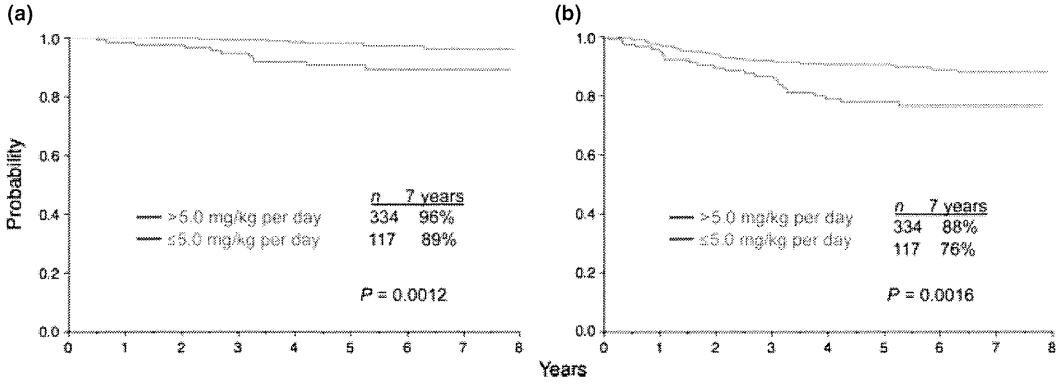

Fig. 4. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months per body weight. The cut-off value was set at >5.0 mg/day/kg (e.g. if a patient whose body weight was <60 kg received imatinib at a mean daily dose of 300 mg).

Table 5. Number of patients and survival according to the mean daily dose of imatinib during the first 24 months per body weight

	Mean daily dose/body weight (mg/day/kg)				
	>5.0†		≤5.0		*P*-value
	Actual bodyweight (kg)	No. patients	Actual bodyweight (kg)	No. patients	
Imatinib daily dose group‡					
400 mg	<80	266	≥80	28	
300 mg	<60	63	≥60	27	
200 mg	<40	5	≥40	62	
Estimated 7-year OS	96%		89%		0.0012
Estimated 7-year EFS	88%		76%		0.0016

†The cut-off value was set at >5.0 mg/day/kg (e.g. the mean daily dose of imatinib during the first 24 months (300 mg) divided by body weight [<60 kg]). ‡Mean daily doses in the 400-, 300-, and 200-mg groups were ≥360, 270–359, and <270 mg imatinib, respectively. Patients who discontinued imatinib were not included in the analysis. EFS, event-free survival; OS, overall survival.

to the mean daily dose during the first 6, 12, and 24 months of treatment. The rate of achieving CCyR or MMR differed significantly between the 300- and 400-mg groups during the first 24 months. Even so, there were no significant differences in OS, PFS, and EFS between the 300- and 400-mg groups during the first 6, 12, or 24 months of treatment. Conversely, the 200-mg group showed markedly inferior cytogenetic and/or molecular responses, as well as inferior survival, compared with the 300- and 400-mg groups. We also analyzed outcomes according to the mean daily dosage during the first 24 months per BW, with the results suggesting that patients who had relatively high daily dosage per BW were likely to have better OS and EFS even though the actual daily dose had been lower than 400 mg imatinib. The OS and EFS in the 300-mg group in the present study were not inferior compared with rates reported in the IRIS study (85% at 7 years vs. 83% at 6 years), which suggests that a considerable number of Japanese patients who received doses lower than 400 mg demonstrated an adequate response. A prospective comparative study would be necessary to confirm this observation.

Two recent studies showed a correlation between the plasma trough levels (C_{min}) and response, suggesting that maintaining C_{min} above approximately 1000 ng/mL was associated with improved outcomes.[22,23] In the present study, the mean daily dose was 331 ± 108 mg during the first 24 months and the relatively high dosage of imatinib per BW was associated with better OS and EFS, whereas in the IRIS study the mean daily dose among the patients who continued receiving imatinib was 382 ± 50 mg.[1] On the basis of our results, we assume that the relatively small body size of Japanese patients compared with their Western counterparts may have affected C_{min}, although differences in the metabolism of imatinib because of ethnicity cannot be ruled out either. Therefore, we measured the C_{min} of imatinib in a group of patients who had received imatinib continuously at a daily dose of either 300 or 400 mg. The patients from whom blood samples were collected showed almost similar background characteristics to the entire study population. There was no significant difference in the mean C_{min} between patients receiving 300 or 400 mg imatinib, and there was no significant difference in the ratio of patients whose C_{min} was higher than 1000 ng/mL between the two groups. When pharmacokinetic analyses of patients receiving 400 mg imatinib in the present study are compared with the IRIS study, the C_{min} in the present study was distributed at higher concentrations than in the IRIS study (mean C_{min} 1165 vs. 979 ng/mL, respectively); however, the distribution of C_{min} in patients receiving 300 mg imatinib was similar between the studies.[23] Larson *et al.* reported a weak correlation between C_{min} and age, BW, or BSA in the IRIS study, but also suggested that the effects of body size and age on C_{min} were not likely to be of clinical significance because C_{min} showed large interpatient variability.[23] However, the C_{min} in their female patients was significantly higher than that in male patients, and they speculated that this may be due to the small body size of the female patients. The same tendency was seen in the present study, especially in terms of age and gender. Therefore, a small body size among Japanese old and/or female patients may partly account for the higher C_{min} of imatinib. Regarding

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Table 6. Patient characteristics and plasma trough levels of imatinib according to the daily dose of imatinib

	Imatinib daily dose†		*P*-value
	400 mg	300 mg	
No. patients	26	24	
No. men/women	19/7	12/12	0.092
Age (years)	49 (17–79)	58 (33–76)	0.012
Body weight (kg)	65.2 ± 10.6	59.5 ± 10.7	0.062
BSA (m^2)	1.68 ± 0.17	1.57 ± 0.17	0.034
Sokal risk group (*n*)			
Low	18	13	0.357
Intermediate	6	6	
High	2	5	
C_{min} (ng/mL)			
Mean ± SD	1165 ± 445	1113 ± 426	0.673
Median (range)	1035 (710–2420)	1130 (439–2140)	
% Patients on >1000 ng/mL imatinib	57.7 (15/26)	62.5 (15/24)	0.1
Best response (%)			
MCyR	26 (100)	23 (96)	
CCyR	26 (100)	22 (92)	
MMR	24 (92)	23 (96)	

Unless indicated otherwise, data are given as the mean ± SD, as the median with the range given in parentheses, or as the number of patients in each group with percentages given in parentheses, as appropriate. †Imatinib at a daily dose of 400 or 300 mg without any dose modification. BSA, body surface area; CCyR, complete cytogenetic response; C_{min}, plasma trough level; MCyR, major cytogenetic response; MMR, major molecular response.

the plasma concentration of imatinib in Japanese patients, there are other reports showing sufficient C_{min} in patients receiving imatinib at doses lower than 400 mg,[6,24] but it remains uncertain whether there are any individual or ethnic differences in the metabolism of imatinib.[24,25]

Another possible reason for the satisfactory outcomes seen for patients in the 300-mg group could be that, at this dose, imatinib could be administered continuously to some patients without serious adverse events. A recent study regarding imatinib dosage in Japanese patients reported that, based on multivariate analysis, older age and lower BW are significant risk factors for the discontinuation of imatinib therapy and that patients with these factors were less likely to achieve a CCyR.[18] Continuous and adequate dosage is essential for optimal outcome, and adherence to imatinib therapy is critical.[26,27]

In conclusion, the long-term follow-up of the JALSG CML202 study revealed almost similar excellent outcomes to those of the IRIS study and others. There were no significant differences in OS and EFS between the 300- and 400-mg imatinib groups. However, cumulative rates of cytogenetic or molecular responses in the 300-mg group were inferior to those in the 400-mg group. The results of the present study suggest that imatinib at a dose of 400 mg may be optimal for Japanese patients, but that 400 mg imatinib is not tolerable in a considerable number of patients, and that the measurement of C_{min} is useful in finding the optimal dose, especially in elderly and/or female patients. Nevertheless, excessive dose reductions to <300 mg imatinib should be avoided even in patients who are intolerant to 400 mg imatinib or have a small body size. We hope our findings are useful for the treatment of CML patients in other Asian countries.

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Disclosure Statement

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Correlation between Amp-CMLTM (FUJIREBIO Inc., Tokyo, Japan) and Fusion Quant M-BCRTM (Ipsogen, Marseille, France).

Data S1. Measurement of major *BCR-ABL1* transcript.

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ORIGINAL ARTICLE

Pre-transplant imatinib-based therapy improves the outcome of allogeneic hematopoietic stem cell transplantation for *BCR–ABL*-positive acute lymphoblastic leukemia

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A high complete remission (CR) rate has been reported in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) following imatinib-based therapy. However, the overall effect of imatinib on the outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is undetermined. Between 2002 and 2005, 100 newly diagnosed adult patients with Ph + ALL were registered to a phase II study of imatinib-combined chemotherapy (Japan Adult Leukemia Study Group Ph + ALL202 study) and 97 patients achieved CR. We compared clinical outcomes of 51 patients who received allo-HSCT in their first CR (imatinib cohort) with those of 122 historical control patients in the pre-imatinib era (pre-imatinib cohort). The probability of overall survival at 3 years after allo-HSCT was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and 44% (95% CI, 35–52%) for the pre-imatinib cohort. Multivariate analysis confirmed that this difference was statistically significant (adjusted hazard ratio, 0.44, $P = 0.005$). Favorable outcomes of the imatinib cohort were also observed for disease-free survival ($P = 0.007$) and relapse ($P = 0.002$), but not for non-relapse mortality ($P = 0.265$). Imatinib-based therapy is a potentially useful strategy for newly diagnosed patients with Ph + ALL, not only providing them more chance to receive allo-HSCT, but also improving the outcome of allo-HSCT.
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Keywords: Philadelphia chromosome-positive acute lymphoblastic leukemia; imatinib; allogeneic hematopoietic stem cell transplantation

Introduction

The Philadelphia chromosome (Ph) presents in 20–25% of adult patients with acute lymphoblastic leukemia (ALL) and is an extremely unfavorable prognostic factor. The outcome of patients with Ph-positive ALL (Ph + ALL) following conventional chemotherapy is dismal, showing <20% long-term survival.[1–4] Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) has offered a curative option in Ph + ALL,[3–5] relatively high rates of relapse and non-relapse mortality (NRM) impair the treatment success even after allo-HSCT. The International Bone Marrow Transplant Registry reported a leukemia-free survival rate of 38% following human leukocyte antigen (HLA)-identical allo-HSCT for Ph + ALL patients transplanted in the first complete remission (CR).[6] Previously, we and others reported that imatinib-based chemotherapy produced very high CR rate, thus allowing a high proportion of patients to prepare for allo-HSCT.[7,8] However, because of the short observation period, the impact of imatinib-based therapy upon the survival outcomes after allo-HSCT remains unclear. To address whether allo-HSCT after imatinib-based therapy is a superior treatment approach to that after conventional chemotherapy, we conducted a retrospective analysis of Ph + ALL patients who underwent allo-HSCT before and after imatinib became available, using data from the Japan Adult Leukemia Study Group (JALSG) Ph + ALL202 study and from the nationwide database of the Japan Society of Hematopoietic Stem-cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP).

