Original Article

ND4 mutations are more prevalent in patients with acute myeloid leukemia of M2 morphology

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Background: To evaluate the prognostic value of *ND4* gene mutation and other gene mutations in acute myeloid leukemia (AML) patients, especially among those without karyotype abnormalities.

Methods: We analyzed the biological and clinical characteristics of 460 newly diagnosed AML patients. The mutation status and prognostic impact in *FLT3*-ITD, *NPM1*, *c-KIT*, *CEBPA*, *DNMT3A*, and *ND4* genes were investigated.

Results: The frequency of *ND4* gene mutation was 6.6%. *ND4* mutations were prevalent in patients with AML of M2 morphology (P=0.001). About 11.3% patients were diagnosed with core binding factor (CBF) AML and *c-KIT* mutations were most commonly seen in CBF leukemia patients (16.2%). *DNMT3A* mutations were usually found in M5 but not in M4 (P=0.006 and 0.498, respectively). *ND4* germline mutations in non-M3 patients included types of A131V, F149L, A404T, and Y409H, while one M3 patient had type of G242D somatic mutation. Patients with *ND4* mutations were significantly associated with CD19 expression in non-M3 patients (P=0.045). Patients with *ND4* germline mutations may have unfavorable survival, but showed no statistical significance in overall survival (OS) and relapse-free survival (RFS) between patients with germline *ND4* mutations and wild-type (P=0.159 and 0.087, respectively). According to the molecular prognostic factors, patients with normal cytogenetic risk were divided into three groups in the OS and RFS analysis (P=0.017 and 0.025, respectively) as favorable mutational risk has favorable prognosis and unfavorable mutational risk has poor one.

Conclusions: Conventional molecular and cytogenetic factors could divide AML patients into distinctive prognosis groups. *ND4* mutations were prevalent in M2 patients and were associated with higher expression of CD19. Whether patients with germline *ND4* mutations have poorer prognosis still needs to be confirmed.

Keywords: Acute myeloid leukemia (AML); *ND4* gene; gene mutation; prognosis

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Introduction

Acute myeloid leukemia (AML) is a group of heterogeneous diseases with varying clinical characteristics and prognostic implications. Conventional genetic prognostic markers, including *FLT3*-ITD, *NPM1*, *c-KIT*, *CEBPA*, *DNMT3A*, and *MLL*-PTD, combined with cytogenetic abnormalities

are considered to be the important prognostic factors of AML (1,2). In patients with a normal karyotype, specific and accurate predictors may play a key role in the evaluation of prognosis. These normal cytogenetic patients with isolated bi-allelic *CEBPA* (*biCEBPA*) or *NPM1* mutation without *FLT3*-ITD are associated with a favorable prognosis, whereas *FLT3*-ITD or *TP53* mutations are associated with a poor prognosis (3-6). DNA methyltransferase 3A (*DNMT3A*) gene mutations are associated with hyperleukocytosis at disease presentation, the elderly, and a poor prognosis (7). Patients less than 60 years and with *DNMT3A*, *FLT3*-ITD, and *NPM1* mutations had a shorter event-free survival (EFS) (P=0.047). Further, patients with *DNMT3A* and *NPM1* mutations had a significantly shorter overall survival (OS) compared to those with *FLT3*-ITD and *NPM1* mutations (P=0.047) suggesting that the adverse impact of *DNMT3A* mutations is more pronounced than that of *FLT3*-ITD among patients with an *NPM1* mutation (8).

Mitochondrial dysfunction has been found in cancer progression (9). Human mitochondrial DNA (mtDNA) is a 16-kb circle that contains genes encoding 13 electron transport chain proteins, 22 tRNAs, and 2 rRNAs (10). Disrupted electron transport chain function was due to mtDNA mutations, which involve mitochondrial genes encoding components of respiratory Complex I of the electron transport chain. ND4, one of the seven Complex I subunits encoded by mtDNA, is predicted to be important for proton translocation (9). Acquired deletions of mtDNA in the hematopoietic compartment have also been found to occur in association with some hematological diseases (10). Mutations in the mitochondrial NADH dehydrogenase subunit 4 (*ND4*) were described in three of 93 AML patients, but the importance of these mutations is not yet clear (11). Another study noted 29 of 452 patients (6.4%) had *ND4* mutations predicted to affect translation, which implied acquired *ND4* mutations in AML may have a favorable prognostic value. Patients with somatically acquired *ND4* mutations had significantly longer relapse-free survival (RFS) and OS than *ND4* wild-type patients, while germline *ND4* mutations tended to have shorter survival (12). The clinical characteristics of *ND4* gene mutations were not very clear and the prognostic influence in AML was not validated until now. We performed this study to systematically investigate the frequency and the prognostic relevance of new molecular markers and conventional gene mutations in 460 adult AML patients and divide these patients into appropriate prognostic groups using molecular markers.

