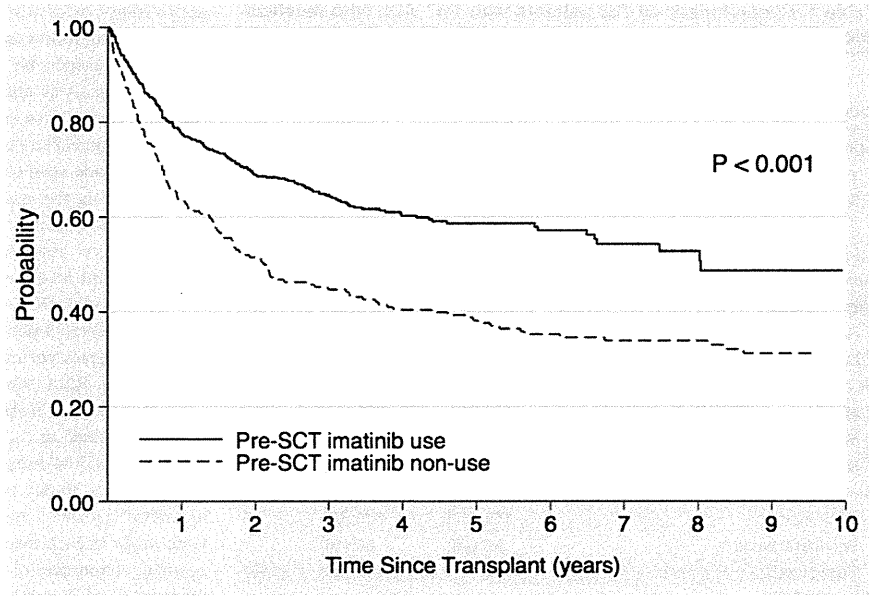

Figure 1. Effects of imatinib administration before stem cell transplantation on the overall survival of patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR period.

HLAs were matched at the A, B, and DRB1 loci, as determined by low-resolution HLA typing in allo-SCT from a related donor or cord blood. For unrelated allo-HSCT, matching at the HLA-A, B, Cw, and DRB1 loci in HLA high-resolution molecular typing was considered matched cases. Mismatches were defined by the presence of ≥1 disparity among these loci. Univariate analysis was performed using Cox regression models or a log-rank analysis. Multivariate analysis was performed using the Cox proportional hazards regression model or the competing risk regression model,[15] as appropriate. Demographic differences among groups were evaluated using the χ^2 or Wilcoxon rank-sum tests as appropriate. All statistical analyses were performed with STATA 11 software (STATA Corp., College Station, TX).

Results

Patient characteristics

The 738 study patients included 402 men and 336 women with a median age of 41 years (range, 16-59 years). HLA matching information was not available for 2 patients. The donor sources included HLA-identical sibling donors (n = 280), unrelated donors (n = 439), and other related donors (n = 19). There were no significant differences between the imatinib and non-imatinib cohorts with respect to gender, PS at allo-HSCT, interval between diagnosis and allo-HSCT, donor-recipient gender match, WBC count at diagnosis, or donor-recipient ABO compatibility, whereas significant differences were observed with respect to the age distribution at allo-HSCT, donor status, HLA disparity, stem cell source, BCR-ABL subtype, conditioning regimen, GVHD prophylaxis, and cytogenetics (Table 1). Of the 196 patients in the non-imatinib cohort, 183 (93%) underwent allo-HSCT between 1990 and 2005. In contrast, 403 of the 542 (74%) patients in the imatinib cohort underwent allo-HSCT between 2006 and 2010.

Outcomes

Overall survival. The median follow-up duration of the allo-HSCT survivors was 1551 days (range, 66-6648 days), and the 3- and 5-year OS rates for all patients were 59% (95% confidence interval [CI], 55-63%) and 53% (95% CI, 49-56%), respectively. The 5-year OS in the imatinib cohort was 59% (95% CI, 54-63%), which was significantly higher than that in the non-imatinib cohort (38%; 95% CI, 31-45%; $P < .001$; Figure 1). Table 2 shows the OS risk factor analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on OS, as revealed by univariate analysis (hazard ratio [HR], 0.56; 95% CI, 0.45-0.70; $P < .001$] and confirmed by multivariate analysis (HR, 0.57; 95% CI, 0.42-0.77; $P < .001$). In addition, age, interval between diagnosis and HSCT, and WBC count at diagnosis were significant prognostic factors for OS in the multivariate analysis.

Relapse. Relapse after allo-HSCT occurred in 116 (21%) and 66 (34%) patients in the imatinib and non-imatinib cohorts, respectively, after median periods of 232 (range, 19-2560 days) and 258 days (range, 42-2350 days), respectively. In the imatinib cohort, the estimated 3-year cumulative incidence of relapse was 23% (95% CI, 20-27%), which was significantly lower than that in the non-imatinib cohort (39%; 95% CI, 31-47%; $P < .001$; Figure 2). Table 3 shows the relapse risk factor analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on relapse, as determined by univariate analysis (HR, 0.52; 95% CI, 0.39-0.71; $P < .001$) and confirmed by multivariate analysis (HR, 0.66; 95% CI, 0.43-0.99; $P = .048$). In addition, the following were significant prognostic factors for relapse: age, 30 to 54 years; HLA disparity; and female-male donor-recipient matching.

NRM. Overall, 207 (38%) patients in the imatinib cohort and 131 (67%) in the non-imatinib cohort died after allo-HSCT within median periods of 178 (range, 8-2935 days) and 177 days (range, 5-4549 days), respectively. Of these, 124 (23%) and 71 (36%) deaths in the former and latter cohorts, respectively, were not related to relapse after allo-HSCT. The major causes of all deaths and their respective frequencies in the imatinib and non-imatinib cohorts were as follows: relapse (40% vs 47%), infection (18% vs 9%), organ failure (12% vs 9%), interstitial pneumonia (6% vs 4%), GVHD (5% vs 9%), transplantation-associated thrombotic microangiopathy (2% vs 2%), bleeding (2% vs 5%), sinusoidal obstruction syndrome (1% vs 5%), and others (13% vs 11%). The estimated cumulative incidence of NRM at 3 years was significantly lower in the imatinib cohort (22%; 95% CI, 18-26%) than in the non-imatinib cohort (30%; 95% CI, 24-37%; $P = .002$; Figure 2). Table 4 shows the NRM risk factor

Table 2. Results of univariate and multivariate analysis of overall survival among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.56 (0.45-0.70)	<.001	0.57 (0.42-0.77)	<.001
Age at SCT (regression)	1.02 (1.01 −1.03)	.002	1.02 (1.01-1.03)	<.001
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	0.89 (0.71-1.11)	.30	0.89 (0.66-1.20)	.430
Stem cell source				
Related bone marrow	1 (Reference)		1 (Reference)	
Unrelated bone marrow	0.81 (0.62-1.10)	.13	0.92 (0.63-1.33)	.640
Related peripheral blood	1.08 (0.79-1.48)	.64	1.27 (0.90-1.78)	.180
Cord blood	0.86 (0.61-1.22)	.39	1.25 (0.78-2.0)	.360
PS at SCT				
0	1 (Reference)	.24	1 (Reference)	
1-4	1.15 (0.91-1.47)		1.05 (0.82-1.36)	.690
Duration from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	1.26 (1.02-1.57)	.03	1.31 (1.03-1.67)	.030
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	0.77 (0.36-1.66)	.51	NA	
Major and minor	0.90 (0.44-1.83)	.78		
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.83 (0.61-1.11)	.21	0.78 (0.57-1.06)	.110
Female-male	0.78 (0.56-1.08)	.13	0.77 (0.55-1.07)	.120
Female-female	0.71 (0.51-0.98)	.03	0.70 (0.50-0.98)	.040
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	0.96 (0.60-1.53)	.87	1.04 (0.64-1.70)	.150
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.29 (1.04-1.61)	.02	1.07 (0.99-1.14)	.053
GVHD prophylaxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	0.78 (0.62-0.98)	.03	0.98 (0.73-1.31)	.899
Cytogenetics				
t(9;22)only	1 (Reference)		1 (Reference)	
Other abnormality	0.97 (0.71-1.34)	.87	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	1.12 (0.83-1.51)	.48	NA	
Major	1.21 (0.93-1.59)	.16	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on NRM, as determined by univariate (HR, 0.65; 95% CI, 0.49-0.88; $P < .001$) and multivariate analyses (HR, 0.55; 95% CI, 0.37-0.83; $P = .005$). Age was also found to be a significant prognostic factor in multivariate analysis.

MRD. Data regarding MRD status before allo-HSCT were available for 67 (34%) patients in the non-imatinib cohort and 400 (74%) patients in the imatinib cohort (Table 1). Among the 467 patients, the MRD negativity rate before allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (64%; 95% CI, 59-69% vs 34%; 23-47%; $P < .001$). The estimated cumulative incidence of relapse at 3 years was significantly lower in the MRD-negative patients than in the MRD-positive patients (20%; 95% CI, 15-25% vs 32%; 95% CI, 25-40%, respectively; $P = .0017$), and this tendency was significant in the imatinib cohort (19%; 95% CI, 15-25% vs 34%; 95% CI, 25-42%, respectively; $P = .0016$), but not in the non-imatinib cohort (27%; 95% CI, 10-48% vs 28%; 95% CI, 15-43%, respectively; $P = .566$). There was no significant difference in NRM between the MRD-negative and MRD-positive patients (19%; 95% CI, 14-24% vs 22%; 95% CI, 16-28% at 3 years, respectively; $P = .0642$).

