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Clinical features and outcome of T-lineage acute lymphoblastic leukemia in adults: A low initial white blood cell count, as well as a high count predict decreased survival rates

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Abstract

Although biological and clinical features differ between B-lineage acute lymphoblastic leukemia (ALL) and T-lineage ALL (T-ALL), there have been few reports that focused on the prognosis for T-ALL in adults, primarily due to its rarity. Here, we studied the long-term outcomes and prognostic factors specific for adult T-ALL by combining patient data from the three prospective trials conducted by the Japan Adult Leukemia Study Group (JALSG). Among 559 patients whose immunophenotypes could be evaluated, 87 (15.6%) were identified as T-ALL. Of them, 66 patients (75.8%) achieved complete remission, and relapse occurred in 41 patients. With a median follow-up for surviving patients of 7.5 years, the probability of overall survival was 35.0% at 5 years. Risk factor analysis revealed that serum albumin levels, initial white blood cell (WBC) counts, and age had independent values for predicting survival. For WBC, not only the high-count group ($50 \times 10^9\,l^{-1}$ or higher), but also the low-count group (less than $3 \times 10^9\,l^{-1}$) showed a significantly lower survival rates than the intermediate-count group ($p = 0.0055$ and 0.0037, respectively). Although our findings need confirmation, these results will be helpful in the identification of prognostically distinct subgroups within adult T-ALL.

Keywords: Acute lymphoblastic leukemia; T-ALL; Survival; Prognostic factor; White blood cell count

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1. Introduction

Recent clinical trials have shown that although 70–90% of adult patients with acute lymphoblastic leukemia (ALL) achieve complete remission (CR), the percentage of long-term survivors is not much improved [1–7]. Despite intensive induction and post-remission chemotherapy, a majority of remitters eventually relapse, and the outcome for relapsed patients is almost exclusively grim. Several factors have been reported as affecting the outcome of the disease, including age, initial white blood cell (WBC) count, time to achieve CR, immunophenotype, and cytogenetics [8,9], most of which were, however, identified from the analysis of the entire cohort of each study. Adult ALL represents a heterogeneous disease, and it is well recognized that biological and clinical features differ between B-lineage ALL and T-lineage ALL (T-ALL) [10–12]. Because T-ALL accounts for only around 20% of adult ALL, commonly used prognostic factors for ALL may not be necessarily applicable to T-ALL. Owing to its rarity, there have been few reports that have studied a large group of adult T-ALL patients. Under these circumstances, we analyzed the data of 87 T-ALL patients that had been entered into previous ALL trials conducted by the Japan Adult Leukemia Study Group (JALSG), investigated clinical features and long-term outcomes, and identified the prognostic factors specific for T-ALL in adults.

2. Patients and methods

2.1. Patients

All patients were subjects of one of the three prospective trials conducted by the JALSG; the ALL90 (1990–1993) [13], ALL93 (1993–1997) [6], and ALL97 studies (1997–2001). For all the trials, newly diagnosed, previously untreated ALL patients were eligible if they were 15 years or older, and showed adequate heart, lung, liver, and renal function. Informed consent was obtained from all participants before their enrollment. Diagnosis of ALL was carried out according to the French–American–British (FAB) classification [14], and confirmed by the Central Review Committee. Patients who were immunophenotyped and met the definition for T-ALL on the basis of the criteria described below were considered for the subsequent analysis.

2.2. Treatment

Details of each treatment schedule are described in Table 1. For the ALL90 study, induction therapy consisted of six drugs; doxorubicin (ADR), vincristine (VCR), cyclophosphamide (CPM), L-asparaginase (L-ASP), prednisolone (PSL), and mitoxantrone (MIT). Patients with CR received four courses of consolidation and maintenance/intensification therapy. For the ALL93 study, instead of omitting MIT for induction, the dose-intensity of ADR was increased by more frequent administration on days 1–3, and 8–10. After completion of three courses of consolidation, patients were randomized to receive early sequential or intermittent intensification during maintenance therapy. For the ALL97 study, induction therapy comprised the five drugs similar to the ALL93 study. After achieving CR, patients received eight courses of consolidation featuring dose-intensified ADR and CPM, and intermediate-dose methotrexate (MTX), followed by maintenance therapy. Central nervous system (CNS) prophylaxis was given by means of intrathecal (IT) injection of MTX, cytarabine (Ara-C) and steroids during both consolidation and intensification courses. Patients with symptomatic or cytological evidence of CNS leukemia received additional IT injections. Prophylactic whole cranial irradiation was given at a total dose of 20–24 Gy to patients either with cytologically diagnosed CNS leukemia or with high initial WBC counts ($100 \times 10^9\,l^{-1}$ or higher for the ALL90/ALL93 studies and $50 \times 10^9\,l^{-1}$ or higher for the ALL97 study).

2.3. Definition

CR was defined as the presence of all of the following: less than 5% of blasts in bone marrow (BM), no leukemic blasts in peripheral blood (PB), recovery of PB values to a neutrophil count of at least $1.5 \times 10^9\,l^{-1}$ and a platelet count of at least $100 \times 10^9\,l^{-1}$, and no evidence of extramedullary leukemia. Patients who failed to attain CR after two courses of induction therapy were regarded as failure cases. Relapse was defined as the presence of at least one of the following; recurrence of more than 10% leukemic cells in BM or of any leukemic cells in PB or extramedullary sites. Performance status was assessed on the basis of criteria from the Eastern Cooperative Oncology Group (ECOG). Surface markers were considered positive when more than 20% of blasts expressed the antigens. The immunophenotype was classified according to criteria from the Cancer and Leukemia Group B (CALGB) [11]. T-lineage ALL was defined as the presence of either (1) CD2 or CD7 positivity combined with positivity of CD1, CD3, CD4, CD5, CD8; or (2) CD5 positivity without CD19 or CD20 positivity. Myeloid antigen positivity was defined as positive expression of either or both of CD13 and CD33.

2.4. Statistical analysis

Kaplan–Meier analysis was used to estimate the probabilities of overall survival (OS) and event-free survival (EFS). OS was defined as the time from the first day of therapy to death or last visit, and EFS as the time from the first day of therapy to induction failure, relapse, death, or last visit. For EFS, patients who failed to achieve CR were categorized as failure cases at time zero. Patients undergoing hematopoietic stem cell transplantation (HSCT) were not censored at the time of transplantation unless indicated. Differences

