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ORIGINAL ARTICLE

The prognostic impact of *FLT3*-ITD, *NPM1* and *CEBPa* in cytogenetically intermediate-risk AML after first relapse

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Abstract

We evaluated the impact of *FLT3*-ITD, *NPM1* mutations, and double mutant *CEBPa* (*dmCEBPa*) on overall survival (OS) after relapse in patients with cytogenetically intermediate-risk acute myeloid leukemia (AML) who were treated with chemotherapy alone in the first remission (CR1). Patients aged 16–65 years diagnosed with cytogenetically intermediate-risk AML, and who achieved CR1 were included. We retrospectively analyzed *FLT3*-ITD, *NPM1* mutations and *CEBPa* using samples obtained at diagnosis, which therefore did not affect the therapeutic decisions. Among 235 patients who had achieved CR1, 152 relapsed, and 52% of them achieved second CR. The rate of achieving second CR was significantly higher (85%) in those with *dmCEBPa*. Patients with *FLT3*-ITD had significantly worse OS after relapse than those without (19% vs 41%, $p=0.002$), while OS was comparable between patients with and without *NPM1* mutations (37% vs 34%, $p=0.309$). Patients with dm*CEBPa* had improved OS than those without (61% vs 32%, $p=0.006$). By multivariate analysis, *FLT3*-ITD was independently associated with worse OS after relapse [hazard ratio (HR) 1.99, 95% CI 1.27–3.12, $p=0.003$], and *dmCEBPa* with improved OS (HR 0.40, 95% CI 0.17–0.93, $p=0.033$). Our data show that screening for these mutations at diagnosis is useful for facilitating effective therapeutic decision-making even after relapse.

Keywords Acute myeloid leukemia · FLT3-ITD · NPM1 · CEBPa · First relapse

Introduction

Acute myeloid leukemia (AML) is a molecularly heterogeneous hematological malignancy [1–4]. Although pretreatment cytogenetic classification has traditionally been the most potent prognostic factor [5–8], the importance of considering genetic profiles when formulating post-remission treatment strategies has been recognized [9]. Regarding the role of allogeneic hematopoietic cell transplantation (allo-HCT), genetic profiles may be particularly useful in cytogenetically intermediate-risk AML, which accounts for 46–67% of AML cases.

The National Comprehensive Cancer Network (NCCN) [10] and European LeukemiaNet (ELN) [11] have included three major mutations, namely Fms-like tyrosine kinase 3-internal tandem duplication (*FLT3*-ITD), mutant nucleophosmin (*NPM1*), and double mutant CCAAT/enhancer binding protein alpha (*dmCEBPa*), in the risk stratification of AML. Exploration of these molecular markers in the initial work-up is strongly encouraged, especially in cytogenetically intermediate-risk AML. *FLT3*-ITD is associated with poor prognosis [12–15], and we previously showed that allo-HCT during first complete remission (CR1) improved the outcomes of patients with *FLT3*-ITD [16]. On the other hand, *dmCEBPa* has been shown to carry a favorable prognosis [17, 18], and we showed in the same analysis that allo-HCT in CR1 was not recommended in patients with *dmCEBPa*. Regarding *NPM1*, Schlenk et al. indicated that patients with mutant *NPM1* without *FLT3*-ITD had a favorable prognosis and did not benefit from allo-HCT [19]. However, an Eastern Cooperative Oncology Group trial indicated

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that the favorable effect of *NPM1* mutations was restricted to patients who had co-occurring mutations in isocitrate dehydrogenase (*IDH*) [2], suggesting that possible heterogeneities in *NPM1*-positive patients may require the examination of background co-mutations [20–22]. NCCN and ELN both regard *NPM1* and the allelic ratio of *FLT3*-ITD [23–25] as potent prognostic factors, and include them in risk stratification: mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD of low allelic ratio indicates low risk; mutated *NPM1* and *FLT3*-ITD of high allelic ratio, and wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD of low allelic ratio indicates intermediate risk; and wild-type *NPM1* with *FLT3*-ITD of high allelic ratio indicates poor risk.