Discussion

Although many studies have confirmed the beneficial effects of imatinib on the clinical outcomes of patients with Ph$^+$ ALL,[1-6] the potential benefits of pretransplant imatinib administration has not been investigated in a sufficient number of patients. In our study, which is the largest of its type to date, we analyzed the records of 738 patients during a long-term follow-up period to analyze the benefits of pretransplant imatinib administration in patients with Ph$^+$ ALL. We observed significant improvements in the relapse rate and NRM in patients who received imatinib before allo-HSCT compared with those who did not receive imatinib (23% vs 39%; $P < .001$ and 22% vs 30%; $P = .002$, respectively). In the MVA, pretransplant imatinib administration was shown to have a significant favorable effect on both relapse and NRM after allo-HSCT.

Some investigators have reported that MRD before HSCT can serve as a powerful predictor of a lower relapse rate. In an analysis of the outcomes of 95 patients with Ph$^+$ ALL who received pretransplant imatinib-based therapy, Lee et al showed that the strongest predictor of relapse was the patient's MRD status at the end of 2 courses of pretransplant imatinib-based chemotherapy.[16] In the present study, patients who were MRD negative before HSCT had a significantly lower relapse rate after HSCT compared with those who were initially MRD positive (20.0% vs 32%, $P = .0017$), and this tendency was remarkable in the imatinib cohort (19% vs 34%, $P = .0016$). Moreover, the MRD negativity rate for *BCR-ABL* patients before allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (62% vs 37%, $P < .001$). These data suggest that in the imatinib cohort, the powerful antileukemia activity associated with pretransplant imatinib administration extensively decreased the MRD before allo-HSCT and prevented subsequent relapse after allo-HSCT.

The Ph chromosome is an adverse prognostic factor in patients with ALL, and only allo-HSCT offers a curative option for patients with Ph ALL. However, the probability of NRM in patients who undergo transplantation during the initial CR is relatively high; therefore, methods to decrease NRM were investigated. Recently, the UKALLXII/ECOG2993 study confirmed the superiority of allogeneic transplantation over chemotherapy on the basis of prospective outcome data from 267 unselected adult patients and reported that high NRM remained a significant problem in the pre-imatinib era.[17] Patient age, donor status, and HLA disparity are well-known prognostic factors for NRM after allo-HSCT.[1,3,17-19] In the present study, the risk of NRM was significantly lower in the imatinib cohort than in the non-imatinib cohort ($P = .002$), despite the former comprising significantly larger proportions of older recipients and unrelated and/or HLA-mismatched donors ($P < .001$, $P < .001$, and $P = .01$, respectively). Imatinib-based therapy has increased the proportion of patients who achieve sustained remission, thus providing additional

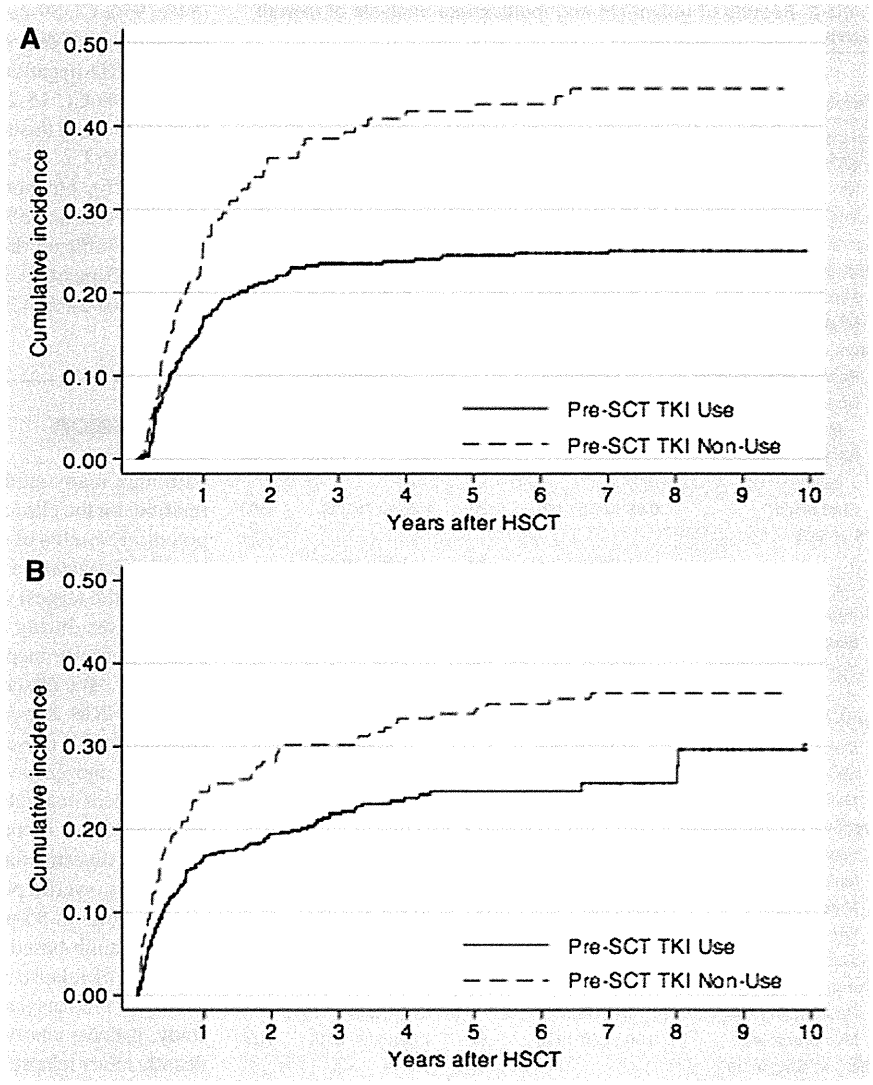

Figure 2. Cumulative incidence of relapse- or nonrelapse-related mortality of patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR period. (A) Relapse mortality. (B) NRM.

time for suitable donor selection and allo-HSCT and enabling individualized treatment approaches.[1-3] These secondary benefits may have contributed to the lower NRM in the imatinib cohort. Moreover, several recent studies have reported improved NRM following the incorporation or dose escalation of imatinib before allo-HSCT.[18-22] Given these findings, we believe that imatinib administration has allowed more patients with Ph$^+$ ALL to undergo allo-HSCT while in a better condition, resulting in the achievement of a lower NRM.

Over the last few decades, there have been many attempts to improve patient outcomes after allo-HSCT, including changes in the conditioning regimens and donor selection and the prophylaxis and treatment of organ complications, GVHD, and infectious diseases. In Japan, the period of 1990 to 2005 marked a pioneering era of cord blood transplantation, during which the relevance of cell doses and HLA matching had not yet been recognized. Laport et al reported their experiences with 79 patients with Ph$^+$ ALL who underwent allo-SCT with matched sibling donors; in these patients, the 5-year OS and NRM were examined according to the decade in which SCT was performed (1985-1995 vs 1996-2005), and no significant difference were observed between these 2 time periods.[23] In Japan, Kurosawa et al used a nationwide registry database of >6000 patients to retrospectively assess changes in the incidence and causes of NRM during 3 consecutive 4-year periods (1997-2000, 2001-2004, and 2005-2008).[24] The authors reported that the incidence of NRM after allo-HCT had significantly decreased during the entire 12-year period, which led to improvements in OS and decreases in NRM in subgroups comprising older patients (50-70 years of age) and/or those who received unrelated bone marrow transplants.[24] According to the present study, patients who underwent allo-HSCT with alternative donors and/or elderly patients would benefit from recent improvements in transplantation procedures, and this progress in transplantation may have partly contributed to the improved NRM in the imatinib cohort.

A strength of the present study was its large sample size; this permitted a more accurate estimation of the end points and added statistical power to the analyses. However, because this was a retrospective multicenter study, our results may be susceptible to the disadvantages of any retrospective study, such as heterogeneity in the treatment strategies selected by the physicians. With regard to patient selection bias, changes in patient selection and transplantation procedures throughout the study period (1990-2010) should also be considered. In Japan, the widespread use of alternative donors after 2000 facilitated the extension of allo-HSCT eligibility. Furthermore, cord blood cells were more frequently used in the imatinib cohort (20%) than in the non-imatinib

Table 3. Results of univariate and multivariate analysis of relapse among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.52 (0.39-0.71)	<.001	0.66 (0.43-0.99)	.048
Age at SCT (years)				
≤29	1 (Reference)		1 (Reference)	
30-54	0.58 (0.42-0.81)	.001	0.63 (0.45-0.89)	.009
≥55	0.60 (0.36-1.03)	.062	0.71 (0.40-1.30)	.250
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	0.52 (0.3-0.74)	<.001	0.48 (0.29-0.80)	.005
Stem cell source				
Related bone marrow	1 (Reference)			
Unrelated bone marrow	0.50 (0.35-0.70)	<.001	0.76 (0.48-1.21)	.251
Related peripheral blood	0.76 (0.50-1.16)	.202	0.91 (0.58-1.42)	.670
Cord blood	0.51 (0.31-0.84)	.008	1.2 (0.58-2.5)	.618
PS at SCT				
0	1 (Reference)		1 (Reference)	
1-4	1.038 (0.75-1.44)	.821	NA	
Days from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	0.91 (.68-1.22)	.538	1.16 (0.84-1.61)	.366
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	0.61 (0.20-1.91)	.400	NA	
Major and minor	1.22 (0.44-3.39)	.703	NA	
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.95 (0.65-1.40)	.790	1.02 (0.69-1.52)	.908
Female-male	0.59 (0.37-0.93)	.024	0.51 (0.32-0.81)	.004
Female-female	0.49 (0.30-0.80)	.004	0.53 (0.33-0.87)	.013
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	1.15 (0.61-2.17)	.675	0.95 (0.51-1.79)	.864
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.39 (1.03-1.87)	.029	1.08 (1.00-1.16)	.057
GVHD prophylaxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	0.56 (0.40-0.77)	<.001	0.74 (0.50-1.09)	.135
Cytogenetics				
t(9;22) only	1 (Reference)		1 (Reference)	
Other abnormality	1.01 (0.65-1.57)	.955	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	0.75 (0.49-1.16)	.199	NA	
Major	0.86 (0.59-1.24)	.413	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

cohort (5%). These discrepancies resulted in different donor status, HLA disparity, and stem cell source frequencies in the present study.

