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Introduction

The translocation t(1;19)(q23;p13) and its unbalanced variant der(19)t(1;19)(q23;p13) are well-known chromosomal abnormalities in pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) [1, 2]. This translocation results in the fusion of *TCF3* on 19p13 with *PBX1* on 1q23, generating the fusion gene *TCF3-PBX1* on derivative chromosome 19 [3].

Although t(1;19)(q23;p13) was initially associated with poor prognosis in pediatric BCP-ALL, the recent development of intensified chemotherapy regimens has improved the outcome of this subgroup, resulting in a 5-year event-free survival (EFS) rate of ~85–90% in western countries, which is similar to that of *TEL-AML1* positive or high hyperdiploid BCP-ALL [2, 4–6]. However, ~10% of patients experience relapse with dismal prognosis [2, 4], underscoring the importance of identifying reliable prognostic markers to improve the treatment of these patients. In the last decades, several studies have attempted to identify prognostic markers for this subgroup of pediatric BCP-ALL with unsatisfactory results [4, 5, 7]. Classic prognostic factors, such as age at onset, initial white blood cell (WBC) count, National Cancer Institute (NCI) risk group, and type of chromosomal abnormality [balanced t(1;19) and unbalanced t(1;19)], did not have prognostic value in recent studies [4, 5]. Genetic analysis to identify alterations related to poor prognosis in pediatric BCP-ALL patients with *TCF3-PBX1* fusion has not been performed to date, with the exception of one study that analyzed the relationship between *TP53* mutation and poor prognosis in a small number of patients [8]. Herein, we reviewed the clinical data of 112 pediatric BCP-ALL patients with *TCF3-PBX1* fusion, which is the largest such cohort reported to date. Additionally, we performed genetic analyses, including *IKZF1* and *TP53*, to determine the prognostic value of these genetic alterations in pediatric BCP-ALL with *TCF3-PBX1*.

Design and Methods

Patient cohorts and samples

From April 2002 to May 2008, 1138 patients aged 1–18 years with newly diagnosed BCP-ALL were enrolled in the Japan Association of Childhood Leukemia Study (JACLS) ALL02 study [9–11]. The diagnosis of BCP-ALL was based on morphological findings of bone marrow (BM) aspirates and immuno-phenotypic analysis of leukemic cells by flow cytometry. Conventional cytogenetic analysis using G-banding was performed as part of the routine workup. Molecular studies using quantitative RT-PCR (RQ-PCR) for the detection of *TCF3-PBX1* were also performed as part of the routine workup (Table S1). Ph + ALL and infantile ALL patients were excluded from the study. Patients with Down syndrome were also excluded.

Bone marrow smears were examined under the microscope on days 15 and 33 (at the end of the induction phase) to evaluate the treatment response. M1, M2, and M3 marrow were defined as fewer than 5%, 5–25%, and more than 25% blast cells in the BM aspirate, respectively. Complete remission (CR) was defined as the absence of blast cells in the peripheral blood, fewer than 5% blast cells in the BM aspirate, normal cellularity and trilineage hematopoiesis, and absence of blast cells in the cerebrospinal fluid and elsewhere. RQ-PCR for *TCF3-PBX1* was also performed on days 15, 33, and 71 (at the end of consolidation) to determine minimal residual disease (MRD). The *GAPDH* gene was amplified as an internal control of RNA quality.

An independent validation cohort of 30 pediatric BCP-ALL patients with *TCF3-PBX1* fusion was enrolled from the Children's Cancer and Leukemia Study Group (CCLSG) ALL 2004 protocol between June 2004 and May 2009 [12]. The diagnosis of BCP-ALL was based on morphological and immuno-phenotypic analyses as described for the JACLS cohort. Patients with t(1;19)/der(19)t(1;19) determined by G-banding analysis or *TCF3-PBX1* fusion determined by RQ-PCR in the JACLS or CCLSG cohorts were enrolled in this analysis. Informed consent was obtained from the patients' guardians according to the Declaration of Helsinki; treatment and genetic study protocols were approved by the Institutional Review Boards of the participating institutions.

Determination of *IKZF1* deletion by multiplex ligation-dependent probe amplification analysis

Genomic DNA was isolated from diagnostic BM or peripheral blood samples using the Qiagen DNeasy tissue

and blood kit according to the manufacturer's instructions (Qiagen, Venio, the Netherlands). DNA specimens of 53 patients in the JACLS cohort and 22 patients in the CCLSG cohort were analyzed using the SALSA multiplex ligation-dependent probe amplification (MLPA) kit P335-A4 according to the manufacturer's instructions (MRC Holland, Amsterdam, the Netherlands) as described elsewhere [11, 13].

