International Journal of Hematology (2023) 118:36–46
https://doi.org/10.1007/s12185-023-03567-1

ORIGINAL ARTICLE

Prognostic impact of *FLT3*-ITD, *NPM1* mutation and *CEBPA* bZIP domain mutation in cytogenetically normal acute myeloid leukemia: a Hokkaido Leukemia Net study

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Received: 24 November 2022 / Revised: 14 February 2023 / Accepted: 14 February 2023 / Published online: 28 February 2023

Abstract

Mutation status of FLT3, NPM1, and CEBPA is used to classify the prognosis of acute myeloid leukemia, but its significance in patients with cytogenetically normal (CN) AML is unclear. We prospectively analyzed these genes in 295 patients with CN-AML and identified 76 (25.8%) FLT3-ITD, 113 (38.3%) NPM1 mutations, and 30 (10.2%) CEBPA biallelic mutations. We found that patients with FLT3-ITD had a poor prognosis at any age, while patients with CEBPA biallelic mutation were younger and had a better prognosis. FLT3-ITD and NPM1 mutations were correlated, and the favorable prognostic impact of being FLT3-ITD negative and NPM1 mutation positive was evident only in patients aged 65 years or more. For CEBPA, 86.7% of the patients with biallelic mutation and 9.1% of patients with the single allele mutation had in-frame mutations in the bZIP domain, which were strongly associated with a favorable prognosis. Multivariate analysis showed that age < 65 years, FLT3-ITD and CEBPA bZIP in-frame mutation were independent prognostic factors. The results suggest that analyzing these gene mutations at diagnosis can inform selection of the optimal intensity of therapy for patients with CN-AML.

Keywords AML · *FLT3* · *NPM1* · *CEBPA* bZIP · Hokkaido Leukemia Net (HLN) · North Japan Hematology Study Group (NJHSG)

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Introduction

Prognostic risk classification of acute myeloid leukemia (AML) has been subdivided according to chromosomal abnormalities and genetic mutations. The National Comprehensive Center Network (NCCN) and European LeukemiaNet (ELN) continue to revise their prognostic risk classifications for AML [1–7]. In NCCN guidelines 2017 AML classification, cytogenetically normal (CN)-AML is prognostically stratified by Fms-like tyrosine kinase 3-internal tandem duplication (*FLT3*-ITD), mutations of nucleophosmin 1 (*NPM1*) and biallelic mutation of CCAAT/enhancer-binding protein alpha (*CEBPA*) [2]. Although biallelic mutation of *CEBPA* (*CEBPA*-bm) has long been considered to be a better prognostic marker [2, 6, 8], recent studies have shown that a single mutation in the C-terminus basic leucine zipper domain (bZIP) is strongly associated with better prognosis [9, 10]. We determined the clinical features and prognosis of CN-AML by analyzing *FLT3*-ITD, *NPM1* mutation, and *CEBPA* mutation based on our regional prospective AML cohort.

Patients and methods

Patients

Hokkaido Leukemia Net (HLN) is a multicentric prospective observational cohort study collecting acute leukemia samples from affiliated hospitals of the North Japan Hematology Study Group (NJHSG) covering all of Hokkaido (UMIN000048611). In the HLN prospective protocol, newly diagnosed cases of acute myeloid leukemia excluding acute promyelocytic leukemia (APL) are analyzed for the presence of *FLT3*-ITD and Wilms tumor gene (WT1) expression, preserving genome DNA and complementary DNA (cDNA), respectively. The results are returned to the clinician in charge and then the clinician reports the patient's mutation and clinical information including final diagnosis and karyotype. *NPM1* and *CEBPA* or *KIT* mutations are additionally analyzed for CN-AML or core-binding factor AML, respectively (Fig. 1A). The results of these genetic analyses are returned to the clinician, and this information enables the clinician to stratify AML into three prognostic groups based on the NCCN 2017 prognostic criteria (Table 1). In the HLN protocol, patients aged 16 years or older are eligible for registration without an upper age limit, reflecting regional real-world cohort. Each patient's treatment is at the physician's discretion. We retrospectively analyzed *FLT3*-ITD, *NPM1* mutation, and the mutation pattern of *CEBPA* and prognosis in patients

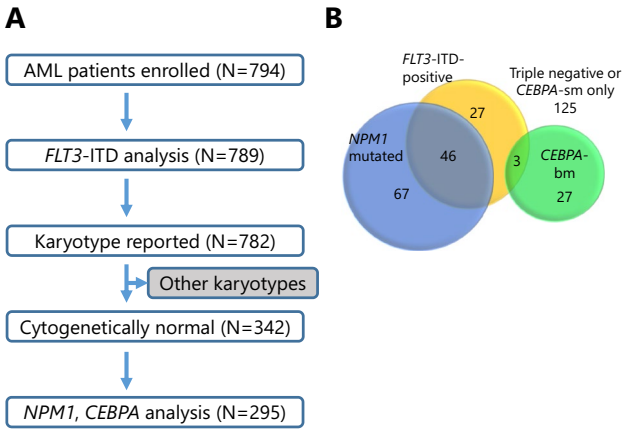


Fig. 1 **A** Flow of analysis of AML patients in HLN. **B** Venn diagram of *FLT3*-ITD, *NPM1* and *CEBPA* mutations

with CN-AML. This study was conducted in accordance with the Helsinki Declaration and was approved by the institutional review boards of Hokkaido University Hospital (#015-0344). Written consent was obtained from all patients.