An important difference in the pretransplant chemotherapy regimens should also be noted. Although detailed information about pretransplant chemotherapy was not available, the majority of the non-imatinib cohort was likely treated according to the JALSG ALL93[25] or JALSG ALL97 protocols,[26] whereas most of the imatinib cohort was likely to be treated according to the JALSG ALL202 protocols,[4] in which the chemotherapeutic regimen was similar to that used in the earlier protocols, except for the use of imatinib, because these were widely used regimens in Japan during the study period. Therefore, the influence of pretransplant chemotherapy appears to be limited.

In conclusion, our study involving a large number of patients observed over a long-term follow-up period clearly demonstrates that imatinib administration before allo-HSCT had advantageous effects on the clinical outcomes of patients with Ph$^+$ ALL. This finding encourages us to consider allo-HSCT for patients with Ph$^+$ ALL even during the imatinib era; however, we should continue to investigate

Table 4. Results of univariate and multivariate analysis of NRM among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.65 (0.49-0.88)	<.001	0.55 (0.37-0.83)	.005
Age at SCT (years)				
≤29	1 (Reference)		1.03 (1.02-1.05)	<.001
30-54	1.77 (1.16-2.70)	.008	(Regression)	
≥55	2.54 (1.51-4.30)	<.001		
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	1.15 (0.86-1.54)	.330	1.27 (0.87-1.87)	.219
Stem cell source				
Related bone marrow	1 (Reference)		1 (Reference)	
Unrelated bone marrow	1.47 (1.01-2.13)	.044	1.32 (0.76-2.32)	.327
Related peripheral blood	1.37 (0.87-2.14)	.174	1.55 (0.95-2.53)	.081
Cord blood	1.49 (0.93-2.38)	.097	1.59 (0.81-3.11)	.181
PS at SCT				
0	1 (Reference)		1 (Reference)	
1-4	1.04 (0.76-1.42)	.810	0.90 (0.64-1.26)	.542
Days from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	1.60 (1.20-2.13)	.001	1.35 (0.97-1.88)	.075
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	1.20 (0.40-3.62)	.750	NA	
Major and minor	0.96 (0.33-2.76)	.940	NA	
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.83 (0.55-1.25)	.380	0.73 (0.48-1.12)	.150
Female-male	1.01 (0.67-1.51)	.970	1.07 (0.71-1.62)	.737
Female-female	0.95 (0.63-1.42)	.790	0.85 (0.55-1.30)	.446
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	0.76 (0.44-1.31)	.328	0.93 (0.52-1.67)	.819
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.04 (0.78-1.38)	.810	1.03 (0.94-1.14)	.468
GVHD profiraxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	1.19 (0.89-1.60)	.250	1.23 (0.84-1.81)	.287
Cytogenetics				
t(9;22) only	1 (Reference)		1 (Reference)	
Other abnormality	1.03 (0.68-1.54)	.900	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	1.31 (0.89-1.92)	.170	NA	
Major	1.28 (0.91-1.82)	.160	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

alternative treatment options for patients who are not eligible for allo-HSCT because of older age and/or comorbidity. For example, in recent years, MRD monitoring has been increasingly used as an independent prognostic factor in response to a number of studies that have demonstrated its importance. Ravandi et al analyzed the clinical outcomes of patients with Ph^+ ALL treated with TKI combined chemotherapy without allogeneic SCT and demonstrated that the achievement of a major molecular response status at 3 months (and beyond) after treatment initiation was associated with a decreased likelihood of relapse and a longer OS.[27] Bachanova et al used data from the Center for International Bone Marrow Transplant Research to analyze 197 patients with Ph^+ ALL and reported that the achievement of a MRD-negative status may lead to a low relapse rate and prolonged survival in response to either myeloablative conditioning or decreased-intensity conditioning HSCT. They also reported that MRD status may be more helpful than a predefined age cutoff in guiding decisions regarding the conditioning intensity before allo-HSCT.[28] In the TKI era, the potential of MRD monitoring via PCR was demonstrated; this technique allows us to identify patients who would benefit from treatment intensification and to select continued therapy without transplantation in older patients with poorer conditions. In addition, recent studies have shown that imatinib therapy before autologous HSCT is also beneficial.[7,29] The clinical relevance of autologous HSCT in patients with Ph^+ ALL should also be investigated as an alternative stem cell source in the TKI era.

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Authorship

Contribution: S.M., S.N., K.I., and J.T. designed the study and wrote the manuscript; S.M., Y.A., and K. Matsuo performed the statistical analysis and interpreted the data; H.K., K.O., T.F., Y.O., K. Miyamura, S.T., and M.O. provided the patient data; and Y.A., R.S., Y.M., K.K., and H.S. collected the patient data.

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Tamibarotene As Maintenance Therapy for Acute Promyelocytic Leukemia: Results From a Randomized Controlled Trial

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See accompanying articles on pages 3692 and 3723

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ABSTRACT

Purpose
The introduction of all-*trans*-retinoic acid (ATRA) has significantly improved outcomes for acute promyelocytic leukemia (APL), although a subset of patients still suffer relapse. The purpose of this study was to evaluate the role of maintenance therapy with the synthetic retinoid tamibarotene in APL.

Patients and Methods
Patients with newly diagnosed APL in molecular remission at the end of consolidation therapy were randomly assigned to receive ATRA or tamibarotene, both orally, for 14 days every 3 months for up to 2 years.

Results
A total of 347 patients were enrolled. Of the 344 eligible patients, 319 (93%) achieved complete remission. After completing three courses of consolidation therapy, 269 patients underwent maintenance random assignment. The relapse-free survival (RFS) rate at 4 years was 84% for the ATRA arm and 91% for the tamibarotene arm (hazard ratio [HR], 0.54; 95% CI, 0.26 to 1.13). When the analysis was restricted to 52 high-risk patients with an initial WBC count $\geq 10.0 \times 10^9$/L, the intergroup difference was statistically significant, with 4-year RFS rates of 58% for the ATRA arm and 87% for the tamibarotene arm (HR, 0.26; 95% CI, 0.07 to 0.95). For patients with non–high-risk disease, the HR was 0.82 (95% CI, 0.32 to 2.01). The test for interaction between treatment effects and these subgroups resulted in $P = .075$. Both treatments were generally well tolerated.

Conclusion
In this trial, no difference was detected between ATRA and tamibarotene for maintenance therapy. In an exploratory analysis, there was a suggestion of improved efficacy of tamibarotene in high-risk patients, but this requires further study.

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INTRODUCTION

Front-line therapy combining all-*trans*-retinoic acid (ATRA) and chemotherapy has significantly improved outcomes for patients with acute promyelocytic leukemia (APL).[1-14] Nevertheless, up to 30% of patients still experience relapse and die of the disease.

Maintenance therapy is a therapeutic component that potentially reduces the risk of APL relapse. Randomized controlled trials conducted by the North American Intergroup[6] and the European APL Group[11] both demonstrated the beneficial effects of maintenance therapy. In contrast to these studies, we showed that six courses of intensified maintenance chemotherapy did not reduce relapse, but rather worsened overall survival (OS) as a result of higher incidences of late relapse, therapy-related leukemia, and failure to respond to reinduction therapy.[8] More recently, the Gruppo Italiano Malattie Ematologiche dell'Adulto reported that maintenance therapy did not yield any clinical benefits.[14] In view of these conflicting findings, maintenance therapy for APL remains an issue in need of further investigation.[15]

Tamibarotene (formerly Am80) is a synthetic retinoid that induces differentiation of HL-60 and NB-4 cells, with in vitro activity approximately 10 times more potent than that of ATRA.[16,17] This drug has a low affinity for the cellular retinoic acid binding protein, the overexpression of which is known to be associated with ATRA resistance.[18] In addition, unlike ATRA, tamibarotene has a favorable pharmacokinetic profile because the plasma level does not decline after daily administration.[19] These properties suggest that tamibarotene could be superior to ATRA. We previously conducted a phase II study of tamibarotene in patients with APL who had experienced relapse after ATRA-containing therapy and demonstrated the efficacy and safety of this agent in a relapse setting.[19]

These findings prompted the Japan Adult Leukemia Study Group to test tamibarotene as maintenance therapy for APL. To this end, a phase III study, designated APL204, was initiated with the aim of comparing tamibarotene with ATRA as maintenance therapy for patients with newly diagnosed APL. We report here the results of this study.

PATIENTS AND METHODS

Patients

Patients enrolled onto this study had been newly diagnosed with APL with documented cytogenetic and/or molecular evidence of t(15;17)/*PML-RARA*. Other eligibility criteria included age between 15 and 70 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 (partial pressure of oxygen in arterial blood ≥ 60 Torr or saturation of peripheral oxygen $\geq 93\%$), and heart (no severe abnormalities detected on ECG and/or echocardiograms). Written informed consent was obtained from all patients before registration. The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki. This study is registered at the University Hospital Medical Information Network Clinical Trials Registry as C000000154.