Table 1
Treatment schedules

ALL90 study	ALL93 study	ALL97 study	
Remission induction			
VCR 1.4 mg m^{-2} IV × 3–4	VCR 1.3 mg m^{-2} IV × 4	VCR 1.3 mg m^{-2} IV × 4	
ADR 25 mg m^{-2} IV × 1–3	ADR 30 mg m^{-2} IV × 6	DNR 45 mg m^{-2} IV × 3	
CPM 600 mg m^{-2} IV × 1–2	CPM 600 mg m^{-2} IV × 1	CPM 1200 mg m^{-2} IV × 1	
PSL 40–60 mg m^{-2} PO × 14	PSL 40 mg m^{-2} PO × 10	PSL 60 mg m^{-2} PO × 14	
L-ASP 5000 IU m^{-2} IV or SC × 0–2	L-ASP 6000 IU m^{-2} IV × 7	L-ASP 3000 IU m^{-2} IV × 6	
MIT 6 mg m^{-2} IV × 1–3			
Consolidation			
ETP 100 mg m^{-2} IV × 5	MIT 6 mg m^{-2} IV × 3	C-1	C-5
BHAC 200 mg m^{-2} IV × 5	ETP 100 mg m^{-2} IV × 5	VCR 1.3 mg m^{-2} IV × 1	Same as C-1 except for substituting
VDS 2 mg m^{-2} IV × 1	Ara-C 100 mg m^{-2} IV × 6	ADR 60 mg m^{-2} IV × 1	DEX 10 mg m^{-2} PO × 3 for PSL
PSL 40 mg m^{-2} PO × 5	IT × 1	CPM 1000 mg m^{-2} IV × 1	
IT × 1		PSL 60 mg m^{-2} PO × 3	
	MTX 600 mg m^{-2} IV × 2	IT × 1	C-6
MIT 7 mg m^{-2} IV × 3	L-ASP 10000 IU m^{-2} IV × 2		Same as C-2 except for substituting
BHAC 200 mg m^{-2} IV × 5	IT × 1	C-2	DEX 10 mg m^{-2} PO × 3 for PSL
IT × 1		MTX 500 mg m^{-2} IV × 1	
	ACR 14 mg m^{-2} IV × 8	VCR 1.3 mg m^{-2} IV × 1	
CPM 800 mg m^{-2} IV × 1	Ara-C 70 mg m^{-2} IV × 7	ADR 45 mg m^{-2} IV × 1	C-7
ACR 50 mg m^{-2} IV × 2	PSL 40 mg m^{-2} PO × 7	PSL 60 mg m^{-2} PO × 3	Same as C-5
VDS 2 mg m^{-2} IV × 1	IT × 1	IT × 1	
PSL 40 mg m^{-2} PO × 5			C-8
IT × 1		C-3	Same as C-4 except for substituting
		Same as C-1	MIT 8 mg m^{-2} IV × 2 for ETP
MTX 400 mg m^{-2} IV × 1			
L-ASP 6000 IU m^{-2} IM or SC × 2		C-4	
		ETP 100 mg m^{-2} IV × 4	
		Ara-C 200 mg m^{-2} IV × 4	
		6MP 60 mg m^{-2} IV × 4	
		PSL 60 mg m^{-2} PO × 4	
		IT × 1	
Intensification			
DNR 30 mg m^{-2} IV × 3	ADR 30 mg m^{-2} IV × 6		
VDS 2 mg m^{-2} IV × 2	VCR 1.3 mg m^{-2} IV × 3		
CPM 700 mg m^{-2} IV × 2	PSL 30 mg m^{-2} PO × 10		
PSL 40–60 mg m^{-2} PO × 14			
IT × 1	MIT 6 mg m^{-2} IV × 3		
	ETP 100 mg m^{-2} IV × 5		
MIT 6 mg m^{-2} IV × 3	Ara-C 100 mg m^{-2} IV × 6		
VDS 2 mg m^{-2} IV × 2	IT × 1		
CPM 700 mg m^{-2} IV × 2			
PSL 40–60 mg m^{-2} PO × 14	MTX 600 mg m^{-2} IV × 2		
IT × 1	L-ASP 10000 IU m^{-2} IV × 2		
	IT × 1		
ADR 20 mg m^{-2} IV × 3			
VDS 2 mg m^{-2} IV × 2	ACR 14 mg m^{-2} IV × 8		
CPM 700 mg m^{-2} IV × 2	Ara-C 70 mg m^{-2} IV × 7		
PSL 40–60 mg m^{-2} PO × 14	PSL 40 mg m^{-2} PO × 7		
IT × 1	IT × 1		
Maintenance			
6MP 60 mg m^{-2} PO daily	6MP 60 mg m^{-2} PO daily	VCR 1.3 mg m^{-2} IV monthly	
MTX 20 mg m^{-2} PO weekly	MTX 20 mg m^{-2} PO weekly	PSL 60 mg m^{-2} PO × 5 monthly	
		6MP 60 mg m^{-2} PO daily	
		MTX 20 mg m^{-2} PO weekly	

Maximum dose of VCR was 2.0 mg/body. For remission induction in the ALL90 study, number of doses for each drug was determined according to the findings of serial bone marrow aspirations. Drugs used for IT injection were MTX 15 mg/body, Ara-C 40 mg/body and PSL 10 mg/body in the ALL90/ALL93 studies, and MTX 15 mg/body and DEX 4 mg/body with or without Ara-C 40 mg/body in the ALL97 study. ALL, acute lymphoblastic leukemia; VCR, vincristine; ADR, doxorubicin; CPM, cyclophosphamide; PSL, prednisolone; L-ASP, L-asparaginase; MIT, mitoxantrone; DNR, daunorubicin; ETP, etoposide; BHAC, behenoyl-ara-C; VDS, vindesine; DEX, dexamethasone; Ara-C, cytarabine; ACR, aclarubicin; MTX, methotrexate; 6MP, 6-mercaptoprine; IV, intravenous; PO, oral; SC, subcutaneous; IM, intramuscular; IT, intrathecal.

between the curves were compared using a log-rank test. For risk factor analysis, a multivariate Cox proportional hazards model was constructed for OS, and a logistic regression model for CR achievement. Variables with p-values of less than 0.10 by log-rank test for OS, and in univariate logistic analysis for CR achievement were included in the respective final multivariate model. A hazard ratio (HR) and an odds ratio (OR) were calculated in conjunction with a 95% confidence interval (CI). Stata Version 8 software (StataCorp, College Station, TX, USA) was used for all statistical analyses.

3. Results

3.1. Patients

Among 559 patients whose immunophenotypes were evaluable, 87 (15.6%) were identified as T-ALL. Baseline characteristics of the 87 patients are summarized in Table 2. The median age was 26 years (range, 15–60 years), with 60 males and 27 females. Involvements in CNS, skin, and mediastinum were detected in 7.0, 4.6 and 17.2%, respectively. Of the 60 patients for which cytogenetic information was available, 34 showed abnormal karyotype, including del(5q) in 4, del(6q) in 3, del(9p) in 3, del(11q) in 3, t(11;14) in 2, t(1;12) in 2, trisomy 8 in 2, and del(12p) in 2 patients.

3.2. Response to induction therapy

In total, 66 patients (75.8%) achieved CR after one course of remission induction therapy ($n=59$), or two courses ($n=7$). Of the remaining 21 patients, toxicity-related death during induction therapy occurred in four (4.6%). Their causes of death were sepsis ($n=2$), intracranial hemorrhage ($n=1$), and liver failure ($n=1$). Multivariate analysis indicated two factors were significantly associated with CR achievement. Patients aged 30 or older had a greater risk of induction failure (OR: 5.13, 95% CI: 1.52–17.5, $p=0.009$), as did those whose serum albumin level was lower than 3.5 g dl^{-1} (OR: 6.71, 95% CI: 1.64–27.4, $p=0.008$). Other variables such as initial WBC count, sex, performance status, and any surface markers did not correlate with CR achievement.

Table 2
Presenting characteristics

	$n=87$
Age (years)	26 (15–60)
Sex: male/female	60/27
FAB type: L1/L2	27/60
WBC count ($\times 10^9$ l^{-1}l)	17.1 (0.3–396)
RBC count ($\times 10^{12}$ l^{-1})	3.20 (1.49–6.33)
Platelet count ($\times 10^9$ l^{-1})	57 (4–341)
Performance status: 0–1/2–3	66/17
CNS involvement: present/absent	6/80
Skin involvement: present/absent	4/83
Mediastinal involvement: present/absent	15/72
Karyotype: normal/abnormal/NE	26/34/27

Values are presented as median (range) unless indicated. FAB, French–American–British; WBC, white blood cell; RBC, red blood cell; CNS, central nervous system; NE, not evaluable (not carried out or failed).

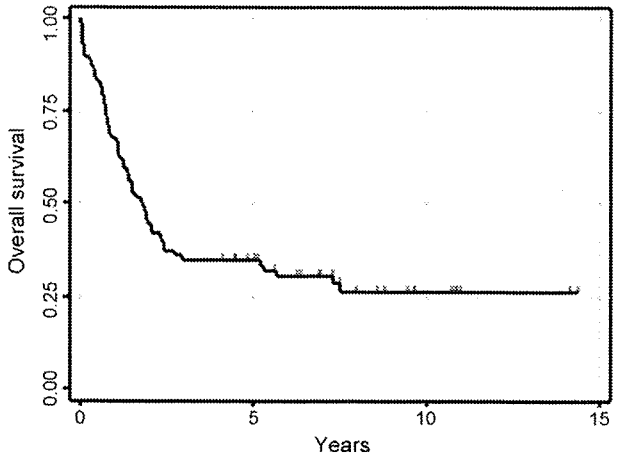

Fig. 1. Kaplan–Meier curve for overall survival. The probability of overall survival was 35.0% at 5 years for the whole population ($n=87$).

3.3. Survival

At the end of observation, 26 patients were alive with a median follow-up of 7.5 years (range, 0.8–14.3 years). Among 66 remitters, relapse occurred in 41 patients. A total of 33 patients underwent allogeneic HSCT, and three underwent autologous HSCT. Disease status at the time of transplantation was first CR for 17, second CR for 7, third CR for 1, non-CR for 9, and unknown for 2.

