

# A randomized study with or without intensified maintenance chemotherapy in patients with acute promyelocytic leukemia who have become negative for *PML-RAR $\alpha$* transcript after consolidation therapy: The Japan Adult Leukemia Study Group (JALSG) APL97 study

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To examine the efficacy of intensified maintenance chemotherapy, we conducted a prospective multicenter trial in adult patients with newly diagnosed acute promyelocytic leukemia treated with all-*trans* retinoic acid and chemotherapy. Of the 302 registered, 283 patients were assessable and 267 (94%) achieved complete remission. Predicted 6-year overall survival in all assessable patients and disease-free survival in patients who achieved complete remission were 83.9% and 68.5%, respectively. A total of 175

patients negative for *PML-RAR $\alpha$*  at the end of consolidation were randomly assigned to receive either intensified maintenance chemotherapy (n = 89) or observation (n = 86). Predicted 6-year disease-free survival was 79.8% for the observation group and 63.1% for the chemotherapy group, showing no statistically significant difference between the 2 groups (P = .20). Predicted 6-year survival of patients assigned to the observation was 98.8%, which was significantly higher than 86.2% in those allocated to

the intensified maintenance (P = .014). These results indicate that the intensified maintenance chemotherapy did not improve disease-free survival, but rather conferred a significantly poorer chance of survival in acute promyelocytic leukemia patients who have become negative for the *PML-RAR $\alpha$*  fusion transcript after 3 courses of intensive consolidation therapy. (Blood. 2007;110:59-66)

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## Introduction

The use of all-*trans* retinoic acid (ATRA) has markedly improved the therapeutic outcome in patients with acute promyelocytic leukemia (APL).<sup>1-3</sup> However, most patients treated with ATRA alone after achievement of complete remission (CR) eventually relapse, indicating that postremission chemotherapy is essential to obtain long-term survival.<sup>2,3</sup> Noncross-resistance between ATRA and chemotherapeutic drugs has contributed to not only a high CR rate but also a decrease in the relapse rate, leading to a significant improvement in disease-free survival (DFS) and overall survival (OS) rates.<sup>4-11</sup> Despite the impact of ATRA in the treatment of APL, approximately 10% to 30% of patients who were given intensive chemotherapy after achievement of CR still experienced relapse in several cooperative group studies.<sup>5,7-12</sup>

Before the introduction of ATRA in the treatment of APL, the efficacy of maintenance chemotherapy had been observed in patients with APL.<sup>13,14</sup> In our previous study, the Japan Adult

Leukemia Study Group (JALSG) APL92 study, patients with newly diagnosed APL received intensified maintenance therapy according to an earlier result of the AML87 study, which was performed before the use of ATRA.<sup>5</sup> The AML87 study showed a significantly better DFS in patients who received 12 courses of intensified maintenance chemotherapy compared with those administered 4 courses of the same chemotherapy.<sup>15</sup> However, it is not clear whether maintenance chemotherapy actually prevents relapse in APL patients treated with ATRA and chemotherapy, especially after they have become negative for the *PML-RAR $\alpha$*  transcript at the end of intensive consolidation chemotherapy. If short-term therapy without maintenance shows DFS rates identical to those for long-term therapy with maintenance, it would be beneficial for patients' quality of life as well as for medical costs. To determine the value of intensified maintenance chemotherapy, this study was

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designed to compare DFS and survival in previously untreated APL patients who had become negative for *PML-RAR $\alpha$*  transcript after 3 courses of intensive consolidation and were randomly allocated to either intensified maintenance chemotherapy or observation.

## Patients and methods

### Eligibility

Adult patients with previously untreated de novo APL were consecutively registered in the JALSG APL97 study. Eligible criteria were a diagnosis of APL with t(15;17) and/or the *PML-RAR $\alpha$*  fusion gene; age from 15 to 70 years; Eastern Cooperative Oncology Group performance status between 0 and 3; and sufficient function of the heart (no severe abnormalities detected on ECGs and echocardiographs), lung ( $\text{PaO}_2 > 60$  mm Hg or  $\text{SpO}_2 > 93\%$ ), liver (serum bilirubin level  $< 2.0$  mg/dL), and kidney (serum creatinine level  $< 2.0$  mg/dL). The study was approved by the Institutional Review Boards at each participating institution. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki.

### Assessment of disease

Morphologic diagnosis of APL was made according to the French-American-British classification and the bone marrow smears were centrally reviewed at the JALSG pathology committee. The diagnosis was confirmed by the presence of t(15;17) and/or the *PML-RAR $\alpha$*  fusion gene. Bone marrow samples were obtained at diagnosis, after induction therapy, after each cycle of consolidation chemotherapy, and periodically during maintenance chemotherapy. The *PML-RAR $\alpha$*  fusion gene was amplified using bone marrow samples obtained at diagnosis and after consolidation therapy by reverse-transcriptase polymerase chain reaction analysis.<sup>16,17</sup> The detection limit of *PML-RAR $\alpha$*  fusion transcript in this assay was  $10^{-4}$ .

### Treatment regimens

**Induction therapy.** Treatment was started as soon as a morphologic diagnosis of APL had been made. For remission induction therapy, patients received 45 mg/m<sup>2</sup>/d of ATRA orally divided into 3 doses given after meals daily until the day before the start of the first consolidation therapy. If patients had leukocyte counts below  $3.0 \times 10^9$ /L and APL cells below  $10^9$ /L at the start of therapy, they were treated with ATRA alone (group A). ATRA at the same dosage combined with idarubicin (12 mg/m<sup>2</sup>/d by 30-minute intravenous infusion on days 1 and 2) plus cytarabine (Ara-C) (80 mg/m<sup>2</sup>/d by continuous intravenous infusion on days 1 through 5) was given to patients with initial leukocyte counts between  $3.0 \times 10^9$ /L and  $10.0 \times 10^9$ /L, and those with leukocyte counts below  $3.0 \times 10^9$ /L and APL cells above  $10^9$ /L (group B). Patients with initial leukocyte counts of  $10.0 \times 10^9$ /L or more received idarubicin (12 mg/m<sup>2</sup> on days 1 to 3) plus Ara-C (100 mg/m<sup>2</sup> on days 1 to 5) in addition to ATRA (group C). During treatment with ATRA, if blast and promyelocyte counts in the peripheral blood were more than  $10^9$ /L, an additional cycle of chemotherapy consisting of idarubicin (12 mg/m<sup>2</sup> for 2 days) and Ara-C (80 mg/m<sup>2</sup> for 5 days) was given. Patients in groups A and B who received an additional cycle of chemotherapy during induction were designated as groups AD and BD, respectively.