Treatments

For remission induction therapy, ATRA was administered to all patients at a daily dose of 45 mg/m^2 until complete remission (CR) or for 60 days, whichever was shorter. The chemotherapy protocol depended on the initial WBC count and blast count in the peripheral blood. If the initial WBC count was less than 3.0×10^9/L and the blast count was less than 1.0×10^9/L (group A), simultaneous chemotherapy was withheld. If the initial WBC count was between 3.0×10^9/L and 10.0×10^9/L and/or the blast count exceeded 1.0×10^9/L (group B), idarubicin (IDA) 12 mg/m^2 was administered on days 1 and 2, and cytarabine (AraC) 100 mg/m^2 was administered on days 1 to 5. If the initial WBC count was 10.0×10^9/L or higher (group C), IDA 12 mg/m^2 was administered on days 1 to 3, and AraC 100 mg/m^2 was administered on days 1 to 7. Patients whose blast counts increased to 1.0×10^9/L during the induction course were given additional chemotherapy consisting of IDA 12 mg/m^2 for 3 days and AraC 100 mg/m^2 for 7 days for group A patients, IDA 12 mg/m^2 for 1 day and AraC 100 mg/m^2 for 2 days for group B patients, and IDA 12 mg/m^2 for 1 day for group C patients. All patients who received additional chemotherapy during the induction course were classified as group D.

Treatment of coagulopathy was risk adapted and stratified into levels 1, 2, and 3. Disseminated intravascular coagulation (DIC) was diagnosed in accordance with the Japanese Ministry of Health and Welfare scoring system.[20] Patients with a diagnosis of DIC were treated as level 1 if they met at least one of the following criteria: less than 7 days had elapsed since the start of chemotherapy; complication with retinoic acid syndrome, pneumonia, or severe hemorrhagic complications, or (3) documented increase of the Japanese Ministry of Health and Welfare DIC score. Otherwise, patients with a diagnosis of DIC were treated as level 2, and those without DIC were treated as level 3. Platelet transfusions were administered to maintain the platelet count greater than 50×10^9/L for level 1, 30×10^9/L for level 2, and 20×10^9/L for level 3. Fresh frozen plasma was transfused to maintain the plasma fibrinogen level greater than 1.5 g/L for level 1, 1.0 g/L for level 2, and as required for level 3. Anticoagulants were used for both levels 1 and 2. Retinoic acid syndrome was treated with high-dose dexamethasone or methylprednisolone along with immediate interruption of ATRA.

Consolidation therapy consisted of three courses of intensive chemotherapy: mitoxantrone 7 mg/m^2 on days 1 to 3 and AraC 200 mg/m^2 on days 1 to 5 for the first course; daunorubicin 50 mg/m^2 on days 1 to 3 and AraC 200 mg/m^2 on days 1 to 5 for the second course; and IDA 12 mg/m^2 on days 1 to 3 and AraC 140 mg/m^2 on days 1 to 5 for the third course. Before the start of the third consolidation course, intrathecal injection of methotrexate, AraC, and prednisolone was used for CNS prophylaxis.

After completion of the third consolidation course, the *PML-RARA* transcript levels in the bone marrow were assessed. Patients in molecular remission at this time were then randomly assigned to oral administration of ATRA at a daily dose of 45 mg/m^2 or to tamibarotene at a daily dose of 6 mg/m^2, both for 14 days every 3 months. Random assignment was stratified according to induction treatment (ie, group A, B, C, or D). Maintenance therapy was continued for up to 2 years for a total of up to eight courses.

Assessments

CR was defined as the presence of all of the following: less than 5% of blasts in the bone marrow, no leukemic blasts in the peripheral blood or extramedullary sites, and recovery of peripheral-blood counts. Hematologic relapse was defined as the presence of at least one of the following: recurrence of more than 5% leukemic cells in the bone marrow, recurrence of any leukemic cells in the peripheral blood, or development of extramedullary

Table 1. Patient Demographics and Clinical Characteristics

Demographic or Clinical Characteristic	No. of Patients (N = 344)
Age, years	
Median	48
Range	15-70
Sex	
Male	183
Female	161
Performance status	
0	188
1	126
2	19
3	11
White blood cell count, $\times 10^9$/L	
Median	1.4
Range	0.1-127
Platelet count, $\times 10^9$/L	
Median	3.1
Range	0.1-47.0
Sanz's risk category	
Low	115
Intermediate	151
High	70
Unknown	8
Morphology	
M3	323
M3v	21
Induction therapy group	
A	133
B	56
C	69
D	86

Abbreviations: ATRA, all-*trans*-retinoic acid; M3v, M3 variant.

disease. Bone marrow was analyzed for *PML-RARA* levels after the end of consolidation therapy, after every two courses during maintenance therapy, and every 6 months thereafter. The *PML-RARA* levels were measured at a single independent laboratory using the real-time quantitative reverse transcription polymerase chain reaction assay as described elsewhere.[8] Levels less than 100 copies/μg RNA were defined as molecular remission for this study. If a patient lost molecular remission, an extra bone marrow examination was performed 1 month later to confirm the results. Molecular relapse was defined as loss of molecular remission confirmed in two consecutive bone marrow samples taken 1 month apart.

Statistical Analyses

The primary end point of this study was relapse-free survival (RFS), which was defined as the time from random assignment to hematologic or molecular relapse, death, or last visit, whichever came first. We aimed to include 240 patients in a maintenance random assignment procedure for the detection of an increase in the RFS probability by 17% in the tamibarotene arm compared with the ATRA arm. This sample size ensured two-tailed $\alpha = .05$, and $1\text{-}\beta = .83$. All the analyses for maintenance comparisons were intent-to-treat analyses.

Distributions of patient characteristics between groups were compared using the Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. The probabilities of RFS and OS were estimated using the Kaplan-Meier method, with differences between groups determined with the log-rank test. OS was defined as the time from registration to death or last visit. The Cox proportional hazards regression model was used for calculating the hazard ratio (HR) in conjunction with the 95% CI. The proportional hazards assumption was tested based on Schoenfeld residuals,[21] and the test for significance showed nonsignificance ($P = .491$), supporting that the proportional hazard assumption was not violated.

RESULTS

Patients

Between April 2004 and December 2010, 347 patients with newly diagnosed APL were enrolled onto this study. Three patients who had subsequently turned out to be negative for *PML-RARA* were excluded, leaving 344 patients eligible for analysis. Table 1 lists the baseline characteristics of the eligible patients.

Figure 1 depicts patient flow in the CONSORT diagram. For remission induction, 133 patients (39%) were included in treatment group A, 56 (16%) in group B, 69 (20%) in group C, and 86 (25%) in group D. Group D consisted of 83 patients who had been initially

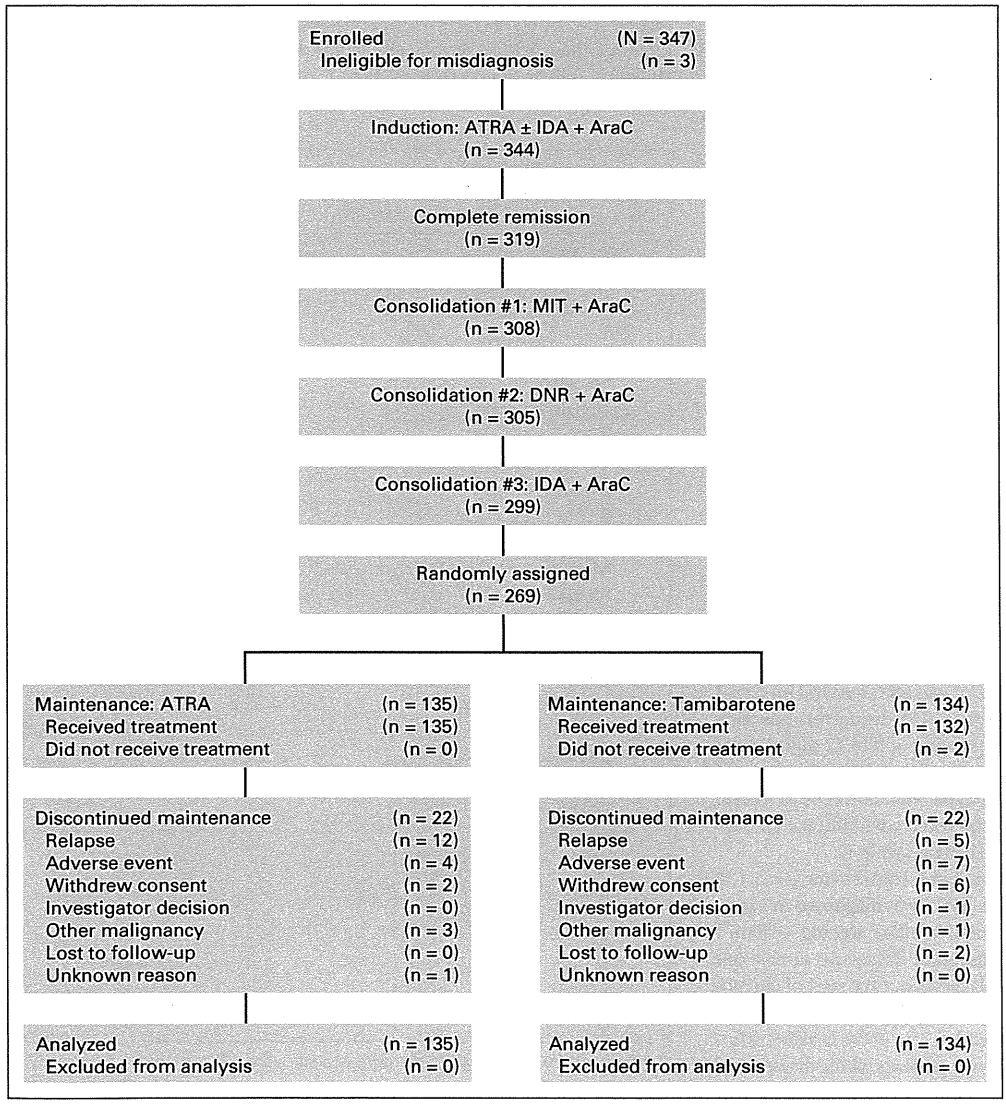

Fig 1. CONSORT diagram and treatment schema. AraC, cytarabine; ATRA, all-*trans*-retinoic acid; DNR, daunorubicin; IDA, idarubicin; MIT, mitoxantrone.