Fig. 1 shows the survivals for all patients. The probability of OS at 5 years was 35.0 ± 5.1% for the whole population. As there was no survival difference among the three studies ($p=0.475$), nor between the two studies (data not shown), all patients were grouped for the risk factor analysis. OS according to patient characteristics is presented in Table 3. Univariate analysis showed that their initial WBC counts and serum albumin levels strongly affected survival. As presented in Fig. 2, patients with a WBC count of 50×10^9 l^{-1} or higher had lower survival (19.2 ± 7.7% at 5 years). Unexpectedly, the outcome for those with a WBC count lower than 3×10^9 l^{-1} was also worse (20.0 ± 10.0% at 5 years) than those with an intermediate count (48.0 ± 7.5% at 5 years). Induction failure or disease recurrence occurred in 14 of 15 patients in the low-count group, in 26 of 45 in the intermediate-count group, and in 22 of 27 in the high-count group. These observations resulted in inferior EFS rates for those in the low- and high-count groups to those in the intermediate-count group, too (Fig. 3). Even when patients undergoing HSCT were analyzed as censored cases at the time of transplantation, differences in terms of both OS and

Table 3
Overall survival at 5 years according to patient characteristics

Characteristics	Number of patients	Overall survival (%)	p-Value
All cases	87	35.0 ± 5.1	
Treatment protocol			0.475
ALL90	21	33.3 ± 10.3	
ALL93	26	44.7 ± 9.9	
ALL97	40	30.0 ± 7.2	
Age			0.054
Younger than 30	50	42.0 ± 7.0	
30 or older	37	25.4 ± 7.3	
Sex			0.700
Male	60	31.7 ± 6.0	
Female	27	42.9 ± 9.7	
WBC count			0.003
Lower than $3 \times 10^9\ l^{-1}$	15	20.0 ± 10.0	
3×10^9–$50 \times 10^9\ l^{-1}$	45	48.0 ± 7.5	
$50 \times 10^9\ l^{-1}$ or higher	27	19.2 ± 7.7	
Serum albumin			<0.001
Lower than 3.5 g dl^{-1}	14	7.1 ± 6.9	
3.5 g dl^{-1} or higher	68	39.0 ± 6.0	
Performance status			0.577
0–1	66	37.1 ± 6.0	
2–3	17	29.4 ± 11.1	
CNS involvement			<0.001
Present	6	0.0 ± 0.0	
Absent	80	38.1 ± 5.5	
Skin involvement			<0.001
Present	4	0.0 ± 0.0	
Absent	83	36.7 ± 5.3	
Mediastinal involvement			0.077
Present	15	53.3 ± 12.9	
Absent	72	31.2 ± 5.5	
No. of induction course[a]			0.972
1 Course	59	44.9 ± 6.5	
2 Courses	7	42.9 ± 18.7	

ALL, acute lymphoblastic leukemia; WBC, white blood cell; CNS, central nervous system. Values are presented with standard errors.
[a] Only patients who achieved complete remission are considered.

EFS remained statistically significant (data not shown). Of the 14 patients with serum albumin lower than 3.5 g dl^{-1}, seven failed to obtain CR, and all of the remaining patients with CR had a relapse. The probability of survival for these patients was only 7.1 ± 6.9% at 5 years. An age of 30 or older, and the presence of mediastinal involvement were also associated with a trend in favor of survival. Although the number was small, patients who presented CNS or skin involvement had an extremely poor prognosis, and no long-term survivors existed. We failed to detect a significant effect of sex, performance status, or number of induction courses on survival. Neither did surface markers including CD2, CD3, CD34, or myeloid antigens have any prognostic significance. Based on these results, serum albumin levels, initial WBC counts, age, and mediastinal involvement were subjected to a multivariate analysis. The results are shown in Table 4. Lower albumin levels, too low or too high WBC counts, and older age were identified as independently associated with lower survival.

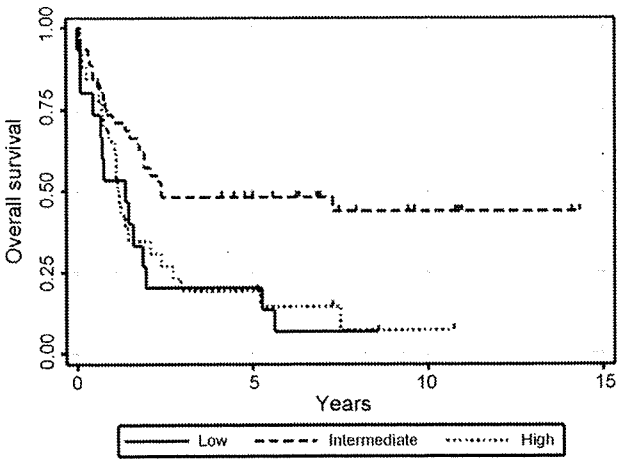

Fig. 2. Kaplan–Meier curves for overall survival according to the initial white blood cell count. The low-count group (less than $3 \times 10^9\ l^{-1}$, $n = 15$) as well as the high-count group ($50 \times 10^9\ l^{-1}$ or higher, $n = 27$) showed significantly worse overall survival than the intermediate-count group ($3–50 \times 10^9\ l^{-1}$, $n = 45$: $p = 0.0037$ and 0.0055, respectively).

3.4. Outcome after relapse

A total of 41 remitters had a disease recurrence after a median CR duration of 8.6 months (range, 0.6–79.4 months). The sites of relapse were BM in 28, CNS in 9, concurrent BM and CNS in 2, intraocular area in one, and mamma in one. Among 11 cases whose disease recurred in CNS, none had CNS involvement at presentation. The probabilities of OS for the whole recurred patients were 26.7 ± 7.1% at 1 year, and 11.9 ± 5.4% at 5 years after relapse. The survival curves are shown in Fig. 4. No patients could survive long-term unless they underwent HSCT after relapse.

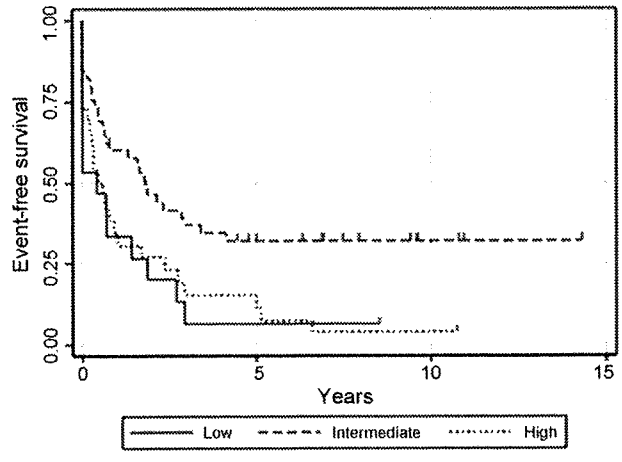

Fig. 3. Kaplan–Meier curves for event-free survival according to the initial white blood cell count. The low-count group (less than $3 \times 10^9\ l^{-1}$, $n = 15$) as well as the high-count group ($50 \times 10^9\ l^{-1}$ or higher, $n = 27$) showed significantly worse event-free survival than the intermediate-count group ($3–50 \times 10^9\ l^{-1}$, $n = 45$: $p = 0.0146$ and 0.0221, respectively).

Table 4
Factors associated with overall survival

Univariate analysis p-value	Multivariate analysis		
	p-Value	HR (95% CI)	Factor
Serum albumin			
<0.001	0.013	2.25 (1.18–4.28)	Lower than 3.5 g dl^{-1}
		1.00	3.5 g dl^{-1} or higher
WBC count			
0.004	0.018	2.34 (1.16–4.73)	50×10^9 l^{-1} or higher
0.005	0.036	1.91 (1.04–3.53)	Lower than 3×10^9 l^{-1}
		1.00	3×10^9–50×10^9 l^{-1}
Age			
0.057	0.049	1.70 (1.00–2.89)	30 or older
		1.00	Younger than 30
Mediastinal involvement			
0.084	0.212	1.68 (0.74–3.80)	Absent
		1.00	Present

An HR higher than unity indicates worse survival for patients with the factor. HR, hazard ratio; 95% CI, 95% confidence interval; WBC, white blood cell.

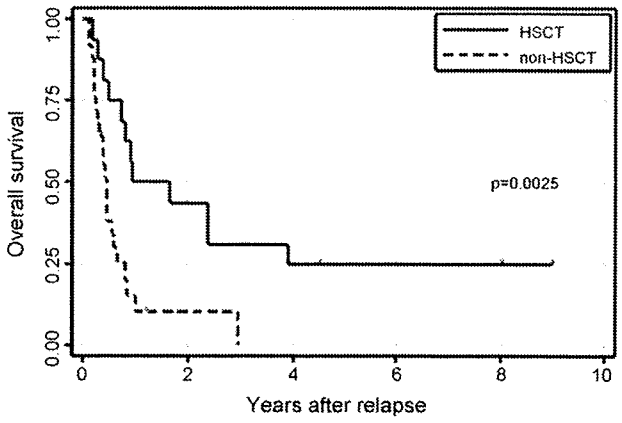

Fig. 4. Kaplan–Meier curves for survival after relapse The 5-year probability of overall survival after relapse was 25.0% for patients who underwent transplantation thereafter ($n = 16$), and 0% for those who did not ($n = 25$).