For prevention of bleeding, patients received transfusions of platelets and fresh frozen plasma to maintain platelet counts above  $30 \times 10^9$ /L or more and plasma fibrinogen level above 4.4  $\mu\text{mol/L}$  (150 mg/dL) or more, respectively. If coagulation studies were abnormal, prophylactic use of heparin and/or other antifibrinolysis agents (dalteparin, gabexate mesilate, or nafamostat mesilate) was recommended. When retinoic acid (RA) syndrome occurred, ATRA was discontinued and 20 mg/kg of methylprednisolone was administered by 1-hour intravenous infusions for at least 3 days. RA syndrome was diagnosed in patients with unexplained fever, respiratory distress, weight gain, interstitial pulmonary infiltrate, and pleural or pericardial effusions, as previously described.<sup>18-20</sup> After resolution of the syndrome, ATRA was resumed at the same dosage.

**Consolidation therapy.** After achieving CR, patients received 3 courses of consolidation chemotherapy. The first consolidation consisted of mitoxantrone (7 mg/m<sup>2</sup>) by 30-minute intravenous infusion on days 1 to 3, and Ara-C (200 mg/m<sup>2</sup>) by continuous infusion on days 1 to 5. The second consolidation contained Ara-C (140 mg/m<sup>2</sup>) for 5 days, etoposide (100 mg/m<sup>2</sup>) by 1-hour intravenous infusion for 5 days, and daunorubicin (50 mg/m<sup>2</sup>) by 30-minute infusion on days 1 through 3. The third consolidation consisted of Ara-C (140 mg/m<sup>2</sup>) for 5 days and idarubicin (12 mg/m<sup>2</sup>) for 3 days. Each consolidation course was given after recovery from the previous course, when polymorphonuclear cells were  $1.5 \times 10^9$ /L or more and platelets were  $100 \times 10^9$ /L or more. All patients received an intrathecal administration of methotrexate (MTX) (15 mg), Ara-C (40 mg), and prednisolone (10 mg) at the end of the second consolidation therapy.

**Intensified maintenance chemotherapy.** After completion of consolidation therapy, patients negative for the *PML-RAR $\alpha$*  transcript were randomly allocated either to receive 6 courses of intensified maintenance chemotherapy every 6 weeks or to observation. Randomization was stratified by age and initial leukocyte count, both of which were prognostic factors for DFS in the JALSG APL92 study.<sup>21</sup> The first course of intensified maintenance therapy consisted of behenoyl Ara-C (BHAC) (170 mg/m<sup>2</sup>, 2-hour infusion, days 1 through 5), daunorubicin (30 mg/m<sup>2</sup>, 30-minute infusion, days 1 and 4) and mercaptopurine (6MP; 70 mg/m<sup>2</sup>, orally, days 1 through 7). The second consisted of BHAC and mitoxantrone (5 mg/m<sup>2</sup>, 30-minute infusion, days 1 and 2). The third consisted of BHAC, etoposide (80 mg/m<sup>2</sup>, 1-hour infusion, days 1, 3, and 5), and vindesine (2 mg/m<sup>2</sup>, bolus infusion, days 1 and 8). The fourth consisted of BHAC, aclarubicin (14 mg/m<sup>2</sup>, 30-minute infusion, days 1 through 4), and 6MP. The fifth and sixth courses were the same as the first and third, respectively. Patients who were positive for the *PML-RAR $\alpha$*  fusion transcript at the end of consolidation chemotherapy received late ATRA therapy (45 mg/m<sup>2</sup>/day, orally after meals for 4 weeks) followed by maintenance therapy. These patients were also scheduled to receive allogeneic hematopoietic stem cell transplantation (HSCT) if there was a human leukocyte antigen-identical donor.

### Definition and study end points

Hematologic response was evaluated by standard criteria generally used for chemotherapy.<sup>22,23</sup> CR was defined as less than 5% of blasts and promyelocytes with normal erythropoiesis, thrombopoiesis, and granulopoiesis in the bone marrow, and neutrophil counts of more than  $1.5 \times 10^9$ /L and platelet counts of more than  $100 \times 10^9$ /L in the peripheral blood. Hematologic relapse was defined as the presence of more than 10% blasts plus abnormal promyelocytes in the marrow or the presence of any those cells in the peripheral blood or extramedullary sites. In addition, molecular relapse detected by the reverse-transcriptase polymerase chain reaction analysis of *PML-RAR $\alpha$*  was also considered as a relapse event.

The primary end point of this study was survival and DFS of patients in CR who had become negative for the *PML-RAR $\alpha$*  fusion transcript after the consolidation therapy and who were registered in the randomized study of the maintenance chemotherapy. OS for all patients was calculated from the first day of therapy to death or last visit. DFS for patients who had achieved CR was measured from the date of CR to relapse, death from any cause, or last visit. Survival and DFS in patients who were randomized to either observation or maintenance chemotherapy groups were measured from the date of random assignment to the same end points of these mentioned.