Mutational analysis

Patient samples were collected at diagnosis, and genomic DNA was extracted from bone marrow or peripheral blood. *FLT3*-ITD, *NPM1 mutation*, and *CEBPA* mutation were analyzed using a genome DNA template. The relevant region of *FLT3* was amplified by polymerase chain reaction (PCR) and gel electrophoresis to verify the presence of the ITD band. *NPM1* exon 12 and full-length *CEBPA* were analyzed by Sanger sequencing. Primers used for PCR and Sanger sequencing are listed in Supplemental Table 1. Sanger sequencing was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) and analyzed by Applied Biosystems SeqStudio Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). The 6-bp duplication polymorphism within the *CEBPA* TAD2 domain (c.589_590 ins ACC CGC; rs762459325) and single-nucleotide polymorphism (c.690 G>T; rs34529039) were not considered as mutations. Although AML with *TP53* mutation is classified as a poor prognosis group in the NCCN 2017 guidelines, mutation analysis of *TP53* was omitted because the frequency of *TP53* mutations is rare (2–3%) in CN-AML [11, 12].

Statistical analysis

Clinical variables across groups were compared using the χ^2 test or 2-sided Fisher's exact test for categorial variables. The nonparametric Mann–Whitney *U* test and Kruskal–Wallis

Table 1 NCCN guidelines 2017

Risk status	*Cytogenetics/Molecular abnormalities
Favorable	Normal cytogenetics: *NPM1* mutation in the absence of *FLT3*-ITD or isolated biallelic *CEBPA* mutation
Intermediate	Normal cytogenetics
Poor	Normal cytogenetics: with *FLT3*-ITD mutation *TP53* mutation

*Criteria for CN-AML were extracted

test were used for continuous variables. A P value < 0.05 indicated a significant difference. Prognostic analysis was done excluding patients treated by best supportive care only. The log-rank test was used to evaluate overall survival (OS). For multivariable analysis of prognostic factors, Cox proportional hazards regression models were used. Variables were selected based on previous studies and clinical experience. For multiple comparisons of three or more groups, p-values were adjusted using Bonferroni–Holm correction. All statistical analyses were performed with EZR software ver.1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13].

Results

A total of 794 patients with AML were enrolled in the HLN from January 2009 to December 2021. CN-AML accounted for 43.7% (342 cases) of the 782 karyotype-identified cases. Of those, 295 patients for whom all data were available were included in the study (Fig. 1A). Patients' characteristics are shown in Table 2. The median age of the 295 CN-AML patients was 64 years (range, 18–93 years). Age distribution was shown on Supplemental Fig. 1. The patients included 155 males and 140 females. Intensive chemotherapy with anthracycline plus cytarabine was performed in 195 patients (66.1%). After induction chemotherapy, complete remission was achieved in 207 patients (70.2%). Allogeneic hematopoietic stem cell transplantation (allo-SCT) was performed in 97 patients (32.9%). The median follow-up period was 446.5 days (range 0–4219). We excluded patients who did not receive chemotherapy from the prognostic analysis.

FLT3-ITD, *NPM1* mutation, and *CEBPA*-bm were positive in 76 patients (25.8%), 113 patients (38.3%), and 30 patients (25.1%), respectively. Cases with *NPM1* mutation and *CEBPA*-bm were completely exclusive (Fig. 1B). The proportion of patients with *NPM1* mutations increased with age, while the proportion of patients with *CEBPA*-bm decreased with age (Fig. 2A). Patients with *CEBPA*-bm were significantly younger at diagnosis than the other patients (median age: 46 vs 65 years, $P < 0.001$).

The prognostic impacts of *FLT3*-ITD, *NPM1* mutation, and *CEBPA* mutations were different between age groups (Fig. 2B, C). Patients who had *FLT3*-ITD-positive showed significantly poor survival compared to those patients who were *FLT3*-ITD-negative, whereas the difference is more prominent in patients younger than 65 years. Then, we analyzed the effect of the *FLT3*-ITD allelic ratio (AR) on the

Table 2 Patient's characteristics ($N = 295$)

	N (%)
Age	
Median, (range)	64 (18–93)
Gender	
Male	155 (52.5)
Female	140 (47.5)
WBC at diagnosis	
Median, (range) (/μL)	13,300 (340–496,000)
FLT3-ITD	
Positive	76 (25.8)
Negative	219 (74.2)
NPM1	
Mutated	113 (38.3)
Wild type	182 (61.7)
CEBPA	
Biallelic mutation	30 (10.2)
Single mutation	44 (14.9)
Wild type	221 (74.9)
Chemotherapy	
Intensive	195 (66.1)
*Reduced intensity	83 (28.1)
BSC	17 (5.8)
Achieved CR by induction chemotherapy	
Yes	207 (70.2)
No	75 (25.4)
NA	13 (4.4)
Underwent Allo-SCT	
Upfront	67 (22.7)
After relapse	30 (10.2)
No	198 (67.1)

CR complete remission, *BSC* best supportive care, *NA* not available, *Allo-SCT* allogeneic hematopoietic stem cell transplantation

*Reduced intensity chemotherapy: Azacitidine ± Venetoclax, Low-dose Cytarabine ± anthracycline, Behenoyl cytarabine + anthracycline, Hydroxyurea, Daunorubicin monotherapy

