Clinical Implications of Non-A-Type *NPM1* and *FLT3* Mutations in Patients with Normal Karyotype Acute Myeloid Leukemia

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Key Words

Acute myeloid leukemia · *FLT3* · Nucleophosmin · Prognosis

Abstract

Mutations in the nucleophosmin (*NPM1*) and fms-like tyrosine kinase-3 (*FLT3*) genes are the most commonly observed mutations in patients with normal-karyotype acute myeloid leukemia (AML-NK). We analyzed the prognostic effects and interactions of these mutations in 201 AML-NK patients. *NPM1* and *FLT3* mutations were found in 38.3 and 24.9% of AML-NK patients, respectively. *NPM1* mutations (*NPM1*mut), especially in patients without *FLT3* mutations (*FLT3*mut), were associated with a favorable outcome. However, *NPM1*mut did not affect survival. *FLT3*mut tended to be associated with a poor survival outcome. *FLT3*mut showed no prognostic effects in patients with A-type *NPM1*mut. However, *FLT3*mut were associated with a significantly worse prognosis in patients with non-A-type *NPM1*mut. The prognostic interaction between the *NPM1* and *FLT3* mutations was significant in patients with non-A-type *NPM1*mut.

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Introduction

New molecular markers have been used to determine the prognosis of patients with acute myeloid leukemia (AML), especially patients with normal-karyotype AML (AML-NK). These patients constitute approximately 40% of patients with AML and are regarded as an intermediate-risk group [1, 2]. In addition, these patients harbor specific mutations in genes that serve as prognostic markers for survival outcomes after adequate therapy [1, 2]. Prognostically important mutations in patients with AML-NK include mutations in the nucleophosmin (*NPM1*), fms-related tyrosine kinase-3 (*FLT3*) and CCAAT/enhancer binding protein α (*CEBPA*) genes [3]. For example, *NPM1* mutations (*NPM1*mut) are observed in 50–60% of patients with AML-NK and are included in the 2008 World Health Organization classification of myeloid neoplasms as provisional entities [3]. In contrast, *FLT3* mutations (*FLT3*mut) are not included as defining criteria for distinct entities because they often accompany other genetic abnormalities. However, *FLT3* internal tandem duplications (ITD) are the second most common mutation in patients with AML-NK [3–5]. *NPM1*mut are associated with a better patient prognosis, whereas *FLT3*-ITD mutations (*FLT3*-ITDmut) are associated with shorter disease-free survival and overall survival (OS) [4, 5]. Approximately

Table 1. Clinical and demographic characteristics of patients with AML-NK

	<i>NPM1</i> wt	A-type <i>NPM1</i> mut	Non-A-type <i>NPM1</i> mut	Total	p ^a	p ^b
Patients	124	61	16	201		
Age, years	49.9 ± 14.7	54.0 ± 15.0	52.6 ± 14.6	51.4 ± 14.9	0.075	0.514
Sex					0.142	0.099
Male	68 (54.8)	24 (39.3)	10 (62.5)	102 (50.7)		
Female	56 (45.2)	37 (60.7)	6 (37.5)	99 (49.3)		
FAB subtype					0.068	0.586
M0	3 (2.4)	1 (1.6)	0	4 (2.0)		
M1	26 (21.0)	18 (29.5)	7 (43.8)	51 (25.4)		
M2	39 (31.5)	19 (31.1)	1 (6.3)	59 (29.4)		
M4	16 (12.9)	13 (21.3)	3 (18.8)	32 (15.9)		
M5	3 (2.4)	5 (8.2)	0	8 (4.0)		
M6	7 (5.6)	1 (1.6)	0	8 (4.0)		
M7	5 (4.0)	2 (3.3)	1 (6.3)	8 (4.0)		
M8	1 (0.8)	0	0	1 (0.5)		
AML-MRC	22 (17.7)	2 (3.3)	4 (25.0)	28 (13.9)		
tAML	2 (1.6)	0	0	2 (1.0)		
WBC, × 10 ⁹ /l	46.98 ± 77.75	49.23 ± 60.25	38.60 ± 46.51	46.99 ± 70.54	0.107	0.490
Hb, g/dl	8.8 ± 1.9	8.8 ± 1.4	9.3 ± 1.4	8.8 ± 1.7	0.451	0.173
Platelets, × 10 ⁹ /l	71.5 ± 64.1	83.5 ± 71.4	79.2 ± 72.4	75.7 ± 66.9	0.228	0.589
BM blasts, %	53.2 ± 22.9	66.7 ± 20.5	42.3 ± 40.0	43.5 ± 35.6	0.000	0.594
<i>FLT3</i> mut	23 (18.5)	23 (37.7)	4 (25.0)	50 (24.9)	0.009	0.346
<i>FLT3</i> -ITDmut	22	20	3	45		
<i>FLT3</i> -TKDmut	1	3	1	5		

