

Treatment outcome of all-*trans* retinoic acid/anthracycline combination chemotherapy and the prognostic impact of *FLT3*/ITD mutation in acute promyelocytic leukemia patients

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Background

All-*trans* retinoic acid (ATRA)/anthracycline chemotherapy is beneficial in newly diagnosed acute promyelocytic leukemia (APL); however, it is important to identify patients with high-risk disease to increase the cure rate. We investigated the outcome of ATRA/anthracycline chemotherapy and clinicobiological correlations of *FLT3*/ITD and *NPM1* mutations in APL patients.

Methods

Induction therapy included oral ATRA (45 mg/m²/day) and idarubicin (12 mg/m²/day, intravenous, on days 2, 4, and 6). Patients achieving complete remission (CR) received 3 courses of ATRA combined with reinforced consolidation therapy. Mutations were analyzed using GeneScan and polymerase chain reaction assays of bone marrow samples obtained from patients at diagnosis.

Results

Forty-five (84.9%) of 53 eligible patients achieved CR. The overall relapse rate was 8.9%, and the 3-year overall survival (OS) and leukemia-free survival (LFS) were 84.9±4.9% and 77.5±6.0%, respectively. The *NPM1* mutation was not found in any patient, while the *FLT3*/ITD mutation was found in 10 (20.0%) patients. Of the *FLT3*/ITD+ patients, 80% belonged to the high-risk group, defined according to the presenting WBC and platelet counts. Among the patients who achieved CR, those who were *FLT3*/ITD+ had a higher relapse rate than those *FLT3*/ITD-. *FLT3*/ITD+ patients also had a significantly lower 3-year LFS than *FLT3*/ITD- patients. Multivariate analysis of the LFS showed that the *FLT3*/ITD mutation was independently associated with a shorter overall LFS, after adjusting for pre-treatment risk stratification.

Conclusion

This study investigated the clinical outcome of newly diagnosed APL patients treated with ATRA/anthracycline chemotherapy. Patients carrying the *FLT3*/ITD mutation had more aggressive clinical features and a poorer clinical outcome.

Key Words Acute promyelocytic leukemia, *FLT3*, Prognosis

INTRODUCTION

Most patients with acute promyelocytic leukemia (APL) are now successfully treated with all-*trans* retinoic acid

(ATRA) and anthracycline-based chemotherapy as first line therapy. A randomized study comparing the simultaneous administration of ATRA and anthracycline chemotherapy with the sequential administration of these therapeutic agents revealed that the former was more effective than



the latter [1]. The complete remission (CR) rates were 92% in both study arms, but the relapse rate at 2 years was 6% in the ATRA plus chemotherapy group versus 16% in the sequential group.

While the survival in most APL patients treated with ATRA plus chemotherapy is excellent, relapse or failure to achieve molecular remission is observed in 20-30% patients [2]. Several pretreatment characteristics of APL patients have been identified as having prognostic value [3]. Among them, the presenting white blood cell (WBC) count has the highest impact on the patient outcome [4].

The cooperative group PETHEMA (Programa de Estudio y Tratamiento de las Hemopatías Maligna) reported a risk-adapted treatment strategy of combining ATRA and anthracycline monochemotherapy for both induction and consolidation, followed by maintenance with ATRA and low-dose methotrexate and mercaptopurine [5, 6]. With the previously used treatment regimen, the number of deaths during induction and the relapse rates were higher in patients having an elevated WBC [4]. Therefore, PETHEMA recommended risk stratification based on the WBC and platelet counts at presentation (low-risk group, presenting WBC count of $\leq 10 \times 10^9/L$ and platelet count of $> 40 \times 10^9/L$; intermediate-risk group, presenting WBC of $\leq 10 \times 10^9/L$ and platelets $\leq 40 \times 10^9/L$; and high-risk group: presenting WBC count of $> 10 \times 10^9/L$). Recently, the French APL 2000 and PETHEMA 99 trials showed that a cytarabine-containing regimen resulted in a better CR rate and longer survival in patients with a high WBC count at presentation [7].