Patients and methods

Data source and patient selection criteria

We compared the transplantation outcome of patients treated by the JALSG Ph + ALL 202 study (imatinib cohort) with those in the historical control data in the pre-imatinib era from the JSHCT and JMDP (pre-imatinib cohort), in which information on patient survival, disease status and long-term complications, including chronic graft-versus-host disease (cGVHD) and second malignancies, is renewed annually using follow-up forms.[9,10] To

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attain an adequate level of comparability in terms of allo-HSCT, patients were selected according to the following criteria: (1) patients with *de novo* Ph + ALL; (2) age range of 15–65 years and (3) allo-HSCT during their first CR. A total of 122 patients who received allo-HSCT between January 1995 and December 2001 (before the approval of imatinib by the Japanese government) were selected. This study period of the pre-imatinib cohort included the pioneering period of cord blood transplantation (CBT) when the relevance of cell dose and HLA matching had not yet been recognized. Thus, the subjects were limited to those who received bone marrow (BM) or peripheral blood (PB) as a treatment graft.

Patients

Between September 2002 and May 2005, 100 newly diagnosed patients with Ph + ALL were registered to the JALSG Ph + ALL202 study, and received a phase 2 imatinib-combined chemotherapy as described previously.[7] Ph + ALL was diagnosed by the presence of Ph through chromosome and/or FISH analysis, and positivity for *BCR–ABL* fusion transcripts detection by real-time quantitative polymerase chain reaction (RQ–PCR) analysis.

Of 97 patients who achieved CR, 60 patients received allo-HSCT in their first CR. Of these 60 patients, 9 patients who received unrelated CBT were excluded in this analysis because of the reason as described at the selection criteria for control patients in the pre-imatinib era. Thus, 51 patients transplanted between February 2003 and December 2005 were analyzed. In the JALSG Ph + ALL202 study, allo-HSCT was recommended after achieving CR if an HLA-identical donor was available. The stem cell source for allo-HSCT was chosen in the following order: (1) matched-related allo-HSCT; (2) HLA-A, B and DRB1 allele matched (6/6) or DRB1 one-allele mismatched-unrelated allo-BMT, if patients had no HLA-matched-related donor and (3) unrelated CBT or HLA-mismatched-related allo-HSCT, if they had no donors described in (1) and (2). A prophylaxis for GVHD was determined by each institute, but did not include T-cell depletion. The study was approved by the institutional review board of each participating center and conducted in accordance with the Declaration of Helsinki.

Definition of engraftment and GVHD

Engraftment day was defined as the first day of three consecutive days when the absolute neutrophil count was $\geqslant 0.5 \times 10^9$/l. Graft failure was defined as the lack of any sign of neutrophil recovery. Engraftment that occurred after day 60 was also considered to be a graft failure. Patients who died early (<day 29) were excluded from the analysis of engraftment. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined according to previously described standard criteria.[11]

Quantitation of BCR–ABL transcripts

The copy number of *BCR–ABL* transcripts in BM was determined at a central laboratory using the RQ–PCR as described previously.[7] To minimize the variability in the results because of differences in the efficiency of cDNA synthesis and RNA integrity among the patient samples, the copy number of the *BCR–ABL* transcripts was converted to molecules per microgram RNA after being normalized by means of *GAPDH*. The normalized values of the *BCR–ABL* copies in each sample were reported as *BCR–ABL* number of copies. At least 5.7×10^5 copies/μg RNA *GAPDH* levels were required in a sample to consider a negative PCR result valid; otherwise, the sample was not useful for minimal residual studies. The threshold for quantification was 50 copies/μg RNA. The levels below this threshold were designated as 'not detected' or '<50 copies/μg'. In this study, the former was categorized as PCR negativity.

Minimal residual disease (MRD) at the time of HSCT was evaluated by the result of RQ–PCR within 30 days prior to transplantation.

Statistical considerations

The primary end point of this study was overall survival (OS) after allo-HSCT. Secondary end points included disease-free survival (DFS) and the incidence of aGVHD, cGVHD, NRM and relapse. We defined DFS events as relapse or death, whichever occurred earlier. The observation periods for OS were calculated from the date of transplantation until the date of the event or last known date of follow-up. The probabilities of OS and DFS were estimated using the Kaplan–Meier product limit method. The cumulative incidences of NRM, relapse, aGVHD and cGVHD were estimated as described elsewhere, taking the competing risk into account.[12] In each estimation of the cumulative incidence of an event, death without an event was defined as a competing risk. Risk factors for OS and DFS were evaluated by a combination of uni- and multivariate analyses. The following variables were evaluated for each analysis: imatinib-based therapy prior to HSCT, age group (under 40 versus 40 to 54 versus 55 and older), stem cell source (BM versus PB), HLA disparity (matched (HLA-identical siblings or 6/6 allele matched unrelated) versus mismatched), duration from diagnosis to HSCT and cGVHD as time-varying covariate (yes versus no). Univariate analysis was performed using Cox regression models or log-rank test. Multivariate analysis was performed using Cox proportional hazards regression model or competing risk regression model[13] as appropriate. For the evaluation of time-varying events, such as aGVHD or cGVHD, upon clinical outcomes, we treated these as time-varying covariates. Differences among groups in terms of demographic characteristics were tested using the χ^2 or Mann–Whitney tests as appropriate. All statistical analyses were conducted using STATA 11 (STATA Corp., College Station, TX, USA).

Results

Patient characteristics

In the imatinib cohort, there were 29 males and 22 females, with a median age of 38 years (range, 15–64 years). Regarding transcript types, 36 patients had minor *BCR–ABL* and 15 had major *BCR–ABL*. In 5 patients, pre-treatment cytogenetic data were not available, and of the remaining 46 patients, 8 showed t(9;22) only, 36 had additional chromosome aberrations and 2 showed normal karyotype. Of 48 patients who were evaluable for MRD analysis, 36 patients achieved PCR negativity at the time of HSCT.

Some of the clinical and biological features (such as presence of additional chromosome aberrations, *BCR–ABL* subtype, MRD status at HSCT and performance status at HSCT) were not available in the pre-imatinib cohort and not included in the present analysis.

Table 1 lists the characteristics of patients included in this comparative analysis. Some of the clinical features were significantly different between two cohorts: age distribution at HSCT ($P=0.048$), conditioning regimens ($P<0.001$), GVHD prophylaxis ($P<0.001$) and duration from diagnosis to HSCT ($P=0.041$). The majority of patients received the preparatory

Table 1 Patient characteristics ($N=173$)

Characteristic	*Imatinib cohort*	*Pre-imatinib cohort*	P
No. of transplantations	51	122	
Age, n (%)			0.048
−39	27 (53)	71 (58)	
40–54	17 (33)	49 (40)	
55–	7 (14)	2 (2)	
Median (range)	38 (15–64)	38 (15–57)	
Gender (male/female)	29/22	73/49	0.717
HSCT donor, n *(%)*			0.460
Related	24 (47)	73 (60)	
Unrelated	21 (41)	43 (35)	
HLA-mismatched related	6 (12)	6 (5)	
Hematopoietic cell source, n *(%)*			0.246
Bone marrow	35 (69)	94 (77)	
Peripheral blood	16 (31)	28 (23)	
Conditioning regimen, n *(%)*			<0.001
CY+TBI	24 (47)	26 (22)	
CY+CA+TBI	14 (27)	37 (31)	
CY+VP+TBI	2 (4)	21 (17)	
CY+TESPA+TBI	—	7 (6)	
CY+BU+TBI	—	6 (5)	
Flu+BU	3 (6)	—	
Flu+ LPAM ± TBI	2 (4)	—	
Others	6 (12)	25 (20)	
GVHD prophylaxis, n *(%)*			<0.001
Cyclosporine + sMTX	24 (47)	95 (80)	
Cyclosporine ± other	3 (6)	3 (2)	
Tacrolimus + sMTX	22 (43)	17 (14)	
Tacrolimus + other	—	4 (3)	
Median days from diagnosis to HSCT (range)	162 (67–512)	182 (66–834)	0.041

Abbreviations: BU, oral busulfan; CA, cytarabine; CY, cyclophosphamide; Flu, fuludarabine; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; LPAM, melphalan; sMTX, short-term methotrexate; TBI, total body irradiation; TESPA, tespamine; VP, etoposide.

regimen of total body irradiation followed by cyclophosphamide and/or cytarabine. Five patients aged >55 in the imatinib cohort were given a reduced intensity regimen consisting of fludarabine and melphalan or busulfan. In the pre-imatinib cohort, a combination of cyclosporine (CsA) and short-term methotrexate (sMTX) was mostly used in the prophylaxis of GVHD. On the other hand, both CsA + sMTX and tacrolimus (FK506) + sMTX combinations were commonly used in the imatinib cohort. In both cohorts, none of the patients received imatinib therapy after HSCT in their first CR. In the imatinib cohort, all patients who showed hematologic relapse after HSCT received salvage treatment comprising of imatinib and/or chemotherapy. As for the pre-imatinib cohort, 13 patients relapsed after the approval of imatinib by the Japanese government (beyond December 2001). However, we have no information on how many patients received imatinib-based therapy after their relapse. The median follow-up period for survivors was 2.6 years (range, 1.0–4.6 years) for the imatinib cohort and 6.9 years (range, 0.1–11.4 years) for the pre-imatinib cohort.