Values represent either numbers (percentages) or means ± standard deviation. FAB = French-American-British classification; AML-MRC = AML with myelodysplasia-related change; tAML = treatment-related AML.

^a *NPM1*wt versus *NPM1*mut. ^b A-type versus non-A-type *NPM1*mut.

80% of *NPM1*mut are A-type (tandem duplication of TCTG) [6], whereas the remaining 20% are non-A-type (exon 12 mutations), which have been associated with poor prognosis [7]. Mutations in the *FLT3* tyrosine kinase domain (TKD) have been observed in approximately 4–7% of patients with AML; however, little is known about the clinical impact of these *FLT3*-TKD mutations [8–10].

It has not been determined whether or not these mutations exert positive or negative effects on prognosis after treatment [11]. Several Japanese and Korean studies have described the prognostic significance of *NPM1*mut and *FLT3*mut in various clinical settings. However, these studies did not focus on AML-NK [7, 10, 12, 13]. The prognostic effects of these mutations in AML-NK have been emphasized. Therefore, to further investigate the effects of these mutations on survival in AML-NK patients, we assessed the frequencies and prognostic effects of *NPM1*mut and *FLT3*mut, including non-A-type *NPM1* and *FLT3*-TKD mutations, and their interactions in AML-NK patients.

Patients and Methods

Patients

Our patient population comprised 201 adults (102 men, 99 women) who were aged 15 years or older (median age 53 years, range 15–81 years, mean ± SD 51.4 ± 14.9 years) and had been newly diagnosed with AML-NK at the Asan Medical Center between January 2002 and March 2010 (table 1). Of these 201 patients, 153 (76.1%; 77 men and 76 women; median age 48 years, range 15–79 years, mean ± SD 48.2 ± 14.1 years) received standard induction chemotherapy consisting of 7 days of cytarabine at 200 mg/m²/day (100 mg/m²/day for patients aged >60 years) and 3 days of daunorubicin at 45 mg/m²/day. Patients who failed to achieve complete remission (CR) but attained partial remission received a second, identical cycle of induction chemotherapy. Consolidation chemotherapy was followed by anthracycline and cytarabine (or cytarabine and daunorubicin for patients aged >60 years) according to the standard regimen of our institution [14]. CR was defined as the presence of <5% blast cells with more than 20% cellularity in a standardized bone marrow (BM) aspirate sample after the first or second course of induction chemotherapy according to our institution's criteria [15]. The study protocol was approved by our institutional review board, and all patients provided written informed consent for additional genetic analyses at diagnosis.

Table 2. List of *NPM1* exon 12 mutations that were observed in our patients with AML-NK

Type of mutation ^a	Nucleotide sequence of insertion at c.860_863	Patients	
		n	%
A type	TCTG	61	79.2
Non-A type			
B	CATG	5	6.5
D	CCTG	3	3.9
Qm	TCGG	2	2.6
Nm	CCAG	1	1.3
C	CGTG	1	1.3
4	CTTG	1	1.3
NR	TCGA	1	1.3
NR	AGGC	1	1.3
NR	CCGC	1	1.3

NR = Not reported.

^a The type of mutation was defined according to previously published data [17].

Analyses of *NPM1mut* and *FLT3mut*

BM samples that were obtained at diagnosis were retrospectively analyzed for *NPM1mut* and *FLT3mut*. Genomic DNA was extracted from BM smears or cell pellets using QIAamp DNA Blood Mini Kits (Qiagen, Hilden, Germany).