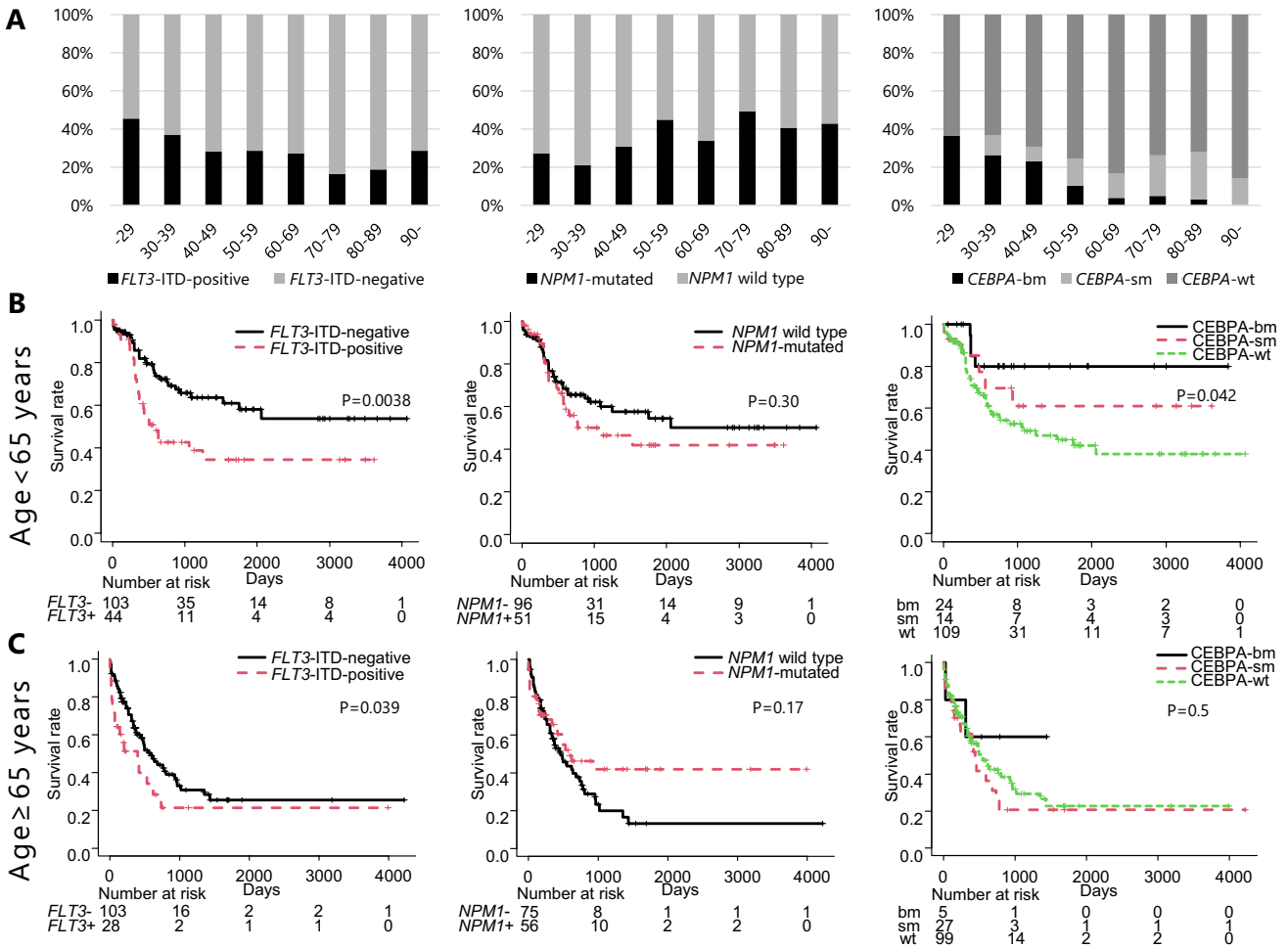


Fig. 2 **A** Ratios of mutations in *FLT3*-ITD, *NPM1*, and *CEBPA* by age. **B** Survival curves stratified by *FLT3*-ITD status, *NPM1* mutation status, and *CEBPA* mutation status for patients younger than 65 years. **C** Survival curves stratified by *FLT3*-ITD status, *NPM1* mutation status, and *CEBPA* mutation status for patients aged 65 years or more

prognosis of *FLT3*-ITD positive patients. *FLT3*-ITD AR was retrospectively analyzed for 58 patients. The WBC count at diagnosis was higher in the AR^{high} ($AR \geq 0.5$) group, but there were no statistically significant differences in other patient characteristics (Supplemental Table 2). Prognostic analysis of 56 patients who underwent induction chemotherapy showed no difference in prognosis between the AR^{high} and AR^{low} ($AR < 0.5$) groups (Supplemental Fig. 2A). Even combined with *NPM1* mutation, the better prognostic impact of AR^{low} was not evident (Supplemental Fig. 2B).

Although there was no statistically significant difference in prognosis by the *NPM1* mutation status alone, *NPM1*-mutated patients tend to show better prognosis only in patients aged 65 years or more. The 295 CN-AML patients were divided into three groups depending on *CEBPA* mutation; *CEBPA*-bm, *CEBPA* single mutation (*CEBPA*-sm), and *CEBPA* wild type (*CEBPA*-wt). *CEBPA*-bm showed significantly better survival in patients younger than 65 years; however, the statistical significance is not clear for patients aged 65 years or more due to the limited number of *CEBPA*-mutated cases. As reported previously [14], patients with *CEBPA*-bm had a significantly higher rate of complete remission (CR) (*CEBPA*-bm, 90.0% vs the other patients, 68.0%; $P = 0.025$) (Supplemental Table 3) as well as a better 5-year OS (*CEBPA*-bm, 76.4% vs *CEBPA*-sm, 35.0% vs *CEBPA*-wt, 32.7%; $P = 0.0074$).