4. Discussion

A recent meta-analysis [15] showed that patients with high-risk ALL benefit from allogeneic HSCT during first CR, whereas its efficacy is unclear for those with standard-risk ALL, suggesting the importance of prognostic prediction at diagnosis or soon thereafter. Risk stratification for ALL has been traditionally studied without distinguishing T-ALL from B-lineage ALL; however, because they are two distinct clinical entities, determining prognostic factors separately should mean more accuracy. But the relatively small number of patients, especially those with T-ALL, available for a single trial has made such an analytical examination difficult. In this study, through combining data for patients from the three prospective JALSG trials, we studied long-term outcomes and prognostic factors specific for T-ALL.

Given that highly intensive regimens in recent reports have increased survival rates for adult T-ALL up to 40–60% [8–11], the 5-year survival rate of 35% for our patients was somewhat low. As it is suggested that CPM and Ara-C may play an important role in the treatment of T-ALL [8], our results would leave room for improvement through the use of treatment that features intensified administration of such agents. Risk factor analysis identified serum albumin levels, initial WBC counts, and age as having independent values for predicting survival. As reported previously, an initial WBC count had significant influence on survival, but the striking finding was that not only the high-count group (50×10^9 l^{-1} or higher) but also the low-count group (less than 3×10^9 l^{-1}) showed a significantly worse survival rate than the intermediate-count group ($p = 0.0055$ and 0.0037, respectively). This observation is in accordance with the report from the Pediatric Oncology Group (POG) [16]. They described that the subgroup of patients with T-ALL who had an initial WBC count of less than 10×10^9 l^{-1} at diagnosis fared worse than those with a WBC count between 10×10^9 and 50×10^9 l^{-1}. It is an accepted concept that a high WBC count has less influence on the prognosis of T-ALL than of B-lineage ALL [8]. Many investigators have pursued the upper cut-off points to discriminate outcomes, with these cut-off points recently set at 100×10^9 l^{-1} in adults [7,8], which is much higher than for B-lineage ALL. From the results of both POG and our studies, it can be assumed that a poor prognosis for T-ALL patients with a low WBC count may partly offset the prognostic significance of the high WBC count. Possible reasons why a low WBC count affected survival adversely could not be identified from a careful examination of our patient data. A French group showed that the T-cell receptor (TCR) status could stratify T-ALL into four groups, and patients in the immature subset presented a lower WBC count, and had inferior survival mainly due to a lower CR rate [17]. However, for our patients, a low WBC count did not exert any significant effect on CR achievement, but was associated with shorter survival due to a higher rate of relapse. Immunophenotypic maturation stages have also been indicated in correlating with the outcome for T-ALL [16,18–21], although

interactions between such maturation stages and initial WBC counts have not been established. Lack of information for CD1a expression in our dataset enabled us to classify our patients according to the criteria by the European Group for the Immunological Characterization of Leukemias (EGIL) [22]; however, the observations that expression of surface markers including CD3 and CD34 revealed no prognostic relevance seem to show that worse outcomes for patients with low WBC counts cannot be explained by maturation stages. More recently, risk assessment for T-ALL has been investigated based on the genetic characteristics of leukemic cells, including the expression of specific genes such as HOX11 [23,24] and HOX11L2 [25], gene expression profiles using microarray technology [26], and DNA methylation profiles [27]. Also, prognostic significance of minimal residual disease (MRD) during or after treatment has been vigorously studied, and several groups showed clinical utility of MRD quantification by flow cytometry [28,29] or polymerase chain reaction [30–32]. Although such research should be continued, risk assessment according to information commonly available at all hospitals remains important in clinical practice. It should be noted that our study has several limitations, and the results must be interpreted with caution. The limitations include the retrospective nature of the study, and the relatively small number of patients, especially of those in the low WBC count group ($n = 15$). Validations for a larger number of patients will be needed.

In summary, from the analysis of a relatively large cohort of 87 adult patients with T-ALL, serum albumin levels, initial WBC counts, and age were identified as prognostic factors for survival. For WBC counts, not only patients with a high count, but also those with a low count had significantly worse outcomes than patients with an intermediate count. Although our findings need confirmation, these results will be helpful in the identification of prognostically distinct subgroups within adult T-ALL.

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Brief report

Concurrent transcriptional deregulation of AML1/RUNX1 and GATA factors by the *AML1-TRPS1* chimeric gene in t(8;21)(q24;q22) acute myeloid leukemia

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The Runt domain transcription factor AML1/RUNX1 is essential for the generation of hematopoietic stem cells and is the most frequent target of chromosomal translocations associated with leukemia. Here, we present a new *AML1* translocation found in a patient with acute myeloid leukemia M4 with t(8;21)(q24;q22) at the time of relapse. This translocation generated an in-frame chimeric gene consisting of the N-terminal portion of *AML1*, retaining the Runt domain, fused to the entire length of *TRPS1* on the C-terminus. *TRPS1* encodes a putative multitype zinc finger (ZF) protein containing 9 C2H2 type ZFs and 1 GATA type ZF. AML1-TRPS1 stimulated proliferation of hematopoietic colony-forming cells and repressed the transcriptional activity of AML1 and GATA-1 by 2 different mechanisms: competition at their cognate DNA-binding sites and physical sequestrations of AML1 and GATA-1, suggesting that simultaneous deregulation of AML1 and GATA factors constitutes a basis for leukemogenesis. (Blood. 2007;109:4023-4027)

Introduction

AML1/RUNX1 encodes the DNA-binding α subunit of the heterodimeric transcription factor PEBP2/CBF, which interacts with the partner β subunit (PEBP2β/CBFβ) through its evolutionarily conserved Runt domain.[1] *AML1* is one of the most frequently mutated genes in human leukemia,[2-4] and was originally identified as a gene on chromosome 21 involved in t(8;21)(q22:q22).[5] To date, 11 *AML1*-related translocations are known that produce chimeric proteins such as AML1-MTG8/ETO in t(8;21), AML1-EVI1 in t(3;21), and TEL-AML1 in t(12;21).[2,6,7] All these AML1 chimeric proteins retain the Runt domain and inhibit transcriptional activity of wild-type AML1 in a dominant-negative manner.[2-4] However, the functional contributions of partner moieties in leukemogenesis remain largely undetermined. Here, we report a new *AML1* translocation, t(8;21)(q24;q22), in a patient with acute myeloid leukemia (AML), and present results from functional analyses of the chimeric protein AML1-TRPS1.

Patient, materials, and methods

Patient profile

A 56-year-old Japanese man was diagnosed with AML M4 with a normal karyotype in October 1997. He was treated with idarubicin and cytarabine, followed by postremission therapy according to the Japan Adult Leukemia Study Group (JALSG) AML97.[8] In July 1999, his marrow showed 55.6% blasts with t(8;21)(q24;q22) at relapse. Bone marrow cells at diagnosis and relapse showed the similar morphology and immunophenotypes positive for CD13, CD33, CD4, and HLA-DR, but negative for CD34, which were consistent with AML M4.[9] The study was approved by the Institutional Review Board of Kumamoto University School of Medicine, Japan, and informed consent was obtained from the patient, according to the Declaration of Helsinki.

Molecular cloning

The fusion partner gene was cloned by long-distance 3′ rapid amplification of cDNA ends (RACE) using the SMART RACE kit (Clontech Labs, Mountain View, CA).

Plasmid constructions

The cloned fusion gene *AML1-TRPS1* was inserted into the pEF-Bos expression plasmid[10] or MIG retroviral vector.[11] Mutant constructs were generated by polymerase chain reaction (PCR)–based site-directed mutagenesis.

EMSA

Electrophoretic mobility shift assays (EMSAs) were performed using biotin-labeled probes containing the AML1[12] or GATA factor-binding sites.[13] The specificity of the probes are shown in Figure S1, available on the *Blood* website (see the Supplemental Figure link at the top of the online article). Whole-cell extracts of COS7 cells (1×10^6) transfected with pEF-Bos expression vectors were subjected to the assay.

