NPM Mutations in *de novo* Acute Myeloid Leukemia: the Mutation Occurs Much More Frequently in Adults than in Children and Is Closely Associated with Disease Status

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Running title: *NPM* mutation in AML

Key words: *NPM*, AML, *CEBPA*, *FLT3*/ITD, immunophenotype

Abstract

NPM mutations have been found in a significant proportion of adults with *de novo* acute myeloid leukemia (AML), especially in those of a normal karyotype. These results provide a basis for studies of the pathogenesis in this specific subgroup of AML. In this study, *NPM* mutations were analyzed in 173 *de novo* AML patients including adults and children. We found *NPM* mutations were present in 19.1% of the overall population and 40.3% of those with a normal karyotype. Adults had a significantly higher incidence of *NPM* mutations than children (32/126, 25.4% vs. 1/47, 2.1%, $p<0.001$). *NPM* mutations were closely associated with normal karyotype ($p<0.001$) and *FLT*3/ITD ($p=0.002$), but negatively associated with *CEBPA* mutations ($p=0.032$) and expression of CD34 ($p<0.001$) and HLA-DR ($p=0.003$). Serial analyses of *NPM* mutations showed the mutation disappeared at complete remission, but the same mutation reappeared at relapse, except for one who lost the mutation at the second relapse, when new cytogenetic abnormalities emerged. None acquired novel mutations during the follow-up period. In conclusion, *NPM* mutations occur in an age-dependent fashion. The findings that *NPM* mutations are stable during disease evolution and closely associated with disease status make it a potential marker for monitoring minimal residual disease.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of hematological malignancy. Despite that recurrent chromosomal abnormalities are present in a significant proportion of AML patients, leukemia cells from about 40% to 50% of the patients have a normal karyotype and lack a reliable biological marker, thus making difficult the investigation of the etiologies and monitoring of residual disease in some of the *de novo* AML patients [1].

Recently, *nucleophosmin* (*NPM*) exon 12 mutations, which resulted in shift of the *NPM* protein from the nucleus to cytoplasm, were found in about 30% of *de novo* AML patients [2-8]. The mutations were even more prominent in those with a normal karyotype, and were frequently associated with *FLT3* mutation. The *NPM* encoding protein shuttles between the nuclei and cytoplasm and is involved in ribosomal biogenesis [9,10], centrosome duplication [11], and regulation of the functions of tumor suppressor proteins such as p53 and alternative reading frame (ARF) [12,13]. Although it is frequently a partner of translocations in various hematological malignancies such as anaplastic large cell lymphoma (*NPM-ALK*) [14], acute promyelocytic leukemia (*NPM-RARα*) [15], myelodysplastic syndrome [16], and AML (*NPM-MLF-1*) [16], the contribution of the mutated *NPM* protein to the leukemogenesis remains undetermined. The frequent mutation of *NPM* in *de novo* AML provides a basis for investigation of the pathogenesis and monitoring of residual disease of AML with a

normal karyotype.

Most reports of *NPM* mutations came from Europe and focused mainly on adults [2-7]. In the current study, we investigated the mutation in 173 *de novo* AML patients, a population of mixed Chinese adults and children and correlated the results with clinical features, cytogenetics, immunophenotyping and other genetic alterations. Sequential analyses of the *NPM* mutation during the clinical course were also performed in some of them.

Materials and methods

Patients and sample collection

The *NPM* mutation was analyzed in the bone marrow cells form 156 unselected patients with *de novo* AML diagnosed based on the FAB criteria [17] at the National Taiwan University Hospital from 1995 to 2000. We subsequently recruited another 17 children with AML diagnosed outside the period (1995-2000) to increase the number of pediatric patients. The bone marrow mononuclear cells were collected by heparinization followed by Ficoll-Hypaque gradient centrifugation. The isolated cells were frozen and stored at -80°C.