We previously showed that achieving second complete remission (CR2) and performing allo-HCT in CR2 were crucial for improving the prognosis after relapse [26]. Considering the risks of not only acute but late side effects [27], and possible changes in quality of life [28], the decision to perform allo-HCT in CR1 should be made by carefully evaluating the risk of relapse and the probability of being rescued after relapse [9]. In the abovementioned study [26], we only evaluated the cytogenetic risk and did not consider genetic heterogeneities. The aim of the present study was to evaluate the impact of *FLT3*-ITD, mutated *NPM1*, and *dmCEBPa* on outcomes of patients with cytogenetically intermediate-risk AML who were treated with chemotherapy alone in CR1, as well as the impact of genetic profiles on outcomes after the first relapse.

Patients and methods

Patients and mutational analysis

Adult patients with AML who had achieved CR1 were retrospectively registered in a nation-wide database that formed the basis of this study. We included patients aged 18–65 years who were diagnosed between 1999 and 2010 with intermediate- or unknown-risk AML according to the Southwest Oncology Group (SWOG) cytogenetic classification, and who had achieved CR1 with one or two courses of chemotherapy. We excluded patients with AML with myelodysplasia-related changes.

For eligible patients registered in the database, we retrospectively collected clinical information as well as bone marrow or peripheral blood samples obtained at diagnosis. Genomic DNA extraction and mutational analyses of *FLT3*-ITD, *NPM1*, and *CEBPA* were conducted as previously reported [22, 29]. Chemotherapy regimens were chosen at the discretion of physicians. Mutational status was examined retrospectively, so the mutational profile was not available when treatment strategy was determined. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by institutional review boards of the National Cancer Center Hospital and all the participating institutions.

Statistical analyses

Distributions of patient characteristics between groups were compared using the chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables. The unadjusted probabilities of overall survival (OS) and relapse-free survival (RFS) were estimated using Kaplan–Meier survival analysis. The log-rank test was used to compare OS and RFS among groups, and Gray's test was used to compare the cumulative incidence of relapse (CIR). To compare the OS of patients who received allo-HCT and those who did not, we performed landmark analyses by excluding patients who died within 150 days after relapse; 150 days was the median day for receiving allo-HCT from an unrelated donor registered in the Japan Marrow Donor Program [30]. A Cox proportional hazard regression model was used to estimate relative hazard ratios (HRs) for OS. The analyses were performed using SPSS software (IBM, SPSS Statistics 22) and EZR version 1.36 (Saitama Medical Center, Jichi Medical University), the latter of which is a graphical user interface for R (the R Foundation for Statistical Computing, version 3.3.2) [31]. For original data, please contact skurosaw@inahp.jp.

Results

Patients

A total of 480 patients with cytogenetically intermediate-risk AML who had achieved CR1 were retrospectively registered in the database. DNA extraction and analysis of *FLT3*-ITD status were successfully conducted in 296 patients (62%, Fig. 1). Most of the patients had received conventional anthracycline-AraC-based induction therapy (94% in 480 patients originally registered, and 95% in 296 with successful genetic data). Patients with available mutational data had a shorter follow-up period and a higher WBC count at diagnosis compared to those without available mutational data, but the two groups were similar in other characteristics including probabilities of OS [16]. In the 296 patients with cytogenetically intermediate-risk AML, 61 received allo-HCT in CR1. In further analyses, we included the 235 patients who were treated with chemotherapy alone during CR1 (Table 1); their median age at diagnosis was 51 years, the median follow-up was 3.2 years among survivors, and 152 of the 235 patients relapsed. After relapse, 77 patients (51%) received allo-HCT.

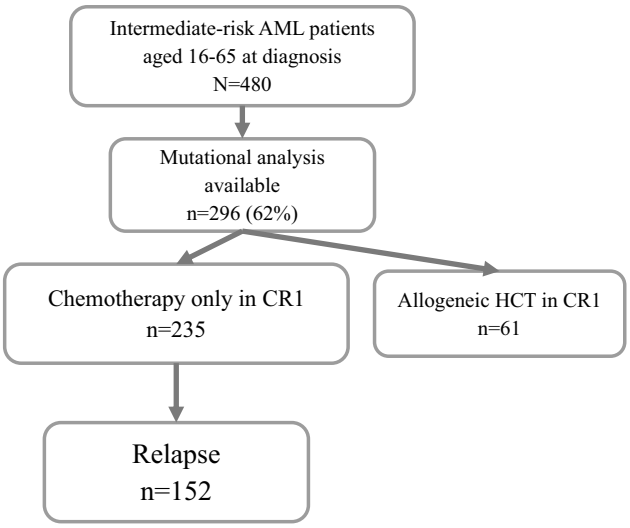

Fig. 1 Flow diagram of study participants. Among 296 patients whose mutational status was available, 235 were treated with chemotherapy alone, and 152 relapsed