Because there was no statistically significant difference in 5-year OS for the *NPM1* mutation alone, we combined *NPM1* mutation with *FLT3*-ITD status determined in 2 age groups (Age < 65 years, ≥ 65 years) (Fig. 3A, B). *FLT3*-ITD was a poor prognostic factor in both age groups, but the favorable prognostic impact of *NPM1* was more evident in patients aged 65 years or more.

Patients were classified according to the NCCN 2017 guidelines (Fig. 4A, B). Patients' characteristics are shown on Table 3. Patients younger than 65 years were

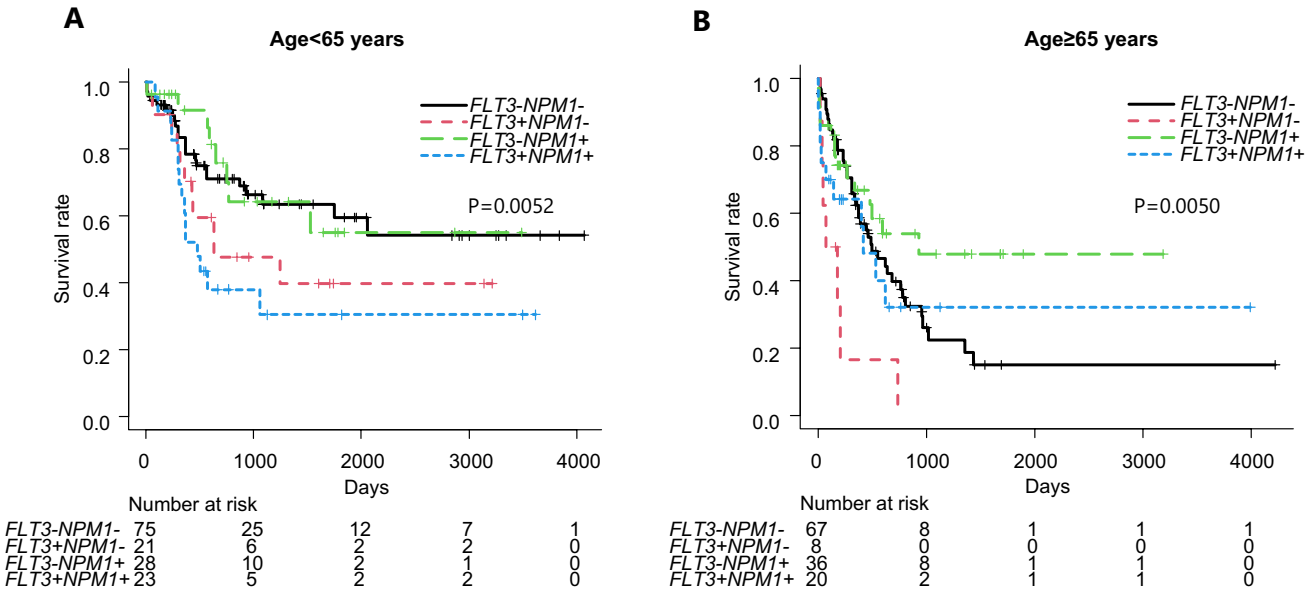


Fig. 3 **A** Survival curves stratified by the presence or absence of *FLT3*-ITD and *NPM1* mutation for patients younger than 65 years. **B** Survival curves stratified by the presence or absence of *FLT3*-ITD and NPM1 mutation for patients aged 65 years or more