Methods

Patients and treatment

From 2004 to 2016, bone marrow (BM) and peripheral blood (PB) samples were collected from 460 patients diagnosed with *de novo* AML according to the French-American-British (FAB) criteria admitted to the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital. Two hundred and sixty patients were males and 200 were females, with a median age of 47 years (range, 18–86 years). Median follow-up time was 18 months (range, 1–144 months). The diagnosis of core binding factor (CBF) AML was based on cytogenetic findings of karyotype t(8;21) and inv[16] or detection of the fusion transcripts *AML1-ETO* and *CBFβ-MYH11* by reverse transcription polymerase chain reaction (RT-PCR). This study was approved by the ethics board of the hospital.

Gene mutations and cytogenetic analysis

Genomic DNA was isolated according to standard protocol. For mutation analysis, the whole amplicon or hot spot of *FLT3*-ITD, *NPM1*, *c-KIT*, *CEBPA*, *DNMT3A*, and *ND4* genes were amplified using standard PCR conditions (*Table S1*). Somatic or germline status of *ND4* mutations was established by evaluating matched samples, with follow-up samples obtained when patients were in complete remission (CR). The mutational status of these genes was determined by Sanger sequencing. The BM samples of the patients were studied mostly by R-banding analysis, and chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature 2013 (ISCN2013).

Flow cytometric analysis

Cell surface antigens were analyzed by four-color flow cytometry. Anti-CD34, human leukocyte antigen (HLA)-DR, CD117, CD13, CD33, CD14, CD15, CD10, CD19, CD20, CD22, CD2, CD3, CD5, CD7, CD4, and CD8 were purchased from BD Pharmingen (San Diego, CA, USA). All cell surface antigens were detected following the manufacturer's instructions and the data were analyzed using CELLQuest software (Becton-Dickinson).

Treatment protocols

For acute promyelocytic leukemia (APL) patients with *PML-RARα* fusion gene or t(15;17), all-trans retinoic acid

and arsenic trioxide -based treatment was given for the induction and consolidation therapy. Other AML patients received standard first-line treatment. In the consolidation therapy group, patients were additionally treated with high-dose cytarabine-based chemotherapy (13).

Statistical analysis

Quantitative data were described in the form of mean ± standard deviation (SD). Statistical analysis was done by comparison between groups using one-way ANOVA regarding quantitative data and chi-square test regarding qualitative data while quantitative nonparametric data comparison was performed using Mann-Whitney U. Kaplan-Meier analysis was used to calculate the distribution of OS and RFS. All P values <0.05 (two-tailed) were considered statistically significant. The statistical analysis of data was done by using SPSS version 17.0.

Results

Frequencies of gene mutations

Among 460 AML patients, *FLT3*-ITD mutations were found in 48 (48/385, 12.5%), *NPM1* in 67 (67/428, 15.7%), *c-KIT* in 7 (7/312, 2.2%), mono-allelic *CEBPA* (*monoCEBPA*) in 37 (37/287, 12.9%), bi-allelic *CEBPA* (*biCEBPA*) in 10 (10/287, 3.5%), *DNMT3A* in 20 (20/252, 7.9%), and *ND4* in 10 (10/152, 6.6%).