### Statistical analyses

Baseline characteristics of the 2 randomized groups were compared using the chi-square test or Fisher exact test for categorical data, and the Wilcoxon rank-sum test for continuous data. Probabilities of survival and DFS were estimated using the Kaplan-Meier method and compared by the log-rank test. The follow-ups on these patients were updated on September 30, 2004. Patients who were lost to follow-up or were still alive at the time of data cutoff were censored at the last date they were known to be alive. Patients who underwent HSCT were also censored at the date of HSCT. Factors affecting survival and DFS were analyzed by the use of the Cox regression model to estimate a hazard ratio with 95% confidence intervals (CI). All analyses were performed according to the intent-to-treat principle.

All statistical tests were 2-sided, and the significance level was set at .05. Statistical analyses were performed using SAS 8.2 (SAS Institute Japan, Tokyo, Japan).

## Results

### Patient characteristics

Between May 1997 and June 2002, 302 patients from 92 institutes participating in the JALSG were consecutively enrolled in the study. Of these, 19 were excluded because 4 were misdiagnosed, 2 were not consistent with the eligibility criteria, 7 were negative for *t(15;17)* or *PML-RAR $\alpha$* , and 6 had no test for *t(15;17)* or *PML-RAR $\alpha$* . Early death was not excluded, although 5 patients died of hemorrhage within 7 days. The characteristics of the 283 evaluable patients are listed in Table 1. Ages ranged from 15 to 70 years, with a median of 48 years. Eighteen patients (6%) had a variant form of French-American-British morphology (M3v). The median leukocyte count was  $1.7 \times 10^9/L$  (range, 0.03 to  $257 \times 10^9/L$ ) on admission. One hundred fifty-one patients started on ATRA alone during induction, and in 66 of these, chemotherapy was later added because of increased blasts and promyelocytes according to the protocol (groups A and AD; Table 1). One hundred twenty-five patients received both ATRA and chemotherapy from the beginning of therapy (groups B and C), and in 4 of group B an additional cycle of chemotherapy was later added because of increased blasts and promyelocytes (group BD).

**Treatment outcome.** Of the 283 evaluable patients, 267 (94.3%) had CR at a median of 42 days (range, 14 to 98) after the start of

therapy. During induction therapy, 60 (21%) patients showed signs of RA syndrome and 2 died of the syndrome. In addition, 65 (23%) patients developed organ bleeding, and 9 patients had fatal bleeding, including 5 early deaths within 7 days (Table 2). Thus, early death caused by bleeding was a major cause of induction failure. Although one patient had resistant leukemia, this patient received ATRA for only 16 days because of RA syndrome. Of the 267 patients who achieved CR, 258 (97%) completed the first course of consolidation, 250 (94%) completed the second, and 235 (88%) patients completed the third (Table 2 and Figure 1). After the consolidation, 5 patients underwent allogeneic HSCT at their first CR and 30 patients underwent HSCT after relapse. At a median follow-up of 64 months (range, 27 to 88 months), 60 (22%) of the 267 patients had relapsed and 18 had died. A further 16 (6%) patients died in CR, and 10 of those died of infection during myelosuppression after consolidation therapy (Table 2). The predicted 6-year OS rate in all 283 assessable patients was 83.9% (95% confidence interval [CI], 79.2% to 88.6%; Figure 2A). The predicted 6-year DFS rate in 265 CR cases was 68.5% (95% CI, 62.1% to 74.9%; Figure 2B).

**Randomized study with or without intensified maintenance therapy.** Among the 235 patients who completed 3 courses of consolidation and were evaluated for minimal residual disease, 5 (2.1%) were positive for the *PML-RAR $\alpha$*  fusion transcript. Three of these subsequently relapsed and another patient received allogeneic HSCT. However, 230 patients (97.9%) showed no *PML-RAR $\alpha$*  transcript in the bone marrow cells at the end of consolidation. A total of 55 patients negative for *PML-RAR $\alpha$*  were not included in the randomized study for a variety of reasons. Of these, 33 patients refused the randomization because 20 did not want to receive

**Table 1. Clinical features of patients at diagnosis**

Parameters	Total		Maintenance chemotherapy		Observation		P*
	No. (%)	Median (range)	No. (%)	Median (range)	No. (%)	Median (range)	
No. of patients	283		89		86		
<b>Sex</b>							
Male	158 (56)		53 (60)		47 (55)		.51
Female	125 (44)		36 (40)		39 (45)		
<b>Age, years</b>		48 (15-70)		49 (15-70)		46 (16-67)	.70
15-29	49 (17)		17 (19)		15 (17)		
30-49	106 (37)		32 (36)		34 (40)		.88
50-70	128 (45)		40 (45)		37 (43)		
<b>FAB Morphology</b>							
M3	265 (93)		80 (90)		82 (95)		.25
M3v	18 (6)		9 (10)		4 (5)		
<b>Leukocyte count, <math>\times 10^9/L</math></b>		1.7 (0.03-257)		1.9 (0.03-152)		2.1 (0.1-98)	.95
Less than 3.0	174 (61)		50 (56)		47 (55)		
3.0-10.0	58 (20)		21 (24)		20 (23)		.95
10.0 or higher	51 (18)		18 (20)		19 (22)		
<b>Platelet count, <math>\times 10^9/L</math></b>		30 (2-238)		31 (4-230)		23 (2-238)	.10
Less than 10	39 (14)		8 (9)		16 (19)		
10-40	140 (49)		47 (53)		41 (48)		.18
40 or higher	104 (37)		34 (38)		29 (34)		
<b>Induction therapy#</b>							
Group A	85 (30)		29 (33)		29 (34)		1.0
Group AD	66 (23)		17 (19)		16 (19)		
Group B+BD	73 (26)		23 (26)		22 (26)		
Group C	52 (18)		20 (22)		19 (22)		
Unknown	7 (2)		NA		NA		

\*Baseline characteristics of the two randomized groups were compared with Chi-square test or Wilcoxon rank-sum test.