Mutation analysis

Mutation analyses were performed by PCR followed by direct sequencing according to the methods described previously [18]. For *NPM* exon 12 mutation, the primers NPM-F 5'-TTAACTCTCTGGTGGTAGAATGAA-3' and NPM-R

5’-CAAGACTATTTGCCATTCCTAAC-3’ as designed by Falini *et. al* [2] were used. The final volume for PCR reaction was 35μL containing 200 ng DNA, 200 nM dNTP, 2 mM $MgSO_4$, 140 nM each primer, and 1 unit of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). The PCR was performed by heating at 95°C for 10 min, followed by 35 cycles of 95°C for 45 sec, 49°C for 1 min, and 72°C for 1 min, with a final step for 10 min at 72°C. PCR products were electrophoresed on 2% agarose gels, purified and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit, which contained AmpliTaq DNA polymerase FS (Applied Biosystems), on an automated ABI-3100 Genetic Analyzer (Applied Biosystems). Abnormal sequencing results were confirmed by at least two repeated analyses. The DNA extracted from the peripheral blood mononuclear cells of 13 healthy persons was used as normal controls.

Point mutations at codons 12, 13 (exon 1) and 61 (exon 2) of the *N-RAS* and *K-RAS* genes were analyzed by PCR on genomic DNA and direct sequencing [19]. Methylation status of *SOCS1* (suppressor of cytokine signaling-1) and *SHP1* (protein-tyrosine phosphatase containing 2 Src homology domains), both encoding proteins functioning as negative regulators of signal transduction, was analyzed by methylation-specific PCR as described [19-23]. Analyses of mutations of *CEBPA* and internal tandem duplication of *FLT3* (F*LT3*/ITD) were performed according to previous studies [24,25].

TA cloning

We chose the PCR products of all four types of mutation and cloned with pGEM-T Easy TA cloning kit (Promega, Madison, WI), followed by sequencing to identify the specific mutation in a single allele as described previously [23].

Immunophenotyping

A panel of monoclonal antibodies to myeloid-associated antigens including CD13, CD33, CD11b, CD15, CD14, and CD41a, as well as lymphoid-associated antigens including CD2, CD5, CD7, CD19, CD10, and CD20, and lineage nonspecific antigens HLA-DR, CD34, and CD56 were used to characterize the phenotypes of the leukemia cells. Expression of surface antigens on the leukemia cells was shown by an indirect immunoalkaline phosphatase method as described before [26]. The cutoff value for positive result of the markers was more than 20% [26]. Since 1998, immunophenotyping was performed by flow cytometry.

Cytogenetic study

Bone marrow samples were aspirated into heparinized syringes and chromosomal analyses by G-banding method was performed on BM cells after 1–3 days of unstimulated culture as described previously [26].

Statistics

Chi square test was employed to calculate the significance of association between *NPM* mutation and other discrete parameters, such as expression of antigens, cytogenetics, mutation or methylation of a specific gene etc., and Mann-Whitney tests were used to compare continuous variables. Kaplan-Meier curve was calculated by

SPSS software (Chicago, IL).

Results

NPM exon 12 mutations

A total of 173 *de novo* AML patients including 105 males and 68 females, with a median age of 41 years (range, 0-85), were analyzed. There were 126 adults and 47 pediatric patients (<=18y/o). Overall, the *NPM* mutation occurred in 33 (19.1%) of all *de novo* AML patients. There were 4 types of mutations, all involving the C terminal portion of the transcript of *NPM* with a 4 nucleotides insertion between positions 960 and 961. The frequencies and resulting changes of amino acid sequences are shown in Fig 1 (A-E). All cases with the mutation were heterozygous and retained a wildtype allele. We also detected a polymorphism of nucleotide T deletion at position 1146 in the 3' untranslated region (Fig. 1F). The polymorphism was detected in 123 patients (71.1%), homozygous in 27 and heterozygous in 96, and in 11 of the 13 healthy persons.

Sequential studies of NPM mutations

NPM exon 12 mutations were serially studied in 41 patients, including 13 patients with and 28 patients without *NPM* mutation at diagnosis; 20 of the latter patients had 1146(-T) polymorphism at the *NPM* gene (Table 1 and Supplementary Table 1). None of these 41 patients acquired a new mutation after a median follow-up time of 16 months (range 1-60 months) although eight of them showed karyotypic evolution

and two had different clonal abnormalities at relapse. The *NPM* mutations disappeared at complete remission (CR) in all patients who harbored the mutations at diagnosis and had available DNA samples for analysis (Table 1). The same mutations as those at diagnosis were detected at first relapse in all five patients analyzed (patients 22, 55, 63, 129 and 147). However, no mutation was detected at second relapse in patient 63; clonal karyotypic evolution was found at that time. In one patient (no. 32), the mutation remained detectable after chemotherapy when BM blasts were 7.2%. In the 20 patients with 1146 (-T) polymorphism but without mutation at the *NPM* gene, the nucleotide T deletion persisted at CR and also at relapse with the exception of one patient (no. 69) in whom the homozygous polymorphism turned to heterozygous status after allogeneic bone marrow transplantation (Supplementary Table 1). In all patients without *NPM* mutation at diagnosis and having subsequent samples for analysis, none acquired mutation at relapse.