FLT3 belongs to the class III receptor tyrosine kinase family. It is expressed in early hematopoietic progenitors, and its dimerization by the *FLT3* ligand induces growth-control signals in normal hematopoiesis. The gene encoding *FLT3* maps to chromosome band 13q12 [8], and an internal tandem duplication (ITD) of the gene (*FLT3*ITD) is detected in 20-30% of young adults with acute myeloid leukemia (AML) [9]. The duplication involves a segment of the juxta-membrane-domain coding sequence, which is always in-frame and frequently involves exon 14 and rarely intron 14 or exon 15 [9]. *FLT3*ITD from AML patients has been shown to induce autonomous proliferation in cytokine-dependent cell lines; this finding supports the potential role of this mutation in leukemogenesis. AML patients with an isolated *FLT3*ITD mutation have poor clinical outcomes, similar to those with poor-risk cytogenetic features [9-11]. Recent reports suggest that quantitative analysis of *FLT3*ITD mutation status may be useful to predict the prognosis [10, 12] and assess the minimal residual disease [13] in AML patients.

The frequency of the *FLT3*ITD mutation varies dramatically across cytogenetically and molecularly defined subsets of AML and is particularly prevalent in patients with t(15;17) [14-16]. Although previous studies on APL patients have revealed that the *FLT3*ITD mutation is associated with an elevated presenting WBC count, hypogranular variant (M3v) morphology, and the short (bcr3) isoform of *PML-RARA*, the prognostic significance of *FLT3* mutations in APL has

not been firmly established [14].

The *NPM1* gene frequently acts as a target of chromosomal translocations and causes the cytoplasmic dislocation of proteins in various hematological malignancies, thereby indicating its role in malignant transformation. Mutations in *NPM1* exon-12 are found in approximately 35% of adult AML patients, and AML with the *NPM1* mutation is preferentially associated with monocytic differentiation, lack of CD34, normal cytogenetics, *FLT3* gene mutations, and tendency to a favorable clinical outcome [17]. In previous studies, however, *NPM1* mutations were not detected in APL patients [18, 19].

In this study, we assessed the treatment outcome of combined ATRA/anthracycline chemotherapy administered as induction and consolidation chemotherapy in APL patients and investigated a series of uniformly treated APL patients to identify the prognostic relevance of various factors present at diagnosis.

MATERIALS AND METHODS

1. Patients and treatment protocol

Induction therapy consisted of oral ATRA (45 mg/m² per day in 2 divided doses), which was maintained for a median 45 days or until complete hematologic remission and idarubicin (12 mg/m² per day) was administered as an intravenous bolus on days 2, 4, and 6. Unlike the protocol followed in previous studies [5-7], ours did not include administration of idarubicin on day 8; this was to eliminate the risk of severe myelosuppression during the treatment for remission induction. Treatment with ATRA was started as soon as APL was diagnosed on the basis of the morphological criteria. For patients in whom the diagnosis was not confirmed by genetic studies, ATRA treatment was withdrawn, and alternative chemotherapy was administered at the physician's discretion. Patients achieving CR received 3 courses of ATRA (45 mg/m²/d, days 1-15) combined with reinforced consolidation chemotherapy, which consisted of idarubicin (7 mg/m²/d for 4 d), mitoxantrone (10 mg/m²/d for 5 d), and idarubicin (12 mg/m²/d for 2 d). Risk stratification was defined using the WBC and platelet counts at presentation, as described in previous studies [5, 6, 20]. For maintenance therapy, ATRA (45 mg/m²/d, days 1-15) was administered every 3 months for 2 years. As supportive care, platelets were transfused to maintain a platelet count of $> 30 \times 10^9/L$ until resolution of coagulopathy, if any. Once the coagulopathy was under control, platelets were transfused only if the patient had infectious or hemorrhagic manifestations or if the platelet count dropped to below $20 \times 10^9/L$. Patients with active coagulopathy were treated with fresh frozen plasma or cryoprecipitate to maintain the fibrinogen level at > 1.5 mg/L and hemostatic levels of coagulation factors.

2. Cytogenetic analysis/detection of PML-RARA fusion

All patients were confirmed to have t(15;17) or PML-RARA fusion transcript by using conventional cytogenetic techni-

ques or nested reverse transcriptase-polymerase chain reaction (RT-PCR), respectively.