JAK2 and *TP53* mutations analysis

To screen for the *JAK2* mutation in exons 16, 20, and 21 (accession number NM 004972), genomic DNA was extracted from diagnostic BM samples of two patients in the JACLS cohort harboring *IKZF1* deletions. Because the frequency of *JAK2* mutation has been reported to be quite rare and clustered in the patients with *IKZF1* deletion [11], we analyzed *JAK2* mutation in the patients with *IKZF1* deletion. To screen for *TP53* mutations in exons 5, 6, 7, 8, and 9 (accession number NM 000546), genomic DNA was also extracted from diagnostic BM samples of eight patients in the JACLS cohort who experienced relapse. Because *TP53* mutation was associated with relapsed *TCF3-PBX1* positive ALL [8], genomic DNA extracted from relapsed BM samples of four of the eight cases in the JACLS cohort was also used for *TP53* mutation screening. The primers used for *JAK2* mutation screening were as previously described [11]. The primers used for *TP53* mutation screening are listed in Table S1. The PCR product was analyzed by direct sequencing using a BigDye Terminator sequencing kit (Applied Biosystems, Foster City, CA).

Statistical analysis

Estimation of survival distributions was performed using the Kaplan–Meier method and differences were compared using the log-rank test. A *P*-value of <0.05 (two-sided) was considered significant. EFS and overall survival (OS) were defined as the times from diagnosis to event (any death, relapse, secondary malignancy, and failure to therapy) and from diagnosis to death from any cause or the last follow up. Patients without an event of interest were censored at the date of last contact. The median follow-up time for EFS and OS was 5.2 years. Hazard ratios for probability of relapse between subgroups were calculated using univariate Cox models. Other comparisons were performed using the χ^2 test, Fisher's exact test, and Mann–Whitney *U*-test, as appropriate.

Results

Patient characteristics and basic cytogenetic data in JACLS cohort

The fusion transcript of *TCF3-PBX1* was detected in 82 (7.2%) of the 1138 patients in the JACLS cohort. The characteristics of these 82 patients are summarized in

Table 1. Comparison of the characteristics of 112 BCP-ALL patients with *TCF3-PBX1* fusion between those included and not included in the genetic analyses.

Study protocol	JACLS ALL02			CCLSG ALL2004		
Number of patients	53 Analyzed	29 Nonanalyzed	*P*-value	22 Analyzed	8 Nonanalyzed	*P*-value
Sex (male/female)	28/25	10/19	0.16	9/13	3/5	1.0
Age (years) at diagnosis, median (range)	5 (1–14)	6 (1–15)	0.27	7 (1–14)	9 (2–14)	0.57
WBC count, cells/μL median (range)	24,500 (4700–183,300)	16,900 (4700–55,220)	<0.01	17,100 (3200–118,000)	27,800 (9000–137,360)	0.26
NCI risk group, SR/HR	27/26	22/7	0.04	10/12	3/5	1.0
Chromosome			0.23			0.90
Normal karyotype	13	10		3	1	
t(1;19)(q23;p13)	11	6		6	3	
der t(1;19)(q23;p13)	20	5		12	4	
Unknown	9	8		1	0	
SCT in 1st CR (*n*)	2	0		ND	ND	
Observation period, median (range)	5.7 (1.6–8.7)	4.7 (0.1–8.8)	0.47	6.4 (2.3–7.7)	4.5 (3.5–7.4)	0.02
Relapse, *n* (%)	8 (15.1)	1 (3.4)	0.15	4 (18.2)	2 (25.0)	0.65
Survival			1.0			1.0
Alive, *n* (%)	45 (84.9)	25 (86.2)		20 (90.1)	7 (87.5)	
Dead, *n* (%)	8 (15.1)	4 (13.8)		2 (9.9)	1 (12.5)	

JACLS, Japan Association of Childhood Leukemia Study; CCLSG, Children's Cancer and Leukemia Study Group; WBC, white blood cell; NCI, National Cancer Institute; SR, standard risk; HR, high risk. SCT, stem cell transplantation; CR, complete remission; ND, not determined.

Table 2. Summary of the overall results of clinical trials of pediatric BCP-ALL with *TCF3-PBX1* from major research groups.

	n	Sex (male/female)	Age (years) at diagnosis, median (range)	WBC count, cells/μL, median (range)	CNS relapse (%)	EFS (y)	OS (y)	Reference
I-BFM (I-ALL90, 96, 12-ALLIC02)	48	22/26	ND (0.8–16)	ND (1400–434,000)	0	85	ND	6
SJCRH XIIIa-XV	41	18/23	ND	ND	9	84 (5)	96.4 (5)	5
MRC-ALL97/99	50	ND	ND	ND	6	80 (5)	84 (5)	2
NOPHO-ALL1992 and 2000	47	21/26	7 (1–18)	16,000 (1300–159,000)	0	79 (5)	85 (5)	4
CCLSG ALL2004	30	12/18	7 (1–14)	17,550 (3200–137,360)	6.7	82.8 (5)	86.3 (5)	Present study
JACLS ALL02	82	38/44	6 (1–15)	21,750 (2700–183,300)	1.2	85.4 (3)	89.0 (3)	Present study

CNS, central nervous system; EFS, event-free survival; OS, overall survival; I-BFM, International Berlin-Frankfurt-Munster; SJCRH, St. Jude Children's Research Hospital; MRC, Medical Research Council; NOPHO, Nordic Society of Pediatric Hematology Oncology; JACLS, Japan Association of Childhood Leukemia Study; CCLSG, Children's Cancer and Leukemia Study Group ND, not described.