Correlation of NPM mutations with clinical features and biological characteristics

One hundred and sixty-five patients had cytogenetic data. The *NPM* mutation was mainly seen in those of a normal karyotype (Table 2). Among the patients with a normal karyotype, 40.3% showed the *NPM* mutation, compared with 6.1% in those with chromosomal abnormalities. No *NPM* mutation was seen in patients with t(15;17), t(8;21), inv(16), or other recurrent chromosomal abnormalities such as t(6;9), t(7;11), and deletions involving chromosome 5 or 7 (Table 2). The *NPM* mutation

occurred much more frequently in adults than in pediatric patients (32/126, 25.4% vs. 1/47, 2.1%, p<0.001). It was infrequently detected in patients younger than 40 years (3/85, 3.5%, Fig. 2). The lower incidence of the *NPM* mutation in children could not be explained by the lower rate of normal karyotype in pediatric patients than in adults (11/41, 26.8% vs. 56/124, 45.1%, p=0.038) because the *NPM* mutation was still significantly less frequent in children than in adults if only the patients with a normal karyotype were counted (1/11, 9% vs. 26/56, 46.4%, p= 0.021). Comparison of *NPM* mutations in different age groups revealed a gradient increase of mutation rate in the older patient groups (Fig. 2). Patients with AML M4 subtype had a higher *NPM* mutation rate than those with other subtypes, but the difference had not reached statistical significance yet (Table 2). *NPM* mutations were significantly associated with higher initial WBC counts, blast percentages and platelet counts in the peripheral blood (Table 2), but not sex, lactate dehydrogenase (LDH), and hemoglobin levels.

We also analyzed mutations in *CEBPA* , *N-RAS*, and *K-RAS*, *FLT3*/ITD, and hypermethylation in *SHP1* and *SOCS1*, which were frequently detected in AML [21-27]. The comparison of these genetic or epigenetic alterations between the patients with and without *NPM* mutation is summarized in Table 3. *FLT3*/ITD and *CEBPA* mutations were positively and negatively associated with *NPM* mutations, respectively. None of other genetic changes was associated with *NPM* mutation.

For immunophenotyping, the *NPM* mutation was associated with lack of expression of CD34 and HLA-DR on leukemic cells (p<0.001 and p=0.003,

respectively) (Table 4). Other markers such as CD13, CD33, CD11b, CD14, CD15, CD19, CD10, CD7, CD56, and CD2 were not found to be significantly associated with *NPM* mutation statuses. We did not detect significant impacts of the *NPM* mutation on the disease-free or overall survival whether *FLT3* mutation status was considered or not (data not shown), probably because of the small sample size.

Discussion

In this comprehensive analysis of *NPM* mutations in *de novo* AML patients, we found a remarkable difference in the incidence of *NPM* mutation between adult and pediatric patients (25.4% vs. 2.1%). A recent study has reported a low incidence of *NPM* mutation in pediatric patients (7%) [28]. Verhaak et al demonstrated that *NPM* mutations were significantly less frequent in adult patients younger than 35 years [6]. This is the first report to present a side-by-side comparison of the *NPM* mutation rate in children and adults with AML. The difference in the incidence of NPM mutation between these two age groups could not be explained by the lower incidence of normal karyotype in the pediatric patients than in adults since the same finding could be demonstrated even only the patients with a normal karyotype were considered (a mutation rate of 46.4% in adults vs. 9% in children, p= 0.021). This is another proof that childhood leukemia can be very different from that of adults in pathogenesis and biological characteristics [1,29].