Outcome

OS and DFS. In the pre-imatinib cohort, 80 patients died after HSCT: 46 of disease recurrence and 34 of causes other than leukemia. In the imatinib cohort, 35 patients were alive, 32 of them were free of leukemia and 16 patients died after HSCT: 4 of disease recurrence and 12 of causes other than leukemia. The 3-year OS was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and significantly higher than 44% (95% CI, 35–52%) for the pre-imatinib cohort ($P=0.0148$; Figure 1a). The 3-year DFS was 58% (95% CI, 41.8–70.9%) for the imatinib cohort and significantly higher than 37% (95% CI, 28.5–45.6%) for the pre-imatinib cohort ($P=0.039$; Figure 1b).

Table 2 shows the result of risk factor analysis for OS and DFS among all 173 patients. In the multivariate analysis, the only variable found to influence OS and DFS was the pre-transplant imatinib-based therapy (hazard ratio (HR) = 0.44 (95% CI, 0.25–0.77); $P=0.004$ and HR = 0.51 (95% CI, 0.31–0.86); $P=0.011$, respectively). The presence of cGVHD showed a tendency of favorable OS and DFS, but did not reach the statistical significance (HR = 0.66 (95% CI, 0.42–1.06); $P=0.085$ and HR = 0.75 (95% CI, 0.47–1.19); $P=0.217$, respectively).

Other outcomes of transplantation

Relapses. In the pre-imatinib cohort, 48 patients relapsed after HSCT with a median of 240 days (range, 42–2302 days).

In the imatinib cohort, 7 patients (3 of 36 with PCR negative and 4 of 12 with PCR positive at HSCT) relapsed after HSCT with a median of 137 days (range, 68–728 days). The estimated cumulative incidence of relapse at 3 years was 15.0% (95% CI, 6.6–26.7%), and significantly lower than that of the pre-imatinib cohort (50.4% at 3 years (95% CI, 39.6–60.2%); $P=0.002$; Figure 1c). Among patients in the imatinib cohort, patients with PCR negative showed significantly lower relapse rate compared with that of PCR positive (10.0% (95% CI, 2.5–23.6%) versus 41.3% (95% CI, 16.9–64.4%) at 3 years, respectively, $P=0.025$).

Non-relapse mortality. In the pre-imatinib cohort, 34 patients died of non-relapse causes at a median of 159 days (range, 5–2094 days) after HSCT. The estimated cumulative incidence of NRM in the pre-imatinib cohort was 28% (95% CI, 20–36) at 3 years (Figure 2a). In the imatinib cohort, 12 patients died of non-relapse causes at a median of 329 days (range, 41–850 days) after HSCT. The 3-year cumulative incidences of NRM were 21% (95% CI, 11–33%; Figure 2a). There were no significant differences between two cohorts ($P=0.265$).

Cause of death. Recurrence of the primary disease was the leading cause of death in both groups: 55% for the pre-imatinib cohort and 25% for the imatinib cohort. In the pre-imatinib cohort, the causes of NRM were organ failure (11%), infection (9%), GVHD (8%), transplantation-associated thrombotic microangiopathy (TMA) (4%), interstitial pneumonia (3%), graft failure (3%) and other causes (6%). In the imatinib cohort, the causes of NRM included infection (19%), bronchiolitis obliterans with organizing pneumonia (13%), TMA (13%), GVHD (13%), organ failure (6%) and other causes (12%).

Graft-versus-host disease. There was no significant difference in the cumulative incidence of Grades 2–4 aGVHD between two cohorts (31% (95% CI, 19–44%) versus 37% (95% CI, 29–46%), $P=0.391$; Figure 2b). The cumulative incidence of cGVHD at 1 year after HSCT was significantly higher in the imatinib cohort than in the pre-imatinib cohort (49% (95% CI, 31–64%) versus 27% (95% CI, 18–37%), $P=0.0261$; Figure 2c).

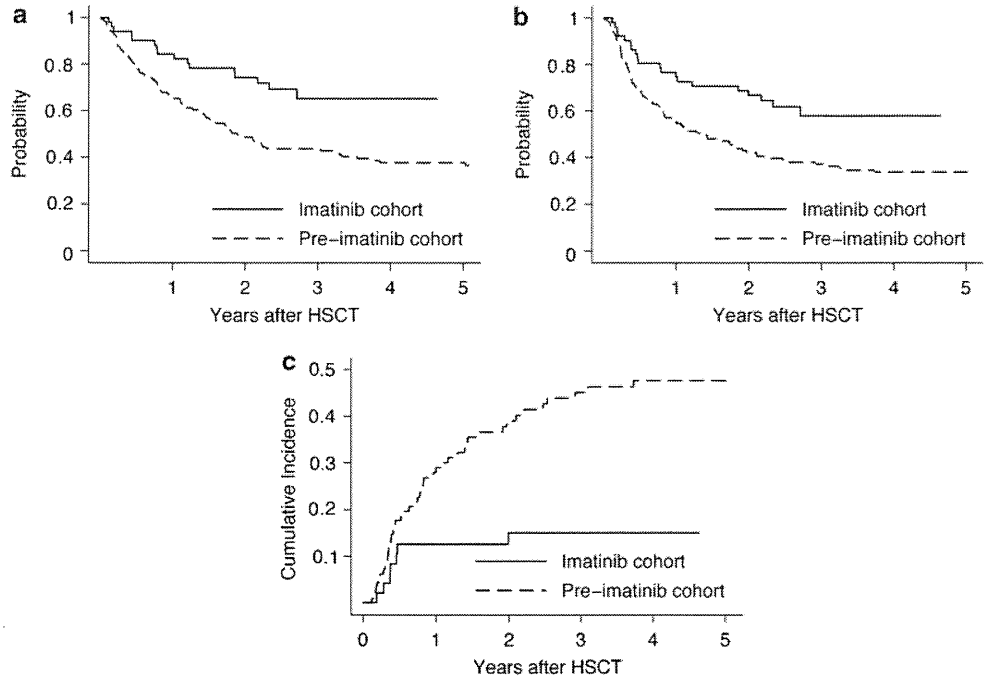

Figure 1 Transplantation outcomes of 51 patients who received imatinib-based therapy and 122 historical patients. (**a**) Overall survival, (**b**) disease-free survival and (**c**) cumulative incidence of relapse.

Table 2 Results of uni- and multivariate analysis of overall survival and disease-free survival among 173 patients with Ph+ALL

Characteristic	*Overall survival*				*Disease-free survival*			
	Univariate analysis		*Multivariate analysis*		*Univariate analysis*		*Multivariate analysis*	
	RR (95% CI)	P	*RR (95% CI)*	P	*RR (95% CI)*	P	*RR (95% CI)*	P
Imatinib-imterim therapy before HSCT	0.45 (0.26–0.77)	0.004	0.44 (0.25–0.77)	0.004	0.51 (0.31–0.83)	0.007	0.51 (0.31–0.86)	0.011
Donor status (RE versus UR)	0.87 (0.57–1.32)	0.521	0.72 (0.40–1.30)	0.275	0.77 (0.51–1.16)	0.211	0.65 (0.37–1.16)	0.147
Age at HSCT (–39 versus 40–55 versus 55–)	1.03 (0.74–1.44)	0.852	1.12 (0.78–1.62)	0.536	0.98 (0.71–1.36)	0.914	1.03 (0.73–1.47)	0.862
HLA-disparity (matched versus mismatched)	0.90 (0.39–2.06)	0.800	0.76 (0.32–1.81)	0.531	1.11 (0.49–2.54)	0.800	1.06 (0.45–2.50)	0.895
Stem-cell source (BM versus PB)	1.15 (0.72–1.82)	0.565	1.23 (0.72–2.10)	0.451	1.30 (0.85–2.00)	0.228	1.34 (0.81–2.20)	0.254
Days from diagnosis to HSCT	1.00 (0.99–1.00)	0.217	1.00 (0.99–1.00)	0.141	1.00 (0.99–1.00)	0.415	1.00 (0.99–1.00)	0.125
cGVHD as time-varying covariate (yes versus no)	0.68 (0.43–1.08)	0.101	0.66 (0.42–1.06)	0.085	0.78 (0.50–1.23)	0.292	0.75 (0.47–1.19)	0.217

Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; CI, confidence interval; cGVHD, chronic graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hemtopoetic stem cell transplantation; PB, peripheral blood; Ph, Philadelphia chromosome; RE, related; RR, relative risk; UR, unrelated.

However, regarding the cumulative incidence of extensive-type cGVHD, there was no significant difference between two cohorts (22% (95% CI, 10–36%) versus 12% (95% CI, 6–20%), $P=0.119$; Figure 2d).

Association between cGVHD and OS/DFS/relapse. To examine the difference of impacts of cGVHD upon clinical outcome in the pre- and imatinib cohorts, we conducted stratified analysis by cohort, treating cGVHD as a time-varying covariate (Table 3). Multivariate analysis revealed that, in the imatinib cohort, there were no significant associations between cGVHD and OS/DFS/relapse ($P=0.707$, 0.332 and 0.713, respectively). On the other hand, in the pre-imatinib cohort, there was a significant association between cGVHD and OS (HR = 0.59 (95% CI, 0.35–1.00), $P=0.048$), but not between cGVHD and DFS/relapse ($P=0.234$ and 0.338, respectively).