Transcription assay

The luciferase reporter constructs pBXH2-LTR-luc and pRBGP3-MαP were used to assay the transcriptional activities mediated by AML1 and GATA-1, respectively. pBXH2-LTR-luc contains the long terminal repeat (LTR) of the BXH2 retrovirus[11] that includes a functional AML1 site, while

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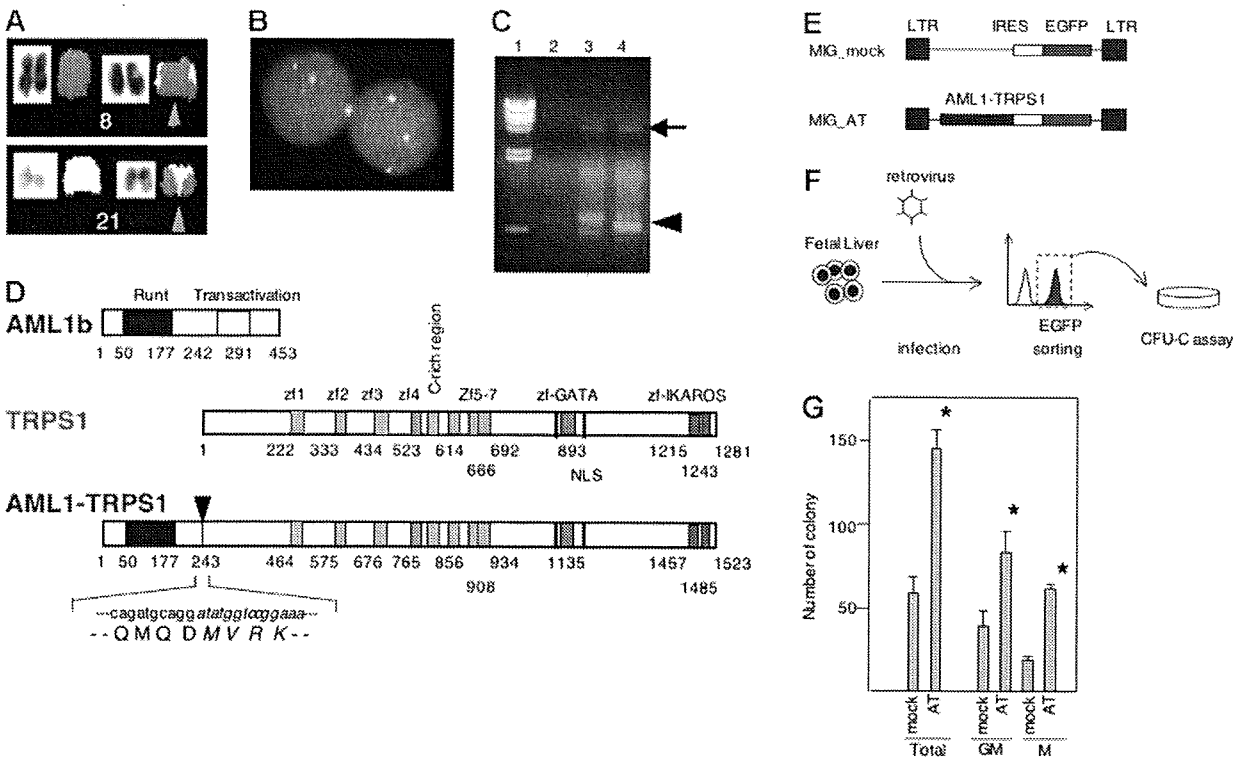

Figure 1. Cloning of the *AML1-TRPS1* that stimulates proliferation. (A) Spectral karyotyping (SKY) showed the balance translocation between chromosomes 8 and 21 (arrowheads). Representative SKY of chromosomes 8 and 21 is indicated as reverse DAPI (left) and SKY (right). (B) Fluorescence in situ hybridization using cosmid clones covering the whole *AML1* gene showed that t(8;21)(q24;q22) involved the *AML1* gene. Nearly half (55 [48.2%] of 114) of the cells had 3 signals of the *AML1* gene. Images were acquired within an SD200 spectral imaging system (Applied Spectral Imaging, Migdal Haemek, Israel) attached to an Optiphot-2 epifluorescence microscope (Nikon, Tokyo, Japan) through a Splan Apo 100×/1.4 NA oil objective lens (Olympus, Tokyo, Japan) and analyzed with SkyView software (Applied Spectral Imaging). (C) The fusion partner gene with AML1 was cloned by long-distance 3′ RACE with 5′ primers on the *AML1* exon 5 (AML1S5: 5′-cacagtggatgggccccgagaacctcg-3′) or exon 6 (AML1S6: 5′-tgcggcgcacagccatgagggtcagc-3′). The arrowhead indicates AML1a; the arrow shows the fusion gene. Lane 1, λ/*Hin*dIII DNA marker; lane 2, Human placental DNA; lane 3, AML cells (AML1S5); lane 4, AML cells (AML1S6). (D) N-terminal portion of *AML1* retaining the Runt domain fused to the whole of *TRPS1*. An in-frame fusion gene consists of exon 6 of *AML1* and *TRPS1*. In addition, sequencing analyses of subclones showed another transcript between exon 5 of *AML1* and *TRPS1*, indicating skipping of exon 6 of *AML1* and generating another in-frame fusion gene. The reciprocal TRPS1-AML1 transcript was not detected in the presenting case. (E) Structures of retrovirus constructs for control (MIG_mock) or AML1-TRPS1 (MIG_AT). AML1-TRPS1 was inserted into the indicated position of the plasmid MIG with internal ribosomal entry site (IRES) and the enhanced green fluorescent protein (EGFP) gene. (F) Schematic depiction of the CFU-C assay. Fetal liver cells from embryonic day–14.5 (E14.5) mouse embryo were infected with the MIG vector. EGFP-positive cells were sorted and subjected to the CFU-C assay, supplemented with interleukin-3, stem cell factor, erythropoietin, and granulocyte colony-stimulating factor, as described previously.[11] (G) The number of all kinds of colony (total), granulocyte-macrophage (GM), and macrophage (M) are shown. Error bars indicate standard deviations for 3 independent experiments. Differences between AML1-TRPS1 (AT) and control (mock) transfectants were statistically significant (*$P < .001$, unpaired student *t* test) in all 3 categories.

the mouse α-1 globin gene promoter in pRBGP3-MαP[14] contains a functional GATA site. The specificity of AML1 and GATA site-dependent activation of each reporter is shown in Figure S2.

Retroviral transduction and CFU-C assay

Retroviral transduction, sorting by FACS Vantage (Becton Dickinson, San Jose, CA), and colony-forming unit-culture (CFU-C) assay were performed as previously described.[11]

Immunoprecipitation

Immunoprecipitation of FLAG-tagged fusion proteins was performed according to the manufacturer's protocol (Sigma, St Louis, MO).

Results and discussion

Spectral karyotyping of the marrow cells at relapse confirmed a balanced translocation between chromosomes 8 and 21 (Figure 1A). Fluorescence in situ hybridization using cosmid clones containing the *AML1* gene showed that this late-appearing translocation involved the *AML1* gene (Figure 1B).[15] We cloned the fusion partner gene with *AML1* exon 6 by using 3′ RACE (Figure 1C). The t(8;21)(q24;q22) generated an in-frame chimeric gene that encoded a fusion protein consisting of the N-terminal portion of AML1, retaining the Runt domain but lacking its C-terminal transactivation domain, fused to the entire TRPS1/GC79 (Figure 1D). *TRPS1* on chromosome 8q24.1 encodes a nuclear transcription factor with 10 zinc finger (ZF) domains, including a single GATA-type ZF.[13,16,17] The gene is widely expressed in human tissues, including prostate, testis, ovary, kidney, lung, mammary gland, and hematopoietic cells.[16,17] *TRPS1* has been identified as a disease gene for tricho-rhino-phalangeal syndrome (TRPS) type I and type III (MIM190350 and MIM190351), which is a dominantly inherited disease characterized by craniofacial and skeletal abnormalities.[16]

To investigate whether the fusion gene *AML1-TRPS1* affects proliferation and differentiation of hematopoietic cells, we introduced AML1-TRPS1 into mouse fetal liver cells and carried out a CFU-C assay (Figure 1E-F). As shown in Figure 1G, *AML1-TRPS1* transfectants gave rise to a higher number of colonies than did the control transfectants, indicating that AML1-TRPS1 stimulated the proliferation of immature hematopoietic cells.