Mutational analysis

The distributions of *FLT3*-ITD, *NPM1* mutations, and *CEBPa* in the 235 patients treated with chemotherapy alone and in the 152 who relapsed afterward are shown in Fig. 2. Forty-five patients (19%) had *dmCEBPA*. Of the 46 patients with *FLT3*-ITD (20%), 26 also had *NPM1* mutations but did not have *dmCEBPa* (11%, *NPM1+/FLT3+*). In the other 20 patients with *FLT3*-ITD, 2 had *dmCEBPa*, while the remaining 18 were categorized as *NPM1-/FLT3+* (8%). Of the 189 patients without *FLT3*-ITD, 60 had *NPM1* mutations, and the 59 who did not also have *dmCEBPa* were categorized as *NPM1+/FLT3-* (25%). The remaining 87 patients did not have *FLT3-ITD*, *NPM1* mutations, or *dmCEBPa*, and were categorized as triple negative (37%). The characteristics of patients based on the presence and absence of *FLT3-ITD*, *NPM1* mutations, and *dmCEBPa* are shown in Supplementary Table 1.

Similarly, the 152 patients who relapsed were categorized based on mutational profile (*dmCEBPa*, $n=20$, 13%; *NPM1+/FLT3-*, $n=32$, 21%; triple negative, $n=64$, 42%; *NPM1+/FLT3+*, $n=22$, 14%; *NPM1-/FLT3+*, $n=14$, 9%) (Supplementary Table 2).

Table 1 Characteristics of patients treated with chemotherapy alone

	Total		Relapsed		No relapse		*P value*
	N=235		*n*=152		*n*=83		
Age	*N*	(%)	*N*	(%)	*N*	(%)	
							0.416
Median, (range)	51	(18–65)	52	(18–65)	49	(18–65)	
Gender							*0.358*
Male	141	(60%)	93	(61%)	48	(58%)	
Female	94	(40%)	59	(39%)	35	(42%)	
Cytogenetics							*0.009*
Normal	189	(80%)	115	(76%)	74	(89%)	
Abnormal	46	(20%)	37	(24%)	9	(11%)	
FAB							*0.422*
M2, 4, and 5	146	(62%)	94	(62%)	52	(63%)	
M0, 1, 6, and 7	81	(34%)	54	(36%)	27	(33%)	
Data not available	8	(3%)	4	(3%)	4	(5%)	
WBC at diagnosis							*0.198*
($\times 10^3/\mu l$)	24.9	(0.3–551.4)	25.0	(0.3–551.4)	24.1	(1.1–259.2)	
Remission induction therapy courses							*0.283*
1 course	204	(87%)	130	(86%)	74	(89%)	
2 courses	31	(13%)	22	(14%)	9	(11%)	
Allo-HCT after relapse							
Yes	–	–	77	(51%)	–	–	
No	–	–	72	(47%)	–	–	
Data not available			3	(2%)			

FAB French-American-British Classification, *allo-HCT* allogeneic hematopoietic cell transplantation