The analysis of *NPM1mut* was performed using fragment analysis as previously described [6]. Genomic DNA was amplified using PCR analysis. The products were subjected to capillary electrophoresis for exact size analysis using an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, Calif., USA) and GeneScan Analysis software (Applied Biosystems). In samples showing an additional peak, which indicated the presence of the 4-bp insertion, the *NPM1* exon 12 was amplified and the products were directly sequenced.

The presence of *FLT3*-ITD and TKD mutations was determined using multiplex PCR analysis with Seeplex *FLT3* Genotyping Kits (Seegene, Korea).

Statistical Analysis

Fisher's exact test was used to compare categorical variables between two groups, and the Mann-Whitney U test was used to compare continuous variables. The Kruskal-Wallis test was used to compare variables among three groups. Relapse-free survival (RFS) was calculated from the time of CR until relapse, death or the end of the study. OS was measured from the date of the first diagnosis to death from any cause during the course of this study. Nonparametric survival curves for RFS and OS were calculated using the Kaplan-Meier method and compared using the log-rank test. Multivariate Cox proportional hazard regression analysis was used to identify independent prognostic factors for RFS and OS. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 12.0 software (SPSS, Chicago, Ill., USA).

Results

Incidence of NPM1mut and FLT3mut in AML-NK Patients

The clinical characteristics of our 201 adult patients with AML-NK are shown in table 1. Of these 201 patients, 77 (38.3%) exhibited *NPM1mut*. When grouped according to patient age, the incidence of *NPM1mut* was 28.6% (14/49) in patients aged <40 years, 42.1% (37/88) in patients aged 41–60 years and 40.6% (26/64) in patients aged >60 years. *NPM1*, especially the A-type *NPM1mut*, was more common in female patients ($p = 0.142$). The incidence of *NPM1mut* was higher in patients with AML-M1, -M4 and -M5 than in patients with other types of leukemia ($p = 0.068$). The complete blood cell counts at diagnosis were not significantly different between patients with and without *NPM1mut*. However, BM blasts were significantly increased in patients with *NPM1mut* ($p = 0.000$; table 1). The most common *NPM1mut* was the type A mutation ($n = 61$, 79.2%), which is a duplication of TCTG at position c.860_863 (NCBI accession number NM_002520.5) [16, 17]. In addition, 5 patients (6.5%) had type B mutations, 3 (3.9%) had type D, 2 (2.6%) had type Qm and 6 (7.8%) had other types of *NPM1mut* (table 2).

We also detected *FLT3mut* in 50 patients (24.9%) and showed that 45 patients (22.4%) exhibited *FLT3*-ITDmut, whereas 5 patients (2.5%) displayed *FLT3*-TKD mutations. The incidence of *FLT3mut*, regardless of the type of mutation, was significantly higher in patients with *NPM1mut* than in patients without *NPM1mut* ($p = 0.009$; table 1).

Prognostic Impact of NPM1mut and FLT3-ITDmut

Of the 153 patients who received standard induction chemotherapy, 121 patients (79.1%) achieved CR. The CR rate was significantly higher in patients with *NPM1mut* than in patients with wild-type *NPM1* (*NPM1wt*; 86.2 vs. 74.7%, $p = 0.007$; table 3). Of the patients who achieved CR, patients with *NPM1mut* had a lower relapse rate and a higher survival rate than patients without *NPM1mut* (relapse rate: 32.8 vs. 41.1%, $p = 0.081$; survival rate: 56.9 vs. 44.2%, $p = 0.129$). However, these differences were not statistically significant (table 3). *NPM1mut* were not independently associated with RFS or OS. Of the 153 patients who received standard induction chemotherapy, 75 (49.0%) underwent hematopoietic stem cell transplantation (HSCT), regardless of whether or not they had *NPM1mut*. Most patients who received allogeneic HSCT, regardless of donor type, underwent re-

Table 3. Clinical characteristics and treatment outcomes based on *NPM1*mut status