Since AML1-TRPS1 contains the Runt and GATA ZF domains, we evaluated the DNA-binding ability of AML1-TRPS1 to both

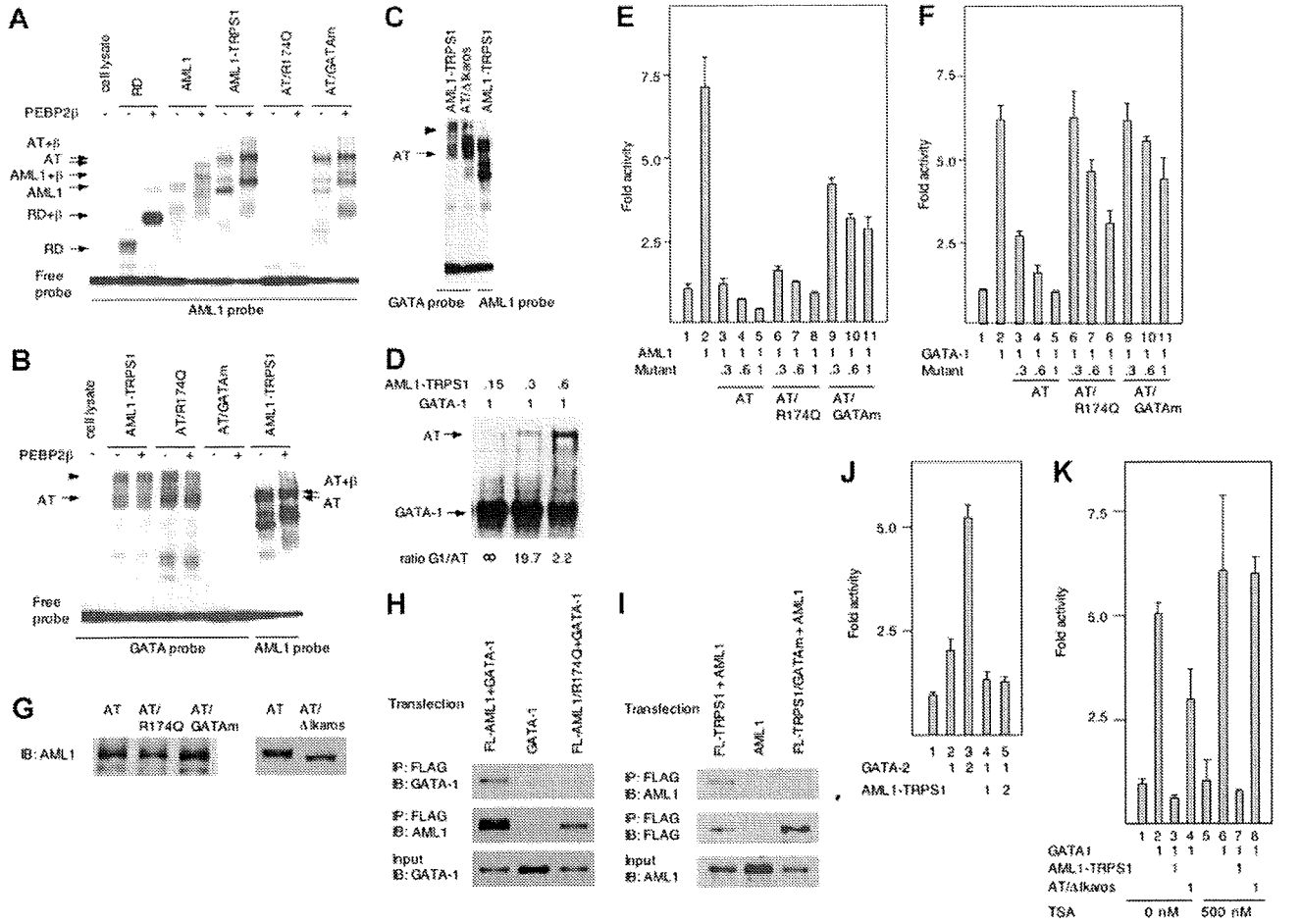

Figure 2. Functional analysis of AML1-TRPS1. (A-B) AML1-TRPS1 and their mutants were subjected to EMSA in the presence (+) or absence (−) of PEBP2β/CBFβ. RD and AT indicate the Runt domain and AML1-TRPS1, respectively. RD and AML1 served as positive controls. The position of the indicated factor or complex with DNA is shown. Mutant constructs of AML1-TRPS1 were generated by PCR-based mutagenesis, using the following primers: AT/R174Q, 5′-gtggatgggccccaagaacctcgaagaca-3′; and AT/GATAm, 5′-ggatatgtaggcaacgcgggtggcctctacc -3′. (C) AML1-TRPS1 seems to form a homodimer through the Ikaros-like ZF domain. The deletion of Ikaros-like ZF (AT/ΔIKAROS) resulted in a decrease in the upper band (arrowhead), which might indicate the homodimer of AML1-TRPS1, and an increase in the lower band (AT, AML1-TRPS1 monomer). The AT/ΔIKAROS mutant was made by PCR-based mutagenesis using 5′-gaagtactcaagatgaactttcaacataatgtgrgcactgtggc-3′ as a primer. (D) A fixed amount of GATA-1 (1 U) and increasing relative amounts (0.15, 0.3 and 0.6 U) of AML1-TRPS1 were subjected to EMSA. Relative amounts of expressed proteins were evaluated by Western blotting. (E-F) Wild-type AML1, GATA-1, AML1-TRPS1, or its mutants were cotransfected with pBXH2-LTR-luc or pRBGP3-MαP reporter at varying relative doses, as indicated, into NIH3T3 cells by the nonliposomal transfection reagent FuGENE6 (Roche Applied Science, Basel, Switzerland). Luciferase activities are expressed as fold changes relative to the control transfected with the backbone expression vector alone. The total input of plasmid DNAs was kept constant (0.6 μg) by supplementing appropriate amounts of the backbone pEF-Bos plasmid so as to avoid potential artifacts due to uneven overall DNA dosages. Error bars indicate standard deviations for 3 independent experiments. (G) Expression levels of AML1-TRPS1 and its mutants were comparable with each other. COS7 cells were transfected with expression plasmids for the indicated genes, and whole-cell extracts were prepared 48 hours after transfection and subjected to Western blotting using rabbit polyclonal anti-AML1 antibody (Active Motif, Carlsbad, CA). (H-I) The Runt domain of AML1 physically interacted with GATA-1 (H), while GATA ZF of TRPS1 interacted with AML1 (I) in immunoprecipitation (IP) assays. COS7 cells were cotransfected with pEF-Bos expression vectors for FLAG-tagged AML1 (FL-AML1), FLAG-tagged AML1 mutant R174Q (FL-AML1/R174Q), and GATA-1, or FLAG-tagged TRPS1 (FL-TRPS1), FLAG-tagged TRPS1 GATA ZF mutant (FL-TRPS1/GATAm), and AML1, as indicated. Cell lysates were immunoprecipitated using anti-FLAG antibody (M2 monoclonal antibody; Sigma). Immunoprecipitates were detected by immunoblotting (IB) using rat monoclonal anti-GATA1 antibody (N6; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal anti-AML1 antibody (Active Motif), or anti-FLAG antibody. (J) AML1-TRPS1 inhibits GATA-2–mediated transcription. GATA-2 and AML1-TRPS1 were cotransfected with MαP reporter at the indicated doses, into HL-60 cells. Luciferase activity is expressed as fold changes relative to the control. (K) HDAC inhibitor TSA did not greatly affect the repression mediated by intact AML1-TRPS1, but dramatically relieved that of AT/Δ Ikaros. GATA-1, AML1-TRPS1, or AT/ΔIKAROS were cotransfected into NIH3T3 cells with the MαP reporter at the indicated doses. Cells were treated with TSA (500 nM) for 36 hours from 12 hours after transfection. Luciferase activity is expressed as fold changes relative to the control. The results represent 3 independent experiments.

AML1 and GATA sites. AML1-TRPS1 bound to the AML1 site and showed heterodimerization activity with the PEBP2β subunit (Figure 2A). AML1-TRPS1 also showed DNA-binding to the GATA site (Figure 2B). However, it did not form a heterodimer with the β subunit at this site. It is interesting to note that AML1-TRPS1 shifted as a doublet of bands with the GATA probe, and the upper band (arrowhead in Figure 2B) was much slower than that of AML1-TRPS1/PEBP2β complex bound to the AML1 probe (right end lane). Since the Ikaros-like ZF in the C-terminal TRPS1 moiety is known to serve as a domain to form homo- or heterodimers of factors containing this motif,[18] the slower migrating band might indicate the formation of homodimer by AML1-TRPS1. In fact, the deletion of the Ikaros-like ZF resulted in a significant decrease in the intensity of the upper band, accompanied by an increase in that of the lower band (Figure 2C).