# Patients in Group A were treated with ATRA alone; patients in Groups B and C were treated with ATRA plus idarubicin and cytarabine. Patients in Groups A and B who received an additional cycle of chemotherapy due to increased leukemic cells during induction were designated as Groups AD and BD, respectively. Four patients were in Group BD.

NA indicates not applicable.

**Table 2. Events occurring during the induction and consolidation therapy**

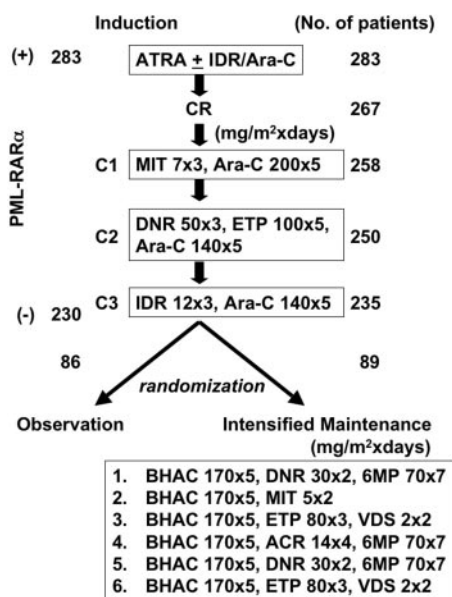
	Induction	Consolidation 1	Consolidation 2	Consolidation 3
No. of registered patients	283	267	258	250
Death during treatment	13	0	4	6
Infection	1	0	4	6
Bleeding	9	0	0	0
RA syndrome	2	0	0	0
Other	1	0	0	0
Going off study by toxicity	0	3	0	0
Lost to follow-up	2	6	3	7
Relapse	0	0	1	2
Refractory	1	0	0	0
Stem cell transplantation	0	0	0	0
No. of completed patients	267	258	250	235

further therapy and 13 wanted to receive additional chemotherapy; another 13 had residual toxicity from the consolidation and were considered as lack of tolerance to subsequent therapy (10 myelosuppression, 2 general fungal infection, and 1 heart disease); lost to follow-up in 3 patients; and unknown causes or no report in 6 patients. There was no significant difference in the 6-year DFS between 175 patients included in the randomized study (70.8%; 95% CI, 62.7% to 78.8%) and 55 patients not included (76.7%; 95% CI, 65.1 to 88.3%;  $P = .87$ ). The 6-year OS was 92.1% (95% CI, 87.2% to 97.1%) in the patients enrolled in the randomized study and 93.1% (95% CI, 85.3% to 100%) in the patients not enrolled ( $P = .97$ ).

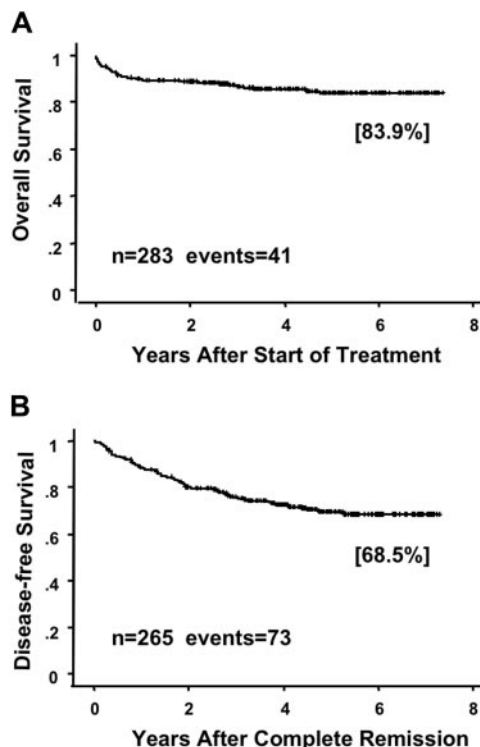
A total of 175 patients who were negative for *PML-RAR $\alpha$*  at the end of consolidation were randomly assigned to either observation ( $n = 86$ ) or intensified maintenance chemotherapy ( $n = 89$ ; Figure 1). Median interval from the recovery of myelosuppression after the third course of consolidation to the randomization was 20 days in both the maintenance and observation groups ( $P = .35$ ). More than 90% of patients were allocated to either intensified maintenance chemotherapy or observation groups within 2 months after

the consolidation. There was no significant difference between the 2 groups in patient profiles, including sex, age, French-American-British morphology, initial leukocyte count, platelet count, and induction therapy (Table 1).

At a median follow-up time of 49 months (range, 24 to 81 months) after randomization, there were 25 (28%) relapses and 13 (15%) deaths among the 89 patients who were allocated to the intensified maintenance chemotherapy. Of the 86 patients who were assigned to the observation, 17 (20%) relapsed and 3 (3%) died. There was no therapy-related mortality during the intensified maintenance chemotherapy. All but 2 patients in the maintenance group died after relapse. In the chemotherapy group, one patient developed therapy-related myelodysplastic syndrome and another developed acute myeloid leukemia during their first CR of APL. By contrast, none of patients in the observation group developed therapy-related leukemia and all 3 patients died after relapse. A second CR was achieved in 13 of 24 (54%) in the chemotherapy group and 13 of 17 (76%) in the observation group ( $P = .19$ ). The predicted 6-year DFS rates were 63.1% (95% CI, 50.2 to 76.0%) for