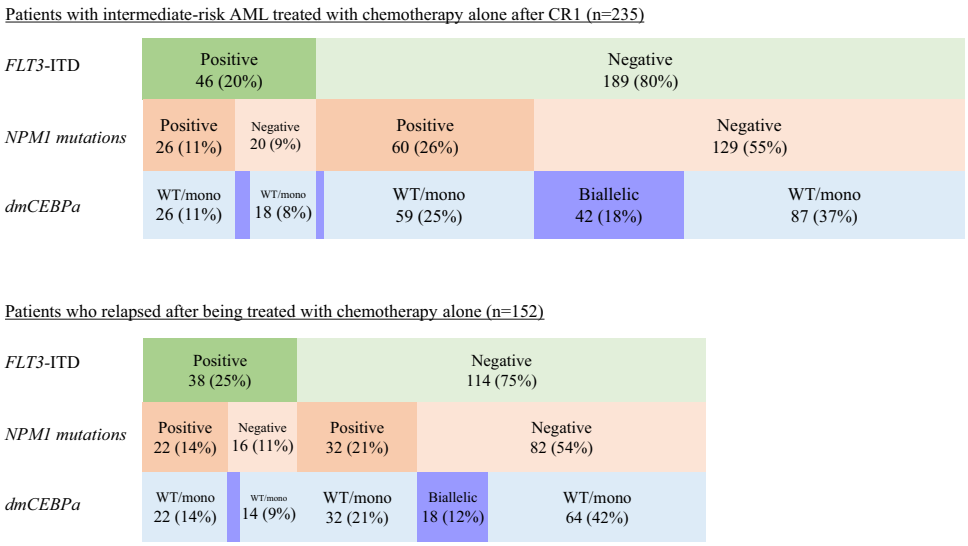

Fig. 2 Distribution of *FLT3*-ITD, *NPM1* mutations, and *CEBPa*. The distribution of *FLT3*-ITD, *NPM1* mutations, and *CEBPa* are shown for 235 patients treated with chemotherapy and 152 who relapsed afterwards

Outcomes after treatment with chemotherapy alone

As shown in Supplementary Fig. 1A, OS, RFS, and CIR in the 235 patients who were treated with chemotherapy alone during CR1 were 63%, 36%, and 61%, respectively, at 2 years after CR1. When stratified by the five groups of genetic profile groups, namely *dmCEBPa*, *NPM1+/FLT3-*, triple negative, *NPM1+/FLT3+*, and *NPM1-/FLT3+*, OS, RFS, and CIR at 2 years after CR1 were 88%/74%/57%/46%/29%, 54%/48%/26%/31%/6%, and 44%/49%/71%/69%/87%, respectively (Fig. 3). Statistically significant differences in OS and RFS after CR1 were seen versus *dmCEBPa* in the triple-negative, *NPM1+/FLT3+*, and *NPM1-/FLT3+* groups, but there were no significant differences between the *dmCEBPa* and *NPM1+/FLT3-* groups (Supplementary Table 3). Similarly, the *NPM1+/FLT3-* group had significantly better OS and RFS than the triple-negative, *NPM1+/FLT3+*, and *NPM1-/FLT3+* groups. OS and RFS in the triple-negative group were significantly better than in the *NPM1-/FLT3+* group, and were significantly worse than in the *dmCEBPa* and *NPM1+FLT3-* groups. The *NPM1-/FLT3+* group had significantly worse OS and RFS compared to the *dmCEBPa*, *NPM1+/FLT3-*, and triple-negative groups; however, there was no significant difference in OS or RFS between the *NPM1-/FLT3+* and *NPM1+/FLT3+* groups. OS values based on the presence or absence of *FLT3*-ITD, *NPM1* mutations, and *dmCEBPa* are shown in Supplementary Fig. 1B.

Outcomes after relapse based on genetic profiles at diagnosis

In 152 patients who relapsed, the median duration of CR1 was 247 days. Information on CR2 status was available for 133 of the 152 patients who relapsed after being treated with chemotherapy alone, and showed that 69 patients had achieved CR2 (52%). The rates of CR2 achievement were 85%, 48%, 52%, 41%, and 23%, respectively, in the *dmCEBPa*, *NPM1+/FLT3-*, triple-negative, *NPM1+/FLT3+*, and *NPM1-/FLT3+* groups. Patients

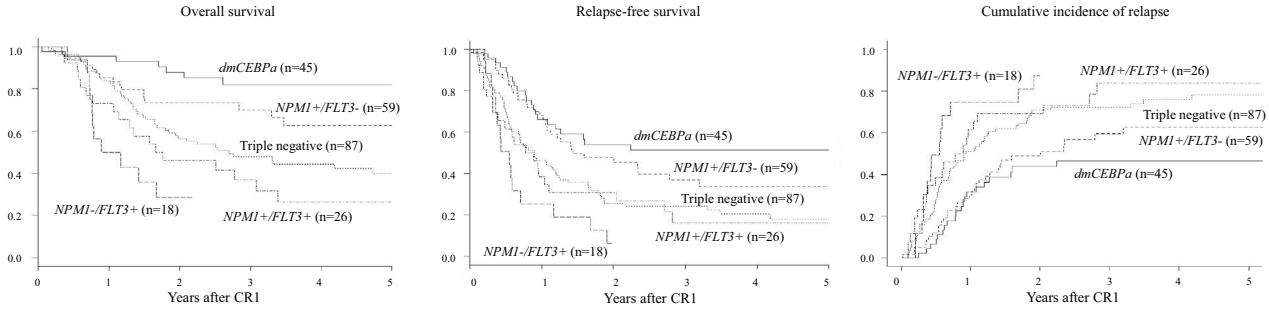

Fig. 3 Overall survival, relapse-free survival, and cumulative incidence of relapse after chemotherapy. Overall survival, relapse-free survival, and cumulative incidence of relapse in 235 patients treated with chemotherapy alone during CR1 are shown, stratified by five genetic profile groups: *dmCEBPa*, *NPM1+/FLT3-*, triple negative, *NPM1+/FLT3+*, and *NPM1-/FLT3+*