The facts that *NPM* mutation is not present in leukemia with recurrent

cytogenetic abnormalities and that the mutation disappears at complete remission imply a critical role of *NPM* mutation in leukemogenesis. Furthermore, we demonstrated that none of the patients without the *NPM* mutation at diagnosis acquired the mutation during the follow-up period, suggesting that the *NPM* mutation might play little role in the progression of AML. In the sequential studies on a smaller population of patients, Boissel et al and Suzuki et al also found that none of their patients without *NPM* mutation at diagnosis acquired *NPM* mutation at relapse [4,8]. Although the same *NPM* mutation as that detected at diagnosis could be identified again at first relapse in the *NPM*-mutated patients in our study, one patient lost the mutation at second relapse (patient 63, Table 1). The bone marrow blasts in this patient were 5.5% when the second relapse was diagnosed, but the relapse status was confirmed by the clonal chromosomal abnormalities of -7 and t(12;18). The disappearance of *NPM* mutation in this patient could be due to suppression of PCR amplification of mutant gene when there were only a small number of mutated cells within a major population of normal cells. Analysis by more sensitive quantitative real-time PCR may overcome this problem. Another possible explanation was the emergence of a different clone of leukemia cells at that time. The presence of -7, a chromosomal abnormality commonly seen in secondary leukemia, and the long interval (58 months) between initial diagnosis and this relapse (Table 1) supported the latter possibility. In the report of Boissel et al, all 10 patients with *NPM* mutations at diagnosis still displayed *NPM* mutations at relapse [4]. Of the 17 patients with *NPM*

mutations at diagnosis reported by Suzuki et al [8], 15 carried the same mutation at relapse. Two patients in that study lost the mutation at relapse; one of them acquired a chromosomal abnormality that was not present at diagnosis, and the other showed normal karyotype at both diagnosis and relapse. *NPM* gene status seems relatively stable in contrast to *FLT3*/ITD[8,25]. However, further studies are needed to determine whether *NPM* mutation can be a good marker for monitoring minimal residual disease. Analysis using quantitative real-time PCR designed according to the mutation pattern at diagnosis in individual patient may be helpful to answer this question.

We found *NPM* mutations were mutually exclusive with *CEBPA* mutations, but highly associated with *FLT3*/ITD. The same findings have been reported [4,6]. Recent studies argue for a multi-step pathogenesis of AML [30]. The genetic events underlying AML pathogenesis appear to fall into two broadly defined complementation groups: one comprises mutations that confer proliferation and/or survival advantage to hematopoietic cells, such as *FLT3* and *RAS* mutations, and the other comprises mutations that affect transcription factors resulting in impaired hematopoietic differentiation, such as t(8;21)/*AML1-ETO*, inv(16)/*CBFβ-SMMHC*, and t(15;17)/*PML-RARα* fusions as well as *CEBPA* and *AML*1 mutations [30,31]. Like *CEBPA, NPM* mutation is not present in AML with t(8;21) t(15;17) or inv(16) [24,31,32]. The mutation of *NPM* results in cytoplasmic translocation of *NPM* protein which is normally located in the nucleoli [33], where it may serve as a chaperone to prevent protein aggregation [34]. Though the pathogenetic role of *NPM* mutation in AML is

not clear, the findings that *NPM* mutation is inversely associated with *CEBPA* mutation and chromosomal abnormalities t(8;21), t(15;17) and inv(16) suggest that mutant *NPM* may serve to impair differentiation of hematopoietic cells in the multi-step pathogenesis model of AML, like *CEBPA* and *AML*1 [6,30,31]. Further functional studies of *NPM* mutant are necessary to elucidate this point.

In summary, *NPM* mutations were detected in 19.1% of a total of 173 *de novo* AML patients and 40.3% of those with a normal karyotype. Children had a significantly lower incidence of *NPM* mutations than adults. The mutation occurred infrequently in AML patients younger than 40 years. The same was also true if only the patients with normal karyotype were analyzed. The genetic changes underlying the childhood AML seem quite different from those in adult AML. Serial studies showed that the *NPM* mutation detected at diagnosis disappeared at complete remission and the same mutation reappeared at relapse. One patient lost the mutation at the second relapse. Whether this is due to an emergence of a new clone of leukemia cells or low sensitivity of the method to detect the mutation remains to be determined. No one acquired a new mutation during a median follow-up time of 16 months (range 1-60 months), suggesting that the *NPM* mutation is probably an early event in the development of AML, but may play little role in the progression of the disease. The findings that the *NPM* mutation was inversely associated with the *CEBPA* mutation and chromosomal abnormalities t(8;21), t(15;17), and inv(16), and was closely correlated with *FLT3*/ITD infer that the *NPM* mutant might impair the

differentiation of hematopoietic cells. Further comprehensive studies on the biological effects of *NPM* mutants are needed to disclose the role of *NPM* mutations in the pathogenesis of AML and their interactions with other genetic alterations.