Table 1 and 2. The median age at diagnosis was 6 years (range, 1–15 years), and 38 were males and 44 were females. The median leukocyte count at diagnosis was 21,750 × 10^9/L (range 2,700–183,300). Forty-nine patients were classified as NCI-SR and 33 patients as NCI-HR. Seventy-three patients were included in the prednisolone good responder (PGR) group and nine patients in the prednisolone poor responder (PPR) group. The translocation was balanced in 17 (20.1%) cases and unbalanced in 25 (30%) cases. Either balanced or unbalanced translocation was not determined in 17 cases, including seven cases with karyotypic failure. Twenty-three (28.1%) cases with normal karyotype and seven (8.5%) cases with karyotypic failure were identified as *TCF3-PBX1* positive by RT-PCR. Fifty-three (64.6%) of the 82 patients were selected for further genetic analysis on the basis of the availability of material for testing. A comparison of the clinical characteristics of patients with and without available DNA/RNA specimens is shown in Table 1. No major differences were observed between the analyzed and nonanalyzed cohorts except for initial WBC count and NCI risk group.

Patient characteristics and basic cytogenetic data in CCLSG cohort

The fusion transcript of *TCF3-PBX1* was detected in 30 (11.4%) of the 264 patients in the CCLSG cohort. The characteristics of these 30 patients are also summarized in Table 1 and 2. The median age at diagnosis was 7 years (range, 1–14 years), and 12 were males and 18 were females. The median leukocyte count at diagnosis was 17,550 × 10^9/L (range 3200–137,360). Thirteen patients were classified as NCI-SR and 17 patients as NCI-HR. All evaluable 27 patients were included in the PGR group. The translocation was balanced in 9 (30%) cases and unbalanced in 16 (53.3%) cases. Four (13.3%) cases with normal karyotype and 1 (0.03%) case with karyotypic failure were identified as *TCF3-PBX1* positive by RT-PCR. Twenty-two (73.3%) of the 30 patients were selected for further genetic analysis on the basis of the availability of material for testing. A comparison of the clinical characteristics of patients with and without available DNA/RNA specimens is shown in Table 1. No major differences were observed between the analyzed and nonanalyzed cohorts.

Conventional adverse prognostic factors were not associated with poor OS in the patients with *TCF3-PBX1* fusion

The predicted 3-year EFS and OS rates in the 82 *TCF3-PBX1* positive patients were 85.4 ± 3.9% and 89.0 ± 3.5% in JACLS cohort, and the 5-yerar EFS and OS were 82.8 ± 7.0% and 86.3 ± 6.4% in CCLSG cohort, respectively, which were comparable to the rates reported in western countries (Fig. 1 and Table 2). In JACLS cohort, nine (11%) of the 82 patients experienced relapse. The site of relapse was the BM in eight patients and the central nervous system (CNS) in one patient. Eight of nine patients experienced relapse during maintenance therapy, and one after allogeneic stem cell transplantation (allo-SCT) during the first CR. Four of the nine patients died of disease progression. The remaining five patients received allo-SCT. However, two of them died of transplant-related complications, and the remaining three eventually experienced relapse and died of disease progression. A comparison of the characteristics of patients according to the occurrence of relapse is shown in Table 3. The results of univariate analysis showed that none of the conventional prognostic factors, including