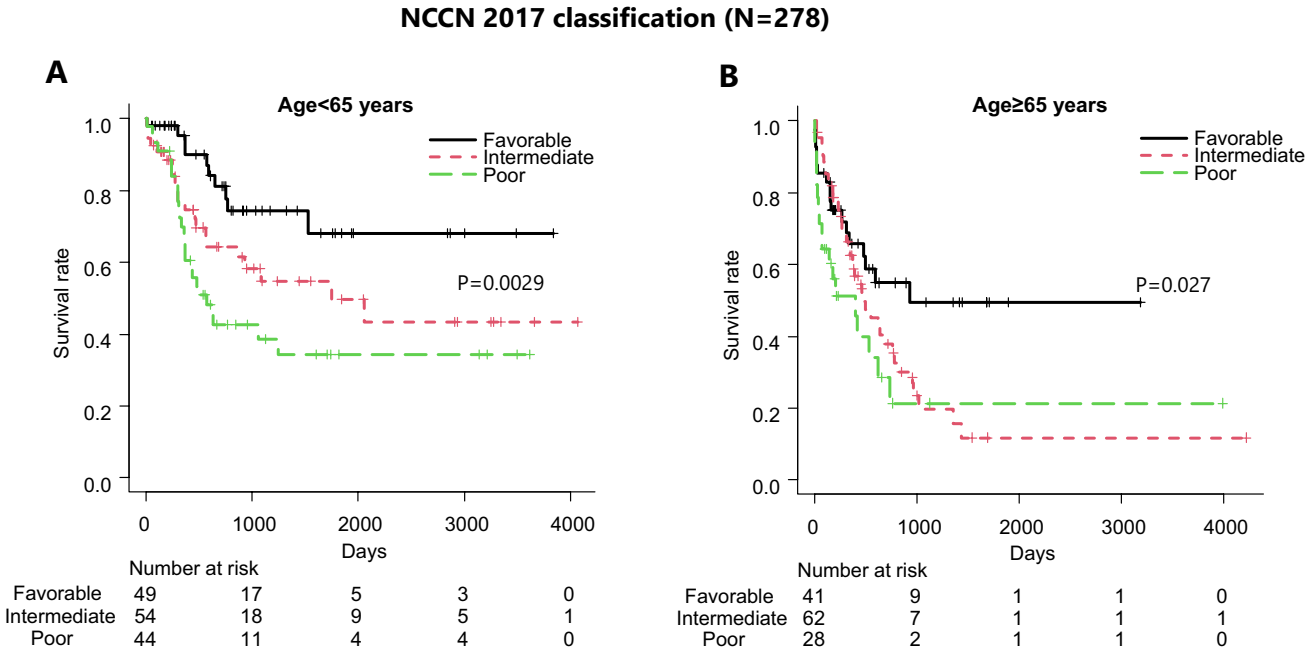


Fig. 4 **A** Survival curves based on the NCCN guidelines 2017 in patients younger than 65 years. **B** Survival curves based on the NCCN guidelines 2017 in patients aged 65 years or more

clearly stratified into 3 prognostic groups. For patients aged 65 years or more, the favorable-risk group had a significantly better prognosis than the intermediate- and poor-risk groups, but there was no difference in prognosis between the intermediate-risk and poor-risk groups in this cohort.

CEBPA bZIP in-frame mutation is good prognostic factor

The domain arrangement of the CEBPA protein is shown in Fig. 5. The CEBPA protein has a TAD1 domain, a TAD2 domain from the N-terminal side and a bZIP domain

Table 3 Patient's characteristics by risk status based on the NCCN guidelines 2017

	Favorable	Intermediate	Poor		*P* value
	N = 94	*N* = 125	*N* = 76		
	N (%)	*N* (%)	*N* (%)		
Age					
Median, (range)	64 (18–93)	66 (26–93)	60 (21–90)		0.06
Gender					
Male	46 (48.9)	68 (54.4)	41 (53.9)		0.72
Female	48 (51.1)	57 (45.6)	35 (46.1)		
WBC at diagnosis					
Median, (range) (/μL)	14,800 (580–316,790)	4450 (340–450,400)	55,135 (680–496,000)		< 0.001
Chemotherapy					
Intensive	66 (70.2)	70 (56.0)	59 (77.6)		0.021
*Reduced intensity	24 (25.5)	46 (36.8)	13 (17.1)		
BSC	4 (4.3)	9 (7.2)	4 (5.3)		
Achieved CR by induction chemotherapy					
Yes	76 (80.9)	80 (64.0)		51 (67.1)	0.0081
No	12 (12.8)	40 (32.0)		23 (30.3)	
NA	6 (6.4)	5 (4.0)		2 (2.6)	
Underwent Allo-SCT					
Upfront	10 (10.6)	27 (21.6)	30 (39.5)		0.062
After relapse	11 (11.7)	10 (8.0)	9 (11.8)		
No	73 (77.7)	88 (70.4)	37 (48.7)		

CR complete remission, *BSC* best supportive care, *NA* not available, *Allo-SCT* allogeneic hematopoietic stem cell transplantation

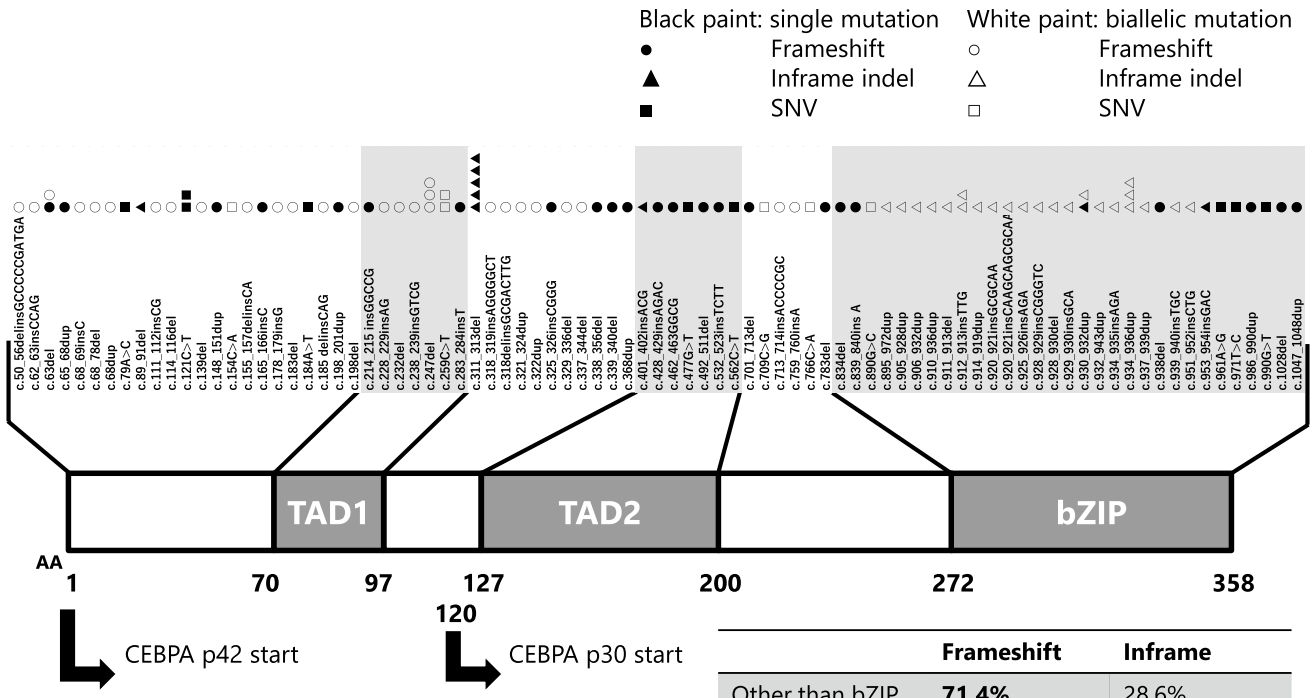
*Reduced chemotherapy; Azacitidine ± Venetoclax, Low-dose Cytarabine ± anthracycline, Behenoyl cytarabine + anthracycline, Hydroxyurea, Daunorubicin monotherapy