Fifty-two patients (11.3%) were diagnosed with CBF AML. There were 6 patients with inv[16] or *CBFβ-MYH11* and 46 patients with t(8;21) or *AML1-ETO*. *FLT3*-ITD (4/20, 20.0%) were frequently present in addition to the *PML-RARα* fusion in the M3 subtype, but there was no significant difference among different groups (P>0.05 for all comparisons). Conversely, *c-KIT* mutations were most commonly seen in CBF leukemia patients (6 of 37, 16.2%) and all the cases were of the M2 type. Patients with *FLT3*-ITD, *NPM1*, *CEBPA*, or *DNMT3A* mutations had no significant difference in the distribution of CBF, M3, or non-CBF and M3 patients (P>0.05) while patients with *c-KIT* mutations were more frequent in CBF patients than in non-CBF and M3 patients (P<0.001). *DNMT3A* mutations were found in 18.4% (7/38) and 10.7% (3/28) of M5 and M4 subtype, respectively. There was a significant difference of *DNMT3A* mutations in M5 but not in M4 (P=0.006 and 0.498, respectively). In patients with intermediate cytogenetic risk, there were six cases of M5, five cases of M2, two cases of M1, and two cases of M4 with *DNMT3A* mutations. There was no significant difference of distribution in M5 and M4 between patients with *DNMT3A* mutations and patients with intermediate cytogenetic risk and *DNMT3A* mutations (P=0.134 and 1.0, respectively).

Types of ND4 mutations and correlations with other molecular markers

The mutation types of *ND4* were A131V, F149L, G242D, A404T, and Y409H. The most common amino-acid change of *ND4* mutation was the A404T substitution, which was observed in four cases (4/10, 40.0%). The A131V mutation was observed in three patients, while F149L, G242D, and Y409H mutations were only detected in a single case. The germline mutations included were types of A131V, F149L, A404T, and Y409H in non-M3 patients, while one M3 patient had somatic mutation with type of G242D. These mutations were established by evaluating matched samples, with follow-up samples obtained when patients were in CR.

In the *ND4* mutated patients, three patients had molecular abnormalities including *monoCEBPA* and *biCEBPA* mutations. One patient had the A131V mutation, while another one patient had the Y409H mutation, and both these patients had an additional *monoCEBPA* mutation. One patient with A404T mutation had a *biCEBPA* mutation, while the other mutated patients had no additional mutations.

Clinical characteristics of patients with gene mutations

In non-M3 patients, *NPM1* (P<0.001) and *DNMT3A* (P=0.012) mutations were older than patients with the wild type, while patients with *c-KIT* mutations were younger (P=0.003) (*Table 1*). *FLT3*-ITD (P<0.001) and *NPM1* (P=0.003) mutations were associated with high white blood cell (WBC) counts, while *c-KIT* (P<0.001) mutations were associated with low WBC counts. *FLT3*-ITD (P=0.005), *NPM1* (P=0.001), and *monoCEBPA* (P=0.013) mutations were associated with a higher percentage of blasts in the BM (*Table 1*). There was no significant difference in any of the gene mutations according to gender, level of hemoglobin (HB) or platelet (PLT) counts (P>0.05 for all comparisons).

Of a total of ten patients with *ND4* mutations, three had normal cytogenetics, three others had favorable cytogenetics including t(8;21) or t(15;17), one patient had a complex karyotype, another one patient had a non-defined karyotype, while no karyotype was found in the remaining two patients. Six patients were males and four were females.