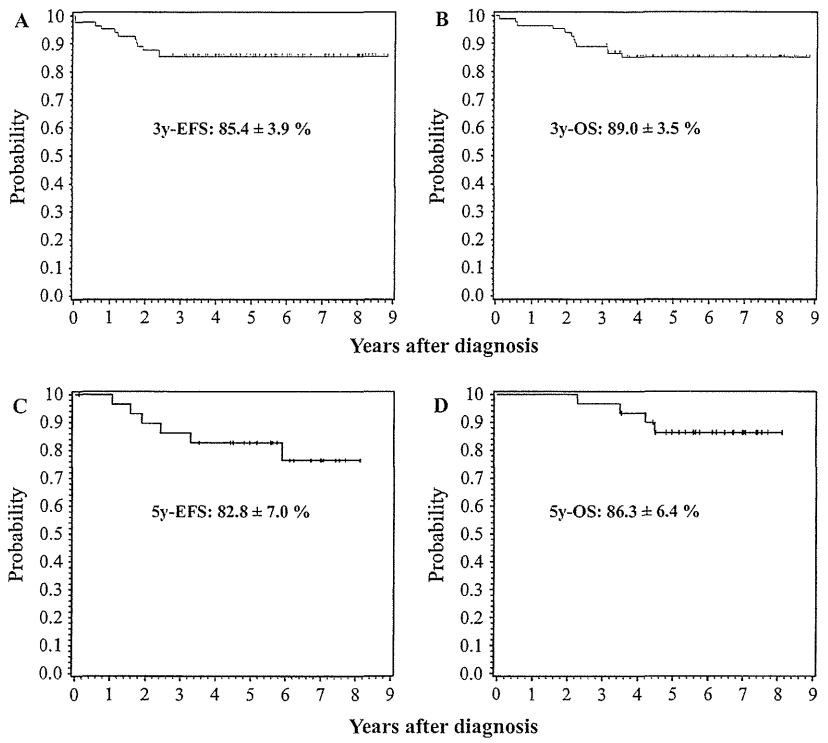

Figure 1. Probability of event-free survival (EFS) and overall survival (OS) in pediatric B-cell precursor acute lymphoblastic leukemia patients with *TCF3-PBX1* fusion treated according to the JACLS ALL02 (n = 82, A and B) and CCLSG ALL2004 protocol (n = 30, C and D). (A and C) EFS, (B and D) OS. JACLS, Japan Association Childhood Leukemia Study; CCLSG, Children's Cancer and Leukemia Study Group.

Table 3. Comparison of the characteristics of BCP-ALL patients with *TCF3-PBX1* fusion according to relapse status in JACLS ALL02 and CCLSG ALL 2004 cohorts.

	JACLS ALL02			CCLSG ALL2004		
	Relapsed	Nonrelapsed	*P*-value	Relapsed	Nonrelapsed	*P*-value
Number of patients	9	73		5	25	
Gender (male/female)	3/6	35/38	0.49	1/4	11/14	0.32
Age (years) at diagnosis, median (range)	7 (1–14)	5 (1–15)	0.81	11 (4–14)	6 (1–14)	0.05
WBC count, cells/μL median (range)	24,500 (9700–70,400)	21,750 (2700–183,300)	0.08	14,500 (9400–26,600)	17,100 (3200–137,360)	0.88
NCI risk group, SR/HR	4/5	45/28	0.53	1/4	12/13	0.25
Chromosome			0.12			0.95
Normal karyotype	4	19		1	3	
t(1;19)(q23;p13)	1	16		2	7	
der t(1;19)(q23;p13)	2	23		1	12	
Unknown	2	15		1	3	
SCT in 1st CR (n)	1	1	0.21	ND	ND	
Survival			<0.01			<0.01
Alive, n (%)	0 (0)	70 (95.6)		1 (25)	25 (100)	
Dead, n (%)	9 (100)	3 (4.4)		4 (75)	0 (0)	

JACLS, Japan Association of Childhood Leukemia Study; CCLSG, Children's Cancer and Leukemia Study Group; WBC, white blood cell; NCI, National Cancer Institute; SR, standard risk; HR, high risk. SCT, stem cell transplantation; CR, complete remission; ND, not determined.

age at onset, initial WBC count, NCI risk group, and BM status on days 15 and 33, were associated with poor EFS/OS in the 82 patients (Table S2). Although data on MRD on days 15 and 33 were not available for all patients, no statistically significant differences in MRD on days 15 and 33 were detected between nonrelapsed and relapsed patients (Figure S1).

In CCLSG cohort, five (16.7%) of the 30 patients experienced relapse. The site of relapse was the BM in four patients and the CNS combined with BM in one patient. Three of five patients experienced relapse during maintenance therapy. Four of the five patients eventually died. A comparison of the characteristics of patients according to the occurrence of relapse is shown in Table 3. The results of univariate analysis also showed that none of the conventional prognostic factors, including age at onset, initial WBC count, and NCI risk group were associated with poor OS in the 30 patients (Table S2).

IKZF1 deletion is identified in relapsed BCP-ALL patients with *TCF3-PBX1* in the JACLS ALL02 cohort

The results of the MLPA analysis were summarized in Tables 4 and 5. Deletion of the *IKZF1* gene was detected in 2 (3.8%) of 53 patients with available DNA sample of diagnostic leukemic blasts, which was a significantly lower frequency than that of pediatric BCP-ALL patients without *TCF3-PBX1* fusion (3.8% vs. 10.4%, $P < 0.001$, Table 4). The *JAK2* mutation was not identified in the two patients with *IKZF1* deletion. Deletions of *PAX5*, *CDKN2A*, and *CDKN2B* were also less frequent in BCP-ALL patients with than in those without *TCF3-PBX1* fusion. However, deletion of *RB1* was detected in nine (17.0%) of 53 patients, which was a higher rate than that of patients without *TCF3-PBX1* fusion (3.0%) (Table 4). In terms of the prognostic impact of these micro deletions, none were associated with an increase of relapse except the *IKZF1* deletion (Table 5).