located at the C-terminus. The location and type of the mutation in the *CEBPA* gene in this study are also shown. Most of the non-bZIP mutations (71.4%) were frameshift mutations, and most of the mutations in the bZIP domain (82.9%) were in-frame mutations (Fig. 5). *CEBPA* bZIP mutation was found in 26 (86.7%) of the 30 *CEBPA*-bm cases and in 4 (9.1%) of the 44 *CEBPA*-sm cases (Fig. 6A).

Then we reclassified the patients with *CEBPA* bZIP in-frame mutations as "*CEBPA*-bZIP inf" and those with other mutations as "*CEBPA*-other" (Fig. 6A). Patients with *CEBPA*-bZIP inf were significantly younger at diagnosis than the other patients (median age: 46 vs 65 years, $P < 0.001$). However, there were no differences in other characteristics (gender, WBC count at diagnosis, blast percentage in bone marrow, *FLT3*-ITD-positive ratio, intensity of induction chemotherapy, and number of patients who received allo-SCT) (Supplemental Table 3). Patients with *CEBPA*-bZIP inf had a significantly higher rate of CR (*CEBPA*-bZIP inf vs the other patients, 90.0 vs 68.0%; $P = 0.025$) (Supplemental Table 3) as well as a higher 5-year OS (*CEBPA*-bZIP inf, 80.2% vs *CEBPA*-other, 34.7% vs *CEBPA*-wt, 33.5%; $P = 0.0068$) (Fig. 6B).

Factors associated with OS

Table 4 shows the results of univariate and multivariate analyses for OS. Univariate analysis was carried out for age, gender, WBC count, *FLT3*-ITD, *NPM1* mutation, *CEBPA*-bZIP inf, and intensity of induction chemotherapy, whether CR was achieved by induction chemotherapy, and allo-SCT. Male gender and *FLT3*-ITD-positive were associated with worse OS ($P = 0.020$ and 0.0080, respectively; log-rank test). Age < 65 years, intensive chemotherapy, achievement of CR by induction chemotherapy, allo-SCT, and *CEBPA*-bZIP inf were associated with better OS ($P < 0.001$, < 0.001, < 0.001, < 0.001, and 0.0036, respectively; log-rank test). Multivariate analysis showed that age < 65 years and *CEBPA*-bZIP inf were independently associated with better OS (age < 65 years: HR, 0.45; 95% CI 0.31–0.64, $P < 0.001$; *CEBPA*-bZIP inf: HR 0.31; 95% CI 0.13–0.78, $P = 0.013$) and that *FLT3*-ITD-positive was independently associated with worse OS (*FLT3*-ITD: HR, 1.77; 95% CI 1.16–2.70, $P < 0.001$).



	Frameshift	Inframe
Other than bZIP	**71.4%**	28.6%
bZIP	17.1%	**82.9%**

Fig. 5 Distribution and types of *CEBPA* mutations detected in this study

Discussion

CN-AML represents the most frequent category of AML cytogenetics, accounting for 40–50% of all cases of AML [15–21]. CN-AML is considered as an intermediate-risk group if the risk is classified only by karyotype. As a result of advances in genetic analysis, the prognostic classification of AML has been updated frequently and has become more detailed. Thus, searching for these genetic alterations in addition to identifying the karyotype is very important for prognostic stratification and selection of patient treatment strategies. According to the NCCN AML guidelines 2017, CN-AML cases with biallelic mutations of *CEBPA* or with *NPM1* mutation without *FLT3*-ITD are classified as a favorable-risk group [2]. However, a comprehensive panel sequence has not been clinically approved in Japan. Therefore, physicians have not been able to use genetic stratification according to international guidelines, and prognostic risk has been evaluated only by cytogenetics and treatment response in daily practice. In this study, without comprehensive genome panel analysis, we showed that mutation analysis of only three genes could clearly stratify a favorable prognostic group in CN-AML. Patients with *CEBPA*-bm or bZIP inframe mutation had a remarkably favorable prognosis. Patients with *NPM1* mutation without *FLT3*-ITD showed better prognosis than other groups in patients aged 65 years or more. If those patients were evaluated only by cytogenetics, they could be overtreated by upfront allo-SCT. In HLN, the prospective feedback of results of *FLT3*-ITD, *NPM1*, and *CEBPA* analysis to the attending physician would have resulted in a lower rate of upfront allo-SCT in the favorable-risk group and, conversely, a higher rate in the poor-risk group as shown in Table 2. The favorable risk group clearly showed a better prognosis despite a lower allo-SCT rate. On the other hand, we could not find a difference in OS between the intermediate- and poor-risk groups. The lack of statistical difference in prognosis between the intermediate-risk and poor-risk groups may be due to the older age of patients and the lower rate of intense chemotherapy in the intermediate-risk group. The higher allo-SCT rate in the poor-risk group might have improved OS in that group and abolished the difference between the poor-risk and intermediate-risk groups.