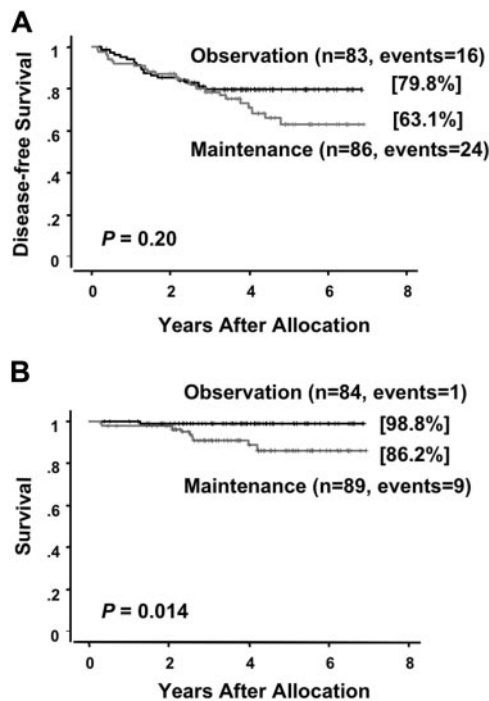


**Figure 1. Study design.** The number of patients who completed each step is indicated. C1, C2, and C3 were consolidation courses 1, 2, and 3. A total of 283 patients had t(15;17) and/or the *PML-RAR $\alpha$*  transcript at the time of diagnosis, and 230 patients were negative for *PML-RAR $\alpha$*  at the end of 3 courses of consolidation therapy. After completion of consolidation therapy, 175 patients who showed absence of *PML-RAR $\alpha$*  transcript were randomized either to receive 6 courses of intensified maintenance chemotherapy ( $n = 89$ ) or to observation ( $n = 86$ ).



**Figure 2. Overall survival and disease-free survival in patients enrolled in the JALSG APL97 study.** Overall survival (A) in all assessable patients and disease-free survival (B) in patients who achieved CR are estimated by the Kaplan-Meier method.





**Figure 3.** Disease-free survival and survival of randomized patients in the maintenance phase. Disease-free survival (A) and survival (B) are estimated from the date of randomization.

patients assigned to the maintenance chemotherapy and 79.8% (95% CI, 71.0 to 88.7%) for patients assigned to the observation (Figure 3A). No statistically significant difference in DFS was observed in patients treated with or without the maintenance chemotherapy ( $P = .20$ ). In the chemotherapy group, 8 patients showed late relapses occurring after at least 3 years of continuous CR, whereas no patients in the observation group showed a late relapse (Figure 3A;  $P = .006$ ). Univariate analysis showed that an initial leukocyte count of more than  $10.0 \times 10^9/L$  and induction group C trended to be unfavorable prognostic factors for DFS (Table 3). The predicted 6-year survival in the observation group was 98.8% (95% CI, 96.3 to 100%), which was significantly higher than 86.2% (95% CI, 77.3 to 95.0%) in the intensified maintenance group ( $P = .014$ ; Figure 3B). Univariate analysis revealed that induction group C and maintenance chemotherapy were significant unfavorable prognostic factors for survival (Table 4). Patients with initial leukocyte counts above  $10.0 \times 10^9/L$  showed a trend toward unfavorable DFS and survival, although this cohort was small (Figure 4A,B).

## Discussion

The present randomized study demonstrated that intermittent intensified maintenance chemotherapy did not improve DFS, but rather worsened survival in patients with newly diagnosed APL who had become negative for the *PML-RAR $\alpha$*  fusion transcript at the end of consolidation therapy.

In this study, ATRA and chemotherapy resulted in a high CR rate, improved OS, and DFS in patients with previously untreated APL. In our previous APL92 study, in which ATRA was used for the first time in the JALSG studies to newly diagnosed APL, the combination of ATRA plus chemotherapy induced CR in 333 of 369 (90%) assessable patients.<sup>24</sup> The 6-year OS rate of all evaluable patients and the 6-year DFS rate of CR cases in the APL92 study were 65% and 59%, respectively. In both APL92 and

**Table 3.** Effects of factors on disease-free survival

Parameters	No. of patients	No. of relapses	Univariate analysis	
			HR (95%CI)	P*
<b>Sex</b>				
Female	100	16	1	
Male	75	26	1.4 (0.7-2.7)	.29
<b>Age, years</b>				
15-50	98	24	1	
50-70	77	18	0.9 (0.5-1.7)	.75
<b>Leukocyte count, <math>\times 10^9/L</math></b>				
Less than 10.0	138	29	1	
10.0 or higher	37	13	1.7 (0.9-3.5)	.12
<b>Platelet count, <math>\times 10^9/L</math></b>				
Less than 40	112	25	1	
40 or higher	63	17	1.4 (0.7-2.6)	.32
<b>Induction therapy</b>				
Group A	58	10	1	
Group AD	33	6	1.0 (0.3-2.9)	.97
Group B, BD	45	12	1.8 (0.8-4.1)	.17
Group C	39	14	2.3 (1.0-5.3)	.05
<b>Maintenance chemotherapy</b>				
No maintenance	86	17	1	
Maintenance	89	25	1.5 (0.8-2.8)	.20

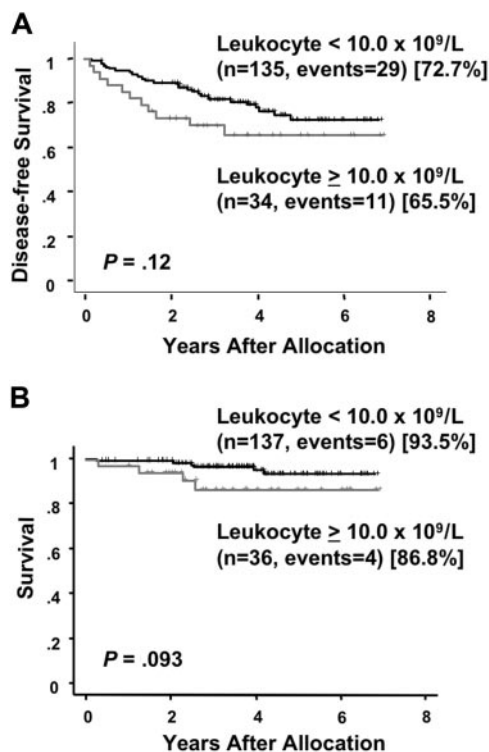
\*Factors affected on disease-free survival were analyzed by the Cox hazard regression model.