TP53 mutation has been associated with relapse in BCP-ALL patients with *TCF3-PBX1* fusion [8]. Therefore, we screened for mutations in *TP53* in the diagnostic samples of eight cases experiencing relapse. In addition, *TP53* mutation screening was performed in relapsed leukemic samples from four of the eight relapsed patients. However, *TP53* mutations were not observed in these 12 samples.

IKZF1 deletion is also identified in relapsed patient with *TCF3-PBX1* positive BCP-ALL in the validation cohort (CCLSG cohort)

To confirm the significance of the *IKZF1* deletion in BCP-ALL with *TCF3-PBX1*, 22 diagnostic leukemic samples with *TCF3-PBX1* fusion obtained from the patients registered in the CCLSG ALL2004 protocol were analyzed by MLPA. Although only 1 (4.5%) of 22 patients had the *IKZF1* deletion, this patient experienced relapse and died of the disease (Tables 4 and 5).

Discussion

In this study, we showed that *TCF3-PBX1* positive pediatric BCP-ALL patients treated according to the JACLS ALL02 and CCLSG ALL2004 protocol had favorable outcomes similar to those reported in western countries (Table 2) [2, 4–6]. A low frequency of CNS relapse (1 in 82 patients, 1.2%) was observed in JACLS ALL02 cohort, showing a more favorable result than those reported in the St. Jude and MRC cohorts (Table 2) [2, 5]. However, 9 (11%) of 82 in JACLS and 5 (16.7%) of 30 patients in CCLSG cohort experienced relapse and most of them died of disease progression or transplant-related complications. The clinical and biological features of the relapsed cases

Table 4. Summary of the results of MLPA analyses of *TCF3-PBX1* positive and negative BCP-ALL patients in the JACLS ALL02 and CCLSG cohorts.

	JACLS ALL02 cohort			CCLSG cohort		
	TCF3-PBX1 (+)	*TCF3-PBX1* (−)	*P*-value	*TCF3-PBX1* (+)	*TCF3-PBX1* (−)	*P*-value
Number of patients	53	163		22	155	
IKZF1 deletion (%)	2 (3.8)	17 (10.4)	<0.001	1 (4.5)	21 (13.5)	0.23
CDKN2A deletion (%)	10 (18.9)	71 (43.6)	<0.001	6 (27.3)	37 (23.9)	0.73
CDKN2B deletion (%)	8 (15.1)	61 (37.4)	<0.001	5 (22.7)	40 (25.8)	0.76
PAX5 deletion (%)	12 (22.6)	47 (28.8)	0.37	9 (40.9)	28 (18.1)	0.014
ETV6 deletion (%)	1 (1.9)	46 (28.2)	<0.001	2 (9.1)	40 (25.8)	0.085
RB1 deletion (%)	9 (17.0)	3 (1.8)	<0.001	4 (18.2)	20 (12.9)	0.50
BTG1 deletion (%)	1 (1.9)	14 (8.6)	<0.001	1 (4.5)	20 (12.9)	0.26
EBF1 deletion (%)	2 (3.8)	20 (12.3)	<0.001	2 (9.1)	17 (10.9)	0.79

JACLS, Japan Association of Childhood Leukemia Study; CCLSG, Children's Cancer and Leukemia Study Group.

Table 5. Impact of genetic alterations on the outcomes of patients with BCP-ALL with *TCF3-PBX1*.

	JACLS ALL02 cohort			CCLSG cohort		
	Relapse	Nonrelapse	*P*-value	Relapse	Nonrelapse	*P*-value
Number of patients	8	45		4	18	
IKZF1 deletion (%)	2 (25)	0	<0.01	1 (25)	0	0.03
CDKN2A deletion (%)	1 (12.5)	9 (20)	0.62	2 (50)	4 (22.2)	0.26
CDKN2B deletion (%)	1 (12.5)	7 (15.6)	0.99	2 (50)	3 (16.7)	0.15
PAX5 deletion (%)	2 (25)	10 (22.2)	0.72	2 (50)	7 (38.9)	0.68
ETV6 deletion (%)	0	1 (2.2)	0.67	0	2 (11.1)	0.48
RB1 deletion (%)	2 (25)	7 (15.6)	0.51	0	4 (22.2)	0.30
BTG1 deletion (%)	0	1 (12.5)	0.67	1 (12.5)	0	0.18
EBF1 deletion (%)	0	2 (25)	0.54	0	2 (11.1)	0.48