with *dmCEBPa* showed a significantly higher rate of CR2 achievement compared to those without *dmCEBPa*. The CR2 rates based on the presence or absence of *FLT3*-ITD, *NPM1* mutations, and dm*CEBPa* are shown in Supplementary Table 4.

OS after the first relapse was 35% at 2 years (Supplementary Fig. 2A). OS after relapse in patients without *FLT3*-ITD was significantly better than in those with *FLT3*-ITD (41% vs 19% at 2 years after relapse, respectively, $p = 0.002$, Fig. 4A). On the other hand, OS after relapse was similar in patients without and with *NPM1* mutations (34% vs 37%, $p = 0.309$). OS was significantly better in patients with *dmCEBPa* than in those without *dmCEBPa*, and in those without *dmCEBPa*, there was no significant difference in OS between patients with monoallelic *CEBPa* and wild type (61%, 40%, and 30%, $p = 0.022$). Among the five genetic profile groups (Fig. 4b), a significant difference in OS was seen between the *dmCEBPa* group and each of the triple-negative, *NPM1 + /FLT3 +* , and *NPM1-/FLT3 +* groups (*dmCEBPa* vs *NPM1 + /FLT3-*, $p = 0.079$). The *NPM1-/FLT3 +* group showed significantly inferior OS compared to the triple-negative and *dmCEBPa* groups (*NPM1-/FLT3 +* vs *NPM1 + /FLT3-*, $p = 0.057$), and there was no significant difference between the *NPM1 + / FLT3 +* and *NPM1-/FLT3 +* groups.

Mutational status at the time of relapse was obtained for 44 patients, and different profile was observed in 6 patients. Five of them showed triple-negative at the time of relapse (mutational status at diagnosis: two patients had *dmCEBPa* and one patient for each of *NPM1 + /FLT3-*, *NPM1 + /FLT3 +* , and *NPM1-/FLT3 +*), and one patient who had triple-negative at diagnosis showed *dmCEBPa* at the time of relapse. All of the six patients achieved CR2, and significant difference in OS was not seen between those with and without different mutational profile.

Role of allo-HCT after relapse and outcomes based on genetic profile at diagnosis

After relapse, 77 patients received allo-HCT; 24 patients received allo-HCT in the first relapse, 38 in CR2, and the other 15 were in other statuses. We compared OS after relapse in patients who received allo-HCT and those who did not using a landmark analysis at 150 days after relapse, which excluded 40 patients (6 of them had received allo-HCT). Patients who received allo-HCT after relapse had a significantly better OS compared to those who did not (55% and 24%, respectively, $p < 0.001$, Supplementary Fig. 2B). The advantage of allo-HCT after relapse was observed in most of the genetic profiles (Supplementary Fig. 2C).