To assess the effect of AML1-TRPS1 on transcription, we next performed a reporter assay. While AML1 and GATA-1 activated BXH2-LTR and MαP reporters, respectively, AML1-TRPS1 inhibited transactivation activity of both AML1 and GATA-1 in a dose-dependent manner (Figure 2E-F; bars 3-5). AT/R174Q has an amino acid substitution in the Runt domain and resulted in the loss of AML1 site-binding activity (Figure 2A).[19] AT/R174Q showed reduced ability to suppress wild-type AML1 in cotransfection assays (Figure 2E; bars 6-8 vs 3-5), suggesting that AML1-TRPS1

inhibits wild-type AML1 transactivation through DNA binding of its Runt domain. Likewise, AT/GATAm lost its DNA-binding function for the GATA site (Figure 2B) due to changes in 2 amino acids that contribute to its structural integrity,[13] and demonstrated weaker inhibition of wild-type GATA-1 transactivation than did AML1-TRPS1 (Figure 2F; bars 9-11 vs 3-5). As the DNA-binding affinity of AML1-TRPS1 was comparable with that of GATA-1 (Figure 2D), this competition for GATA-binding sites is considered to play a significant role in transcriptional suppression mediated by AML1-TRPS1. However, these DNA-binding mutants still retained significant repressive activity (compare bars 2, 5, and 8 in Figure 2E and bars 2, 5, and 11 in Figure 2F), and AT/R174Q and AT/GATAm even showed decreased suppression in the MαP and BXH2-LTR reporter assays, respectively, in a dose-dependent manner (bars 6-8 in Figure 2F and bars 9-11 in Figure 2E). Since AML1 is known to physically interact with GATA-1 through its Runt domain,[20-22] the chimera may also bind to GATA-1. As expected, in an immunoprecipitation experiment, wild-type AML1 interacted with GATA-1, while the AML1 R174Q mutant did not (Figure 2H). On the other hand, GATA ZF in GATA-1 is one of the domains responsible for the interaction with the Runt domain.[21] It is therefore plausible that GATA ZF in TRPS1 is also capable of interacting with AML1. Indeed, we demonstrated that wild-type TRPS1 bound to AML1, whereas the GATA ZF mutant of TRPS1 did not (Figure 2I). Taken together, we postulate that AML1-TRPS1 compromises AML1 and GATA-1 functions through 2 distinct mechanisms: (1) as a dominant-negative protein competing for their cognate binding sites; and (2) via the physical sequestration of both AML1 and GATA-1 proteins. Collectively, these 2 functions may enable AML1-TRPS1 to simultaneously disrupt AML1 and GATA factor-driven genetic programs. Since AML1-TRPS1 showed the inhibition of GATA-2 mediated transactivation (Figure 2J), the inhibitory mechanism may be extended further to other GATA factors.

In an attempt to further investigate the AML1-TRPS1–mediated transcriptional inhibitory mechanism, we carried out a reporter assay in the presence of histone deacetylase (HDAC) inhibitor trichostatin A (TSA). TSA did not affect greatly the repression mediated by intact AML1-TRPS1 (Figure 2K; bar 3 vs bar 7), but dramatically relieved that of AT/Δ Ikaros (Figure 2K; bar 4 vs bar 8), suggesting that the dimerization of AML1-TRPS1 through Ikaros-like ZF confers potent recruitment of corepressors such as HDAC. AML1-TRPS1 seems to serve as another example confirming the emerging hypothesis that most of the chimeric proteins possess the property of forming a dimer (or a multimer), thereby leading to aberrant transcriptional regulation.[2,23]

Although there is only 1 additional reported patient with AML with t(8;21)(q24;q22) probably carrying AML1-TRPS1,[6,7] a similar fusion, AML1-FOG2, that represses both AML1- and GATA-1–mediated transactivation, was found in a patient with myelodysplastic syndrome.[24] Moreover, the most pervasive fusion gene in AML, *AML1-MTG8*, has also been shown to repress GATA-1 function in addition to its dominant-negative effect on AML1.[20,25] Collectively, the concurrent transcriptional deregulation of AML1 and GATA factors in leukemia seems relatively common. This novel chimeric gene *AML1-TRPS1* could serve as a tool for elucidating the details of the interplay between AML1 and GATA factors and its disruption in leukemogenesis.

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Authorship

Author contributions: N.A. and M. Yanagida contributed equally to this study. N.A. participated in the study design, molecular cloning, and writing of the manuscript. M. Yanagida performed the molecular analyses and wrote the manuscript. L.H. and M. Yamamoto provided vital analysis tools. K.S., H.M., and Y.I. contributed to the writing of the manuscript and discussions on the experimental design. M.O. participated in the study design, analysis of the experiments, and writing of the manuscript. All authors have reviewed the manuscript.

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A randomized study with or without intensified maintenance chemotherapy in patients with acute promyelocytic leukemia who have become negative for *PML-RARα* 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-RARα* 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-RARα* fusion transcript after 3 courses of intensive consolidation therapy. (Blood. 2007;110:59-66)

Introduction

The use of all-*trans* retinoic acid (ATRA) has markedly improved the therapeutic outcome in patients with acute promyelocytic leukemia (APL).[1-3] 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.[2,3] 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.[4-11] 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.[5,7-12]

Before the introduction of ATRA in the treatment of APL, the efficacy of maintenance chemotherapy had been observed in patients with APL.[13,14] 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.[5] 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.[15] 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-RARα* 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-RARα* 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-RARα* 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 ($PaO_2 > 60$ mm Hg or $SpO_2 > 93\%$), 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-RARα* 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-RARα* fusion gene was amplified using bone marrow samples obtained at diagnosis and after consolidation therapy by reverse-transcriptase polymerase chain reaction analysis.[16,17] The detection limit of *PML-RARα* 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^2/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 3.0×10^9/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^2/d by 30-minute intravenous infusion on days 1 and 2) plus cytarabine (Ara-C) (80 mg/m^2/d by continuous intravenous infusion on days 1 through 5) was given to patients with initial leukocyte counts between 3.0×10^9/L and 10.0×10^9/L, and those with leukocyte counts below 3.0×10^9/L and APL cells above 10^9/L (group B). Patients with initial leukocyte counts of 10.0×10^9/L or more received idarubicin (12 mg/m^2 on days 1 to 3) plus Ara-C (100 mg/m^2 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^2 for 2 days) and Ara-C (80 mg/m^2 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 30×10^9/L or more and plasma fibrinogen level above 4.4 μmol/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.[18-20] 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^2) by 30-minute intravenous infusion on days 1 to 3, and Ara-C (200 mg/m^2) by continuous infusion on days 1 to 5. The second consolidation contained Ara-C (140 mg/m^2) for 5 days, etoposide (100 mg/m^2) by 1-hour intravenous infusion for 5 days, and daunorubicin (50 mg/m^2) by 30-minute infusion on days 1 through 3. The third consolidation consisted of Ara-C (140 mg/m^2) for 5 days and idarubicin (12 mg/m^2) for 3 days. Each consolidation course was given after recovery from the previous course, when polymorphonuclear cells were 1.5×10^9/L or more and platelets were 100×10^9/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-RARα* 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.[21] The first course of intensified maintenance therapy consisted of behenoyl Ara-C (BHAC) (170 mg/m^2, 2-hour infusion, days 1 through 5), daunorubicin (30 mg/m^2, 30-minute infusion, days 1 and 4) and mercaptopurine (6MP; 70 mg/m^2, orally, days 1 through 7). The second consisted of BHAC and mitoxantrone (5 mg/m^2, 30-minute infusion, days 1 and 2). The third consisted of BHAC, etoposide (80 mg/m^2, 1-hour infusion, days 1, 3, and 5), and vindesine (2 mg/m^2, bolus infusion, days 1 and 8). The fourth consisted of BHAC, aclarubicin (14 mg/m^2, 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-RARα* fusion transcript at the end of consolidation chemotherapy received late ATRA therapy (45 mg/m^2/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.[22,23] 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 1.5×10^9/L and platelet counts of more than 100×10^9/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-RARα* 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-RARα* 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-RARα*, and 6 had no test for t(15;17) or *PML-RARα*. 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 1.7×10^9/L (range, 0.03 to 257×10^9/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-RARα* fusion transcript. Three of these subsequently relapsed and another patient received allogeneic HSCT. However, 230 patients (97.9%) showed no *PML-RARα* transcript in the bone marrow cells at the end of consolidation. A total of 55 patients negative for *PML-RARα* 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		
Sex							
Male	158 (56)		53 (60)		47 (55)		.51
Female	125 (44)		36 (40)		39 (45)		
Age, years		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)		
FAB Morphology							
M3	265 (93)		80 (90)		82 (95)		.25
M3v	18 (6)		9 (10)		4 (5)		
Leukocyte count, $\times 10^9$/L		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)		
Platelet count, $\times 10^9$/L		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)		
Induction therapy#							
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-squqre 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-RARα* 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