JACLS, Japan Association of Childhood Leukemia Study; CCLSG, Children's Cancer and Leukemia Study Group.

should be evaluated to further improve the prognosis of pediatric BCP-ALL with *TCF3-PBX1* fusion. To the best of our knowledge, a reliable prognostic marker has not been identified in this subgroup [4, 5, 7]. In this study, conventional prognostic markers, such as age at onset, initial WBC count, and NCI risk group, were not associated with poor prognosis, which was in agreement with previous studies. Furthermore, we found that the type of chromosomal abnormality had no prognostic impact [4, 5]. However, the pattern of relapse was unique in the present study, especially in JACLS cohort: eight of nine patients relapsed during the maintenance phase and one patient had primary induction failure, suggesting that leukemic blasts in these patients initially showed resistance to the chemotherapeutic agents used. However, BM status and assessment of MRD on days 15 and 33 did not identify those patients who experienced very early relapse, suggesting that a small fraction of leukemic cells contributed to relapse.

Few studies have investigated the association between genetic alterations and prognosis in BCP-ALL with *TCF3-PBX1*. Kawamura et al. identified point mutations of *TP53* in two of 20 initial patients and in four relapsed patients with BCP-ALL expressing *TCF3-PBX1* and suggested a possible relationship between *TP53* mutation and disease progression in BCP-ALL patients with *TCF3-PBX1* fusion [8]. Therefore, in this study, we screened for *TP53* mutations in *TCF3-PBX1* positive BCP-ALL patients who experienced relapse. However, no mutations in *TP53* (exons 5–9) were detected in four relapsed leukemia samples of eight relapsed patients. Furthermore, *TP53* mutation analysis of the diagnostic samples of eight relapsed patients did not show any mutations. However, because the prognosis of BCP-ALL with *TCF3-PBX1* fusion has improved dramatically in the last decades, *TP53* mutations might not be associated with relapse in this subgroup.

IKZF1 deletion is known to be a strong prognostic factor associated with poor outcome in pediatric BCP-ALL [11, 14–16]. However, the significance of *IKZF1* deletion in BCP-ALL with *TCF3-PBX1* fusion has not been determined. Previous studies have shown that *IKZF1* deletion is less frequent in BCP-ALL with recurrent chromosomal abnormalities [11, 14–16], which is in agreement with our results showing that the frequency of *IKZF1* deletion was lower in the *TCF3-PBX1* fusion positive subgroup than in the other subgroups (Table 4). However, *IKZF1* deletion was detected in 2 (25%) of eight evaluable relapsed patients, whereas it was not present in any of the patients maintaining continuous CR (Table 5). In addition, MLPA analysis of 22 diagnostic samples of BCP-ALL with *TCF3-PBX1* in the independent CCLSG cohort identified one patient with *IKZF1* deletion who eventually relapsed and died. Although our observation may be potentially interesting, further study employing larger cohort is warranted to determine the prognostic impact of *IKZF1* deletion in this subgroup.

Ferreiros-Vidal et al. reported that Ikaros-regulated genes are highly represented in pre-Bcell receptor signaling, cell cycle regulation, and the somatic rearrangement of Ig genes, which are key to the differentiation of B-cell progenitors [17]. These authors showed that inducible Ikaros expression in cycling pre-B cells was sufficient to drive transcriptional changes, such as repression of *Myc*, *Cdk6*, *Ccnd2*, *Cdkn1a*, and *Cdkn1b*, resembling the differentiation of cycling to resting pre-B cells in vivo. These findings suggested that haplo-insufficiency of *IKZF1* might be associated with the differentiation block and accelerated cell cycle progression in BCP-ALL. In that study, 52% of TCF3 target genes were also bound by Ikaros, suggesting that these two transcriptional factors collaborate to regulate B-cell specification. These findings indicate that a severe impairment in the differentiation of cycling to resting pre-B cells in BCP-ALL with *IKZF1* deletion and *TCF3-PBX1* fusion may be associated with poor prognosis.

In this study, no additional genetic alterations were detected in ~75% of relapsed patients with *TCF3-PBX1*.

To identify additional genetic alterations related to poor prognosis, a comprehensive genomic analysis using matched pair samples of onset and relapse might be useful. Our group is therefore planning to perform exome analysis of relapsed and nonrelapsed patients with BCP-ALL expressing *TCF3-PBX1*.

In conclusion, the prognosis of pediatric BCP-ALL patients with *TCF3-PBX1* fusion treated according to the JACLS ALL02 and CCLSG ALL2004 protocol was similar to that reported in studies performed in Western countries. Although concomitant *IKZF1* deletion may account for ~25% of treatment failure in this subgroup, further study of larger cohort is warranted to determine the prognostic impact of *IKZF1* deletion in this subgroup.