3. Mutational analysis

Genomic DNA was extracted from cells, such as cryopreserved mononuclear cells, which were harvested from BM samples by using a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's protocol. For *FLT3*/ITD mutational analysis, wild-type *FLT3* gene exon 11 and 12 were amplified by genomic PCR using the primers 5'-CAATTTAGGTATGAAAGCC-3' (sense) and 5'-CTTTCAGCATTGACGGCAACC-3' (anti-sense). Each reaction contained 2.5 mM dNTP, 2.5 mM MgCl₂, 0.5 μM of each primer, and 0.5 U *f-Taq* polymerase in a total volume of 20 μL. Samples were amplified by initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 53°C for 1 min, and 72°C for 2 min, with a final 10-min extension at 72°C. PCR products (10 μL) were resolved on 6% polyacrylamide gels, stained with ethidium bromide, and photographed under ultraviolet light.

For *NPM1* mutational analysis, *NPM1* gene exon-12 was amplified by genomic PCR using the primers 5'-TCTGAGTATAAATTTCTTGGAGTCA-3' (sense) and 5'-ACCAAGCAAA GGGTGGAGTT-3' (anti-sense). The reaction contained 1.25 pmol of each primer, 50 ng genomic DNA, 250 μM dNTPs, and 0.5 U *f-Taq* polymerase (SolGent, Daejeon, Korea) in the buffer provided by the manufacturer. Amplification was performed with a PTC 200 thermal cycler (MJ Research, Waltham, MA, USA). The PCR fragments were purified using the GeneAll PCR Purification Kit (General Biosystem, Seoul, Korea). Sequencing reactions were analyzed using an ABI 3100 sequencer and BigDye Terminator kit (Applied Biosys-

tems, Foster City, CA, USA).

4. Statistical analyses

CR was defined as per the criteria recommended previously [21]. Relapse was defined as the reappearance of blasts after CR in peripheral blood or BM. Leukemia-free survival (LFS) end points, measured from the date of documented CR, included relapse, patient death from any cause, and alive in CR at last follow-up (censored). The overall survival (OS) end points, measured from the date of diagnosis, were death from any cause and alive at last follow-up (censored) [21].

For intergroup comparisons, Fisher's exact test (categorical data) and the Mann-Whitney *U*-test (continuous data) were used. OS and LFS were analyzed using Kaplan-Meier survival curve estimates and log-rank tests to compare the differences between the distributions of survival in the patient groups. Multivariate analysis using forward conditional selection of variables was performed using Cox's proportional-hazards model. A *P*-value of less than 0.05 was considered to indicate statistical significance. All statistical computations were performed using the SPSS software (ver. 18.0; SPSS, Chicago, IL, USA).

RESULTS

1. Patient characteristics

The median age of the 53 APL patients was 48 years (range, 18-75 years), with 7 patients being over 60 years.

Table 1. Demographic data of 53 acute promyelocytic leukemia patients.

	Median (range)	N (%)
Age	48 (18-75) yr	
Aged over 60 yr		7 (13.2)
Sex		
Male		24 (45.3)
Female		29 (53.7)
FAB subtypes		
Typical		49 (92.5)
Variant		4 (7.5)
WBC, ×10 ⁹ /L	2.0 (0.5-102.8)	
Hb, g/dL	8.0 (5.0-13.9)	
Platelets, ×10 ⁹ /L	33.0 (5.0-135.0)	
BM blasts, %	83 (4-95)	
Risk group		
Low		14 (26.4)
Intermediate		23 (43.4)
High		16 (30.2)
<i>FLT3</i> /ITD+		10 (20.0)
(available in 50 patients)		

Abbreviations: FAB, French-American-British; WBC, white blood cell; Hb, hemoglobin; BM, bone marrow.

Table 2. Patient characteristics according to *FLT3*/ITD mutation status (N=50).