Engraftment. In the pre-imatinib cohort, three patients experienced graft failure. The median periods to reach the neutrophil count of $>0.5 \times 10^6$/l and platelet count of 50×10^6/l were 15 days (range, 8–49 days) and 25 days (range, 9–120 days), respectively, for evaluable patients. In the imatinib cohort, all 51 patients were engrafted. The median period to reach a neutrophil count of $>0.5 \times 10^6$/l and platelet count of 50×10^9/l was 15 days (range, 5–41 days) and 25 days (range, 11–504 days), respectively, for evaluable patients. There was no

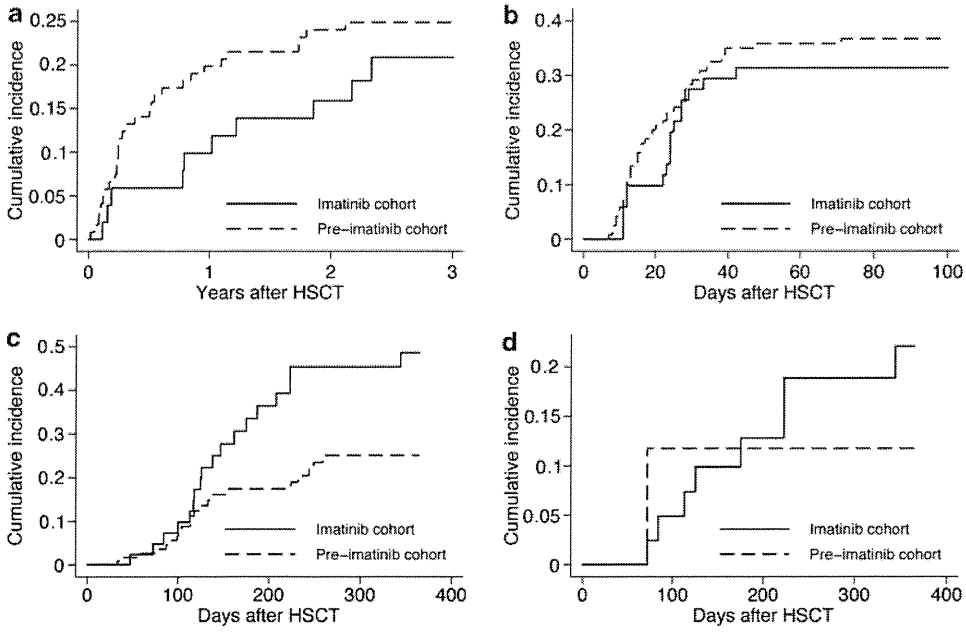

Figure 2 Cumulative incidence of GVHD or NRM. (**a**) Non-relapse mortality, (**b**) Grade 2–4 acute GVHD, (**c**) chronic GVHD and (**d**) extensive-type chronic GVHD.

Table 3 Impact of overall cGVHD on OS, DFS and relapse in multivariate analysis using cGVHD as a time-varying covariate

Cohort	*OS*			*DFS*			*Relapse*		
	Relative risk	*95% CI*	P	*Relative risk*	*95% CI*	P	*Relative risk*	*95% CI*	P
Imatinib cohort	0.80	(0.26–2.51)	0.707	0.59	(0.21–1.71)	0.332	0.74	(0.15–3.67)	0.713
Pre-imatinib cohort	0.59	(0.35–1.00)	0.048	0.73	(0.43–1.23)	0.234	0.75	(0.39–1.44)	0.388

Abbreviations: CI, confidence interval; cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; HLA, human leukocyte antigen; OS, overall survival; PBSC, peripheral blood stem cell.
Data were adjusted for age categories, donors from unrelated subjects, HLA-matching status, PBSC graft and days to transplantation. Cox proportional hazard models were applied to OS and DFS, and a competing risk regression model was applied to relapse.

significant difference in neutrophil and platelet recovery between two cohorts ($P=0.201$ and 0.783, respectively).

Discussion

This study showed that patients with Ph + ALL who achieved CR by imatinib-based therapy and subsequently received allo-HSCT in their first CR showed significantly superior survival outcome to those in the pre-imatinib era. To our knowledge, our current report is the first to describe the superiority of imatinib-based therapy for this disease by analyzing a substantial number of patients with sufficient follow-up period. The treatment of Ph + ALL has changed dramatically since the introduction of imatinib and >90% of patients have achieved CR,[7,14,15] and allows SCT to be performed in a substantial proportion of patients in major or complete molecular remission.[8,16–18] Actually, in the imatinib cohort, 97 of 100 patients (97%) achieved CR and 60 (60%) could receive allo-HSCT in their first CR. Several studies reported improved OS rates compared with that in the pre-imatinib era by incorporation of imatinib-based therapy.[14,15,19,20] However, there had been few reports focusing on the clinical impact of pre-transplant imatinib administration on the outcome of HSCT. Lee *et al*.[8] reported superior outcome of HSCT by imatinib-based therapy compared with the historical control data, in which 29 patients with prior imatinib treatment showed better outcomes in terms of relapse, DFS and OS than the historical control patients. However, their comparative analysis included patients who received HSCT for refractory disease or beyond their first CR (4 of 29 patients in the imatinib group and 16 of 33 patients in the historical group). Several studies showed that remission status at the time of HSCT was one of the most important prognostic factors for outcome.[21,22] Therefore, we contend that it would be better to assess a greater number of patients and exclude patients with advanced stage at HSCT to accurately compare the clinical impact of imatinib-based therapy on the outcome of HSCT. To our knowledge, this study has the largest number of Ph + ALL patients receiving allo-HSCT in their first CR with the longest follow-up duration yet reported.

It is noteworthy from our findings that a lower rate of relapse was found in the imatinib cohort. Our results thus suggest that an imatinib-based therapy provides a survival benefit for newly diagnosed Ph + ALL patients by lowering the rate of subsequent relapse after HSCT. Despite the lack of comparative data of MRD in the pre-imatinib cohort, 75% of patients in the imatinib cohort achieved RQ–PCR negativity for *BCR/ABL* at the time of HSCT. Moreover, the relapse rate was significantly lower among

patients with PCR negative. From these, we believe that a powerful anti-leukemia activity of the imatinib-based therapy mostly contributed to the prevention of subsequent relapse after HSCT in the present analysis. Thinking of the reduced relapse rate after HSCT, impact of cGVHD should also be considered. Several studies in the pre-imatinib era reported beneficial impact of cGVHD on relapse incidence and survival.[23–25] In this study, the incidence of cGVHD was significantly higher in the imatinib cohort compared with that in the pre-imatinib cohort. In the imatinib cohort, more patients received PB as a stem cell source, which might have contributed to the high frequency of cGVHD. Besides, longer leukemia-free survival period in the imatinib cohort might have contributed to the increased frequency of cGVHD, which is a late complication often observed in the recipients of allo-HSCT who had survived without disease for at least 3 months after transplantation. One could argue that this observation could be related to a stronger graft versus leukemia effect and contribute to the lower relapse rate. However, the presence of cGVHD had no significant impact on the OS/DFS/relapse rate in our imatinib cohort by multivariate analysis.

To assist the proper interpretation of our current results, the strengths and limitations need to be considered. As discussed earlier, one of the strengths of this study is the large sample size for the imatinib cohort, which gives us a better estimation of the end points and also adds statistical power to the analyses. In addition, adjustments for potential confounders in the comparisons with the pre-imatinib cohort from a nationwide registry allow unbiased estimates to be made, at least in Japan. Given the evidence for a substantial impact of imatinib in Ph + ALL patients,[7,14–16] it is unrealistic to conduct a prospective study comparing treatments with or without imatinib. Hence, a retrospective cohort design could be suboptimal to address the key questions.

One of the possible limitations of our current analysis could be the presence of residual confounding factors both of known and unknown. Among the known factors, a difference in the conditioning regimens could be noted. The City of Hope National Medical Center reported a favorable result from the use of a fractionated TBI–etoposide regimen in the treatment of Ph + ALL.[26] However, in the comparative analysis, the clinical advantage of this approach seemed to be established mostly among patients transplanted in their second CR.[27] Moreover, this approach was commonly applied in our pre-imatinib cohort rather than in the imatinib cohort (22 and 4%, respectively). Differences in GVHD prophylaxes should also be considered. Tacrolimus was more frequently used in the imatinib cohort than in the pre-imatinib cohort, which reflects the change in practice within the field of allo-HCT in Japan as tacrolimus was widely used for unrelated allo-HSCT since 2000. Nevertheless, the lack of any differences in the incidence of aGVHD between two cohorts indicates that this factor had minimal impact in our analysis.

It may be argued that the improved outcome of the imatinib cohort have been influenced by the pre-transplant chemotherapy in the JALSG Ph + ALL 202 study. Although detailed information on the pre-transplant chemotherapy in the pre-imatinib cohort was not available, it was clear that the majority of patients were most likely treated by the JALSG ALL93 or JALSG ALL97 protocols as pre-transplant chemotherapy,[2] as these were widely used regimens in Japan at the time. The chemotherapeutic regimen in the JALSG Ph + ALL202 study was similar to those used in these protocols. Thus, the effectiveness on Ph + ALL would have been similar between the two cohorts. At least in JALSG, there had been neither remarkable progress in the chemotherapy of Ph + ALL until the clinical introduction of imatinib, nor in other groups including the MD Anderson Cancer Center.[28] Thus, in the present analysis, the influence of pre-transplant chemotherapy appears to be quite limited.

The difference of transplant year between the two cohorts (1995–2001 and 2002–2005, respectively) could have affected the outcome of HSCT, and the improvement of transplantation procedure might have contributed to the favorable outcome in the imatinib cohort. However, Nishiwaki *et al.*[29] analyzed the clinical outcome of 641 Japanese patients with Ph-negative ALL who had received allo-HSCT in their first CR in 1993–1997, 1998–2002 and 2003–2007, and reported that there was no statistical difference in OS and NRM between three periods. In this study, the incidence of NRM was lower in the imatinib cohort, but did not reach the statistical significance. Therefore, the influence of transplantation year is thought to be limited in this study.