Table 1 Clinical characteristics of gene mutations in non-M3 AML patients

Gene mutations	Gender (n)		Median age (years)	WBC (×10^9/L)	HB (g/L)	PLT (×10^9/L)	BM blasts (%)
	Male	Female					
FLT3-ITD							
Mutated	28	16	51.84±17.46	98.52±89.11	83.00±26.75	48.03±47.78	66.27±26.81
Wild type	171	150	47.80±18.41	30.85±56.52	82.81±25.87	66.76±73.23	53.21±28.52
P	0.195		0.170	<0.001	0.966	0.122	0.005
NPM1							
Mutated	34	33	55.34±17.53	65.11±79.80	87.71±27.50	63.74±47.41	63.74±24.56
Wild type	150	130	46.22±19.03	32.06±58.23	82.60±25.90	66.59±77.95	51.97±29.22
P	0.677		<0.001	0.003	0.177	0.788	0.001
c-KIT							
Mutated	5	2	30.14±10.61	10.67±3.18	80.67±24.71	21.67±11.47	68.20±26.87
Wild type	158	136	48.22±19.06	39.83±64.11	83.38±25.82	65.41±74.65	54.22±28.92
P	0.586		0.003	<0.001	0.799	0.153	0.242
monoCEBPA							
Mutated	23	14	44.30±16.85	49.26±63.42	86.31±26.56	57.34±61.63	64.60±24.37
Wild type	120	114	48.52±19.51	38.25±65.62	80.98±24.87	63.33±75.32	52.91±29.37
P	0.218		0.215	0.357	0.245	0.656	0.013
biCEBPA							
Mutated	6	4	44.44±19.79	29.29±32.29	96.50±24.58	50.20±28.26	48.82±26.73
Wild type	120	114	48.52±19.51	38.25±65.62	80.98±24.87	63.33±75.32	52.91±29.36
P	0.828		0.540	0.669	0.055	0.584	0.666
DNMT3A							
Mutated	11	9	58.55±13.57	55.27±74.32	87.20±28.77	64.70±54.97	52.39±26.19
Wild type	116	104	47.48±19.22	38.54±62.54	83.41±25.53	65.98±76.50	54.60±29.28
P	0.845		0.012	0.263	0.532	0.942	0.756
ND4							
Mutated	5	4	40.00±19.91	38.26±72.89	85.06±28.51	58.89±86.38	56.55±28.14
Wild type	70	69	50.09±19.28	41.71±70.94	83.06±27.07	63.02±73.18	54.27±29.35
P	1.000		0.131	0.888	0.831	0.872	0.831

WBC, white blood cell; HB, hemoglobin; PLT, platelet; *monoCEBPA*, mono-allelic *CEBPA*; *biCEBPA*, bi-allelic *CEBPA*.

Patients with *ND4* mutations were M1 (2/10, 20.0%), M2 (7/10, 70.0%), and M3 (1/10, 10.0%) types based on the FAB classification: three patients with A131V were M2; four patients with A404T were: three M2 and one M1; one patient with F149L was M2; one patient with G242D was M3, and one patient with Y409H was M1. *ND4* mutations were prevalent in patients with AML of M2 morphology (P=0.001). Flow cytometric analysis of leukemic cells showed CD34 positive (7/10, 70.0%), CD117 positive (10/10, 100%), HLA-DR positive (9/10, 90.0%), CD13 positive (9/10, 90.0%), CD33 positive (9/10, 90.0%), CD15 positive (7/10, 70.0%), and T- or B-associated markers

CD4 and/or CD7 and/or CD19 positive (5/10, 50.0%) (data not shown). The median age of *ND4* mutated patients *vs.* *ND4* wild-type patients in non-M3 patients was 40 *vs.* 50 years (P=0.131). In non-M3 patients, patients with *ND4* mutations were associated with higher expression of CD19 (P=0.045). Compared with patients with *ND4* wild-type, patients with *ND4* mutations were not associated with *FLT3*-ITD, *NPM1*, *c-KIT*, *CEBPA*, and *DNMT3A* mutations in non-M3 patients (P>0.05).

Response to induction therapy and survival analysis

Rate of CR was 62.9%, and older patients (P=0.032), and males (P=0.003) were significantly associated with lower rates of CR in non-M3 patients. Relapse rate (RR) of non-M3 patients was 25.4% (P=0.001) and CBF patients was 24.1% (P=0.004), while it was 0 in M3 patients.

About 60% of patients (6/10) with *ND4* mutations achieved CR, and of the 10 patients, 4 patients had a relapse. Compared with patients with *ND4* wild-type, patients with *ND4* mutations had no significant difference in CR rate or RRs (P>0.05).

In the OS and RFS analysis for patients with *ND4* mutations and wild-type *ND4*, the median OS were 13 and 18 months, respectively, while the RFS median were 11 and 16 months, respectively. In non-M3 patients, patients with *ND4* germline mutations may have unfavorable prognosis, but there was no statistical significance in OS and RFS between patients with germline *ND4* mutations and wild-type patients (P=0.159 and 0.087, respectively) (*Figure 1A*,*B*).

We then compared the OS and RFS of CBF AML patients according to *c-KIT* mutation status. There was a significant difference in OS and RFS between *c-KIT*-mutated and wild-type patients in Kaplan-Meier analysis (P=0.023 and 0.044, respectively) (*Figure 1C*,*D*). CBF AML patients with *c-KIT* mutations had poor prognosis compared with patients of wild-type *c-KIT*.

