insTTG	K304_Q305insL	Insertion in DBD		
2	F/15	M1	c.65_68dupCCCC	H24fs*85	Frameshift in N-terminal and stop before TAD2	45, XX, -I, inv(2)(P?,q?), -7, +11	-
			c.948_950dupGCT	L317dup	Duplication in ZIP		
3	M/19	M1	c.208_209insGG	P70fs*90	Frameshift in TAD1 and stop in TAD2	46, XY, t(10;11)(p12;q23)	NRAS (+)
			c.933_935dupGCA	K313dup	Duplication in ZIP		
4	F/61	M2	c.246_247insGC	Q83fs*78	Frameshift in TAD1 and stop in TAD2	N	-
			c.935_937dupAGA	K313dup	Duplication in ZIP		
5	F/30	M2	c.246_247insGC	Q83fs*78	Frameshift in TAD1 and stop in TAD2	N	-
			c.935_937dupAGA	K313dup	Duplication in ZIP		
6	M/49	M2	c.246delC	Q83fs*77	Frameshift in TAD1 and stop in TAD2	N	FLT3 (+), DNMT3A (+)
			c.890G>C	R297P	Substitution in DBD		
7	F/47	M2	c.252delC	S85fs*24	Frameshift in TAD1 and stop before TAD2	46, XX, t(2;11)(q32;p15)	-
			c.890G>C	R297P	Substitution in DBD		
8	M/35	M1	c.252delC	S85fs*24	Frameshift in TAD1 and stop before TAD2	N	-
			c.916_933del	R306_Q311del	Deletion between DBD and ZIP		
9	F/33	M2	c.197_206dup	P70fs*41	Frameshift in TAD1 and stop before TAD2	N	-
			c.929_934del	T310fs	Frameshift after DBD		
10	M/39	M2	c.100C > T	P34S	Substitution in N-terminal	N	-
			c.200_201dupAC	I68fs*93	Frameshift in N-terminal and stop in TAD2		
			c.577dupC	H193fs*128	Frameshift in TAD2 and stop in ZIP		
			c.925_926insCAC	E309delinsAQ	Substitution and insertion between DBD and ZIP		
11	F/29	M	c.87delC	A30fs*130	Frameshift in N-terminal and stop in TAD2	N	-
			c.[87delC; 113G > A]	A30fs*130, A38T	Frameshift and substitution in N-terminal and stop in TAD2		
12	F/65	M2	c.64_68dup	H24fs*138	Frameshift in N-terminal and stop in TAD2	N	-
13	F/2	M2	c.64_68dup	H24fs*138	Frameshift in N-terminal and stop in TAD2	N	-
14	F/27	M1	c.68dupC	H24fs*84	Frameshift in N-terminal and stop in TAD2	N	-
15	M/62	M2	c.68dupC	H24fs*84	Frameshift in N-terminal and stop in TAD2	N	-
16	M/53	M5	c.68dupC	H24fs*84	Frameshift in N-terminal and stop in TAD2	N	KRAS (+)
17	M/56	M6	c.68dupC	H24fs*84	Frameshift in N-terminal and stop in TAD2	N	-
18	M/35	M1	c.252delC	S85fs*24	Frameshift in TAD1 and stop before TAD2	N	FLT3 (+)
19	M/60	M2	c.262C>T	Q88fs*	Stop in TAD1	N	-
20	F/53	M2	c.611delC	P204fs*114	Frameshift between TAD2 and bZIP and stop in ZIP	N	-
21	F/85	M2	c.611delC	P204fs*114	Frameshift between TAD2 and ZIP and stop in ZIP	N	-
22	F/28	M2	c.611delC	P204fs*114	Frameshift between TAD2 and ZIP and stop in ZIP	45, XX, -8	DNMT3A (+)
23	F/70	M5	c793G>A	A265T	Substitution between TAD2 and DBD	N	NPM1 (+), NRAS (+), IDH1 (+), DNMT3A (+)

24	F/39	M1	c.890G>C	R297P	Substitution in DBD	N	FLT3 (+)
25	M/57	M2	c.890G>C	R297P	Substitution in DBD	N	-
26	M/42	M6	c.890G>C	R297P	Substitution in DBD	N	NPM1 (+)
27	M/78	M2	c.912_913insTTG	K304_Q305insL	Insertion in DBD	N	-
28	M/41	M2	c.912_913insTTG	K304_Q305insL	Insertion in DBD	47, XY, +17	-
9	M/55	M1	c.912_913insTTG	K304_Q305insL	Insertion in DBD	N	NRAS (+)
30	M/69	M2	c.912_913insTTG	K304_Q305insL	Insertion in DBD	49, XY, +3, +8, +11	-
31	F/41	M5	c.918_919dupAAGGCCAAGCAGCGC	K302_R306dup	Duplication in DBD	ND	KRAS (+)
32	M/51	M2	c.918_919dupAAGGCCAAGCAGCGC	K302_R306dup	Duplication in DBD	N	-
33	M/22	M1	c.918_919dupAAGGCCAAGCAGCGC	K302_R306dup	Duplication in DBD	N	-
34	M/31	M4	c.918_919dupAAGGCCAAGCAGCGC	K302_R306dup	Duplication in DBD	N	-
35	F/66	M1	c.935_937dupAGA	K313dup	Duplication in ZIP	N	NPM1 (+)
36	M/28	M5	c.935_937dupAGA	K313dup	Duplication in ZIP	47, XY, del(1)(p22), +1	-
37	M/17	M4	c.935_937dupAGA	K313dup	Duplication in ZIP	N	-