	<i>FLT3</i> /ITD-	<i>FLT3</i> /ITD+	<i>P</i>
No. of patients	40 (80.0%)	10 (20.0%)	
Age, median (range)	47 (18-75) yr	51 (40-63) yr	0.331
Aged over 60 yr	5 (12.5%)	2 (20.0%)	0.541
Sex			0.776
Male	18 (45.0%)	4 (40.0%)	
Female	22 (55.0%)	6 (60.0%)	
FAB subtypes			0.098
Typical	39 (97.5%)	8 (80.0%)	
Variant	1 (2.5%)	2 (20.0%)	
WBC, ×10 ⁹ /L, median (range)	1.7 (0.5-49.7)	20.4 (0.8-102.8)	0.000
Hb, g/dL, median (range)	8.0 (5.0-12.7)	9.9 (6.7-13.9)	0.018
Platelets, ×10 ⁹ /L, median (range)	36.0 (5.0-135.0)	33.0 (10.0-55.0)	0.306
BM blasts, %, median (range)	80.0 (4.0-94.0)	85.5 (70-95)	0.004
Risk group			0.001
Low	13 (32.5%)	1 (10.0%)	
Intermediate	20 (50.0%)	1 (10.0%)	
High	7 (17.5%)	8 (80.0%)	

Abbreviations: WBC, white blood cell; Hb, hemoglobin; BM, bone marrow.

Low-, intermediate-, and high-risk groups, according to the risk stratification, accounted for 26.4%, 43.4%, and 30.2% of the cases, respectively (Table 1). All patients had received intensive remission-induction chemotherapy. The median follow-up period was 30.8 months (range, 0.2-73.6 months).

BM samples of 50 patients were evaluated for *NPM1* and *FLT3*/ITD mutations. No *NPM1* mutation was found in any patient, while *FLT3*/ITD mutations were detected in 10 patients (20.0%). Details of the presenting features of *FLT3*/ITD+ patients are provided in Table 2. The median peripheral WBC count at diagnosis was significantly higher in *FLT3*/ITD+ patients than in *FLT3*/ITD- patients ($P < 0.001$). The number of BM blasts was also higher in *FLT3*/ITD+ patients than in *FLT3*/ITD- patients ($P = 0.004$). Of the *FLT3*/ITD+ patients, 80% belonged to the high-risk group ($P = 0.001$; Table 2).

2. Treatment outcome of ATRA combined chemotherapy

Of the 53 eligible patients, 45 (84.9%) achieved CR. One patient (1.9%) showed resistance to induction chemotherapy,

and the remaining 7 (13.2%) died during the induction chemotherapy. Of the 7 early deaths, 3 were due to the differentiation syndrome and 4 due to cerebral or pulmonary hemorrhage. Four (8.9%) patients had clinical relapse 7-15 months after achieving CR. The 3-year Kaplan-Meier

Table 3. Treatment outcomes according to risk group and *FLT3*/ITD status.

	N	CR (%)	P	Relapse (%)	P
<i>FLT3</i> /ITD					
Wild-type	40	35 (87.5%)	0.034	1 (2.5%)	0.001
Mutated	10	7 (70.0%)		3 (30.0%)	
Risk group					
Low	14	13 (92.9%)	0.311	0	0.090
Intermediate	23	19 (82.6%)		1 (4.3%)	
High	16	13 (81.3%)		3 (18.8%)	

Abbreviation: CR, complete remission.