APL97 studies, patients received ATRA only in the induction phase. Therefore, the improvement of OS and DFS in the present study can mostly be attributed to the intensification of chemotherapy during induction and consolidation. In the present study, idarubicin and Ara-C were used instead of daunorubicin and BHAC in the induction, and one of the anthracyclines in combination with Ara-C was given in each consolidation.<sup>25</sup> Thus, the OS and DFS appear to depend on the intensities of chemotherapy in the treatments of APL. The high sensitivity of APL to anthracyclines is well-documented by several cooperative groups.<sup>26,27</sup> In addition, there was a hypothesis that an anthracycline alone may be as effective as combinations of anthracycline and Ara-C.<sup>8,27,28</sup> However, the interim analysis of the European APL2000 study showed

**Table 4.** Effects of factors on survival

Parameters	No. of patients	No. of deaths	Univariate analysis	
			HR (95%CI)	P*
<b>Sex</b>				
Female	100	12	1	
Male	75	4	2.1 (0.6-8.3)	.27
<b>Age, years</b>				
15-50	98	8	1	
50-70	77	8	2.6 (0.7-10)	.16
<b>Leukocyte count, <math>\times 10^9/L</math></b>				
Less than 10.0	138	11	1	
10.0 or higher	37	5	2.8 (0.8-10)	.11
<b>Platelet count, <math>\times 10^9/L</math></b>				
Less than 40	112	12	1	
40 or higher	63	4	0.2 (0.03-1.8)	.16
<b>Induction therapy</b>				
Group A	58	1	1	
Group AD	33	4	3.8 (0.3-41)	.28
Group B, BD	45	5	2.8 (0.3-31)	.4
Group C	39	6	8.9 (1.0-76)	.05
<b>Maintenance chemotherapy</b>				
No maintenance	86	3	1	
Maintenance	89	13	8.6 (1.1-68)	.04

\*Factors affected on survival were analyzed by the Cox hazard regression model.



**Figure 4.** Disease-free survival and survival by initial leukocyte count. Disease-free survival (A) and survival (B) in patients with initial leukocyte counts above or below  $10.0 \times 10^9/L$  are estimated from the date of randomization.

the efficacy of Ara-C in induction and consolidation even in patients with leukocyte counts of less than  $10.0 \times 10^9/L$ .<sup>29</sup> Therefore, despite significant improvement of therapeutic outcome in APL, concern still exists regarding which is the best chemotherapeutic strategy for APL.

*PML-RAR $\alpha$*  generated by t(15;17) provides the most clinically relevant information in patients with APL.<sup>2,3,16,17</sup> A number of patients who achieve molecular remission assessed by reverse-transcriptase polymerase chain reaction for *PML-RAR $\alpha$*  after consolidation are predicted to obtain a long-term survival.<sup>7,30</sup> However, detection of *PML-RAR $\alpha$*  identifies patients at risk for relapse after consolidation. In addition, treatment of patients at the time of molecular relapse provides a survival advantage compared with treatment at overt hematologic relapse.<sup>31</sup> In this study, 5 of 235 (2.1%) patients showed the *PML-RAR $\alpha$*  fusion transcript after the consolidation therapy, and 3 of these relapsed subsequently. In contrast, 97.9% of patients were negative for *PML-RAR $\alpha$*  transcript. In the GIMEMA-AIEOP study, 646 of 664 (97.3%) patients were negative for the *PML-RAR $\alpha$*  fusion transcript at the end of consolidation.<sup>32</sup> Because approximately half of APL patients are molecularly positive after induction,<sup>7,9</sup> elimination of *PML-RAR $\alpha$*  positive cells might be associated with intensive consolidation chemotherapy.

Our present results showed no benefit of moderately intensive and intermittent chemotherapy in the maintenance phase. This result is consistent with an earlier GIMEMA study before the availability of ATRA, in which patients randomized to maintenance therapy with low-dose 6MP and MTX did not have better outcomes than those randomized to the observation.<sup>27</sup> However, the North American Intergroup trial showed a benefit for ATRA in both induction and maintenance therapy.<sup>6,12</sup> In addition, the European APL93 study revealed that maintenance therapy with a combination of low-dose chemotherapy (6MP and MTX) and intermittent

ATRA reduced the incidence of relapse.<sup>10</sup> However, the recent GIMEMA-AIEOP study documented no difference in DFS in patients treated with maintenance consisting of either ATRA, 6MP/MTX, ATRA plus 6MP/MTX, or observation.<sup>32</sup> Therefore, the role of maintenance chemotherapy in the treatment of APL remains to be determined. Because intensified maintenance chemotherapy in this study is apparently different from the continuous maintenance with low-dose 6MP and MTX, comparison with other studies of maintenance is difficult. It is very likely that the usefulness of maintenance therapy depends on the intensity of chemotherapy delivered during induction and consolidation phases. In the US Intergroup and European APL93 studies, patients were treated with only 2 cycles of consolidation,<sup>6,10</sup> whereas patients received 3 cycles of consolidation both in the GIMEMA-AIEOP and our studies.<sup>32</sup> Recently, we did not find a benefit for intensified maintenance therapy in patients with acute myeloid leukemia other than APL treated with intensive consolidation therapy.<sup>33</sup> Our present study confirms that there is no beneficial effect of intensified maintenance chemotherapy in previously untreated APL patients who have become negative for the *PML-RAR $\alpha$*  fusion transcript at the end of consolidation. In addition, there was a trend toward better DFS in patients with no maintenance chemotherapy. Patients in the maintenance chemotherapy group showed a significant number of late relapses occurring after at least 3 years of continuous CR compared with the observation group. This was a quite unexpected finding for us. Although the limited number of patients prohibits a robust conclusion, we speculated that intensified maintenance chemotherapy may impair potential immune surveillance to eradicate minimal residual leukemic cells in patients with molecularly undetectable residual leukemia. Further studies are required to investigate whether ATRA has a role in maintenance. The current JALSG APL204 study compares the efficacy of ATRA versus tamibarotene (Am80) in the maintenance phase.