	<i>NPM1</i> wt	A-type <i>NPM1</i> mut	Non-A-type <i>NPM1</i> mut	Total	p ^a	p ^b
Patients	95	45	13	153		
Age, years	47.0 ± 14.1	51.1 ± 14.4	49.0 ± 12.9	48.2 ± 14.1	0.061	0.406
Sex						
Male	50 (52.6)	20 (44.4)	7 (53.8)	77 (50.3)	0.467	0.553
Female	45 (47.4)	25 (55.6)	6 (46.2)	76 (49.7)		
<i>FLT3</i> mut	20 (21.1)	18 (40.0)	4 (30.8)	42 (27.5)	0.024	0.549
<i>FLT3</i> -ITDmut	20	15	3	38		
<i>FLT3</i> -TKDmut	0	3	1	4		
CR	71 (74.7)	38 (84.4)	12 (92.3)	121 (79.1)	0.007	0.219
HSCT	49 (51.6)	20 (44.4)	6 (46.2)	75 (49.0)	0.419	0.914
Relapse	39 (41.1)	16 (35.6)	3 (23.1)	58 (37.9)	0.081	0.320
Mean RFS, months	17.67 ± 24.07	22.31 ± 22.44	24.47 ± 24.49	19.61 ± 23.62	0.073	0.881
Death	53 (55.8)	19 (42.2)	6 (46.2)	78 (51.0)	0.129	0.803
OS, months	24.38 ± 23.93	26.25 ± 22.24	29.45 ± 27.53	25.36 ± 23.65	0.591	0.993

Values represent either numbers (percentages) or means ± standard deviation.

^a *NPM1*wt versus *NPM1*mut.

^b Comparison among *NPM1*wt, A-type *NPM1*mut and non-A-type *NPM1*mut using the Kruskal-Wallis test.

duced-intensity conditioning therapy consisting of busulfan, fludarabine and antithymocyte globulin according to the standard regimen of our institution [18].

Based on their *NPM1* and *FLT3*-ITDmut status, patients were divided into 4 groups. Patients with *NPM1*mut but without *FLT3*-ITDmut (*NPM1*mut/*FLT3*-ITDwt) had the highest CR rate (92.5%; table 4) and significantly longer RFS ($p = 0.004$) and OS ($p = 0.055$; fig. 1) than the other groups. Multivariate analysis using a Cox regression model showed that HSCT was the only factor that was independently associated with longer RFS ($p = 0.000$) and OS ($p = 0.000$). No significant differences among the 4 groups were detected after adjustment for HSCT (table 5). In each group, the OS rate was higher in patients who underwent HSCT than in those who did not (63.4 vs. 26.5%, $p = 0.000$, in *NPM1*wt/*FLT3*-ITDwt patients; 88.9 vs. 40.9%, $p = 0.007$, in *NPM1*mut/*FLT3*-ITDwt patients; 62.5 vs. 16.7%, $p = 0.105$, in *NPM1*wt/*FLT3*-ITDmut patients; 75.0 vs. 20.0%, $p = 0.000$, in *NPM1*mut/*FLT3*-ITDmut patients).

Prognostic Impact and Interaction according to the Type of Mutations

We found that the CR rate tended to be higher in patients with non-A-type *NPM1*mut than in patients with A-type *NPM1*mut (92.3 vs. 84.4%; $p = 0.238$), regardless of the *FLT3*mut status (table 4). Moreover, the CR rate

Table 4. Complete remission rate based on risk factors

Risk factor	Achieved CR, n	Total n	CR rate %
<i>NPM1</i> mut/ <i>FLT3</i> -ITDwt	37	40	92.5
<i>NPM1</i> wt/ <i>FLT3</i> -ITDmut	12	20	60.0
<i>NPM1</i> mut/ <i>FLT3</i> -ITDmut	13	18	72.2
<i>NPM1</i> wt/ <i>FLT3</i> -ITDwt	59	75	78.7
A-type <i>NPM1</i> mut	38	45	84.4
Non-A-type <i>NPM1</i> mut	12	13	92.3
<i>FLT3</i> -ITDmut	25	38	65.8
<i>FLT3</i> -TKDmut	3	4	75.0

CR = Complete remission.