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Conflict of Interest

None declared.

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Supporting Information

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

Table S1. The primer list of *TCF3-PBX1* and *TP53* used in the current study.

Table S2. Univariate Cox model of event-free and overall survival of 112 patients with *TCF3-PBX1*

Figure S1. Comparison of minimal residual disease (MRD) between relapsed and nonrelapsed patients determined by quantitative RT-PCR of the *TCF3-PBX1* transcript on days 15 and 33 of the induction phase in JACLS cohort: (A) day 15 and (B) day 33.

BRIEF REPORT

Loss of Mismatched HLA in Myeloid/NK Cell Precursor Acute Leukemia Relapse After T Cell-Replete Haploidentical Hematopoietic Stem Cell Transplantation

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Myeloid/natural killer cell precursor acute leukemia (MNKL) is an aggressive disease with a high relapse rate even after allogeneic hematopoietic stem cell transplantation (SCT). We report a patient with MNKL who had a donor lymphocyte infusion (DLI) for relapse after T cell-replete human leukocyte antigen (HLA)-haploidentical SCT, but relapsed again 20 months later with loss of mismatched HLA. This case suggests that a strong graft-versus-leukemia effect of haploidentical SCT can be expected in MNKL patients. In the haploidentical setting, DLI should be considered for patients with relapsed leukemia whose leukemic cells have not lost HLA cell surface expression. Pediatr Blood Cancer

Key words: acute; BMT; graft versus host disease; hematology/oncology; leukemias; rare tumors; stem cell transplantation

INTRODUCTION

The efficacy of donor leukocyte infusions (DLI) is excellent in chronic-phase chronic myeloid leukemia (CML) [1–3] but limited in other disorders [1,2,4–8]. We present a male patient who had a leukemic relapse 5 months after T cell-replete human leukocyte antigen (HLA)-haploidentical stem cell transplantation (SCT) for myeloid/natural killer cell precursor acute leukemia (MNKL). He achieved complete remission (CR) with full whole-blood chimerism after DLI without additional chemotherapy; however, he relapsed 20 months after the DLI with loss of mismatched HLA cell surface expression.

CASE REPORT

A 17-year-old male was admitted to his local hospital with headache and stomatorrhagia. Blood count analysis revealed anemia (Hb 4.9 g/dl) and thrombocytopenia (9,000/μl). His white blood cell (WBC) count was 9,600/μl, and 64% of the cells were lymphoblasts. Bone marrow (BM) aspiration revealed a hypercellular marrow with 92.6% myeloperoxidase negative lymphoblasts. Immunophenotypic analysis showed the leukemic blasts to be positive for CD56, CD7, CD33, CD34, CD117, and HLA-DR, but negative for surface CD3 and CD13. Cytogenetic analysis of the bone marrow cells demonstrated complex abnormalities, defined as 46, XY, t(10;11) (p12;q14), add(12)(p13), add(22)(p11.2).

The diagnosis of MNKL was determined and the patient was subsequently treated with induction chemotherapy for acute myeloid leukemia (AML). However, he did not achieve CR and received treatment for acute lymphoblastic leukemia. However, the normal counterpart of peripheral blood increased, 21% leukemic cells persisted in the BM. Thus, a third chemotherapy regimen consisting of high dose cytosine arabinoside (2 g/m^2) for 3 days and VP16 (100 mg/m^2) for 3 days in combination with dexamethasone was administered. After the course of chemotherapy, the patient achieved CR. The patient was transferred to our hospital to receive an allogeneic SCT.

Although the BM aspiration just before starting conditioning regimen showed 20% blasts, the patient underwent haploidentical transplantation using T cell-replete peripheral blood stem cells from his brother with four HLA allele mismatches in the graft-versus-host direction. The conditioning regimen consisted of fractionated total body irradiation (12 Gy total dose), VP16 (1,800 mg/m^2), cyclophosphamide (60 mg/kg × 2) and antithymocyte globulin (1.25 mg/kg × 2). The graft contained 12.7×10^6 CD34$^+$ cells/kg, and 3.7×10^8 CD3$^+$ cells/kg. Graft-versus-host disease (GVHD) prophylaxis consisted of prednisolone (PSL initial dose of 1 mg/kg/day from day +1), tacrolimus and a short course of methotrexate (10 mg/m^2 at day ±1, 7 mg/m^2 at days ±3, ±6). Hematological reconstitution was prompt: a neutrophil count of $>0.5 \times 10^9$/L, and a platelet count of $>50 \times 10^9$/L were observed on day 13 and 24 after transplantation, respectively. The patient achieved CR, and chimerism analysis demonstrated that all BM cells were donor derived on day +33. Grade II acute GVHD (skin rash) observed on day +39 during the tapering process required prolonged immunosuppression, consisting of PSL 0.7 mg/kg and tacrolimus 7 mg daily but subsided with no increase in the dose of immunosuppressive agents which were gradually tapered. PSL was discontinued on day +70, and tacrolimus was tapered to 4 mg/day without the development of GVHD on day +83.