It is interesting to note that patients assigned to the observation group showed a significantly better survival than those randomized to the maintenance group. Because the difference in DFS was not statistically significant and there was no chemotherapy-related death in the latter group, the difference in survival is thought to result from the difference in the second CR rates and CR durations. Although APL cells usually lack p-glycoprotein expression, multi-drug resistance is generally acquired by the use of antileukemic agents.<sup>34</sup> As the chemotherapy in the maintenance phase of this study mainly consisted of one of the anthracyclines and BHAC, accumulation of chemotherapeutic agents in patients in the maintenance group may induce drug resistance to additional chemotherapy. In addition, accumulated chemotherapy may induce an overall increased toxicity and lack of tolerance to subsequent therapy after relapse. Furthermore, it is of note that 2 patients in the maintenance group died of therapy-related leukemia in the first CR of APL. Occurrence of therapy-related leukemia in patients treated for APL is an emerging problem.<sup>35</sup> Chemotherapeutic agents in the maintenance phase seem to increase the risk of therapy-related leukemia.

Although APL has become the most curable subtype of acute leukemia in adults, approximately 20% of patients still die of the disease because of early death or relapse.<sup>2,3</sup> One of the unfavorable prognostic factors for DFS and survival, in the present study as well as in our previous and other studies, was high initial leukocyte count.<sup>9,21,36</sup> In this study, the stratification by intensities of chemotherapy in the induction phase failed to improve DFS in patients with high initial leukocyte count (group C). Thus, patients with

high leukocyte count will require an alternative approach to obtain long-term survival. Use of arsenic trioxide, Am80, and/or gemtuzumab ozogamicin during the front-line therapy may improve DFS and OS in these patients at high risk.<sup>37-41</sup>

In conclusion, we did not find any beneficial effect of intensified maintenance chemotherapy in patients negative for *PML-RAR $\alpha$*  at the end of consolidation chemotherapy. On the contrary, intensified maintenance chemotherapy unexpectedly conferred a significantly poor survival as well as an increased risk of therapy-related leukemia in these patients.

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## Authorship

Contribution: N.A., S.O., T.N., and R.O. participated in the study design, analysis of the experiments, and writing of the manuscript. Y.K., H.K., M.O., Y.K., M.T., K.H., M.M., K.S., T.K., M.N., M.T., F.Y., A.T., and Y.K. were significant clinical contributors to the trial and have reviewed the manuscript. M.I. collected the data and performed statistical analysis.

A complete list of the Japan Adult Leukemia Study Group is provided in Document S1 as a data supplement to the online version of this article.