Considering potential benefit by imatinib, the lack of information about post-transplant imatinib use in the pre-imatinib cohort might have led us to underestimate the difference between two cohorts.

In conclusion, we have found that there is a significant improvement in the OS and DFS of Ph + ALL patients who received allo-HSCT following imatinib-based therapy. Although further validation using larger cohorts from different populations is essential to confirm our findings, imatinib-based therapy is likely to be a useful strategy for not only giving patients with Ph + ALL more chance to receive allo-HSCT, but also for improving their outcome after allo-HSCT.

Conflict of interest

Dr Naoe has received research funding and honoraria from Novartis Japan. Dr Ohnishi has received research funding from Novartis Japan. Dr Miyazaki has received honoraria from Novartis Japan. The remaining authors declare no conflict of interest.

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tions, and the germline sets (A and C) have the advantage of not requiring DNA sequencing or specific custom-made primers.

Two DNA MRD markers with high sensitivity (at least 10^{-4}) are generally required in MRD intervention clinical trials,[1,9] and in a large cohort of 2854 pediatric precursor B ALL patients, 20% of patients had only one sensitive marker and 8% had none.[9] Four of the 16 cases evaluated in this study had only one sensitive Ig/TCR marker so that availability of *IKZF1*-based MRD testing would have been useful for their risk stratification. Using routine PCR, *IKZF1*Δ3–6 rearrangements were identified in 6% of ALL patients in the ANZCHOG cohort in this study, so inclusion of this marker in standard screening for MRD targets would be an easy way to provide more patients with two sensitive markers.

The concept of using disease-related markers for MRD testing has been already established for fusion transcripts such as BCR-ABL and for gene rearrangements such as for *SIL-TAL1* in T-ALL and for *MLL* rearrangements in infant ALLs.[10] Kuiper *et al.*[4] in an analysis of paired diagnosis and relapse samples from 34 patients found *IKZF1* deletions and nonsense mutations in 14 (41%) patients at diagnosis and showed that all were conserved at relapse, in contrast to other recurrent genetic lesions found at diagnosis such as *PAX5*, *CDKN2A* and *EBF1*. It is therefore likely that this *IKZF1* marker will be at least as stable as Ig/TCR rearrangements, although this will need to be confirmed in more extensive studies.

In summary, we have assessed three ways to measure MRD levels by RQ-PCR for the most common deletion of the *IKZF1* gene found in ALL and demonstrated that all three methods provided robust and sensitive MRD assays for patients with this arrangement. The two primer and probe sets based on germline sequences could be used within a few days of diagnosis to provide quantitative measures of very-early responses to therapy. We expect that *IKZF1* gene deletions (*IKZF1*Δ3–6 and probably others) will provide a useful addition to the repertoire of MRD markers currently available for monitoring MRD in ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Prognostic factors for acute myeloid leukemia patients with t(6;9)(p23;q34) who underwent an allogeneic hematopoietic stem cell transplant

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is often selected as a curative treatment strategy for acute myeloid leukemia (AML). In particular, AML patients with poor cytogenetics at diagnosis are considered for allo-HSCT as the first-line therapy.[1–3] Recently, we have reported that AML with the t(6;9)(p23;q34) abnormality, which predicts a very poor prognosis in patients treated with chemotherapy,[4] is associated with an

outcome in patients receiving allo-HSCT that is comparable to that in patients with a normal karyotype.[5] However, 55% of the AML patients with t(6;9)(p23;q34) eventually had a negative outcome. We herein performed a further analysis for AML patients with t(6;9)(p23;q34) who received allo-HSCT to identify the prognostic factors affecting their overall survival (OS).

A total of 64 *de novo* AML patients with t(6;9)(p23;q34) detected in G-band staining at diagnosis, who received their first allo-HSCT between January 1996 and December 2007, were extracted from the databases of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Cord Blood Bank Network. The cytogenetic data were analyzed according to the Southwestern Oncology Group criteria for each institution, instead of by central review.[2] The clinical data were collected using a standardized report form, which was submitted at 100 days, 1 year and annually after HSCT. This study was approved by the Committee for Nationwide Survey Data Management of the JSHCT. Written informed consent was obtained in accordance with the Declaration of Helsinki. The OS was defined as the number of days from HSCT until death from any cause. Non-relapse mortality (NRM) was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. The analysis was performed using the R version 2.13.0 software program (R Foundation for Statistical Computing; www.r-project.org).[6] The probability of OS was calculated using the Kaplan–Meier method and compared using the log-rank test. The probabilities of transplant related mortality and disease relapse were compared using the Grey test[7] and were analyzed using the cumulative incidence analysis,[6] while considering relapse and death without disease relapse as respective competing risks. The following variables related to the survival of the adult patients older than 15 years and their clinical data were compared in a univariate analysis: recipient characteristics (age; younger than 35 vs. older than 35 years, gender, performance status at diagnosis; 0 to 2 vs. 3 or 4, FAB classification; M2 or others, positivity for peroxidase in leukemic blasts at diagnosis; less than 50% vs. greater than 50%, cytogenetic abnormality), donor characteristics (age; younger than 35 vs. older than 35 years, gender, sex compatibility, compatibility of cytomegalovirus antibody serostatus, relationship; related vs. unrelated, and ABO compatibility), transplant characteristics (disease status at HSCT; complete remission (CR) vs. non-CR, use of total body irradiation as a preconditioning regimen, source of the graft; bone marrow, peripheral blood stem cell, cord blood (CB)), graft-versus-host disease prophylaxis; cyclosporine versus tacrolimus and the use of methotrexate. Multivariate Cox models were used to evaluate the hazard ratios associated with the prognosis. Covariates found to be significant in the univariate analyses ($P \leqslant 0.10$) were included in the models. For both the univariate and the multivariate analyses, *P*-values were two sided, and outcomes were considered to be significant for $P \leqslant 0.05$.

The characteristics of the 64 AML patients with t(6;9)(p23;q34) were shown in Table 1a. The OS of the seven pediatric patients younger than 14 years old seemed to be better than the OS of the 57 adult patients older than 15 years, although there were no statistically significant differences between the groups (Figure 1a, the probability of 3-year OS in pediatric patients and adult patients was 83% and 48%, respectively ($P = 0.12$)). We performed a further analysis in the 57 adult patients older than 15 years. The univariate analysis showed that the disease status at HSCT was the sole significant prognostic factor affecting the OS (Figure 1b, the probability of 3-year OS in patients with CR and with non-CR at HSCT was 69% and 29%, respectively ($P < 0.003$)), and the number of HLA disparities, M2 in the FAB classification and CB as the source of the graft were calculated to have a *P*-value < 0.1 (Table 1b). No statistically significant tendencies related to gender, gender mismatch between the donor and recipient, recipient cytomegalovirus serostatus or the use of total body irradiation for the preconditioning regimen were observed. The cumulative incidence of relapse and of NRM are shown in Figure 1c; the cumulative incidence of relapse was significantly lower in patients with a CR at HSCT than in patients without CR, although such differences were not seen in the cumulative incidence of NRM between these two groups (the 3-year cumulative incidence of relapse was 25% in CR patients and 58% in non-CR patients ($P = 0.005$), and the 3-year cumulative incidence of NRM was 10% in CR patients and 16% in non-CR patients ($P = 0.85$)). In the multivariate analysis, the disease status at HSCT and FAB-M2 remained the significant variables associated with the OS (Table 1b). The OS of the patients categorized by the combination of the disease status at HSCT and FAB-M2 showed a favorable outcome in FAB-M2 patients with a CR at HSCT (Figure 1d, the probability of 3-year OS in patients with CR/FAB-M2, CR/non-FAB-M2, non-CR/FAB-M2 and non-CR/non-FAB-M2 was 76%, 60%, 43% and not reached, respectively ($P < 0.001$)). In contrast, the patients who

Table 1a. Characteristics of patients with t(6;9)(p23;q34)

	Children (n = 7)	*Adult* (n = 57)
Age, median (range)	9 (6–14)	35 (17–58)
Gender, male/female	1/6	34/23
FAB classification[a]		
M0	0	1
M1	0	7
M2	5	32
M4	1	13
M5	1	2
Status at HSCT, CR/non-CR	5/2	29/28
HLA disparity[b]		
0	2	24
1	2	5
2	0	10
Graft source		
BM	3	32
PBSC	2	12
CB	2	13

Abbreviations: BM, bone marrow; CB, cord blood; CR, complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; PBSC, peripheral blood stem cells. [a]Data not available in 2 adult patients. [b]Data not available in 3 pediatric patients and 18 adult patients.

Table 1b. Prognostic factors affecting overall survival of adult patients with t(6;9)(p23;q34)

Variables	*Risk factors*	*Univariate*	*Multivariate*		
			HR	*95% CI*	*P-value*
Disease status at HSCT	CR Non-CR	<0.003	1 2.54	1.17-5.51	<0.02
FAB classification	M2 other than M2	0.075	1 3.61	1.59–8.21	<0.003
Number of HLA disparity	0 1 2	0.061		NA	
Source of the graft	BM or PBSC CB	0.076		NA	

Abbreviations: BM, bone marrow; CB, cord blood; CI, confidence interval; CR, complete remission; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; NA, not assessed; PBSC, peripheral blood stem cell.