tended to be lower in patients with *FLT3*-ITDmut than in those with *FLT3*-TKD mutations (65.8 vs. 75.0%, $p = 0.359$; table 4). However, these differences were not statistically significant. Kaplan-Meier survival analyses showed no significant differences in RFS ($p = 0.459$) or OS ($p = 0.847$) between patients with A-type *NPM1*mut and those with non-A-type *NPM1*mut (fig. 2). In addition, no differences were observed in RFS ($p = 0.674$) or OS ($p = 0.897$) between patients with *FLT3*-ITDmut and those with *FLT3*-TKD mutations. A multivariate analysis

Table 5. Cox proportional hazard models for RFS and OS in AML-NK patients based on *NPM1*mut and *FLT3*-ITDmut

Risk factor by survival end points	HR	95% CI	p
RFS			
HSCT	0.302	0.172–0.531	0.000
<i>NPM1</i> mut/ <i>FLT3</i> -ITDwt	0.446	0.176–1.131	0.089
<i>NPM1</i> wt/ <i>FLT3</i> -ITDmut	2.019	0.764–5.333	0.156
<i>NPM1</i> mut/ <i>FLT3</i> -ITDmut	1.634	0.533–5.009	0.390
<i>NPM1</i> wt/ <i>FLT3</i> -ITDwt	0.981	0.393–2.452	0.968
OS			
HSCT	0.229	0.136–0.384	0.000
<i>NPM1</i> mut/ <i>FLT3</i> -ITDwt	0.611	0.242–1.540	0.296
<i>NPM1</i> wt/ <i>FLT3</i> -ITDmut	1.417	0.549–3.657	0.472
<i>NPM1</i> mut/ <i>FLT3</i> -ITDmut	1.594	0.530–4.792	0.407
<i>NPM1</i> wt/ <i>FLT3</i> -ITDwt	1.102	0.451–2.694	0.832
RFS			
HSCT	0.291	0.168–0.503	0.000
A-type <i>NPM1</i> mut	0.533	0.289–0.985	0.045
Non-A-type <i>NPM1</i> mut	0.426	0.131–1.392	0.158
<i>FLT3</i> -ITDmut	3.025	1.687–5.423	0.000
<i>FLT3</i> -TKDmut	1.159	0.259–5.192	0.847
OS			
HSCT	0.234	0.141–0.388	0.000
A-type <i>NPM1</i> mut	0.609	0.352–1.055	0.077
Non-A-type <i>NPM1</i> mut	0.842	0.357–1.985	0.695
<i>FLT3</i> -ITDmut	1.935	1.176–3.182	0.009
<i>FLT3</i> -TKDmut	1.180	0.346–4.026	0.792

HR = Hazard ratio; CI = confidence interval.

using a Cox regression model showed that *FLT3*-ITDmut were independently associated with significantly shorter RFS ($p = 0.001$) and OS ($p = 0.010$) after adjustment for HSCT (table 5). A-type *NPM1*mut and non-A-type *NPM1*mut were associated with longer RFS and OS. *FLT3*-TKD mutations were associated with shorter RFS and OS, although these differences were not statistically significant (table 5).

Only 56 patients were enrolled in this study, and the prognostic study for the interaction between *NPM1*mut and *FLT3*mut showed weaker effects because of the small number of patients. In the 42 patients with A-type *NPM1*mut, the presence of *FLT3*mut, including *FLT3*-TKD mutations, did not significantly affect the RFS ($p = 0.081$) or OS ($p = 0.210$; fig. 2). In the 14 patients with non-A-type *NPM1*mut, the RFS ($p = 0.001$) and OS ($p = 0.062$) tended to decrease in patients with *FLT3*mut compared with patients without *FLT3*mut (fig. 2).

Fig. 1. **a** RFS in patients with AML-NK according to *NPM1* and *FLT3*-ITDmut. **b** OS of patients with AML-NK according to *NPM1* and *FLT3*-ITDmut. Patients with *NPM1*mut but without *FLT3*-ITDmut had significantly better RFS and OS than other patient groups. *NPM1*mut/*FLT3*wt: patients with *NPM1*mut without *FLT3*-ITDmut; *NPM1*mut/*FLT3*mut: patients with both *NPM1* and *FLT3*-ITDmut; *NPM1*wt/*FLT3*wt: patients with neither *NPM1* nor *FLT3*-ITDmut; *NPM1*wt/*FLT3*mut: patients with *FLT3*-ITDmut without *NPM1*mut.