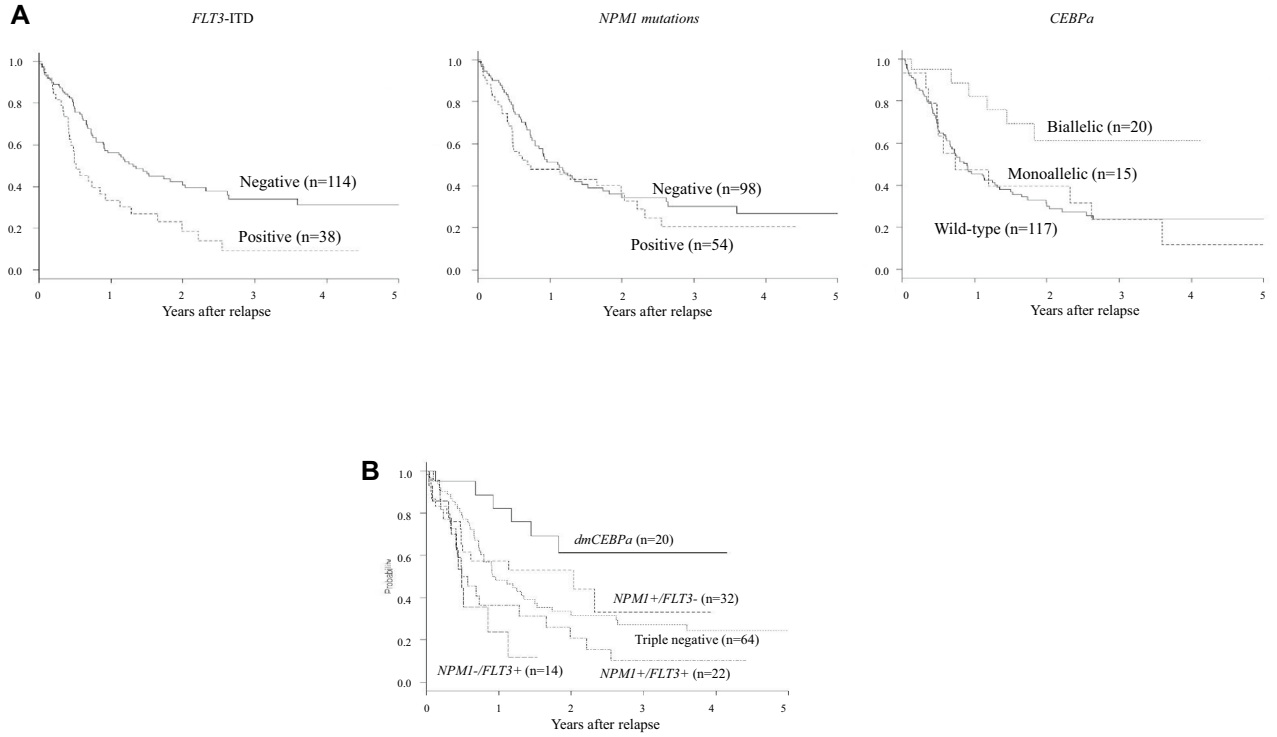

Fig. 4 Overall survival after first relapse. Overall survival in 152 relapsed patients is shown stratified by (**a**) presence or absence of each of *FLT3*-ITD, *NPM1* mutations, and *dmCEBPa*, (**b**) 5 genetic profile groups: *dmCEBPa*, *NPM1 + /FLT3-*, triple negative, *NPM1 + / FLT3 +* , and *NPM1-/FLT3 +*

Patients with *FLT3*-ITD who received allo-HCT had a better OS than those who did not, but the difference was not statistically significant (53% vs 0%, $p = 0.112$); 11 out of 13 patients received HCT in the second relapse or later. In patients with *dmCEBPa*, OS after allo-HCT was 67%; however, the no-HCT group consisted of only two patients, and therefore there was insufficient statistical power to form a definitive conclusion.

Factors associated with OS after relapse

Table 2 shows the results of univariate and multivariate analyses for overall survival after relapse. In univariate analysis, older age (HR 1.03, 95% CI 1.01–1.04, $p < 0.001$), achievement of CR1 after two or more courses of remission induction (HR 1.97, 95% CI 1.17–3.29, $p = 0.010$), a shorter CR1 duration (< 8 months, HR 2.23, 95% CI 1.46–3.41, $p < 0.001$), and *FLT3*-ITD at diagnosis (HR 1.98, 95% CI 1.27–3.06, $p < 0.001$) were associated with a lower OS after relapse, while *dmCEBPa* at diagnosis was associated with better OS after relapse (HR 0.33, 95% CI 0.14–0.75, $p < 0.001$). In multivariate analysis, age, CR1 duration, *FLT3*-ITD, and *dmCEBPA* were independently associated with OS after relapse. When the administration of allo-HCT after relapse was added to the model, allo-HCT was independently associated with better OS after relapse, while *FLT3*-ITD as well as a shorter CR1 duration remained independently associated with worse OS. We also conducted a multivariate analysis that included five subgroups of genetic profile at diagnosis (Supplementary Table 5). Compared to