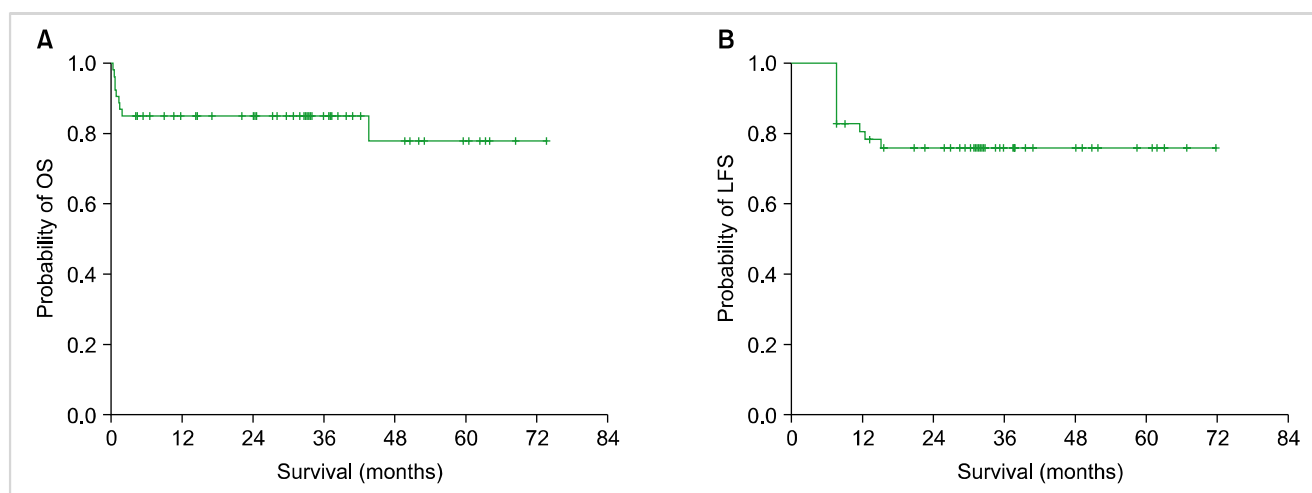


Fig. 1. Kaplan-Meier analysis of the APL patients. (A) Overall survival (OS) and (B) leukemia-free survival (LFS).

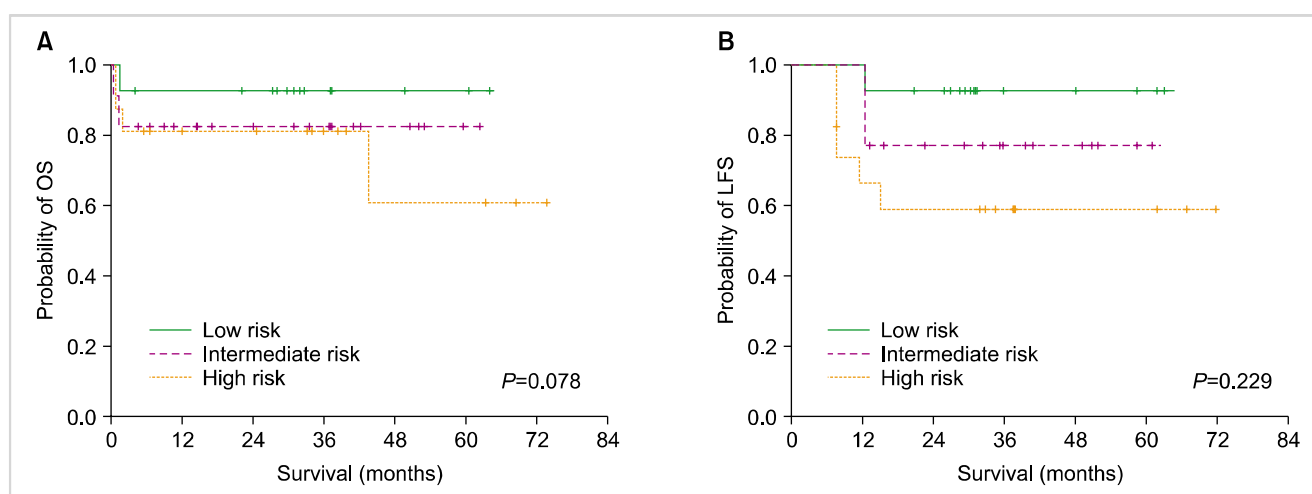


Fig. 2. Kaplan-Meier analysis of APL according to pretreatment risk stratification. (A) Overall survival (OS) and (B) leukemia-free survival (LFS).