treated as group A, two patients who had been treated as group B, and one patient who had been treated as group C. CR was attained in 319 (93%) of the 344 eligible patients; CR rates by group were as follows: 92% for group A, 91% for group B, 87% for group C, and 99% for group D. Sixteen patients (4.7%) died within 30 days, and 14 of these deaths were associated with hemorrhagic complications. During the median follow-up of 4.3 years (range, 1.3 to 8.0 years), 39 relapses and 37 deaths were documented. The probability of OS for the entire cohort was 89% at 4 years.

Maintenance Comparisons

Among 299 patients who received the third consolidation course, 269 eventually underwent maintenance random assignment; 135 patients were randomly assigned to ATRA, and 134 patients were assigned to tamibarotene. All subsequent comparisons between the two arms were conducted by intention to treat. Table 2 lists the characteristics of randomly assigned patients. The main characteristics were equally distributed between the two arms.

Autologous hematopoietic cell transplantation was performed in 23 patients (16 patients in the ATRA arm and seven in the tamibarotene arm), all after relapse, and allogeneic hematopoietic cell transplantation was performed in two patients (one patient in each arm), both in first CR, for the treatment of secondary myelodysplastic syndrome (MDS). These two patients were censored at the time of transplantation. During the entire follow-up period, 30 patients (20 in the ATRA arm and 10 in the tamibarotene arm) suffered relapse, with the

Table 2. Demographics and Clinical Characteristics of Patients Randomly Assigned for Maintenance Therapy

Characteristic	No. of Patients		
	ATRA (n = 135)	Tamibarotene (n = 134)	
Age, years			.597
Median	48	46	
Range	15-70	16-69	
Sex			.807
Male	70	72	
Female	65	62	
Performance status			.840
0	72	78	
1	50	43	
2	8	8	
3	5	5	
White blood cell count, $\times 10^9$/L			.841
Median	1.3	1.4	
Range	0.2-111	0.2-88.5	
Platelet count, $\times 10^9$/L			.343
Median	2.8	3.3	
Range	0.2-20.8	0.1-47.0	
Sanz's risk category			.636
Low	46	44	
Intermediate	59	63	
High	26	26	
Unknown	4	1	
Morphology			.597
M3	126	128	
M3v	9	6	
Induction therapy group			.977
A	49	45	
B	21	22	
C	25	26	
D	40	41	

Abbreviations: ATRA, all-*trans*-retinoic acid; M3v, M3 variant.

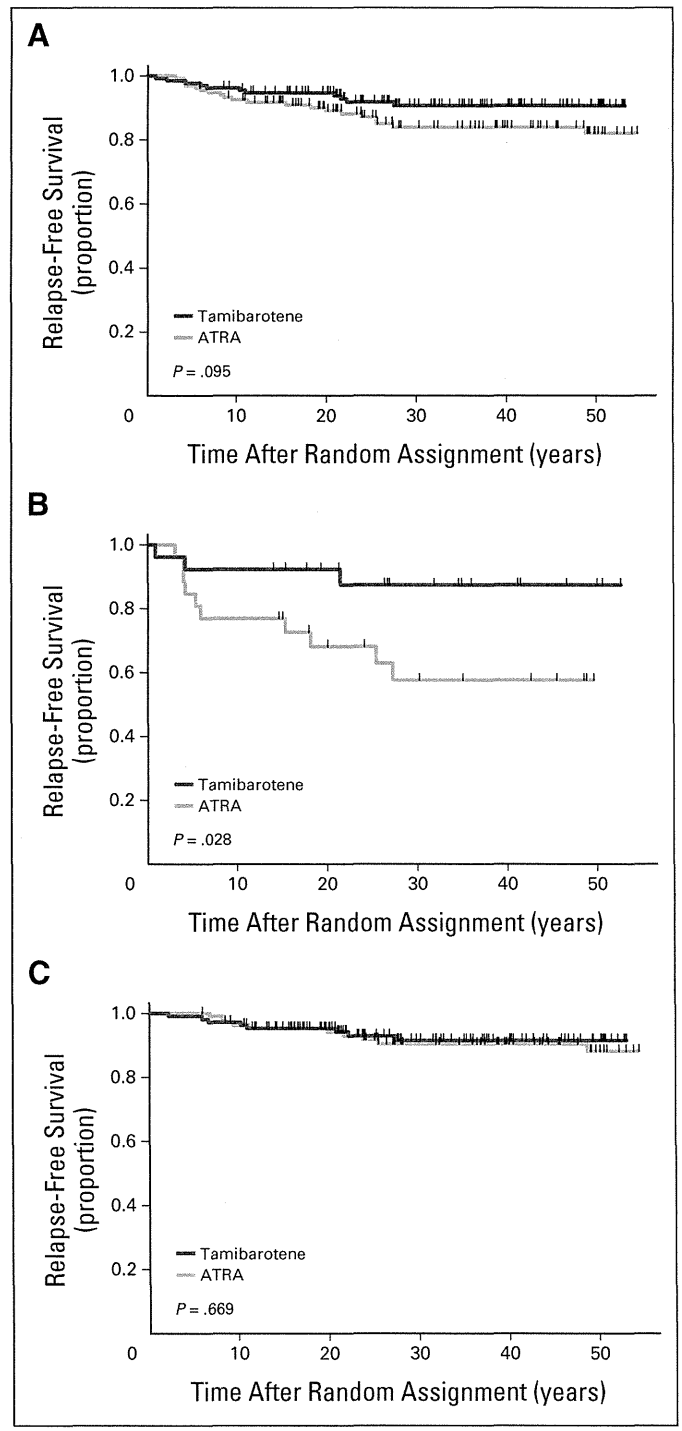

Fig 2. Kaplan-Meier curves for relapse-free survival in relation to maintenance therapy random assignment (A) for all patients (N = 269), (B) for patients with an initial WBC count of ≥ 10.0 × 10^9/L (n = 52), and (C) for patients with an initial WBC count less than 10.0 × 10^9/L (n = 217). ATRA, all-*trans*-retinoic acid.

median time from random assignment to relapse of 1.0 year for the ATRA arm and 0.8 year for the tamibarotene arm. Death occurred in two patients in the ATRA arm and four in the tamibarotene arm; the causes of death were transplant-related mortality (n = 2) in the ATRA arm and APL (n = 3) and secondary MDS (n = 1) in the tamibarotene arm. There was only one death during first CR of a patient in the tamibarotene arm 2.1 years after random assignment as a result of MDS.

Figure 2 compares the RFS curves of the two arms. RFS rates at 4 years were 84% in the ATRA arm and 91% in the tamibarotene arm, and this difference did not reach statistical significance ($P = .095$; HR, 0.54; 95% CI, 0.26 to 1.13; Fig 2A). However, when the analysis was restricted to high-risk patients with an initial WBC count of 10.0×10^9/L or higher, the intergroup difference was statistically significant ($P = .028$; HR, 0.26; 95% CI, 0.07 to 0.95), with 4-year RFS rates of 58% in the ATRA arm and 87% in the tamibarotene arm (Fig 2B). For patients whose initial WBC count was lower than 10.0×10^9/L, RFS was almost identical between the arms (90% for the ATRA arm *v* 92% for the tamibarotene arm; $P = .669$; HR, 0.82; 95% CI, 0.32 to 2.01; Fig 2C). The results of a test for interaction between treatment effects and subgroups suggest a possible difference in treatment effects between these two subgroups ($P = .075$).

Table 3 lists grade 2 or higher drug-related adverse events that were reported at a frequency of greater than 5% in either arm. Both treatments were generally well tolerated, and most of the adverse events were grade 2 or lower except for triglyceride increase. Hyperlipidemia was the most common adverse event in both arms, with a higher frequency in the tamibarotene arm. Skin rash was predominantly seen in the tamibarotene arm. Four patients in the ATRA arm and seven in the tamibarotene arm had to discontinue maintenance therapy because of adverse events. In the ATRA arm, nausea (n = 1), headache (n = 1), liver dysfunction (n = 1), and triglyceride increase (n = 1) resulted in discontinuation, whereas skin rash (n = 5), liver dysfunction (n = 1), and coagulopathy (n = 1) resulted in discontinuation in the tamibarotene arm.

DISCUSSION

To the best of our knowledge, this is the first study to evaluate tamibarotene as maintenance therapy for APL. The study results showed no statistical difference between ATRA and tamibarotene, although there was a suggestion of improved efficacy of tamibarotene in high-risk patients in an exploratory analysis.