Table 2 Factors associated with overall survival after relapse

	Univariate analysis			Multivariate analysis			Multivariate analysis (incl. HCT)		
	HR	(95% CI)	*P*	HR	(95% CI)	*P*	HR	(95% CI)	*P*
Age									
Year	1.03	(1.01–1.04)	*< 0.001*	1.02	(1.01–1.04)	*0.010*	1.01	(0.99–1.03)	*0.361*
Gender									
Female	1.00			1.00			1.00		
Male	1.45	(0.95–2.22)	*0.084*	1.41	(0.90–2.20)	*0.133*	1.33	(0.85–2.08)	*0.221*
FAB									
M2, 4, and 5	1.00								
M0, 1, 6, and 7	1.04	(0.68–1.58)	*0.861*						
WBC at diagnosis									
($\times 10^3$/μl)	1.00	(1.00–1.00)	*0.634*						
Remission induction therapy courses									
1 course	1.00			1.00			1.00		
2 courses	1.97	(1.17–3.29)	*0.010*	1.64	(0.97–2.77)	*0.108*	1.52	(0.89–2.61)	*0.213*
Dysplasia at diagnosis									
No	1.00								
Yes	0.78	(0.38–1.61)	*0.501*						
CR1 duration									
8 months or longer	1.00			1.00			1.00		
< 8 months	2.23	(1.46–3.41)	*< 0.001*	2.17	(1.40–3.35)	*< 0.001*	2.01	(1.30–3.11)	*0.002*
FLT3-ITD at diagnosis									
Negative	1.00			1.00			1.00		
Positive	1.98	(1.27–3.06)	*< 0.001*	1.75	(1.11–2.77)	*0.017*	1.65	(1.04–2.62)	*0.033*
NPM1 at diagnosis									
Wildtype	1.00								
Mutated	1.25	(0.81–1.91)	*0.310*						
CEBPA at diagnosis									
Wildtype/Monoallelic	1.00			1.00			1.00		
Biallelic mutations	0.33	(0.14–0.75)	*< 0.001*	0.41	(0.18–0.96)	*0.040*	0.50	(0.21–1.17)	*0.111*
Allo-HCT after relapse									
No	1.00								
Yes	0.31	(0.20–0.47)	*< 0.001*				0.42	(0.26–0.70)	*0.001*

FAB French-American-British Classification, *CR1* first complete remission, *allo-HCT* allogeneic hematopoietic cell transplantation

dmCEBPA, the *NPM1* + */FLT3* + and *NPM1-/FLT3* + groups were independently associated with a lower OS after relapse, with or without performance of allo-HCT after relapse.

Discussion

The focus of this study was to assess the prognostic impact of *FLT3*-ITD, *NPM1* mutations, and *dmCEBPa* on outcomes of patients with cytogenetically intermediate-risk AML after being treated with chemotherapy alone during CR1, as well as the impact of genetic profiles on outcomes after first relapse. Although we did not evaluate the allelic ratio of *FLT3*-ITD in this cohort, we presented survival probabilities and incidences of relapse after achieving CR1 based on the risk classification recommended by ELN [11] and NCCN [10], and we also demonstrated the relation between genetic profiles and the probabilities of achieving CR2 and survival after relapse.

Patients with *dmCEBPa* treated with chemotherapy alone showed significantly better OS and RFS compared to the triple-negative, *NPM1* + */FLT3* + , and *NPM1-/FLT3* + groups. The risk of relapse in the *dmCEBPa* group was higher than 35%, which according to ELN, is the value at which allo-HCT in CR1 should be considered [9]; however, patients with AML harboring *dmCEBPa* had a high rate of achieving CR2, and consequently had significantly better OS after relapse compared to the other genetic groups. Our sample size was too small to assess the role of allo-HCT after relapse in patients with *dmCEBPa*, but the favorable OS of 67% in patients who received allo-HCT after relapse may have contributed to the improved prognosis.

The favorable prognosis of the *NPM1* + */FLT3*- genetic profile group was previously shown [19], and this profile was categorized as favorable risk both by ELN and NCCN. The patients with *NPM1* + */FLT3*- in our cohort showed better OS and RFS than the triple-negative, *NPM1* + */FLT3* + , and *NPM1-/FLT3* + groups, and the *NPM1* + */FLT3*- group did not differ significantly from the *dmCEBPa* group. However, regarding the prognosis after the first relapse, the *NPM1* + */FLT3*- group demonstrated a significantly lower rate of achieving CR2 than the *dmCEBPa* group, and no significant difference in OS after relapse compared to the triple-negative, *NPM1* + */FLT3* + , and *NPM1-/FLT3* + groups. We also found that allo-HCT after relapse improved the outcomes in patients with mutated *NPM1*. Therefore, *NPM1* + */FLT3*- patients are categorized as favorable risk, but given their modest chance of achieving CR2 and the beneficial effect of allo-HCT after relapse, there may be an increased need to proactively consider the treatment strategy after the first relapse in this group, compared to the *dmCEBPa* group, including administration of allo-HCT in CR2.