Supplementary information is available at *Leukemia*'s website.

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Figure legend

Fig. 1: The *NPM* mutation patterns. The sequences of A-E were derived from single clones by cloning with pGEM-T easy kit. A: wild type *NPM* sequence. B-E: the mutation of *NPM*. The boxed tetranucleotides are the insertion sequences between nucleotide 960 and 961 from the transcription start site. The underlying denotes the corresponding alteration of encoded amino acid sequences and incidences. F: the polymorphism with deletion of T nucleotide at position 1146. The sequences were read forward in A to E, but backward in F.

Fig. 2: Age-dependent incidences of *NPM* mutation. * denotes a p value <0.001 when compared with age groups 0-20 and 21-40. The percentages above the columns represent the incidences of the *NPM* mutation in that specific group of patients.

Fig. 1

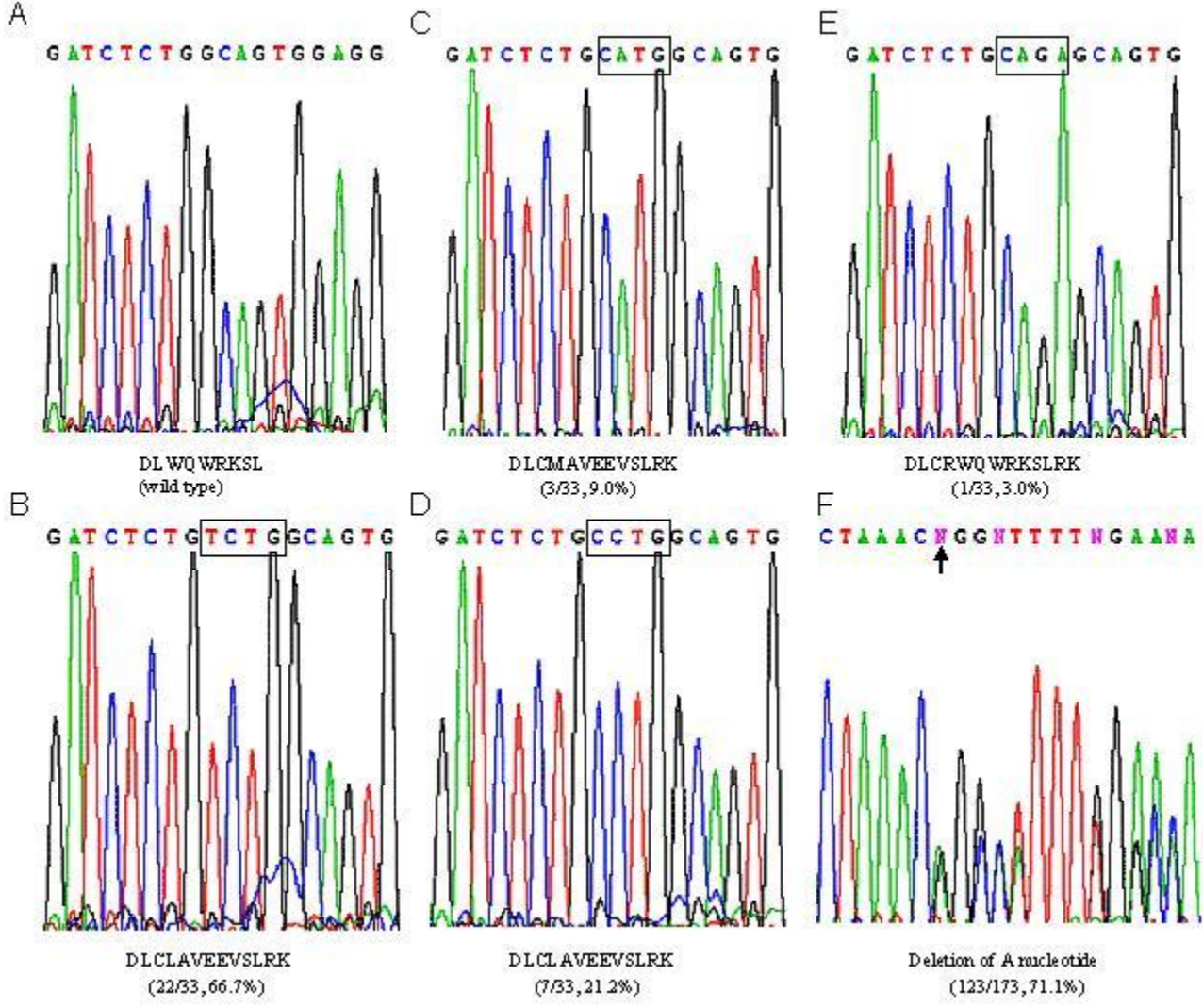
A
GATCTCTGGCAGTGGAGG
DLWQWRKSL
(wild type)
C
GATCTCTGCATGGCAGTG
DLCMAVEEVSLRK
(3/33, 9.0%)
E
GATCTCTGCAGAGCAGTG
DLCRWQWRKSLRK
(1/33, 3.0%)
B
GATCTCTGTCTGGCAGTG
DLCLAVEEVSLRK
(22/33, 66.7%)
D
GATCTCTGCCTGGCAGTG
DLCLAVEEVSLRK
(7/33, 21.2%)
F
CTAAACNGGNTTTTNGAANA
Deletion of A nucleotide
(123/173, 71.1%)