The role of maintenance therapy in APL has been a matter of controversy.[15] To date, several randomized controlled trials have attempted to address this issue, but with conflicting results. Earlier studies showed significant benefits of maintenance,[6,11] whereas more recent studies did not.[8,14] When discussing the role of maintenance, we should keep in mind that whether and to what extent maintenance therapy provides clinical benefits depends on the target patients. For instance, the addition of effective maintenance therapy may improve outcomes for patients with a certain amount of residual disease, whereas the same does not apply to patients without any residual disease at all when maintenance therapy is started. For this reason, inconsistent or even contradictory results reported by various studies could be explained by differences in types of patients enrolled onto each of these studies. In this regard, it is interesting to note that two studies reporting negative results mainly used IDA for anthracycline drugs,[8,14] whereas the two studies with positive results used only daunorubicin.[6,11] In addition, the negative studies used three consolidation courses,[8,14] but positive studies used only two.[6,11] These differences indicate that more intensive treatments were used in the former than in the latter studies, which raises the possibility that the more intensive antileukemic effect of the former may have diminished the proportion of patients who actually benefited from maintenance therapy. Also of interest is the fact that only patients with negative *PML-RARA* at the end of consolidation therapy underwent maintenance random assignment in the two negative studies,[8,14] whereas all patients with hematologic CR did so, irrespective of minimal residual disease status, in the two positive studies.[6,11] These conditions may have led to an increase in the percentage of patients in the negative studies who did not actually benefit from maintenance therapy. Therefore, it is not surprising that maintenance random assignment had no impact on outcomes in our non–high-risk patients, because a substantial fraction of these patients may have done well regardless of which maintenance arm they were assigned to, or even without maintenance in some cases, because our study used IDA as well as three courses of consolidation therapy, and only patients whose *PML-RARA* levels had considerably diminished proceeded to maintenance random assignment.

Our data indicate a possible relationship between the beneficial effects of tamibarotene and the initial WBC count. Patients with an initial WBC count of 10.0×10^9/L or higher are commonly defined as at high risk for relapse.[5,9] Although statistical significance was not reached, there was a trend for a greater effect size for these high-risk patients. Here we should bear in mind that this subgroup analysis

Table 3. Grade 2 or Higher Drug-Related Adverse Events Reported in More Than 5% of Patients in Either Maintenance Arm

	ATRA (n = 135)						Tamibarotene (n = 134)					
	Grade 2		Grade 3		Grade 4		Grade 2		Grade 3		Grade 4	
Adverse Event	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Triglyceride increased	25	19	17	13	6	4.4	28	21	28	21	6	4.5
Cholesterol increased	11	8.1	0	0	2	1.5	20	15	3	2.2	0	0
Rash	2	1.5	1	0.7	0	0	19	14	0	0	0	0
AST/ALT increased	6	4.4	1	0.7	0	0	8	6.0	3	2.2	0	0
Headache	6	4.4	2	1.5	0	0	4	3.0	0	0	0	0

Abbreviation: ATRA, all-*trans*-retinoic acid.

includes several merits and limitations. The result has some authenticity because patients with an initial WBC count of 10.0×10^9/L or higher constitute a clinically significant subgroup in APL[15]; random assignment was stratified in terms of induction therapy, which had been decided based on the initial WBC count; and there was a possible difference of treatment effects between the two subgroups. However, this subgroup analysis was not prespecified in the protocol, and the result therefore needs to be interpreted cautiously. Another issue to note is that RFS for high-risk patients in our control arm seems somewhat lower than the RFS rates reported in previous studies using ATRA in combination with continuous chemotherapy,[11,13,14] suggesting the possibility that maintenance therapy with ATRA alone might not be optimal for these patients. The advantage of tamibarotene over ATRA observed in this study thus may have disappeared if these drugs had been combined with continuous chemotherapy. While acknowledging these notions, our data suggest clinical activity of tamibarotene in APL. The fact that this benefit in terms of RFS did not translate into prolonged survival could be largely a result of availability of effective salvage therapy for relapsed APL, because we recently demonstrated the outstanding efficacy of a sequential treatment consisting of induction and consolidation with arsenic trioxide (ATO), peripheral-blood stem-cell harvest after high-dose AraC chemotherapy and autologous hematopoietic cell transplantation for patients with relapsed APL.[22]

With the accumulation of experience using ATO for relapsed APL, enthusiasm is currently growing for incorporating ATO into front-line therapy in APL clinical trials.[23-26] Some may argue that this development will irrevocably change the role of maintenance therapy, but prognosis for patients with an initial WBC count of 10.0×10^9/L or higher still seems to remain poor even if they are treated with ATO-containing therapy.[23,24] For this reason, tamibarotene may play an important role in the ATO era. Moreover, incorporation of tamibarotene along with ATO into front-line therapy for APL may contribute to a reduction in the use of cytotoxic chemotherapy without impairing outcome, which may constitute a major challenge for the future treatment of APL.

In summary, this randomized controlled trial showed no statistical difference between ATRA and tamibarotene for maintenance therapy, but there was a suggestion of improved efficacy of tamibarotene in high-risk patients. This needs to be confirmed in further studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Prognostic significance of leukopenia in childhood acute lymphoblastic leukemia

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Abstract. Chemotherapy-induced leukopenia has been shown to be associated with the outcomes of several types of cancer, but the association with childhood acute lymphoblastic leukemia (ALL) remains unknown. To elucidate the association of chemotherapy-induced leukopenia with the clinical outcome of childhood ALL, retrospective analysis was performed on 19 child patients with ALL treated according to the ALL-BFM 95 high-risk (HR) protocol. The mean minimum leukocyte count over the first three courses of the consolidation phase was used as the measure of hematological toxicity and ranged between 200 and 1,167/μl. The risk of relapse was significantly higher in patients with a mean minimum leukocyte count above the median of 433/μl (hazard ratio, 6.61; P=0.047). In conclusion, chemotherapy-induced leukopenia was found to correlate with relapse-free survival in childhood HR ALL. Dose escalation based on hematologic toxicity must be prospectively studied.

Introduction

Treatment outcome of childhood acute lymphoblastic leukemia (ALL) has evidently improved, but the prognosis of high-risk (HR) ALL remains unsatisfactory (1). Refinement of risk stratification is required to improve survival by providing intensive treatment to patients at HR of relapse. The clinical outcome of ALL is known to be associated with variable factors, such as demographics, immunophenotype, cytogenetic features and early treatment response (2). In addition, the ability of individual patients to metabolize antileukemic drugs appears to be involved in the prognosis of ALL, but the knowledge of pharmacological features of leukemic cells in childhood ALL is largely limited (3).

Hematological toxicity is the most frequent dose-limiting side effect of combination chemotherapy in the treatment of childhood ALL. The severity of each case of acute hematological toxicity is highly variable despite use of the same regimen. Chemotherapy-induced leukopenia may be a biological measure of drug activities and disease control (4,5). The response of leukemic cells to chemotherapy depends on the level of active drugs reaching the target and the sensitivity to these drugs. These factors also affect the response of non-malignant hematopoietic cells. The availability of active drugs is influenced by pharmacokinetic parameters. In part, sensitivity to the drugs is affected by genetic predisposition, which produces a similar effect in tumor and normal cells, but is also modified by tumor-specific mutations (6).

The association between less chemotherapy-induced leukopenia and poor clinical outcome has been previously reported for several malignancies, including lung cancer, breast cancer, osteosarcoma and Hodgkin lymphoma (6-12). This provides additional prognostic information that may be used to further refine patient stratification and risk-directed therapy. However, the prognostic role of chemotherapy-induced leukopenia in childhood ALL has not been elucidated.

Conventional treatment for ALL consists of induction, consolidation, reinduction and maintenance elements. Cytotoxic agents, dose levels and severity of myelosuppression are significantly different between treatment courses. This makes it difficult to define the measure of hematological toxicity compared with the treatment and to evaluate the prognostic significance of chemotherapy-induced leukopenia. In the ALL-BFM 95 HR protocol for childhood ALL, the consolidation phase consists of a series of intensive treatment courses (block therapy), with an interval of three to four weeks between blocks (13). This repetition of treatments with relatively similar intensity is suitable for the evaluation of chemotherapy-induced leukopenia. Therefore, to investigate the association of leukopenia early in the course of treatment with treatment outcomes of childhood ALL, the current study analyzed ALL patients treated according to the ALL-BFM 95 HR protocol following induction therapy.

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Key words: childhood acute lymphoblastic leukemia, chemotherapy, white blood cell count

Table I. Risk stratification (the TCCSG L99-1502 study).

A, B-lineage ALL

	Years		
Initial risk	1-6	7-9	≥10
Initial leukocyte count, x10^9/l			
<20	SR	IR	IR
20-49	IR	IR	IR
50-99	IR	IR	HR
≥100	HR	HR	HR

B, B-lineage ALL

	Days		
Day 8 risk	1 SR	1 IR	1 HR
Day 8 PB blasts/μl			
0	SR	IR	IR
1-999	SR	IR	HR
≥1,000	IR	HR	Allo-SCT

C, T-lineage ALL

Day 8 risk	All patients
Day 8 PB blasts/μl	
0	IR
1-999	HR
≥1,000	Allo-SCT

PB, peripheral blood; SR, standard-risk; IR, intermediate-risk; HR, high-risk; Allo-SCT, allogeneic stem cell transplantation; ALL; acute lymphoblastic leukemia.

Table II. Risk stratification (the TCCSG L04-16 Study).