In the ELN 2022 classification, *FLT3*-ITD mutation is no longer considered as a poor prognostic factor, and *FLT3*-ITD-positive AML cases are classified as an intermediate-risk group regardless of *NPM1* mutation status [7]. In this study, *FLT3*-ITD was an independent poor prognostic factor for the survival of patients with CN-AML. This may be due to the fact that *FLT3* inhibitors were not available in Japan until 2018, and the agents are currently approved only for relapsed or refractory cases. We found that *FLT3*-ITD AR did not stratify the prognosis

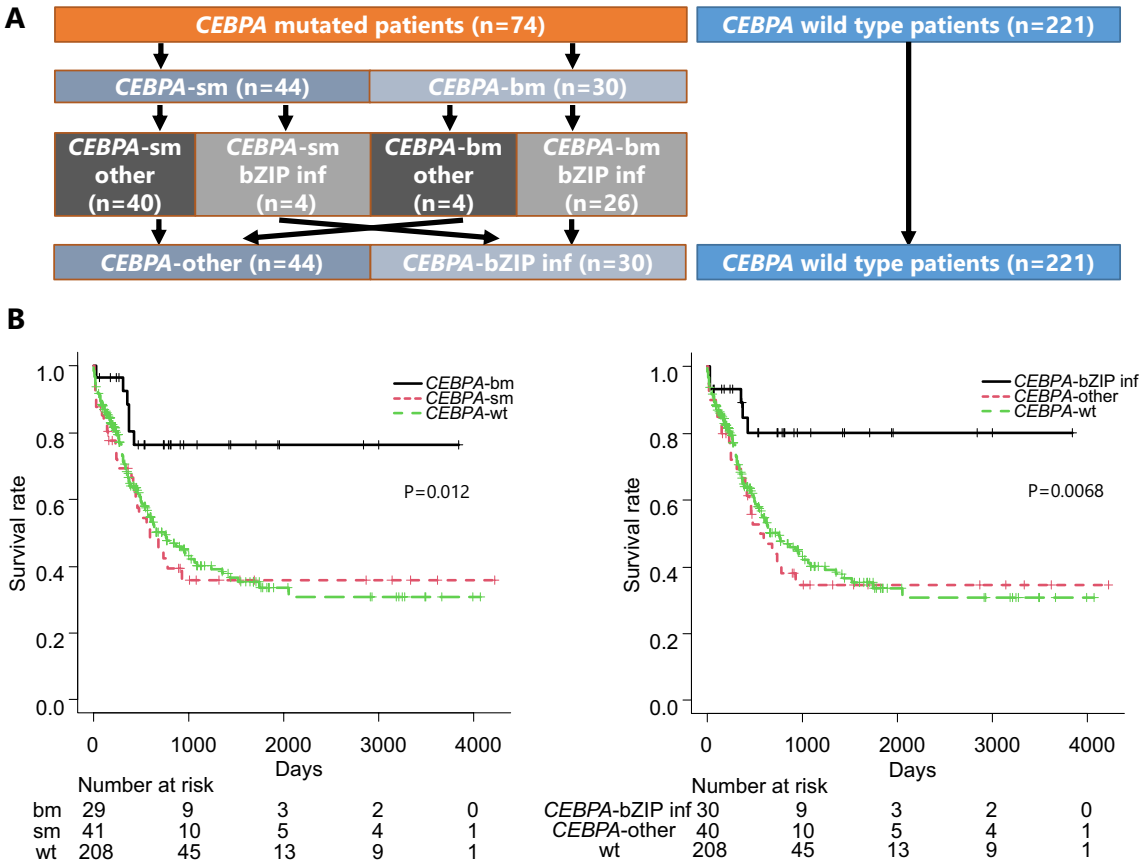


Fig. 6 **A** Reclassification based on mutations in the *CEBPA* bZIP domain. **B** Survival curves based on conventional *CEBPA*-bm (left) and new classification depend on *CEBPA* bZIP inframe mutation (right)

Table 4 Univariate and multivariate analysis for overall survival

	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age < 65	0.47	0.33–0.67	< 0.001	0.45	0.31–0.64	< 0.001
Male gender	1.41	1.07–2.13	0.020	1.30	0.92–1.84	0.14
WBC at diagnosis (/μL) > 12,795	1.34	0.95–1.89	0.090			
FLT3-ITD positive	1.63	1.14–2.34	0.0080	1.77	1.16–2.70	0.0081
NPM1-mutated	1.01	0.71–1.43	0.95			
CEBPA bZIP-inf	0.27	0.11–0.65	0.0036	0.31	0.13–0.78	0.013
Intensive chemotherapy	0.47	0.33–0.68	< 0.001			
Achieved CR by induction chemotherapy	0.27	0.19–0.39	< 0.001			
Underwent Allo-SCT	0.41	0.28–0.61	< 0.001			

HR hazard risk, *CI* confidence interval, *CR* complete remission, *Allo-SCT* allogeneic hematopoietic stem cell transplantation

of *FLT3*-ITD positive patients in our cohort, which is in contrast to the classification of the ELN 2017 guideline [6]. The prognostic significance of *FLT3*-ITD AR in the Japanese cohort is not consistent [22, 23]. Future application of upfront FLT3 inhibitors would abrogate the prognostic importance of *FLT3*-ITD AR.