Fig. 2

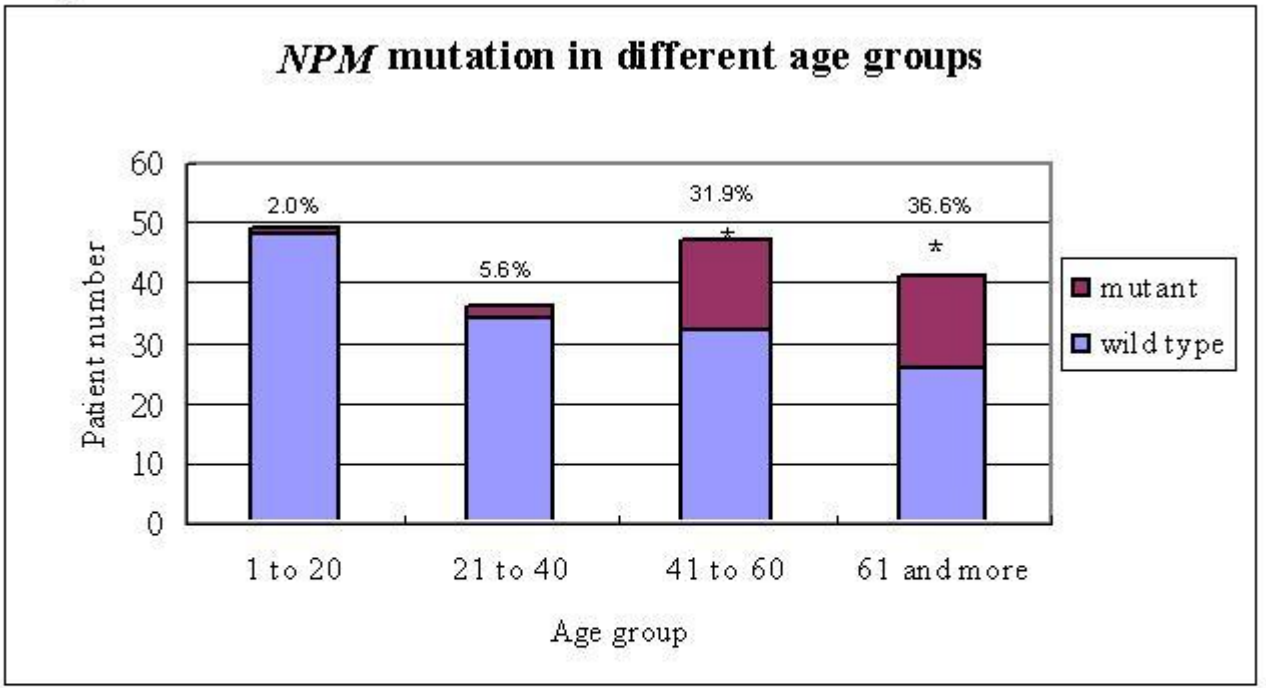
NPM mutation in different age groups
60
50
40
30
20
10
0
Patient number
2.0%
5.6%
31.9%
36.6%
*
*
1 to 20
21 to 40
41 to 60
61 and more
Age group
mutant
wild type

Table 1. Results of sequential studies of *NPM* mutations and chromosomal changes in the patients with the mutation at diagnosis[#]