A, B-lineage ALL

	Years		
Initial risk	1-6	7-9	≥10
Initial leukocyte count, x10^9/l			
<20	SR	IR	IR
20-49	IR	IR	IR
50-99	IR	IR	HR
≥100	HR	HR	HR

B, B-lineage ALL

	Days		
Day 8 risk	1 SR	1 IR	1 HR
Day 8 PB blasts/μl			
0-999	SR[a]	IR[a]	HR
≥1,000	HR	HR	Allo-SCT

C, T-lineage ALL

Day 8 risk	All patients
Day 8 PB blasts/μl	
0-999	HR
≥1,000	Allo-SCT

[a]Patients were assigned to high-risk group if >5 cells/μl were counted in the cerebrospinal fluid and lymphoblasts were identified or if intracranial infiltrates were detected on brain imaging studies. Diagnostic lumbar puncture was performed on day eight in remission induction therapy. PB, peripheral blood; SR, standard-risk; IR, intermediate-risk; HR, high-risk; Allo-SCT, allogeneic stem cell transplantation.

Materials and methods

Study population. In total, 19 patients (age range, 1-18 years) consecutively diagnosed with ALL between November 2003 and September 2010 were studied and uniformly treated according to the ALL-BFM 95 HR protocol following induction therapy at the University of Tokyo Hospital (Tokyo, Japan), Saitama Children's Medical Center (Saitama, Japan) and Gunma Children's Medical Center (Shibukawa, Japan).

Children diagnosed with ALL were enrolled in the Tokyo Children's Cancer Study Group (TCCSG) L99-1502 study between November 2003 and January 2005, and in the L04-16 study between May 2005 and September 2010 (14). The patients were stratified into the following three risk groups: Standard-risk (SR), intermediate-risk (IR) and HR. The initial stratification was based on presenting features (age and white blood cell count prior to initiating treatment) and leukemic blasts in peripheral blood on day eight following prednisolone monotherapy (Tables I and II). The patients were finally stratified based on cytogenetic observations and bone marrow status examined following remission induction therapy. Following induction therapy, patients assigned to the HR group were treated with block chemotherapy regimen of the ALL-BFM 95 HR protocol. Patients who did not achieve remission and those with the Philadelphia chromosome or 11q23 rearrangements (with the exception of MLL/ENL in the L04-16 study) were scheduled for allogeneic stem cell transplantation and were excluded from the present study.

The data regarding chemotherapeutic dosage, dates of administration and leukocyte counts were retrieved from the electronic patient databases of the hospitals involved. The parents of all patients provided written informed consent for the treatment. The current study was approved by the Ethics Committee of the University of Tokyo Hospital.

Treatment protocols. An outline of the treatment regimens is shown in Fig. 1 and the details of each treatment aspect are provided in Table III (13). Following TCCSG induction therapy, patients were uniformly treated according to the ALL-BFM 95

Table III. Details of the treatment regimens.

Therapy	Details
Induction	
Day 8 SR	Pred, 60 mg/m^2 x 5 weeks; VCR, 1.5 mg/m^2 on weeks 1-5; Pirarubicin, 20 mg/m^2 on weeks 3 and 4; and L-asp, 6,000 U/m^2 3 times a week on weeks 2-4
Day 8 IR and HR	Pred, 60 mg/m^2 x 5 weeks; VCR, 1.5 mg/m^2 on weeks 1-5; DNR, 25 mg/m^2 2 times a week on weeks 2 and 5; CY 1,000 mg/m^2 on weeks 2 and 5; and L-asp 6,000 U/m^2 3 times a week on weeks 2-4
HR-1' (two cycles)	Dex, 20 mg/m^2 x 5 days; MTX, 5 g/m^2 on day 1; CY, 200 mg/m^2 once on day 2 and twice on days 3 and 4; Ara-C, 2 g/m^2 twice on day 5; and L-asp, 25,000 U/m^2 on day 6 (VCR 1.5 mg/m^2 on day 1 and 6 only in the second cycle)
HR-2' (two cycles)	Dex, 20 mg/m^2 x 5 days; Vindesine, 3 mg/m^2 on days 1 and 6; MTX, 5 g/m^2; IFO, 800 mg/m^2 once on day 2 and twice on days 3 and 4; DNR, 30 mg/m^2 on day 5; and L-asp, 25,000 U/m^2 on day 6
HR-3' (two cycles)	Dex, 20 mg/m^2 x 5 days; Ara-C, 2 g/m^2 twice on days 1 and 2; VP-16, 100 mg/m^2 once on day 3 and twice on days 4 and 5; and L-asp 25,000 U/m^2 on day 6
Protocol II	
First half	Dex, 10 mg/m^2 x 14 days; VCR, 1.5 mg/m^2 on days 8, 15, 22 and 29; ADR, 30 mg/m^2 on days 8, 15, 22 and 29; and L-asp, 10,000 U/m^2 on days 3, 8, 16 and 21
Second half	6MP, 60 mg/m^2 x 14 days; CY, 1 g/m^2 on day 36; and Ara-C, 75 mg/m^2 x 4 consecutive days for 2 weeks
Cranial irradiation	
Maintenance	6MP/MTX until week 104
Total number of IT therapies	10-17

SR, standard-risk; IR, intermediate-risk; HR, high-risk; Pred, prednisolone; VCR, vincristine; L-Asp, L-asparaginase; DNR, daunorubicin; CY, cyclophosphamide; Dex, dexamethasone; MTX, methotrexate; Ara-C, cytarabine; IFO, ifosfamide; VP-16, etoposide; ADR, adriamycin; 6MP, 6-mercaptopurine; IT, intrathecal.

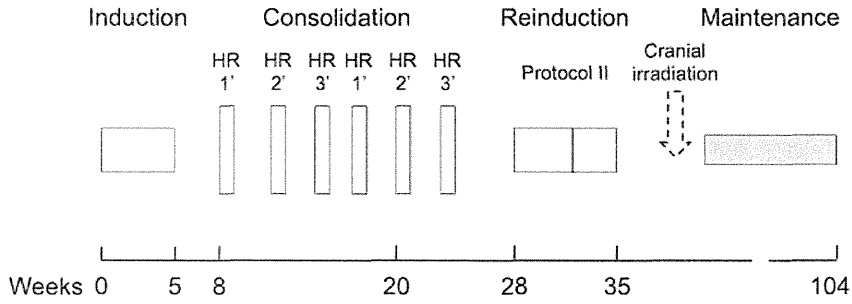

Figure 1. Outline of the treatment regimens. The treatment regimens consisted of induction, consolidation, reinduction and maintenance elements. The mean minimum leukocyte count over the first three courses of the consolidation phase was used as the measure of hematological toxicity. Cranial irradiation was administered only to patients with an initial leukocyte count of >100x10^9/l in the L99-1502 study. Patients aged 1-6 years received 12 Gy and patients >6 years received 18 Gy. The indication of cranial irradiation was limited to patients with central nervous system involvement (12 Gy for patients aged 12-23 months and 18 Gy for patients aged ≥24 months) and T-lineage acute lymphoblastic leukemia patients with <1,000 leukemic blasts/μl on day 8 (12 Gy) in the L04-16 study. Remaining patients received no cranial irradiation. HR, high-risk.

HR protocol; the patients continued on an intensive rotational consolidation schedule consisting of three separate six-day pulses of high-dose chemotherapy, which were each administered twice. Patients were treated according to the reinduction protocol II following the consolidation phase.

Granulocyte colony-stimulating factor (G-CSF) was administered in certain patients with febrile neutropenia and occasionally used prophylactically when severe and prolonged neutropenia was predicted.

Leukocyte count. Blood examination was routinely performed several times a week. The minimum leukocyte count during each course of chemotherapy was recorded. The minimum leukocyte count was averaged over the first three courses of the consolidation phase. The mean was used as the measure of hematological toxicity for each patient. The leukocyte count during the induction phase was excluded from the analysis, since disease status markedly affected the leukocyte count until remission was achieved.

Table IV. Characteristics of the study population.

No.	Age, years	Gender	Immunoph-enotype	Initial WBC, x10^9/l	Day 8 PB blast/μl	Risk group		Mean minimum WBC/μl	Outcome
						Day 1	Day 8		
1	12	M	B	81	63	HR	HR	1,167	RFS
2	11	F	B	581	632	HR	HR	450	Relapsed
3	14	M	T	279	14	HR	HR	433	RFS
4	8	F	B	7.2	7,684	IR	HR	367	RFS
5	9	M	T	430	116	HR	HR	500	Relapsed
6	12	F	B	8.2	1,269	IR	HR	733	Relapsed
7	14	M	T	1.5	0	HR	HR	367	RFS
8	7	M	T	259	20	HR	HR	433	Relapsed
9	12	F	T	147	247	HR	HR	233	RFS
10	15	M	T	42	0	HR	HR	433	RFS
11	6	F	T	11	0	HR	HR	233	RFS
12[a]	3	F	B	8.5	825	SR	SR	667	RFS
13	7	F	B	12	76	HR	HR	300	RFS
14	11	F	B	21	12,802	IR	HR	200	RFS
15	13	M	T	126	[b]ND	HR	HR	633	Relapsed
16	10	M	B	539	7	HR	HR	467	RFS
17	13	M	B	53	81	HR	HR	200	RFS
18	6	M	T	28	28	HR	HR	467	RFS
19	6	M	T	65	459	HR	HR	300	RFS
Median	11			52.9	69.5			433	
IQR	6-13			8.5-278.6	7-825			233-633	

[a]Patient number 12 started treatment with the TCCSG L04-16 SR protocol. Hematological remission was achieved following remission induction, but leukemic infiltration remained in the liver and frontal bone. The patient was finally stratified into high-risk group and received all courses of the ALL-BFM 95 HR protocol with the exception of the induction phase. [b]Unable to determine 'day eight' since prednisolone monotherapy was transiently terminated due to tumor lysis syndrome. The peripheral blood count decreased rapidly to <1,000/μl following initiation of prednisolone. WBC, white blood cell count; PB, peripheral blood; SR, standard-risk; IR, intermediate-risk; HR, high-risk; RFS, relapse-free survival; IQR, interquartile range; ND, no data.