Discussion

We retrospectively investigated the incidence and clinical impact of *NPM1* and *FLT3* mutations in 201 Korean patients with AML-NK. We found that among these patients, 38.3% had *NPM1*mut and 24.9% had *FLT3*mut, which are similar findings to incidences that have been previously reported in Asian populations [8, 19]. Several Japanese studies observed a lower incidence of *NPM1*mut than in our study [12, 13]. However, those studies evaluated AML with or without abnormal karyotype. Al-

Fig. 2. **a** RFS of patients with *NPM1*mut according to the type of *NPM1*mut. **b** OS of patients with *NPM1*mut according to the type of *NPM1*mut. No significant differences in survival outcomes were detected between patients with A-type and non-A-type *NPM1*mut. **c** RFS of patients with A-type *NPM1*mut according to *FLT3*mut. **d** OS of patients with A-type *NPM1*mut according to

*FLT3*mut. No significant differences were detected in survival between patients with *FLT3*wt and *FLT3*mut. **e** RFS of patients with non-A-type *NPM1*mut according to *FLT3*mut. The RFS was significantly increased in patients with *FLT3*wt compared to patients with *FLT3*mut. **f** OS of patients with non-A-type *NPM1* mut according to *FLT3*mut.

though *NPM1*mut was detected in AML with an abnormal karyotype, its prevalence was more frequent in AML-NK. *NPM1*mut has a favorable prognosis in AML-NK [5, 20, 21]. Therefore, a prognostic study for *NPM1*mut must focus on AML-NK. However, most prognostic studies for *NPM1*mut in Asians have been performed in AML with or without abnormal karyotypes [12, 13]. Our study is therefore advantageous from this point of view.

*NPM1*mut is more common in older than in younger patients [19]. We showed that the incidence of *NPM1*mut was lower in adults who were aged <40 years but similar in patients who were aged 40–60 and >60 years. In agreement with previous findings, we found that *NPM1*mut was significantly associated with an increased percentage of BM blasts [19]. However, the increase in BM blasts was not associated with a difference in patient prognosis (data not shown).

NPM1 and *FLT3* mutations have been suggested to be mechanistically linked [16, 22]. Patients with *FLT3*mut are more likely to have *NPM1*mut [8, 9]. We found that *FLT3*-ITD and *FLT3*-TKD mutations were more common in patients with *NPM1*mut than in those with *NPM1*wt. Although 5 of 201 patients (2.5%) had the *FLT3*-TKD mutation, 4 of these 5 patients (80.0%) also carried *NPM1*mut. The interaction between *FLT3*mut and *NPM1*mut may have prognostic impact because patients with *NPM1*mut/*FLT3*-ITDwt had significantly longer RFS and tended to have longer OS than patients with one of the other three genotypes. Moreover, the *NPM1*mut/*FLT3*-ITDwt combination showed a favorable hazard ratio. However, *NPM1*mut/*FLT3*-ITDwt was not a significant prognostic factor in the Cox regression model. Recent studies have demonstrated that patients with *NPM1*mut but without *FLT3*-ITD have a better prognosis [23, 24]. However, most studies on the interaction between *NPM1*mut and *FLT3*mut in AML-NK patients have focused on *FLT3*-ITD. In contrast, we focused on *FLT3*-ITD and *FLT3*-TKD. We observed that patients with *FLT3*-TKD mutations tended to have a poorer outcome, although the difference was not statistically significant because of the small sample size. We determined that the presence of *FLT3*-ITDmut was an independent predictor of poor patient prognosis and that there were no prognostic differences between *FLT3*-ITD and *FLT3*-TKD mutations. These results suggest the possibility that the presence of *FLT3*-TKD mutations predicts poor patient prognosis.

Our findings on the hematologic response of patients following induction chemotherapy correlated with the presence or absence of *NPM1* and *FLT3* mutations. Simi-

lar to previous studies, we found that the CR rate was significantly higher in patients with *NPM1*mut than in patients without *NPM1*mut. This difference is primarily derived from the greater sensitivity of *NPM1*mut-containing blasts to the initial chemotherapy [25]. Indeed, we found that patients with non-A-type *NPM1*mut had superior hematological responses regardless of their *FLT3*mut status. We observed a similar response in patients with *NPM1*mut in the absence of *FLT3*-ITD (table 4).