Case number	Interval (mo)[*]	Status	Karyotype	*NPM* mutation**	Polymorphism***
21		diagnosis	N	+TCTG	-T
	1	CR	N	-	-T
22		diagnosis	N	+TCTG	-
	4	relapse	N	+TCTG	-
23		diagnosis	N	+TCTG	-
	1	CR	ND	-	-
27		diagnosis	N	+CTGC	-T
	56	CR	ND	-	-T
32		diagnosis	N	+TCTG	-T
	2	PR	N	+TCTG	-T
38		diagnosis	N	+CATG	-
	1	CR	ND	-	-
44		diagnosis	N	+TCTG	-T
	4	CR	ND	-	-T
55		diagnosis	+21	+TCTG	-T/-T
	3	CR	N	-	-T/-T
	18	relapse	N	+TCTG	-T/-T
63		diagnosis	N	+CTGC	-
	3	CR1	ND	-	-
	9	relapse 1	ND	+CTGC	-
	19	CR2	ND	-	-
	19	CR2	ND	-	-
	8	relapse 2	-7, t(12;18)	-	-
85		diagnosis	N	+TCTG	-
	1	CR		-	-
120		diagnosis	+4, +8	+TCTG	-T
	14	CR	N	-	-T
129		diagnosis	N	+TCTG	-T/-T
	3	CR	N	-	-T/-T
	3	relapse	N	+TCTG	-T/-T
147		diagnosis	-Y	+CTGC	-T/-T
	25	relapse	-Y	+CTGC	-T/-T

Sequential analyses of *NPM* mutation were performed on 41 patients. The data of the 20 patients without mutation but with polymorphism and the 8 patients with neither mutation nor polymorphism were not shown in this table. None of these 28 patients acquired *NPM* mutation at relapse though 7 of them had karyotypic evolution at relapse and 2 had completely different chromosomal abnormalities from those at diagnosis.

* Interval (month) between the previous study and this study.

** All patients with *NPM* mutations are heterozygous and have 4 bp insertions between positions nt 960 and 961.

*** Deletion of T nucleotide at position 1046 in the 3' untranslated region.

Abbreviations: CR, complete remission; PR, partial remission; N, normal; ND, no data; BMT, bone marrow transplantation.

Table 2. Clinical data, FAB types, and cytogenetic changes in AML patients

Variant	Total (N=173)	Mutation (N=33, 19.1%)		Wild type (N=140, 81.9%)		p value
	Number	Number	%	Number	%	
Age						p<0.001
Adult	126	32	25.4%	94	74.6%	
Children	47	1	2.1%	46	97.9%	
Sex						p=0.230
Male	105	17	16.2%	88	83.8%	
Female	68	16	23.5%	52	76.5%	
Lab data						
LDH (U/L)		1056		954		p=0.640
WBC/μL		39700		21240		p=0.031
Blast %		68		45		p=0.023
Platelet x 1000/μL		57		34.5		p=0.024
Hemoglobin g/dL		8.4		8.0		p=0.146
FAB						p=0.094*
M0	4	0	0.0%	4	100.0%	
M1	43	10	23.3%	33	76.7%	
M2	64	13	20.3%	51	79.7%	
M3	17	0	0.0%	17	100.0%	
M4	26	8	30.8%	18	69.2%	
M5	12	1	8.3%	11	91.7%	
M6	0	0	NA	0	NA	
M7	3	0	0.0%	3	100.0%	
undetermined		1		3		p<0.001**
Cytogenetic						
Abnormal						
t(15;17)	17	0	0.0%	17	100.0%	
t(8;21)	16	0	0.0%	16	100.0%	
Inv(16)	4	0	0.0%	4	100.0%	
t(7;11)	4	0	0.0%	4	100.0%	
t(6;9)	5	0	0.0%	5	100.0%	
del(7)	7	0	0.0%	7	100.0%	
del(5)	84	0	0.0%	4	100.0%	
trisomy 8	8	1	12.5%	7	87.5%	
Others	35	5	14.3%	30	85.7%	
Subtotal	98***	6	6.1%	92	93.9%	
Normal	67	27	40.3%	40	59.7%	

*Comparing M4 subtype and non-M4 subtypes.