Among patients with normal cytogenetic AML, we classified patients into three categories: patients with *NPM1* mutation or *biCEBPA* mutation without *FLT3*-ITD mutations; patients with *FLT3*-ITD mutations; patients with another molecular profile. Mutational status could further stratify AML patients with normal cytogenetic risk into three subgroups, which was a supplement of the cytogenetic prognosis risk (P=0.017 and 0.025 for OS and RFS, respectively) (*Figure 1E*,*F*). The favorable mutational risk demonstrated the longest survival and vice versa with unfavorable mutational risk.

In the univariate analysis, patients older than 60 years [HR for OS, 1.684 (1.097–2.585), P=0.017; HR for RFS, 1.638 (1.067–2.515), P=0.024], *FLT3*-ITD [HR for OS, 2.065 (1.210–3.524), P=0.008; HR for RFS, 2.013 (1.176–3.446), P=0.011], favorable molecular group [HR for OS, 0.641 (0.434–0.948), P=0.026; HR for RFS, 0.662 (0.446–0.983), P=0.041] and normal cytogenetic risk with favorable mutational risk [HR for OS, 0.528 (0.301–0.928), P=0.026; HR for RFS, 0.544 (0.313–0.945), P=0.0.031] were related to prognosis of non-M3 patients. There was no statistical significance in OS and RFS in multivariate analysis of any parameters (P>0.05 for all comparisons).

Discussion

About 50% of AML patients had no cytogenetic markers which are independent predictors for prognosis (14,15), genetic mutations may play an important role in the prognostic value in AML. Different molecular markers usually stratify AML patients into subtypes with distinctive prognosis and response to therapy (16). Recent descriptions of mutations in the mitochondrial *ND4* gene in leukemia patients coupled with altered metabolic function to leukemogenesis (10). In this study, we attempted to study the value of mutation of *ND4* and *DNMT3A* and other conventional factors in the prognosis of AML patients and the role of cytogenetic and molecular markers in the evaluation of prognosis.

Shen *et al.* (13) believed that there was subtype-restricted distribution in AML, such as *c-KIT* mutation, often as the second hit, which plays an important role in the pathogenesis of CBF leukemia. We found that patients with *c-KIT* mutations were more frequent in CBF patients than in non-CBF and M3 patients (*P*<0.001). Yan *et al.* (17) discovered *DNMT3A* mutations were 20.5% and 13.6% of M5 and M4 subtype, respectively. Our results showed that *DNMT3A* mutations were found in M5 but not in M4 (P=0.006 and 0.498, respectively), indicating that *DNMT3A* mutations are restricted to the monocytic lineage involvement in AML. Marková *et al.* (18) showed that occurrence of *DNMT3A* mutations was not associated with particular FAB subtypes in patients with intermediate-risk cytogenetics. Our results also showed that patients with intermediate-risk cytogenetics and with *DNMT3A* mutations had no significant difference in distribution of M5 and M4 subtypes.

ND4 is a part of respiratory Complex I, which leads to decreased Complex I activity and subsequently decreased NAD+ generation may also result in altered α-ketoglutarate production and epigenetic modulation (12). There were 11 predicted transmembrane domains of ND4 may be