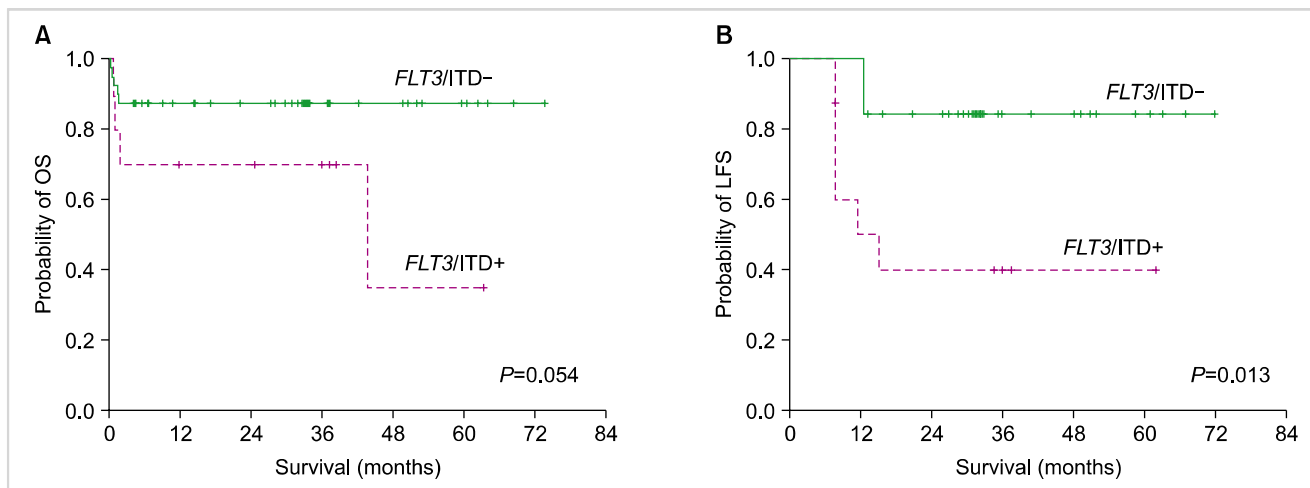


Fig. 3. Kaplan-Meier analysis of APL patients with the *FLT3/ITD* mutation (*FLT3/ITD+*) and without the mutation (*FLT3/ITD-*). **(A)** Overall survival (OS) and **(B)** leukemia-free survival (LFS).

estimates of OS and LFS were $84.9 \pm 4.9\%$ and $77.5 \pm 6.0\%$, respectively (Fig. 1). Patients with the hypogranular variant subtype did not show a significant difference in the treatment outcomes.

3. Prognostic impact of pretreatment risk stratification and *FLT3/ITD* mutations

With induction chemotherapy, the high-risk group, defined using the WBC and platelet counts at presentation, had a lower rate of remission compared to the intermediate- and low-risk groups; however, the difference was not statistically significant (81.3% vs. 82.6% vs. 92.9%, respectively; $P=0.311$; Table 3). Furthermore, a trend to a higher rate of relapse was observed in the high-risk group than in the other risk groups, but the difference was not significant (18.8% vs. 4.3% vs. 0%; $P=0.090$; Table 3). The high-risk group showed a trend to a lower 3-year LFS (60.0% vs. 77.1% vs. 92.9%; $P=0.229$) than the intermediate- and low-risk groups (Fig. 2).

A significant difference was noted between the remission rates achieved by induction chemotherapy in patients with and without *FLT3/ITD* mutations (70.0% vs. 87.5%; $P=0.034$; Table 3). Among the patients who achieved CR, the *FLT3/ITD+* group had a higher relapse rate than the *FLT3/ITD-* group (30.0% vs. 2.5%; $P=0.001$). The former also had a significantly lower 3-year LFS than the latter (44.4%, vs. 84.4%; $P=0.013$). The *FLT3/ITD+* group tended to have a lower 3-year OS than the *FLT3/ITD-* group (70.0% vs. 87.5%; $P=0.054$; Fig. 3), although the difference was not significant.

Multivariate analysis of the LFS revealed that the *FLT3/ITD* mutation was independently associated with a shorter LFS, after adjusting for pretreatment risk stratification (vs. *FLT3/ITD-*, odds ratio, OR=3.840; $P=0.026$; Table 4).

Table 4. Multivariate analyses for leukemia-free and overall survival.

Variables	LFS		OS	
	OR (95% CI)	P	OR (95% CI)	P
<i>FLT3/ITD</i>				
Wild-type				
Mutated	3.840 (1.171-12.593)	0.026	3.372 (0.904-12.573)	0.070
Pretreatment risk group				
Low				
Intermediate	3.396 (0.396-29.121)	0.265	2.651 (0.296-23.729)	0.383
High	5.211 (0.608-44.689)	0.132	3.627 (0.405-32.471)	0.249

Abbreviations: LFS, leukemia-free survival; OS, overall survival; OR, odds ratio; CI, confidence interval.