Study outcomes. To assess the correlation between leukocyte nadir and disease control, relapse-free survival (RFS) from the initiation of chemotherapy was selected as the endpoint.

Statistical analysis. RFS curves were calculated by the Kaplan-Meier method and were compared by means of the log-rank test in a univariate analysis. The minimum leukocyte count in treatment courses with or without the use of G-CSF was compared with the Mann-Whitney U test to assess the effect of G-CSF on leukocyte nadir.

All statistical tests were two-tailed and $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics. In total, 22 patients were assigned to the HR group on day eight in remission induction therapy. One patient in the SR group on day eight was stratified into the HR group due to residual leukemic infiltration in the liver and frontal bone, although, hematological remission was achieved. Finally, 23 patients were stratified into the HR group. Of these, four received allogeneic stem cell transplantation and were excluded from the analysis; one with the Philadelphia chromosome and three with a poor response to prednisolone. The remaining 19 patients were uniformly treated according to the ALL-BFM 95 HR protocol and included in the analysis. The median age was 11 years (range, 1-18 years) and eight (42%) patients were female. All patients were treated with the same dose per body surface area in the consolidation phase with the exception of L-asparaginase, which was not administered to two patients in the third course due to anaphylaxis. Detailed patient characteristics are shown in Table IV.

Treatment outcome. Of the 19 patients, five suffered a relapse: Two during consolidation, one during reinduction and two during maintenance therapy. The median follow-up period of relapse-free patients was 51.5 months (range, 10-85 months). The mean minimum leukocyte count was calculated for the first three courses of the consolidation phase, with the exception of two patients who relapsed during the third course of the consolidation phase; their mean minimum leukocyte count was calculated for the first two courses of the consolidation phase. The median of the mean minimum leukocyte count was 433/μl (range, 200-1,167/μl).

Table V. Results of univariate analysis by log-rank test.

Variables	n	HR (95% CI)	P-value
Age, years			
≤11	11	1	
>11	8	1.26 (0.21-7.40)	0.800
Gender			
Female	8	1	
Male	11	1.07 (0.18-6.37)	0.940
Immunophenotype of leukemic blasts			
B-lineage	9	1	
T-lineage	10	1.37 (0.23-8.02)	0.730
Initial leukocyte count, x10^9/l			
≤52.9	10	1	
>52.9	9	5.45 (0.85-35.0)	0.083
Response to prednisolone monotherapy (day 8 PB blast of <1,000/μl)			
Good	16	1	
Poor	3	1.38 (0.14-13.8)	0.770
Mean minimum leukocyte count/μl			
≤433	11	1	
>433	8	6.61 (1.04-42.1)	0.047

Median age, 11 years; median initial leukocyte count, 52.9x10^9/l; median of the mean minimum leukocyte count, 433/μl. HR, hazard ratio; CI, confidence interval; PB, peripheral blood.

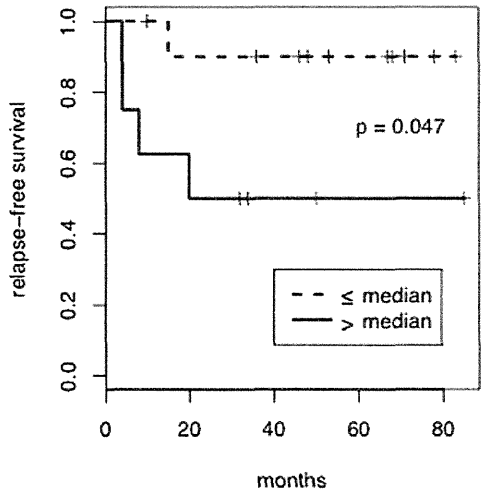

Figure 2. RFS according to the mean minimum leukocyte count. RFS is shown in patients with the mean minimum leukocyte count above the median of 433/μl (solid line) and those at or below the median (dashed line). RFS, relapse-free survival.

Of the 19 patients, 13 received G-CSF at least once during treatment. The minimum leukocyte count was not significantly different between the courses with and without the use of G-CSF (P=0.367; Mann-Whitney U test).

Prognostic factors. RFS curves were compared by means of the log-rank test in a univariate analysis (Table V). Variables included age, gender, immunophenotypes of leukemic blasts (B- or T-lineage), initial leukocyte count, response to prednisolone monotherapy and the mean minimum leukocyte count. Patients were divided at the median values for age, initial leukocyte count and the mean minimum leukocyte count. The risk of relapse was significantly higher in patients with a mean minimum leukocyte count above the median (hazard ratio, 6.61; P=0.047). Fig. 2 shows RFS according to the severity of leukopenia. No other factors were significantly associated with the risk of relapse.

Discussion

The severity of acute hematological toxicity varies considerably in childhood ALL despite the use of the same chemotherapy. The current study analyzed patients with ALL in the same risk group and showed that patients with low hematological toxicity during chemotherapy exhibited a higher rate of relapse. HR of relapse was identified by low hematotoxicity in the first half of the consolidation phase. Early identification of the HR population enables us to intensify treatment in these patients.

Low hematological toxicity has been reported to be associated with a poorer outcome of other malignancies (6-12). This association is predicted to be evident in acute leukemia, considering the common origin of leukemic blasts and normal hematopoietic cells. Previously, Han *et al* showed that a leukocyte nadir of >1,200/μl in induction chemotherapy is associated with poor overall survival in adult patients with acute myeloid leukemia (AML), although, no statistically significant difference was identified (15). This is consistent with the

observations of the current study. On the other hand, previous studies have reported that patients with severe hematological toxicity and a slow rate of myeloid recovery in induction chemotherapy exhibit a poor clinical outcome in adult AML and childhood ALL (15,16). The mechanism underlying this association is unclear, but leukemic blasts in bone marrow are likely to affect the leukocyte count until remission and rate of myeloid recovery following induction therapy. In the present study, chemosensitivity of non-malignant hematopoietic cells were evaluated following remission induction, when the effect of residual leukemic cells may almost be ignored.

A false association between leukopenia and treatment outcome may have been established, since more severe leukopenia was predicted, as the patients had prolonged survival and received more treatment courses. In the present cohort, 17 of the 19 patients completed all three courses of the first half of the consolidation phase. The remaining two patients who relapsed in the third course also received two out of three courses. Low hematological toxicity could not be fully explained by a reduced number of chemotherapy courses.

The results of the present study indicated that leukopenia may be used as a biomarker for effective chemotherapy dose, supporting the theory of individualizing chemotherapy dosage based on hematological toxicity (17). Patients with low acute hematological toxicity may be rapid metabolizers of cytotoxic agents. Considering that the hematopoietic cells of these patients exhibit low sensitivity to cytotoxic agents, corresponding leukemic blasts may also demonstrate low sensitivity to the drugs. Whether the outcome of these patients may be improved by dose-escalation must be prospectively studied in a large clinical trial.

The current study was unable to evaluate the influence of other possible prognostic factors by multivariate analysis, as the number of patients was too small. However, patients in the present cohort were stratified into the same risk group and were roughly adjusted for the conventional factors, including age, leukocyte count at diagnosis, immunophenotypes of leukemic blasts and early treatment response. This may be one of the reasons why these factors were not associated with relapse. In addition, chemotherapy-induced leukopenia is unlike the conventional risk factors, since it reflects the response of normal hematopoietic cells, but not tumor cells. Leukocyte nadir is thus predicted to be an independent prognostic factor. Further investigation in a larger cohort is required to assess this possibility.

In conclusion, the degree of chemotherapy-induced leukopenia was found to correlate with RFS in child patients with ALL. Trials exploring intrapatient dose escalation are warranted.

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ORIGINAL ARTICLE

Treatment outcomes of adolescent acute lymphoblastic leukemia treated on Tokyo Children's Cancer Study Group (TCCSG) clinical trials

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Abstract There is no standard treatment for adolescents aged 15 years or older with acute lymphoblastic leukemia (ALL), although this age group has been reported as having a poorer prognosis compared to younger patients. We retrospectively analyzed the outcomes of three consecutive Tokyo Children's Cancer Study Group ALL trials (1995–2006) of 373 patients aged 10 years or older, with particular focus on adolescents aged 15–18 years (older-adolescents $n = 41$), compared to those aged 10–14 years (younger-adolescents $n = 332$). The probability of event-free survival at 8 years was 67.5 ± 7.4 % for the older-adolescents and 66.5 ± 2.6 % for the younger-adolescents ($p = 0.95$). Overall survival was 70.7 ± 7.1 % for the older-adolescents and 74.3 ± 2.4 % for the younger-adolescents ($p = 0.48$). The differences between groups in relapse incidence, non-relapse mortality, and death rate during induction were not statistically significant, although the older-adolescents trended towards a higher frequency of having stem-cell transplantation during the first remission. In conclusion, our treatment strategy, which consists of intensive induction and block-type consolidation, provided improved outcomes for patients aged 15–18 years, comparable to those for patients aged 10–14 years.

Keywords Acute lymphoblastic leukemia · Adolescents · Clinical trial

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