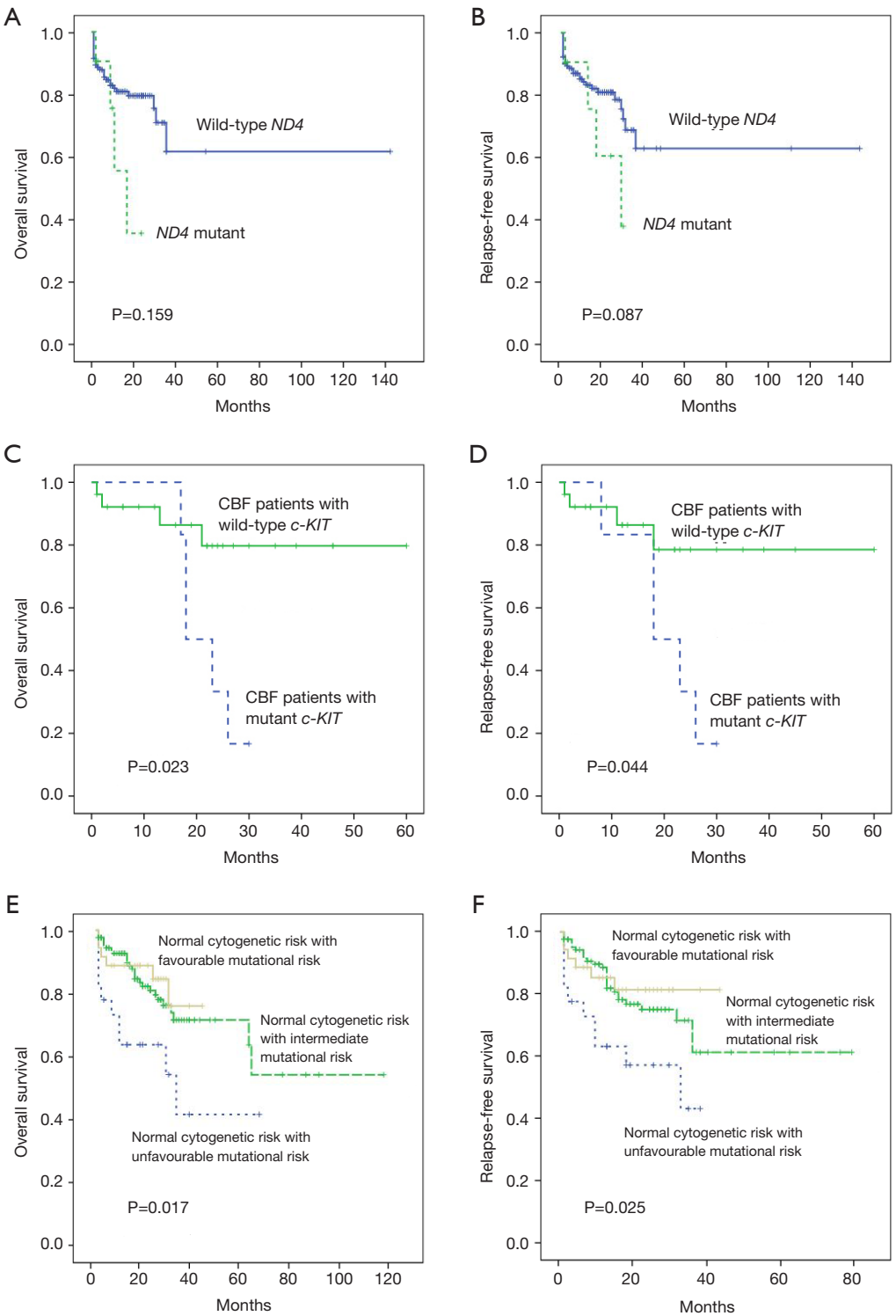

Figure 1 OS and RFS of AML patients in the Kaplan-Meier analysis. (A,B) OS and RFS of *ND4* mutated and wild-type patients in non-M3 patients; (C,D) the OS and RFS of CBF AML patients according to the *c-KIT* mutation status; (E,F) mutational status further stratified AML patients with normal cytogenetic risk into three subgroups. OS, overall survival; RFS, relapse-free survival; AML, acute myeloid leukemia; CBF, core binding factor.

important for mitochondrial proton transport and four types (F149L, G242D, A404T and Y409H) of the mutations we detected were within these domains. *ND4* mutations affect mitochondrial function, and the survival and/or proliferative capacities of leukemia cells may be changed (12). In this study, patients with *ND4* mutations were considered as M1 (20.0%), M2 (70.0%), and M3 (10.0%) types based on FAB classification. *ND4* mutations were prevalent in M2 patients, and this must be further validated. Damm *et al.* (12) observed that *ND4* mutated patients tended to be younger (P=0.059), with a median age of 39 *vs.* 47 years for *ND4* wild-type patients. Our results showed no significant difference in non-M3 patients. The non-M3 patients have germline mutation types of A131V, F149L, A404T, and Y409H, while one M3 patient had somatic mutation with type of G242D. This was in line with the conclusion of the study by Damm *et al.* (12). In non-M3 patients, *ND4* mutations were associated with higher expression of CD19 (P=0.045). A close relationship was observed between the expression of CD19 and t(8;21) (19), and certain *AML1-ETO*-positive cases demonstrated characteristic immunological features (such as CD19 and CD34 expressions, and CD33 negativity) (20). Hence, we speculated that *ND4* mutations were found more often in M1 and M2 patient types in this study, and were associated with the expression of CD19.