DISCUSSION

Because the use of the induction regimen with combined ATRA and anthracycline chemotherapy resulted in high CR rates and good disease-free survival (DFS), the inclusion of cytarabine in addition to daunorubicin or idarubicin for APL patients is considered to have no impact on the treatment outcome [20]. Thus, since many years, ATRA combined with anthracycline monotherapy has been used as a standard treatment for APL. In this study, we assessed the clinical outcome of APL patients treated with ATRA combined with anthracycline chemotherapy.

Previous studies on the use of combined ATRA and chemotherapy without cytarabine for the treatment for newly diagnosed APL showed CR rates ranging from 81 to 95% [1, 20]; the CR rate in this study was 84.9%. Because only 1 patient showed resistance to the induction chemotherapy

regimen, the inferior treatment outcome of our study seemed to result from the high rates of early death during the induction chemotherapy due to fatal bleeding or differentiation syndrome; this suggests that early awareness and management of such complications are important.

This study also demonstrated that the patients in the high-risk group, based on the WBC and platelet counts at presentation, tended to have poorer clinical outcomes, such as lower CR rates, higher relapse rates, and shorter LFS, as compared to those in the low- or intermediate-risk groups; however, the difference between the groups was not statistically significant.

In this series of APL patients, we evaluated the prevalence and clinical impact of *NPM1* and *FLT3*/ITD mutations. As was the case in previous studies [18, 19], none of the 50 patients evaluated in this study harbored the *NPM1* mutation, while 20% exhibited *FLT3*/ITD mutations. The reported frequency of *FLT3*/ITD mutations varies among studies, from 12 to 38% [22-25]. In comparison with the data from Western countries, the reported incidence of *FLT3*/ITD mutations in APL patients is lower in Asian countries such as in Japan and Taiwan (20 and 21%) [24, 25]. For Korea, one study on the prevalence of *FLT3*/ITD mutations in APL patients reported a prevalence of 12% [22]; the results obtained in this study were similar to those reported from other Asian countries. The striking differences in the relative incidence of *FLT3*/ITD between APL patients in Europe/North America and Asia are noteworthy, and the significantly varying propensity for the disease to develop in different ethnic groups [23] suggests that genetic and/or environmental influences play a role in the pathogenesis of APL.

While the prognostic implications of the *FLT3*/ITD mutation status in cytogenetically normal AML are well described [9-11], the prognostic relevance of this mutation in APL patients is less clear. The current literature on the effect of *FLT3* mutations on the outcome in APL is conflicting. Some studies found that the *FLT3* status did not result in any apparent difference in DFS [14], whereas others reported that *FLT3*/ITD mutations were associated with a tendency towards [26, 27] or significantly inferior [28] LFS. In our study, an association between the *FLT3*/ITD mutation and elevated presenting WBC and BM blast counts was noted in APL patients. A previous study indicated that *FLT3* alterations in APL are associated with more aggressive clinical features and suggested that these lesions may not play a major role in leukemia progression [26]. However, in vitro studies of small-molecule *FLT3* inhibitors have shown that they can suppress the survival and proliferation of *FLT3*/ITD+ AML blast cells [29, 30]. In our study, patients carrying this mutation had a lower CR rate, higher rate of relapse, and shorter LFS and OS than those without this mutation. Although the *FLT3*/ITD mutation was associated with high WBC and BM blast counts at presentation, multivariate analysis showed that this mutation was an independent predictor of LFS in APL patients.

In summary, our study examined the clinical outcome of combined ATRA and anthracycline chemotherapy in newly

diagnosed APL patients. Patients with *FLT3*/ITD mutations showed more aggressive clinical features. Further, the *FLT3*/ITD mutation at diagnosis characterizes a small subgroup of patients with extremely poor prognosis. Thus, to increase the cure rates, it would be helpful to identify patients with high-risk disease, such as those carrying *FLT3*/ITD mutations, who could benefit from treatment modification, including the addition of cytarabine or *FLT3* inhibitors.

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