We confirmed that *NPM1* mutation is often coexistent with *FLT3*-ITD as previously reported [19, 24, 25]. Although *NPM1* mutation alone did not show a favorable prognostic impact for all cases of CN-AML, *NPM1* mutation showed a favorable impact for *FLT3*-ITD-negative cases as previously reported in the pre-FLT3 inhibitor

era [19, 26–30]. The results of the analysis classified by age showed that the prognostic impacts of *FLT3*-ITD and *NPM1* mutations were different between age groups as previously reported [31]. In CN-AML, genetic mutations such as *DNMT3A* have been reported to affect prognosis [32], so it is possible that other gene mutations which we did not analyze also contribute to prognosis.

In the ELN 2022 classification, *CEBPA*-bZIP inf rather than *CEBPA*-bm has become a criterion of *CEBPA* as a favorable-risk group. In the 5th edition of the WHO classification, single mutations within the bZIP domain (*CEBPA*-smbZIP) in addition to *CEBPA*-bm defined *CEBPA* criteria for a good prognosis [7, 33]. In our cohort, the *CEBPA*-bm group and the *CEBPA*-bZIP inf group accounted for 87% (26/30) of the cases, and the *CEBPA*-bZIP inf group had an even better prognosis. Further study with a larger number of cases is needed because the number of *CEBPA*-smbZIP cases was small and followed a relatively short period in our cohort. We welcomed the successful simplification of prognostic criteria for *CEBPA* mutation because *CEBPA* is an extremely GC-rich gene (GC: 75%) and a difficult PCR target. Commercially available panel sequences could sometimes fail to detect *CEBPA* mutation due to lower sequence coverage of the region [34]. Targeting only the bZIP domain reduces sequencing effort because the *CEBPA* bZIP domain is less GC-rich (GC: 64%) than domains other than bZIP domain (GC: 78%). As previously reported [35, 36], most of the bZIP domain mutations were inframe mutations, whereas most of the mutations other than bZIP *CEBPA* mutation were frameshift mutations in our series. Frameshift mutation on the N-terminus results in the formation of a short p30-CEBPA protein instead of the normal p42-CEBPA protein. This shortened protein is the dominant-negative form to hamper the function of wild-type CEBPA protein. Most of the C-terminal mutations are in-frame mutations in the bZIP domain, and the DNA-binding and dimer-forming abilities are reduced [37, 38]. In this study, patients with *CEBPA*-bm or *CEBPA*-bZIP inf were significantly younger at diagnosis than patients in the other groups as previously described in several reports [9, 10, 39, 40]. One possible explanation is the presence of a subset of the *CEBPA*-bm population in which one of the *CEBPA* mutations was a germline mutation. It has been reported that more than 10% of cases of AML with biallelic *CEBPA* mutations carried an N-terminal frameshift *CEBPA* germline mutation with acquisition of a C-terminal somatic mutation as a second event in the development of AML [41].

As a limitation, an FLT3 inhibitor was introduced in Japan in the midst of the study period. Application of the FLT3 inhibitor and its future expansion to de-novo *FLT3*-ITD-positive AML would affect the significance of *FLT3*-ITD as a prognostic factor. *CEBPA*-bm out of bZIP and *CEBPA*-smbZIP consisted of a small number of *CEBPA* mutations and it is difficult to compare each group directly.

In conclusion, CN-AML prognostic classification was possible by analyzing mutations in three genes, *FLT3*, *NPM1* and *CEBPA*. Prospective analysis of these gene mutations may enable physicians to choose an appropriate treatment for each patient. *CEBPA*-bZIP inf had a very good prognosis, while *CEBPA* mt other than *CEBPA*-bZIP inf had a prognosis comparable to that of *CEBPA*-wt. *CEBPA*-bZIP inf is a better and simpler prognostic marker than *CEBPA*-bm. The successful simplification of the *CEBPA* mt prognostic criteria will have a great impact on genetic risk stratification of AML and potentially contribute to elucidation of the functional mechanisms of *CEBPA* for tumorigenesis in AML.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12185-023-03567-1.

Acknowledgements We thank all physicians for their contributions to this study and their dedicated care of the patients. We thank Satoshi Oguri and Kaori Sato for *FLT3*-ITD analysis. We thank Chiaki Yokoyama and Fumina Kusaka for management of patients' samples and data integrity.

Author contributions N.M. and M.O. designed the study, analyzed the data and wrote the manuscript; N.M., S.Yoshida, H.K., S.T., S.Yokoyama, and S.F. performed the molecular analysis; T.M., S.H., M.A., S.O., Y.K., Y.T., S.Y., T.M., T.N., M.I., H.K., Y.H., K.F., T.I., H.S., T.K. contributed to the data collection and provided critique to the manuscript; T.T. revised and approved the manuscript; All authors read and approved the final manuscript.

Funding This work was supported by North Japan Hematology Study Group (NJHSG) and partly by Japan Society for the Promotion of Science KAKENHI, Grant-in-Aid for scientific research (20K08745 [M.O.]).

Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing financial interests.

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