The treatment protocol for patients with AML is usually dependent on prognostic factors, including cytogenetic results and age. AML patients with *NPM1*mut/*FLT3*-ITDwt, who have a favorable prognosis, are usually treated with conventional chemotherapy, with or without HSCT [26]. Although *FLT3*mut are regarded as an indication for HSCT, the role of HSCT in patients with *NPM1*mut and *FLT3*mut is unclear [26, 27]. We found that HSCT improved survival in AML-NK patients, including patients with *NPM1*mut/*FLT3*-ITDmut. However, the OS did not exceed 6 years in patients with *NPM1*mut/*FLT3*-ITDmut. Although we did not focus on the detail of HSCT therapy, we found that HSCT improved OS, regardless of the presence of *NPM1*mut and/or *FLT3*-ITDmut. In general, patients in favorable risk groups have not been considered candidates for HSCT [28]. We observed that the presence of *NPM1*mut without *FLT3*-ITDmut was significantly correlated with a higher CR rate and longer OS and that these patients benefited from HSCT. This study could not provide sufficient evidence to justify the benefit of HSCT because of its small sample size and simple retrospective analysis. Because we observed a favorable impact of HSCT in our study, further studies with a larger sample size are warranted to confirm these findings.

Among patients with *NPM1*mut, 80% have A-type *NPM1*mut, which have a favorable impact on patient prognosis [17, 26]. The remaining 20% of patients with *NPM1*mut have non-A-type *NPM1*mut. The clinical impact of non-A-type *NPM1*mut remains unclear. Regardless of the mutation type, mutated *NPM1* encodes cytoplasmic NPM, which acts as an oncoprotein [16, 29]. Therefore, non-A-type *NPM1*mut are believed to have a clinical impact similar to that of A-type *NPM1*mut. Our results indicate that non-A-type *NPM1*mut are associated with favorable outcomes. Patients with A-type and non-A-type *NPM1*mut tended to have more favorable outcomes with no prognostic differences between these two subgroups. Another previous study of 106 patients found that non-A-type *NPM1*mut (5 patients) were associated with a significantly shorter CR rate and shorter OS com-

pared with *NPM1*wt and A-type *NPM1*mut (13 patients) [7]. However, there was a limitation in that karyotype was not specified for all studied patients. In particular, 1 of the 5 patients with non-A-type *NPM1*mut were included in the poor-risk group according to karyotype, for 1 of them there was no information about karyotype and 3 of them were included in the standard-risk group without specific karyotypic information.

Interestingly, we found that the interactions between mutations differed according to the type of *NPM1*mut. Although *FLT3*mut tended to predict poor prognosis in patients with A-type and non-A-type *NPM1*mut, their prognostic impact was significant in patients with non-A-type *NPM1*mut. We included the *FLT3*-TKD mutation in *FLT3*mut in our mutational interaction analysis because we observed that the presence of the *FLT3*-TKD mutation was predictive of poor patient prognosis. Further studies are needed to determine the prognostic interaction between mutations. In addition, our study did not include other molecular markers, such as *CEBPA*

genes, which are known to impact on disease prognosis [3]. Further studies including other prognostic factors are required in the future.

In conclusion, we showed that patients with non-A-type *NPM1*mut, regardless of *FLT3*mut status, and patients with *NPM1*mut in the absence of *FLT3*-ITD have higher CR rates than patients with *NPM1*wt and *FLT3*mut. We found that patients with A-type and non-A-type *NPM1*mut tended to have better prognoses than patients with *NPM1*wt and that *FLT3*-ITD and *FLT3*-TKD mutations were associated with a poor prognosis. The favorable prognostic impact of *NPM1*mut was shown in the univariate analysis, which evaluated the CR rate and RFS. However, in the multivariate analysis, no prognostic impact of *NPM1*mut and *FLT3*mut was detected. Although limited by the small sample size, our study findings on the prognostic interaction between non-A-type *NPM1*mut and *FLT3*mut indicate the importance of assessing the subtypes of *NPM1*mut and *FLT3*mut in patients with AML-NK.

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