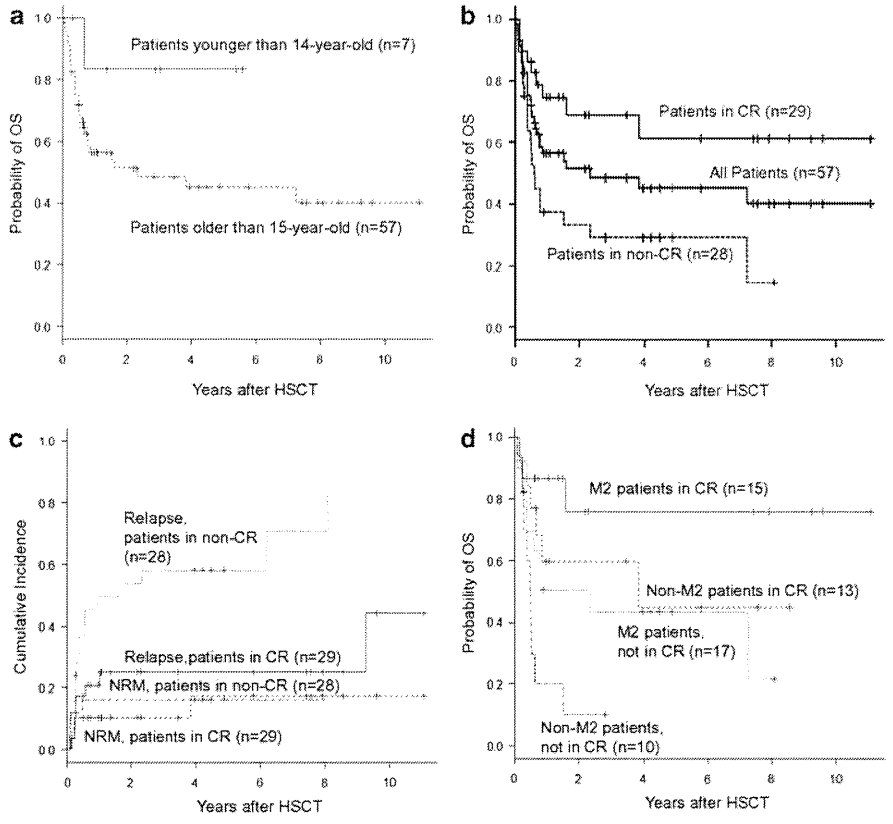

Figure 1. (**a**) The probabilities of OS in the patients with the t(6;9)(p23;q34) abnormality, stratified by age at HSCT. Solid line, pediatric patients younger than 14 years; dotted line, adult patients older than 15 years. (**b**) The probabilities of OS of the patients older than 15 years, stratified by the disease status at HSCT. Bold line, all patients; solid line, patients in CR; dotted line, patients in non-CR. (**c**) The cumulative incidence of events after allo-HSCT stratified by the disease status at the time of HSCT. Solid line, cumulative incidence of relapse of the patients in CR; dashed line, cumulative incidence of NRM of the patients in CR; dotted line, cumulative incidence of relapse of the patients in non-CR; chain line, cumulative incidence of NRM of the patients in non-CR. (**d**) The probability of OS of the patients grouped according to the FAB classification and the disease status at HSCT. Solid line, FAB-M2 patients in CR; dashed line, non-FAB-M2 patients in CR; dotted line, FAB-M2 patients in non-CR; chain line, non-FAB-M2 in non-CR.

were not in remission at the time of HSCT and had non-FAB-M2 showed a poorer outcome; the cause of death in six out of the nine patients was due to a relapse of the AML.

The characteristics of the patients with the t(6;9)(p23;q34) subtype of AML were known to have a poor prognosis and to be associated with development at a younger age, frequent M2 in the FAB classification and achievement of a morphological first CR not predicting a favorable outcome.[8] In this study, we distinguished the seven pediatric patients who seemed to have a superior OS from the adult patients, because the better prognosis in the children might reflect differences in the pathogenesis of the disease, consistent with the better OS in the previous report.[4] The current study revealed that the cumulative incidence of relapse was significantly worse in patients without CR than in patients with CR, although the cumulative incidence of NRM was comparable between these two groups. These results indicate that it is important to have an appropriate treatment strategy, that is, allo-HSCT for the patients who achieved first CR is imperative, while the development of an effective treatment for the refractory/relapsed AML patients is critical. The presence of FLT3-ITD is recognized as a poor prognostic factor in AML patients.[9] As FLT3-ITD was frequently detected in patients with t(6;9)(p23;q34),[4] it has been suggested that the presence of FLT3-ITD might contribute to the poor prognosis of the t(6;9)(p23;q34) patients.[10] With regard to the rate of FLT3-ITD−positive disease, there was no apparent between-group differences in the FAB classification;[11] however, the expression levels of FLT3 were higher in patients with monocytic AML (M4 and M5 in the FAB classification) than in the other patients,[12] and were associated with an unfavorable prognosis.[13] The current study has distinguished FAB-M2 from non-M2, and two-thirds of the non-M2 cases ($n = 23$) in this study consisted of monocytic AML (the number of M4 patients and M5 patients was 13 and 2, respectively). Therefore, the poor prognosis of the non-FAB-M2 patients might be due to the presence of FLT3-ITD. Unfortunately, we could not confirm this hypothesis because this retrospective analysis did not examine the presence of FLT3-ITD. Future studies will be needed to determine whether the FLT3-ITD status was responsible for the poor prognosis in these patients.

In conclusion, this study showed that a CR at the time of HSCT and M2 in the FAB classification are favorable prognostic factors in AML patients with t(6;9)(p23;q34). However, refractoriness to chemotherapy remains an obstacle to a favorable allo-HSCT outcome, especially in non-M2 patients. Novel therapeutic approaches, such as immunotherapy using anti-FLT antibodies combined with HSCT, may also be required for patients expected to have a poor prognosis.[14,15]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Polymorphisms in xenobiotic transporters *ABCB1*, *ABCG2*, *ABCC2*, *ABCC1*, *ABCC3* and multiple myeloma risk: a case–control study in the context of the International Multiple Myeloma rESEarch (IMMEnSE) consortium

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Multiple myeloma (MM) is a hematological neoplasm that arises from a single clone of malignant plasma cells in the bone marrow. In Europe, 4.6/100 000 males and 3.2/100 000 females every year develop MM, with a median age at diagnosis around 60 years.[1]

The observation of a higher risk to develop MM among first-degree relatives of MM patients in several population-based case–control studies supports the idea that genetic factors are involved in MM pathogenesis.[2] Therefore, several studies focusing on various genes and pathways have tried to identify single-nucleotide polymorphisms (SNPs) associated with the susceptibility to the disease.[3,4]

The detoxification and elimination of xenobiotic compounds is one of the most investigated processes in cancer susceptibility, with several evidences of its association with cancer risk.[5] ATP-binding cassette (ABC) subfamily B, member 1 (*ABCB1* or *MDR1*); subfamily G, member 2 (*ABCG2* or *BCRP*); subfamily C, member 2 (*ABCC2* or

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Long-term outcome and prognostic factors of elderly patients with acute promyelocytic leukemia

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Studies focused on elderly acute promyelocytic leukemia (APL) are relatively limited. To evaluate prognostic impact in elderly APL, we compared the long-term outcome of elderly APL patients (60–70 years) with younger patients (15–59 years) treated with *all-trans* retinoic acid combined with anthracycline and cytarabine in the Japan Adult Leukemia Study Group (JALSG) APL97 study. Of 283 evaluable patients, 46 (16.3%) were elderly who had more frequent lower platelet ($P = 0.04$), lower albumin ($P = 0.006$) and performance status 3 ($P = 0.02$), higher induction death rate due to differentiation syndrome ($P = 0.03$), and non-relapse mortality (NRM) during consolidation therapy ($P = 0.001$). Overall survival was significantly inferior in elderly patients ($P = 0.005$), but disease-free survival and cumulative incidence of relapse were not. Better therapeutic approaches should be considered to reduce NRM during induction and consolidation therapy in elderly APL. This study was registered at http://www.umin.ac.jp/ctrj/ under C000000206. (*Cancer Sci* 2012; 103: 1974–1978)

All-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) has dramatically improved the clinical outcome of acute promyelocytic leukemia (APL).[1–6] However, studies focused on elderly APL are relatively limited, and optimal therapeutic approaches for these patients remain to be explored.[7] While the European APL Group (EAG) and Gruppo Italiano Malattie Ematologiche dell' Adulto (GIMEMA), by using ATRA combined with anthracycline ± cytarabine, demonstrated that survival rate of elderly APL was lower than that of younger patients,[8,9] Programa de Estudio y Tratamiento de las Hemopatı'as Malignas (PETHEMA) reported there was no significant difference between them.[10] However, only the EAG report compared directly the clinical characteristics and outcomes between two age groups,[8] and the clinical characteristics and outcomes between them have not been well elucidated in the treatment of APL.

Here we report the long-term outcome and prognostic factors of elderly APL patients who were treated in the Japan Adult Leukemia Study Group (JALSG) APL97 study,[11] by comparing data between the elderly and the younger APL.

Materials and Methods

Patients. Adult patients with previously untreated APL were registered into the JALSG APL97 study between May 1997 and June 2002.[11] Eligibility criteria were: (i) diagnosis of APL with t(15;17) and/or the *PML-RARA* fusion gene amplified by RT-PCR; (ii) age between 15 and 70 years; (iii) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–3; and (iv) sufficient functioning of the heart, liver and kidney. This study was approved by the institutional review boards of each participating institution. Informed consent was obtained from patients before registration in the study in accordance with the Declaration of Helsinki. All data were updated in January 2009.

Study design and treatments. The detail of treatment schedule has been described previously.[11] Briefly, remission induction therapy consisted of ATRA and chemotherapy with idarubicin and cytarabine, with dose and duration determined by initial leukocyte count. After obtaining complete remission (CR) and receiving three courses of intensive consolidation chemotherapy, patients negative for *PML-RARA* were randomly allocated either to receive six courses of intensified maintenance chemotherapy or to observation. Patients, who were positive for the *PML-RARA* fusion transcript, received late ATRA therapy followed by maintenance therapy, and were scheduled to receive allogeneic hematopoietic stem cell transplantation (HSCT) if they had a human leukocyte antigen-identical donor. Elderly patients were treated with the same schedule to younger patients. For prevention of hemorrhage, platelets were transfused to maintain the platelet count above 30×10^9/L, and fresh frozen plasma was transfused to maintain the plasma fibrinogen level above 1.5 g/L. Anticoaglants were used according to the discretion of institutions. Differentiation syndrome (DS) was treated with methylprednisolone and transient discontinuation of ATRA.