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## References

- Huang ME, Ye YC, Chen SR, et al. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood*. 1988;72:567-572.
- Tallman MS, Nabhan C, Feusner JH, Rowe JM. Acute promyelocytic leukemia: evolving therapeutic strategies. *Blood*. 2002;99:759-767.
- Ohno R, Asou N, Ohnishi K. Treatment of acute promyelocytic leukemia: strategy toward further increase of cure rate. *Leukemia*. 2003;17:1454-1463.
- Fenaux P, Le Deley MC, Castaigne S, et al. Effect of all-trans-retinoic acid in newly diagnosed acute promyelocytic leukemia. Results of a multicenter randomized trial. European APL 91 Group. *Blood*. 1993;82:3241-3249.
- Kanamaru A, Takemoto Y, Tanimoto M, et al. All-trans retinoic acid for the treatment of newly diagnosed acute promyelocytic leukemia. Japan Adult Leukemia Study Group. *Blood*. 1995;85:1202-1206.
- Tallman MS, Andersen JW, Schiffer CA, et al. All-trans-retinoic acid in acute promyelocytic leukemia. *N Engl J Med*. 1997;337:1021-1028.
- Mandelli F, Diverio D, Avvisati G, et al. Molecular remission in PML/RAR alpha-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. *Blood*. 1997;90:1014-1021.
- Sanz MA, Martin G, Rayon C, et al. A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RARalpha-positive acute promyelocytic leukemia. PET-HEMA group. *Blood*. 1999;94:3015-3021.
- Burnett AK, Grimwade D, Solomon E, Wheatley K, Goldstone AH. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the Randomized MRC Trial. *Blood*. 1999;93:4131-4143.
- Fenaux P, Chastang C, Chevret S, et al. A randomized comparison of all-trans-retinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. The European APL Group. *Blood*. 1999;94:1192-1200.
- Lengfelder E, Reichert A, Schoch C, et al. Double induction strategy including high dose cytarabine in combination with all-trans retinoic acid: effects in patients with newly diagnosed acute promyelocytic leukemia. German AML Cooperative Group. *Leukemia*. 2000;14:1362-1370.
- Tallman MS, Andersen JW, Schiffer CA, et al. All-trans retinoic acid in acute promyelocytic leukemia: long-term outcome and prognostic factor analysis from the North American Intergroup protocol. *Blood*. 2002;100:4298-4302.
- Marty M, Ganem G, Fischer J, et al. [Acute promyelocytic leukemia: retrospective study of 119 patients treated with daunorubicin]. *Nouv Rev Fr Hematol*. 1984;26:371-378.
- Kantarjian HM, Keating MJ, Walters RS, Smith TL, McCredie KB, Freireich EJ. Role of maintenance chemotherapy in acute promyelocytic leukemia. *Cancer*. 1987;59:1258-1263.
- Ohno R, Kobayashi T, Tanimoto M, et al. Randomized study of individualized induction therapy with or without vincristine, and of maintenance-intensification therapy between 4 or 12 courses in adult acute myeloid leukemia. AML-87 Study of the Japan Adult Leukemia Study Group. *Cancer*. 1993;71:3888-3895.
- Miller WH Jr, Kakizuka A, Frankel SR, et al. Reverse transcription polymerase chain reaction for the rearranged retinoic acid receptor alpha clarifies diagnosis and detects minimal residual disease in acute promyelocytic leukemia. *Proc Natl Acad Sci U S A*. 1992;89:2694-2698.
- Fukutani H, Naoe T, Ohno R, et al. Prognostic significance of the RT-PCR assay of PML-RARA transcripts in acute promyelocytic leukemia. The Leukemia Study Group of the Ministry of Health and Welfare (Kouseisho). *Leukemia*. 1995;9:588-593.
- Frankel SR, Eardley A, Lauwers G, Weiss M, Warrell RP Jr. The "retinoic acid syndrome" in acute promyelocytic leukemia. *Ann Intern Med*. 1992;117:292-296.
- De Botton S, Dombret H, Sanz M, et al. Incidence, clinical features, and outcome of all-trans-retinoic acid syndrome in 413 cases of newly diagnosed acute promyelocytic leukemia. The European APL Group. *Blood*. 1998;92:2712-2718.
- Tallman MS, Andersen JW, Schiffer CA, et al. Clinical description of 44 patients with acute promyelocytic leukemia who developed the retinoic acid syndrome. *Blood*. 2000;95:90-95.
- Asou N, Adachi K, Tamura J, et al. Analysis of prognostic factors in newly diagnosed acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. Japan Adult Leukemia Study Group. *J Clin Oncol*. 1998;16:78-85.
- Kobayashi T, Miyawaki S, Tanimoto M, et al. Randomized trials between behenoyl cytarabine and cytarabine in combination induction and consolidation therapy, and with or without ubenimex after maintenance/intensification therapy in adult acute myeloid leukemia. The Japan Leukemia Study Group. *J Clin Oncol*. 1996;14:204-213.
- Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol*. 2003;21:4642-4649.
- Asou N, Adachi K, Tamura U, et al. Analysis of prognostic factors in newly diagnosed patients with acute promyelocytic leukemia: the APL92 study of the Japan Adult Leukemia Study Group (JALSG). *Cancer Chemother Pharmacol*. 2001;48(Suppl 1):S65-S71.
- Miyawaki S, Kobayashi T, Tanimoto M, et al. Comparison of leukopenia between cytarabine and behenoyl cytarabine in JALSG AML-89 consolidation therapy. The Japan Adult Leukemia Study Group. *Int J Hematol*. 1999;70:56-57.
- Head D, Kopecky KJ, Weick J, et al. Effect of aggressive daunomycin therapy on survival in acute promyelocytic leukemia. *Blood*. 1995;86:1717-1728.
- Avvisati G, Petti MC, Lo-Coco F, et al. Induction therapy with idarubicin alone significantly influences event-free survival duration in patients with newly diagnosed hypergranular acute promyelocytic leukemia: final results of the GIMEMA randomized study LAP 0389 with 7 years of minimal follow-up. *Blood*. 2002;100:3141-3146.
- Estey E, Thall PF, Pierce S, Kantarjian H, Keating M. Treatment of newly diagnosed acute promyelocytic leukemia without cytarabine. *J Clin Oncol*. 1997;15:483-490.
- Ades L, Chevret S, Raffoux E, et al. Is cytarabine useful in the treatment of acute promyelocytic leukemia? Results of a randomized trial from the European Acute Promyelocytic Leukemia Group. *J Clin Oncol*. 2006;24:5703-5710.

30. Diverio D, Rossi V, Avvisati G, et al. Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARalpha fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial. *Blood*. 1998;92:784-789.
31. Lo Coco F, Diverio D, Avvisati G, et al. Therapy of molecular relapse in acute promyelocytic leukemia. *Blood*. 1999;94:2225-2229.
32. Avvisati G, Petti MC, Lo Coco F, et al. AIDA: The Italian Way of treating acute promyelocytic leukemia (APL), Final act. ASH Annual Meeting Abstracts. *Blood*. 2003;102:142a.
33. Miyawaki S, Sakamaki H, Ohtake S, et al. A randomized, postremission comparison of four courses of standard-dose consolidation therapy without maintenance therapy versus three courses of standard-dose consolidation with maintenance therapy in adults with acute myeloid leukemia: the Japan Adult Leukemia Study Group AML 97 Study. *Cancer*. 2005;104:2726-2734.
34. Takeshita A, Shinjo K, Naito K, et al. Role of P-glycoprotein in all-trans retinoic acid (ATRA) resistance in acute promyelocytic leukaemia cells: analysis of intracellular concentration of ATRA. *Br J Haematol*. 2000;108:90-92.
35. Latagliata R, Petti MC, Fenu S, et al. Therapy-related myelodysplastic syndrome-acute myelogenous leukemia in patients treated for acute promyelocytic leukemia: an emerging problem. *Blood*. 2002;99:822-824.
36. Sanz MA, Lo Coco F, Martin G, et al. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. *Blood*. 2000;96:1247-1253.
37. Shen ZX, Chen GQ, Ni JH, et al. Use of arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood*. 1997;89:3354-3360.
38. Soignet SL, Maslak P, Wang ZG, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *N Engl J Med*. 1998;339:1341-1348.
39. Asou N. Arsenic trioxide in the treatment of relapsed and refractory acute promyelocytic leukemia. *Intern Med*. 2005;44:775-776.
40. Tobita T, Takeshita A, Kitamura K, et al. Treatment with a new synthetic retinoid, Am80, of acute promyelocytic leukemia relapsed from complete remission induced by all-trans retinoic acid. *Blood*. 1997;90:967-973.
41. Lo-Coco F, Cimino G, Breccia M, et al. Gemtuzumab ozogamicin (Mylotarg) as a single agent for molecularly relapsed acute promyelocytic leukemia. *Blood*. 2004;104:1995-1999.