In patients who were triple negative for *FLT3*-ITD, *NPM1*, and *dmCEBPa*, OS and RFS after achieving CR1 were significantly worse than patients with *dmCEBPa* and *NPM1* + */FLT3-,* and better than those with *NPM1-/FLT3* + , which may reflect the intermediate risk of this group. In the *NPM1* + */FLT3* + group, which is also categorized as intermediate risk both by ELN and NCCN, OS and RFS were not significantly different from those of the triple-negative group; at the same time, however, the survival rates in the *NPM1* + */FLT3* + group were not different from those in the *NPM1-/FLT3* + group. As for *NPM1*, the presence of minimal residual disease has been reported to be a powerful prognostic factor [32, 33], so we may be able to explore its use when choosing post-remission strategies in patients with mutated *NPM1*.

We confirmed that even after achieving CR1, patients with *NPM1-/FLT3* + had a dismal prognosis when they were treated with chemotherapy alone, with a relapse risk of as high as 87%. As described in the Methods section, the presence or absence of *FLT3*-ITD was analyzed retrospectively, and therefore it did not affect the treatment decisions. After relapse, the rate of achieving CR2 in patients with *FLT3*-ITD was lower than in the other genetic profile groups, and there was a lower likelihood of performing allo-HCT in CR2. Thus *FLT3*-ITD was an independent factor associated with worse prognosis after relapse, and allo-HCT performed after relapse did not seem to adequately improve outcomes. As multiple studies have shown [34, 35], there is a need to consider more proactive and innovative strategies after diagnosis, including *FLT3*-inhibitors [36–39] in addition to allo-HCT in CR1 [16].

We believe that our analysis is informative, but it also has limitations that must be acknowledged. First, our study used a retrospective design and may therefore be susceptible to disadvantages such as patient selection bias. However, participating centers consecutively registered eligible patients. Furthermore, as mutational status was examined retrospectively, physicians did not have molecular results when treatment strategy was chosen. However, a concern about the heterogeneity in the post-remission treatment strategy including re-induction therapy after the first relapse that depended on physicians or institutions must be acknowledged. Second, the relatively small number of patients included in each molecular risk subgroup might have led to failures in distinguishing the prognostic difference of each genetic profile group. Third, we did not assess the allelic ratio of *FLT3*-ITD in this cohort, though it is presently included in risk stratification recommended from ELN and NCCN. We previously reported that mutated *NPM1* with *FLT3*-ITD of low allelic ratio was not associated with favorable outcomes, and patients with this genetic profile benefited from allo-HCT in CR1 [40]. The impact of allele ratio of *FLT3*-ITD may need to be further clarified, and therefore we believe that the

findings of our analysis will help guide post-remission therapeutic decisions. Lastly, this analysis was conducted using a nation-wide database from pre-*FLT3* inhibitor era, therefore, its role in *FLT3*-positive AML need to be evaluated.

In conclusion, this study analyzed the mutation data of 235 patients who achieved CR1, and showed the survival probability and incidence of relapse using the five genetic profile groups defined by ELN and NCCN recommendations. After the first relapse, patients with *dmCEBPa* had a significantly higher rate of achieving CR2 and a remarkably better prognosis even after relapse. For patients with *FLT3*-ITD, we confirmed that the prognosis after treatment with chemotherapy alone was dismal, indicating that more proactive and novel treatment strategies, including *FLT3* inhibitors, are needed. Our data, including the risk of relapse and rate of achieving CR2, show that screening for these risk-related mutations at diagnosis helps to better inform patients of their predicted clinical course after CR1, and is useful in facilitating effective therapeutic decision-making even after relapse.

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Author contribution SK designed the study, manipulated the data file, performed data analysis and wrote the manuscript; HY was primarily responsible for the laboratory work in this study and interpreted data; TY was primarily responsible for the study design, data analysis and interpretation of the data; KF and SY performed laboratory work; HK, KU, NU, MY, JT, IM, JK, HO, SY, HT, TS, SC, and JT provided leukemia samples and clinical data and also interpreted data; and KI and TF interpreted data.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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