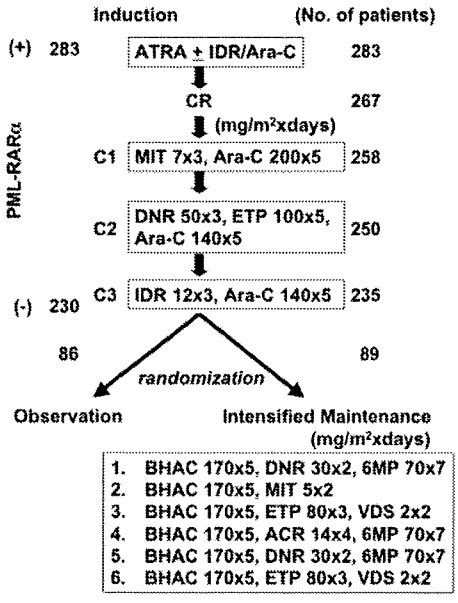

Figure 1. Study design. The number of patients who completed each step is indicated. C1, C2, and C3 were consolidation courses 1, 2, and 3. A total of 283 patients had t(15;17) and/or the *PML-RARα* transcript at the time of diagnosis, and 230 patients were negative for *PML-RARα* at the end of 3 courses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

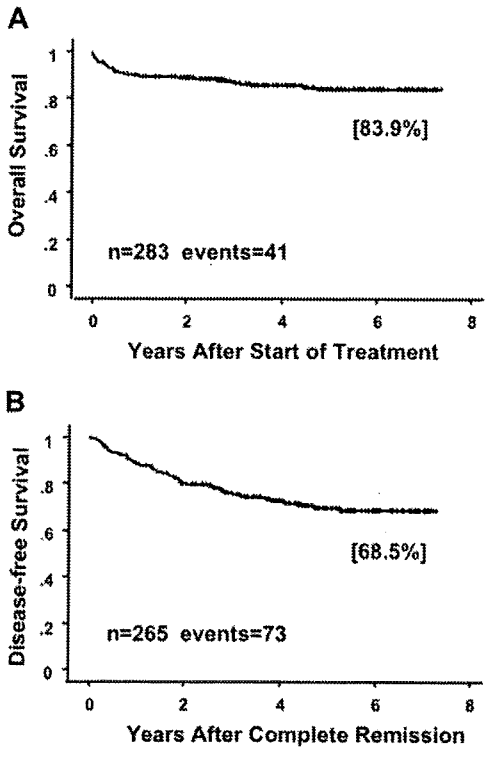

Figure 2. Overall survival and disease-free survival in patients enrolled in the JALSG APL97 study. Overall survival (A) in all assessable patients and disease-free survival (B) in patients who achieved CR are estimated by the Kaplan-Meier method.

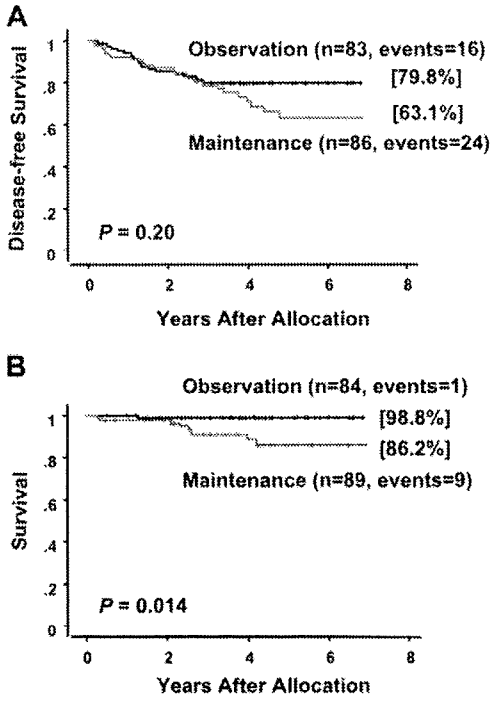

Figure 3. Disease-free survival and survival of randomized patients in the maintenance phase. Disease-free survival (A) and survival (B) are estimated from the date of randomization.

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 10.0×10^9/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 10.0×10^9/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-RARα* 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.[24] 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 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.[25] 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.[26,27] In addition, there was a hypothesis that an anthracycline alone may be as effective as combinations of anthracycline and Ara-C.[8,27,28] However, the interim analysis of the European APL2000 study showed

Table 3. Effects of factors on disease-free survival

Parameters	No. of patients	No. of relapses	Univariate analysis HR (95%CI)	*P**
Sex				
Female	100	16	1	
Male	75	26	1.4 (0.7-2.7)	.29
Age, years				
15-50	98	24	1	
50-70	77	18	0.9 (0.5-1.7)	.75
Leukocyte count, × 10^9/L				
Less than 10.0	138	29	1	
10.0 or higher	37	13	1.7 (0.9-3.5)	.12
Platelet count, × 10^9/L				
Less than 40	112	25	1	
40 or higher	63	17	1.4 (0.7-2.6)	.32
Induction therapy				
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
Maintenance chemotherapy				
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.

Table 4. Effects of factors on survival

Parameters	No. of patients	No. of deaths	Univariate analysis HR (95%CI)	*P**
Sex				
Female	100	12	1	
Male	75	4	2.1 (0.6-8.3)	.27
Age, years				
15-50	98	8	1	
50-70	77	8	2.6 (0.7-10)	.16
Leukocyte count, × 10^9/L				
Less than 10.0	138	11	1	
10.0 or higher	37	5	2.8 (0.8-10)	.11
Platelet count, × 10^9/L				
Less than 40	112	12	1	
40 or higher	63	4	0.2 (0.03-1.8)	.16
Induction therapy				
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
Maintenance chemotherapy				
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.

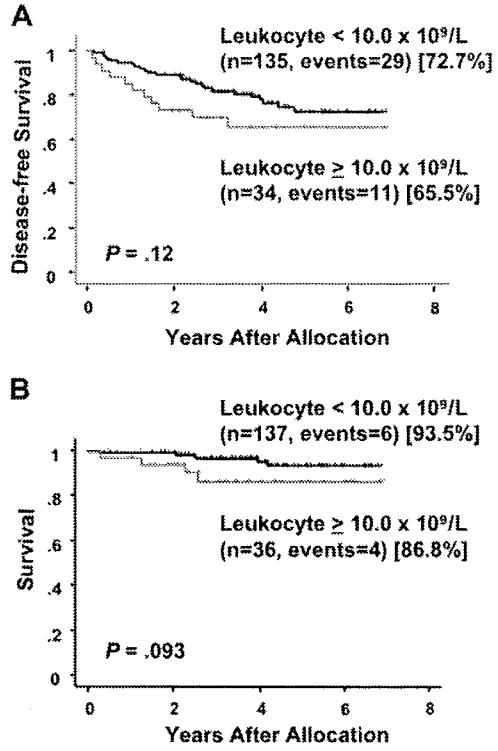

Figure 4. Disease-free survival and survival by initial leukocyte count. Disease-free survival (A) and survival (B) in patients with initial leukocyte counts above or below 10.0 × 10^9/L are estimated from the date of randomization.

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

PML-RARα generated by t(15;17) provides the most clinically relevant information in patients with APL.[2,3,16,17] A number of patients who achieve molecular remission assessed by reverse-transcriptase polymerase chain reaction for *PML-RARα* after consolidation are predicted to obtain a long-term survival.[7,30] However, detection of *PML-RARα* 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.[31] In this study, 5 of 235 (2.1%) patients showed the *PML-RARα* fusion transcript after the consolidation therapy, and 3 of these relapsed subsequently. In contrast, 97.9% of patients were negative for *PML-RARα* transcript. In the GIMEMA-AIEOP study, 646 of 664 (97.3%) patients were negative for the *PML-RARα* fusion transcript at the end of consolidation.[32] Because approximately half of APL patients are molecularly positive after induction,[7,9] elimination of *PML-RARα* 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.[27] However, the North American Intergroup trial showed a benefit for ATRA in both induction and maintenance therapy.[6,12] 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.[10] 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.[32] 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,[6,10] whereas patients received 3 cycles of consolidation both in the GIMEMA-AIEOP and our studies.[32] 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.[33] 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-RARα* 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, multidrug resistance is generally acquired by the use of antileukemic agents.[34] 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.[35] 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.[2,3] 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.[9,21,36] 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.[37-41]

In conclusion, we did not find any beneficial effect of intensified maintenance chemotherapy in patients negative for *PML-RARα* 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.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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