**Comparing normal karyotype and others.

***Including one patient with del(7) plus del(5), and one with del(5) plus trisomy 8.

Table 3. Comparison of other genetic alterations* between AML patients with and without *NPM* mutation

Variant	Total patients		*NPM*-mutated patients		*NPM*-wild patients		*p* value
	No. studied	No. with alteration(%)	No. studied	No. with alteration(%)	No. studied	No. with alteration(%)	
CEBPA	168	26(15.5)	32	1(3.1)	136	25(18.4)	0.032
FLT3/ITD	169	34(20.1)	33	13(39.3)	136	21(15.4)	0.002
N-*RAS*	168	27(16.1)	32	7(21.8)	136	20(14.7)	0.320
K-*RAS*	170	6(3.5)	32	0(0)	138	6(4.3)	0.230
SHP1	128	89(69.5)	30	25(83.3)	98	64(65.3)	0.061
SOCS1	80	45(56.2)	15	6(40)	65	39(60)	0.371

* Mutation in *CEBPA*, *N-RAS*, *K-RAS*, internal tandem duplication (ITD) of *FLT3* (*FLT3*/ITD), and hypermethylation in *SHP1*and *SOCS1*.

Table 4. Comparison of immunophenotyping between AML patients with and without *NPM* mutation

Variant	Percentage of patients with the antigen expression			*p* value
	Total patients	*NPM*-mutated patients	*NPM*-wild patients	
HLA-DR	70.8	48.3	76.0	0.003
CD34	61.6	28.6	68.0	<0.001
CD13	86.6	89.7	85.9	0.595
CD33	92.2	100	90.5	0.089
CD11b	31.2	38.5	29.0	0.365
CD14	15.4	22.2	13.9	0.281
CD15	67.3	64.5	68.1	0.707
CD19	9.8	3.4	11.3	0.201
CD10	1.4	3.6	0.9	0.268
CD7	25.2	25.0	25.2	0.975
CD2	10.5	3.6	12.0	0.188
CD56	28.9	16.7	31.6	0.206

Supplementary Table 1. Results of sequential studies of *NPM* mutations and chromosomal changes in patients without mutation but with polymorphism at diagnosis

Case number	Interval (mo)	Status	Karyotype	*NPM* mutation	Polymorphism
34		diagnosis	N	-	-T
	31	relapse	ND	-	-T
42		diagnosis	N	-	-T/-T
	2	CR1	N	-	-T/-T
	14	relapse	inv(12)	-	-T/-T
	44	CR2	N	-	-T/-T
51		diagnosis	t(15;17)	-	-T
	28	relapse	t(15;17)	-	-T
59		diagnosis	t(15;17)	-	-T/-T
	12	relapse	t(15;17)	-	-T/-T
69		diagnosis	t(9;11)	-	-T/-T
	5	CR (BMT)	N	-	-T
91		diagnosis	N	-	-T
	3	CR1	N	-	-T
	14	relapse	N	-	-T
	5	CR2 (BMT)	N	-	-T
94		diagnosis	del(16q)	-	-T
	2	CR	N	-	-T
97		diagnosis	+8	-	-T/-T
	15	relapsed 1	+8, +15	-	-T/-T
	10	relapsed 2	+X, +15	-	-T/-T
101		diagnosis	N	-	-T
	46	CR	N	-	-T
107		diagnosis	t(15;17)	-	-T
	29	relapse	t(15;17)	-	-T
108		diagnosis	N	-	-T/-T
	6	CR	N	-	-T/-T
118		diagnosis	inv(16)	-	-T
	32	CR	N	-	-T
124		diagnosis	complex	-	-T/-T
	5	relapse	add(2)	-	-T/-T
131		diagnosis	t(15;17)	-	-T
	9	relapse	N	-	-T
136		diagnosis	N	-	-T/-T
	4	CR	N	-	-T/-T
	12	relapsed 1	ND	-	-T/-T
	6	relapsed 2	del(9)	-	-T/-T
139		diagnosis	+8, t(16;21)	-	-T/-T
	2	CR	N	-	-T/-T
	8	relapse	-7, der(18)	-	-T/-T
151		diagnosis	t(11;19)	-	-T
	5	relapse	ND	-	-T
154		diagnosis	complex	-	-T
	21	relapse	complex with evolution	-	-T
	2	relapse	ND	-	-T
165		diagnosis	inv(16)	-	-T/-T
	18	relapse	inv(16), +22	-	-T/-T
167		diagnosis	del(9q)	-	-T
	19	relapse	del(7p), del(9q)	-	-T

Abbreviations: CR, complete remission; N, normal; ND, no data; BMT, bone marrow transplantation.