Definition and evaluation of patients. In this analysis, we set the cut-off age of 60 years to compare our data with previous reports on elderly APL over 60 years old.[5,6,8–10] Hematological response was evaluated by standard criteria generally used for chemotherapy.[12] Molecular relapse detected by RT-PCR analysis of *PML-RARA* was also considered as a relapse. The primary end point of the JALSG APL97 study was overall survival (OS) and disease-free survival (DFS) of patients achieved CR. Overall survival was calculated from the first day of therapy to death or last visit. Disease-free survival was measured from the date of CR to relapse, death from any cause or last

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visit. OS and DFS in patients who were randomized to either observation or maintenance chemotherapy groups were also measured from the date of randomization to the same end points mentioned above.

Statistical analysis. Categorical data were compared using χ^2 test or Fisher's exact test. Continuous data were compared using Wilcoxon rank-sum test. Overall survival and DFS were estimated by Kaplan–Meier methods and compared by the log-rank test. Cumulative incidence of relapse (CIR) was measured from the date of CR to the first relapse, while non-relapse mortality (NRM) was censored as a competing risk event. Gray's test was used to compare the cumulative incidence curves. Univariate and multivariate Cox proportional hazard analyses were performed to determine prognostic indicators of OS. Prognostic variables of univariate significance were selected for inclusion in the multivariate model. Statistical analyses were performed using SPSS 11.0 (SPSS Inc, Chicago, IL, USA) and R 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org/). All hypothesis testing was two-tailed with a significance level of 0.05.

Results

Patient characteristics. Of 302 patients registered, 283 (median age, 48 years; range, 15–70) were evaluated. Nineteen patients were excluded: four misdiagnosis, two inconsistent eligibility, seven negative for t(15;17) and six no test for t(15;17) or *PML-RARA*. The median follow-up period was 7.7 years. Forty-six patients (16.3%) were elderly (median, 63 years; range, 60–70), and 237 were younger (median, 44 years; range, 15–59). Lower platelet count ($<10 \times 10^9$/L), lower serum albumin level (<3.5 g/dL) and PS 3 were significantly more frequent in elderly patients than in younger ($P = 0.04$, $P = 0.006$ and $P = 0.02$, respectively). The distribution of

Table 1. Clinical features of acute promyelocytic leukemia (APL) patients according to age

Parameters	Total		Age (15–59 years)		Age (60–70 years)		*P*
	n (%)	Median (range)	*n* (%)	Median (range)	*n* (%)	Median (range)	
No. patients	283		237		46		
Gender							
Male	158 (56)		128 (54)		30 (65)		0.2
Female	125 (44)		109 (46)		16 (35)		
Leukocyte count, $\times 10^9$/L							0.83
<3.0	174 (61)	1.7 (0.03–257)	143 (60)	1.8 (0.03–152)	31 (67)	1.4 (0.5–257)	0.39
3.0–10.0	58 (21)		52 (22)		6 (13)		
10.0 or higher	51 (18)		42 (18)		9 (20)		
APL cell count, $\times 10^9$/L							0.91
<1.0	238 (84)	0.6 (0–253)	201 (85)	0.6 (0–143)	37 (80)	0.5 (0–253)	0.51
1.0–3.0	23 (8)		20 (8)		3 (7)		
3.0 or higher	19 (7)		14 (6)		5 (11)		
Platelet count, $\times 10^9$/L							0.28
<10	39 (14)	30 (2–238)	28 (12)	30 (2–238)	11 (24)	30 (5–139)	0.04
10–40	140 (49)		119 (50)		21 (46)		
40 or higher	104 (37)		90 (38)		14 (30)		
ECOG performance status score							
0–2	262 (92)		223 (94)		39 (85)		0.02
3	19 (7)		12 (5)		7 (15)		
Albumin level, g/dL							0.89
<3.5	23 (8)	4.2 (2.0–6.1)	14 (6)	4.2 (2.0–6.1)	9 (20)	4.2 (2.3–5.0)	0.006
3.5 or higher	247 (87)		211 (89)		36 (78)		
DIC score*							
0–2	23 (8)		19 (8)		4 (9)		0.97
3–9	215 (76)		182 (77)		34 (74)		
10 or higher	27 (10)		21 (9)		5 (11)		
FAB subtype							
Typical	265 (94)		220 (93)		45 (98)		0.32
Variant	18 (6)		17 (7)		1 (2)		
CD56 expression							
Positive	23 (8)		20 (8)		3 (7)		0.69
Negative	216 (76)		181 (84)		35 (76)		
CD34 expression							
Positive	40 (14)		30 (13)		10 (22)		0.09
Negative	217 (77)		187 (79)		30 (65)		
Past history of malignant disease	11 (4)		10 (4)		1 (2)		0.51
Infectious complications at diagnosis	72 (24)		58 (24)		14 (30)		0.46
Relapse risk (Sanz risk score)							
Low	88 (31)		75 (32)		13 (28)		0.88
Intermediate	145 (51)		121 (51)		24 (52)		
High	50 (18)		41 (17)		9 (20)		

*DIC score[13]; score 3 indicates suspected DIC; score 4–10, definitive DIC; 10 or more, severe DIC. ECOG, Eastern Cooperative Oncology Group; FAB, French-American-British.

Sanz's relapse risk score[14] was not different between the two groups ($P = 0.88$) (Table 1).

Treatment outcome. Elderly patients tended to have lower CR rate and higher incidence of early death during induction therapy (89% *vs* 96%, $P = 0.06$; 11% *vs* 4%, $P = 0.08$; Table 2).

Primary resistant to induction therapy was observed in one patient in the younger group, which was not observed in the elderly patients. Differentiation syndrome was the most frequent cause of early death (4%) in elderly patients, followed by hemorrhage (2%) and infection (2%). Early death rate due to DS was higher in elderly patients (4% *vs* 0%, $P = 0.03$), while DS incidence was similar between two groups ($P = 0.76$). Non-relapse mortality during consolidation therapies was significantly more frequent in elderly patients (13% *vs* 2%, $P = 0.001$), and all were associated with infection (Table 2). Median duration for the recovery of leukocyte over 1.0×10^9/L was significantly longer in elderly patients compared with younger patients in the second consolidation cycle (25 *vs* 22.5 days, $P = 0.03$), and granulocyte colony stimulating factor (G-CSF) was more frequently administered to elderly patients during the first and second cycles (39.5% *vs* 19.6%, $P = 0.007$ and 43.2% *vs* 25.2%, $P = 0.02$, respectively).

Table 2. Cause of death between the elderly and younger acute promyelocytic leukemia (APL) patients

		Age (15–59 years)	Age (60–70 years)	*P* value
		n = 237	*n* = 46	
During induction therapy (*n* = 283)	*n*	*n* (%)	*n* (%)	
Death during induction therapy	15	10 (4)	5 (11)	0.08
Hemorrhage	9	8 (3)	1 (2)	1
Infection	1	0	1 (2)	0.16
Differentiation syndrome	2	0	2 (4)	0.03
Others	3	2 (0.8)	1 (2)	
During consolidation therapy (*n* = 258)		*n* = 222	*n* = 36	
Death during consolidation therapy	10	5 (2)	5 (13)	0.001
Death during C1 therapy	0	0	0	
Death during C2 therapy	4	2	2	
Infection	4	2 (1)	2 (5)	0.1
Death during C3 therapy	6	3	3	
Infection	6	3 (1)	3 (9)	0.04
Post-consolidation therapy (*n* = 258)		*n* = 222	*n* = 36	
Death post-consolidation therapy	35	28 (13)	7 (19)	0.27
After relapse	25	21	4	
Death related to salvage therapy (including HSCT)		11 (5)	1 (3)	
Death related to APL		7 (3)	3 (8)	
Hemorrhage		1 (0.5)	0	
Acute myocardial infarction		1 (0.5)	0	
Secondary leukemia		1 (0.5)	0	
APL in CR	10	7	3	
Secondary leukemia		2 (1)	0	
Pneumonia		1 (0.5)	0	
Unknown		4 (2)	3 (8)	

CR, complete remission; HSCT indicates hematopoietic stem cell transplantation.

Ten-year OS was significantly inferior in elderly patients (63% *vs* 82%, $P = 0.005$) (Fig. 1a). In the previous analyses of the JALSG APL97 study, the predicted 6-year OS in patients with or without the intensified maintenance therapy was 86.2% and 98.8%, which was significantly different ($P = 0.014$).[11] Therefore, we additionally analyzed the influence of age on the long-term outcome between patients with or without the intensified maintenance therapy. In younger patients, 10-year OS in the intensified maintenance therapy group was significantly inferior compared to the observation group (81% *vs* 97%, $P < 0.001$), while OS in the elderly group was not significantly different between patients with and without the intensified maintenance therapy. (86% *vs* 92%, $P = 0.72$). 10-year DFS and CIR were similar between the elderly and younger patients (65% *vs* 67%, $P = 0.70$; 15% *vs* 28%, $P = 0.15$, respectively) (Fig. 1b,c). Ten-year NRM was significantly higher in elderly patients (20% *vs* 7%, $P = 0.007$) (Fig. 1d). Of 17 deaths among elderly patients, 10 (59%) occurred during induction and consolidation therapies (Table 2). In the multivariate analysis for OS, age (more than 60 years) and PS (score 3) were the independent adverse prognostic variable factors (hazard ratio [HR]: 1.95; 95% confidence interval [CI]: 1.10–3.50; $P = 0.02$; HR: 2.48; 95% CI: 1.16–5.29; $P = 0.02$, respectively) (Table 3).

Discussion

We compared the long-term outcome between the elderly and the younger APL patients who were treated with ATRA combined with anthracycline and cytarabine in the JALSG APL97 study, and extracted prognostic factors in elderly APL patients.