In the present study, RR of non-M3 patients was 25.4% (P=0.001) and CBF of patients was 24.1% (P=0.004), while it was 0 in M3 patients, indicating that M3 patients always had a favorable response to therapy. Patients with *ND4* germline mutations may have unfavorable prognosis, but there was no statistical significance in OS and RFS between patients with germline *ND4* mutations and wild-type patients (P=0.159 and 0.087, respectively; *Figure 1A,B*). Patients with somatic *ND4* mutations had favorable prognosis than *ND4* wild-type patients (P<0.05 for both comparisons), while no significant differences were found in patients with *ND4* mutations and *ND4* wild-type patients (12). Obviously, compared to patients with *ND4* wild-type, patients with somatic *ND4* mutations demonstrated favorable prognosis and patients with germline *ND4* mutations tended to have poor prognosis.

Boissel *et al.* (21) found that *c-KIT* mutations were associated with a shorter EFS and RFS (P=0.002 and 0.003) in t(8;21) but not in inv[16] patients. We compared the OS and RFS of CBF AML patients according to *c-KIT* mutation status and found that there was a significant difference in OS and RFS between *c-KIT*-mutated and wild-type patients (P=0.023 and 0.044, respectively) (*Figure 1C,D*). In the present study, we tried to stratify patients with normal cytogenetics into different subgroups. We found that, according to the molecular prognostic factors, patients with normal cytogenetic risk could be divided into three groups in the OS and RFS analysis (P=0.017 and 0.025, respectively) (*Figure 1E,F*). Different mutational risks have distinctive prognosis in these groups, favorable mutational risk had the longest survival and unfavorable mutational risk had the shortest survival. The significance for prognosis could be observed, which in turn could help the physicians to treat patients in an individualized manner.

Conclusions

In summary, combining the cytogenetic risk and the mutational risk, an important role in the distinctive stratification of AML patients is possible. *ND4* mutations were prevalent in M2 patients and were associated with higher expression of CD19. Whether patients with germline *ND4* mutations have poorer prognosis still needs to be confirmed.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.07.21). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Our study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University

(2018-SR-136) and because the medical records/biological specimens were obtained from previous clinical diagnosis, the exemption of informed consent was approved by the IRB.

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Supplementary

Table S1 Primer sequences and amplification conditions of gene mutations

Gene	Primers	Primer sequence (5'→3')	Product (bp)	Annealing temperature (℃)
FLT3-ITD	FLT3-ITD-F	GCAATTTAGGTATGAAAGCCAGC	329	60
	FLT3-ITD-R	CTTTCAGCATTTTGACGGCAACC		
NPM1	NPM1-F	TTAACTCTCTGGTGGTAGAATGAA	550	56
	NPM1-R	CAAGACTATTTGCCATTCCTAAC		
c-KIT	c-KIT-F1	TGAACATCATTCAAGGCGTA	550	56
	c-KIT-R1	TCACATGCCCCAAAATTACA		
	c-KIT-F2	CTCCCTGAAAGCAGAAAC	630	55
	c-KIT-R2	CAGAAAGATAACACCAAAATAG		
CEBPA	CEBPA-F1	TCGGCCGACTTCTACGAG	508	58
	CEBPA-R1	GCTTGGCTTCATCCTCCTC		
	CEBPA-F2	GAGGAGGATGAAGCCAAGC	550	58
	CEBPA-R2	GTTGCCCATGGCCTTGAC		
DNMT3A	DNMT3A-F	TCCTGCTGTGTGGTTAGACG	380	60
	DNMT3A-R	TATTTCCGCCTCTGTGGTTT		
ND4	ND4-F1	GCCAATATTGTGCCTATTGC	680	56
	ND4-R1	TTCTTGGGCAGTGAGAGTGA		
	ND4-F2	TGAACGCAGGCACATACTTC	685	56
	ND4-R2	GGGGGTAAGGCGAGGTTAG		
	ND4-F3	GCCTAGCAAACTCAAACTACGA	499	56
	ND4-R3	GGGGCATGAGTTAGCAGTTC		