Complete remission rates among elderly APL are reportedly lower than younger patients, due to higher early death.[2,8–10] In our study, early deaths by DS were more frequent in elderly patients. Programa de Estudio y Tratamiento de las Hemopatı′as Malignas reported that increased death due to DS was associated with higher PS score and lower serum albumin level.[15] Our study supported their observation, demonstrating significantly more PS 3 and lower albumin level in elderly patients. While number of cases was limited, we should treat them more carefully in future.

Cumulative incidence of relapse in our study was similar between the two groups, as reported by GIMEMA.[9] However, EAG and PETHEMA demonstrated lower CIR in elderly patients compared to younger patients.[8,10] In their studies, Sanz's low relapse-risk features were more frequent in elderly patients,[8,10] which might explain their lower CIR.

European APL Group also reported that 10 year-OS in elderly patients was lower than that of whole population (58.1% *vs* 77%). The major cause of death in their elderly patients was sepsis during myelosuppression.[16] Gruppo Italiano Malattie Ematologiche dell' Adulto reported that AIDA0493 amended protocol in which the intensity of consolidation therapy was reduced for elderly patients improved 5-year OS (76.1%) and 5-year NRM (7.7%) compared with the original protocol (56% and 13%, respectively).[17] Programa de Estudio y Tratamiento de las Hemopatı′as Malignas, using ATRA combined with anthracycline monochemotherapy, showed 6-year DFS in elderly patients to be 79%, which was similar to younger patients.[10] They showed that NRM in elderly patients (60–83 years) was 9.2%, but only 3.2% in patients aged between 60 and 70 years. In our study, NRM (13%) in elderly APL was higher than those of PETHEMA[10] or GIMEMA[17] studies, and NRM during

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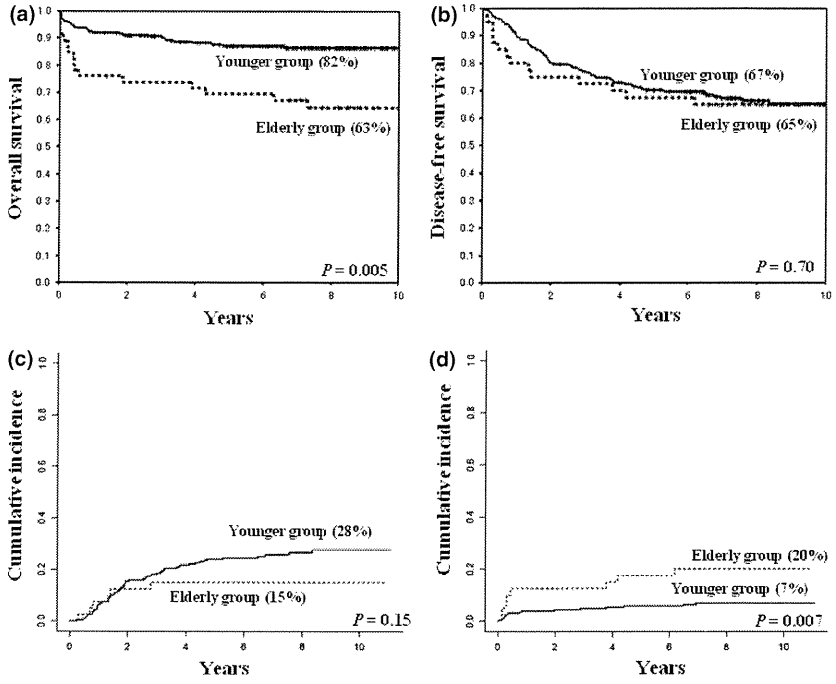

Fig. 1. Overall survival, disease-free survival, cumulative incidence of relapse and non-relapse mortality at 10 years between the elderly and younger acute promyelocytic leukemia (APL) patients. (a) Overall survival (63% *vs* 82%, $P = 0.005$). (b) Disease-free survival (65% *vs* 67%, $P = 0.70$). (c) Cumulative incidence of relapse (15% *vs* 28%, $P = 0.15$). (d) Non-relapse mortality (20% *vs* 7%, $P = 0.007$).

Table 3. Prognostic factors affecting overall survival of acute promyelocytic leukemia (APL) patients

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Initial leukocyte count (more than 10×10^9/L)	1.56 (0.86–2.84)	0.14	1.48 (0.81–2.69)	0.21
Initial platelet count (<10×10^9/L)	1.65 (0.88–3.11)	0.12	1.45 (0.71–2.57)	0.37
Sex (Male)	1.73 (1.01–2.96)	0.05	1.69 (1.01–3.00)	0.05
Albumin (<3.5 g/dL)	1.07 (0.43–2.68)	0.88		
Performance status (score 3)	2.43 (1.15–5.11)	0.02	2.48 (1.16–5.29)	0.02
Age (more than 60 years)	2.20 (1.25–3.85)	0.006	1.95 (1.10–3.50)	0.02

consolidation therapies was the main reason for inferior long-term outcome in elderly patients. On the other hand, our study showed that the difference between OS and DFS in the elderly group was subtle compared to those in the younger group. One of the reasons might be that patients who relapsed in the elderly group died due to APL because no effective salvage therapy was available at that time. In contrast, patients who relapsed in the younger group underwent several effective salvage therapies with or without HSCT.

While the disadvantage of intensified maintenance therapy was shown in the total analysis of JALSG APL97 study,[11] it was not in the analysis focused on elderly patients. We demonstrated that OS in the elderly group was not significantly different between patients with or without the intensified maintenance therapy in this analysis. The frequency of dose reduction in maintenance therapy was not significantly different between elderly and younger groups. (14.2% *vs* 4.9%, $P = 0.44$), and NRM during maintenance therapy was not in the elderly group. Cumulative incidence of relapse in the elderly group was not different between cases with or without the intensified maintenance therapy (0% *vs* 16.7%, $P = 0.27$). These results might be affected by the small number of elderly cases analyzed, because only seven patients (7.9%) of the 89 patients in the maintenance arm were elderly. Further investigation will be required in the issue.

Taking into consideration the factors mentioned above, further refinement will be needed to reduce NRM in elderly patients. Recently, several new agents have been introduced in APL. Arsenic trioxide and gemtuzumab ozogamicin (GO) are reportedly effective and well tolerated by elderly APL.[6,18–20] These agents may be used for elderly patients to reduce the risk of NRM.

In conclusion, elderly APL were more vulnerable to complications such as DS and infection, which resulted in lower OS. Reduction of intensity of induction and post-remission chemotherapy without increasing relapse should be considered. Early intervention against DS and infection may result in lower induction mortality and NRM. ATO and reduced dosage of GO may be incorporated into the therapy for elderly APL.

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Disclosure Statement

The authors have no conflict of interest.

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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Phase 2 study of arsenic trioxide followed by autologous hematopoietic cell transplantation for relapsed acute promyelocytic leukemia

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Key Points

- We conducted a phase 2 study of ATO followed by autologous HCT for relapsed APL.
- This sequential treatment is effective and feasible.

The optimal treatments for relapsed acute promyelocytic leukemia (APL) remain equivocal. We conducted a phase 2 study to evaluate the efficacy and feasibility of a sequential treatment consisting of induction and consolidation with arsenic trioxide (ATO), peripheral blood stem cell (PBSC) harvest after high-dose cytarabine chemotherapy, and autologous hematopoietic cell transplantation (HCT). Between 2005 and 2009, 35 patients (26 with hematologic and 9 with molecular relapse) were enrolled. Induction therapy resulted in complete remission in 81% of those with hematologic relapse, and most patients became negative for *PML-RARα* after the first ATO consolidation course, but 4 remained positive. Administration of the second ATO consolidation course further decreased the transcript levels in 3 patients. In total, 25 patients proceeded to PBSC harvest, all of whom successfully achieved the target CD34+ cell doses, and 23 underwent autologous HCT with *PML-RARα*–negative PBSC graft. Posttransplant relapse occurred in 3 patients, and there was no transplant-related mortality. With a median follow-up of 4.9 years, the 5-year event-free and overall survival rates were 65% and 77%, respectively. These findings demonstrate the outstanding efficacy and feasibility of the sequential treatment featuring ATO and autologous HCT for relapsed APL. This study was registered at http://www.umin.ac.jp/ctr/ as #C000000302. (*Blood.* 2013;121(16):3095-3102)

Introduction

Outcomes for acute promyelocytic leukemia (APL) have improved significantly since the advent of all-*trans* retinoic acid (ATRA), and the recently introduced frontline therapy that combines ATRA and chemotherapy can provide long-term complete remission (CR) for a majority of patients with newly diagnosed APL.[1-6] Nevertheless, relapse still occurs in ~20% of cases, for which arsenic trioxide (ATO) has been shown to provide high CR rates exceeding 80%,[7-9] thus making it a current recommendation for reinduction therapy.[10,11] After returning to CR, autologous or allogeneic hematopoietic cell transplantation (HCT) for consolidating the CR status is generally considered if the patient is eligible for the procedure.[10-12] However, because there have been few prospective studies for this very small patient population, the therapeutic approach after achievement of second or subsequent CR is mostly based on findings from retrospective studies.

In 2005, the Japan Adult Leukemia Study Group (JALSG) initiated a phase 2 study entitled APL205R for patients with relapsed APL. The main purpose of this study was to evaluate the efficacy and feasibility of a sequential treatment consisting of induction and consolidation with ATO, peripheral blood stem cell (PBSC) harvest after chemotherapy using high-dose cytarabine (AraC), and autologous HCT. This report presents and discusses the results of this study.

Methods

Patients

This study enrolled patients with relapsed APL between December 2005 and June 2009. At least a single documentation of cytogenetic and/or molecular evidence of t(15;17)/*PML-RARα* was required at the time of entry. Eligibility criteria consisted of age between 18 and 65 years; an Eastern Cooperative Oncology Group performance status between 0 and 3; and adequate functioning of the liver (serum bilirubin level <2.0 mg/L), kidneys (serum creatinine level <2.0 mg/dL), lungs ($Pao_2 \geq 60$ mm Hg or $SpO_2 \geq 93\%$), and heart (no severe abnormalities detected on electrocardiograms). Patients who

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