However, 5 months after transplantation, he had a BM relapse of the leukemia. Surface marker analysis showed that the leukemic cells had the same phenotype as the previously relapsed tumor cells. Tacrolimus was discontinued, and 7 days later the patient received DLI at a dose of 1×10^6 CD3$^+$ cells/kg without any prior therapy

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Conflict of interest: Nothing to declare.

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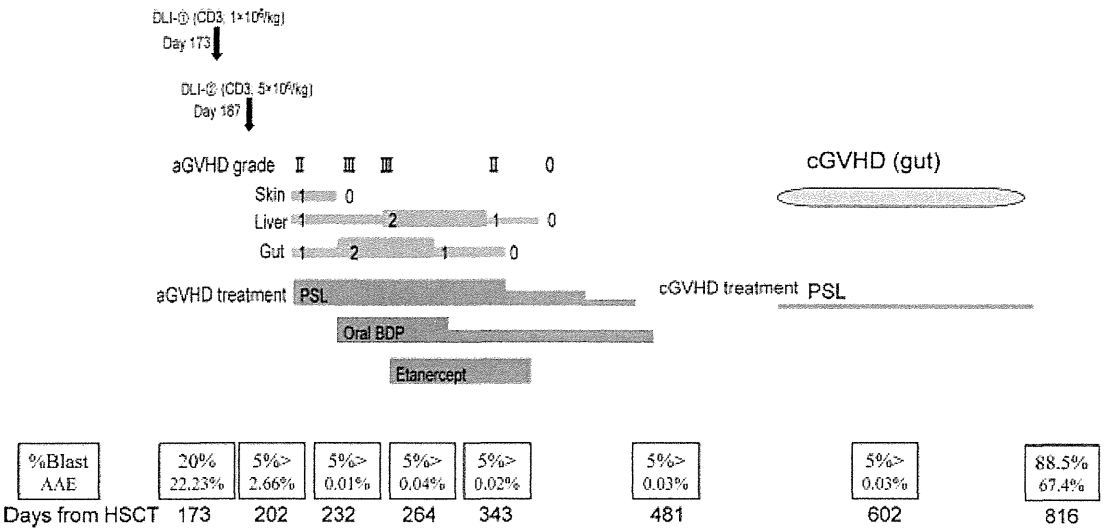

Fig. 1. Clinical course after the first DLI. T lymphocytes (CD3+) normalized and CD4+ T cell counts of more than 150/μl by 6 months after DLI. DLI, donor lymphocyte infusion; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; PSL, prednisolone; BDP, beclomethasone dipropionate; AAE, aberrant antigen expression using CD45 gating for minimal residual disease.

and GVHD prophylaxis on day +173 (Fig. 1). It took 2 weeks until there were no signs of GVHD, and the second DLI was performed. The dose of $CD3^+$ cells was increased to 5×10^6 cells/kg. BM aspirate performed 29 days after the first DLI showed a reduction in blasts to less than 5%. Thirty days after the first DLI, acute GVHD of the skin (grade I), liver (grades II–III) and gut (grades II–III) appeared, and oral beclomethasone dipropionate was administered, together with PSL (1 mg/kg/day). The patient's skin and gut GVHD reduced; however, his liver GVHD did not. Thus, we started therapy with etanercept at a dose of 0.4 mg/kg subcutaneously twice weekly for 8 weeks from day ±237. Liver GVHD progressively improved from grade III (total bilirubin level: 3.0–4.2 mg/dl) to grade I (total bilirubin level: 1.0–1.6 mg/dl) by day +278, which permitted the tapering of PSL. Mild chronic GVHD of the gut was treated with a low dose of PSL 5 mg/day while leukemia was in CR without additional therapy. However, leukemia relapsed on day ±816. HLA molecular typing was performed at relapse, and the genomic alteration resulted in the loss of HLA haplotype (Fig. 2).

DISCUSSION

MNKL is an uncommon entity and was originally proposed by Suzuki et al. [9] in 1997. In this report, seven cases were characterized by immature lymphoblastoid morphology without myeloperoxidase reactivity, $CD7^+$, $CD33^+$, $CD34^+$, and $CD56^+$ phenotype.

MNKL generally shows poor response to chemotherapy and the prognosis is poor [9–16]. Suzuki et al. [17] reported on 40 patients with neoplasms of NK-cell origin, who underwent SCT, including MNKL. The 4-year survival was better than that of patients who did not undergo SCT (39% vs. 21%, $P = 0.0003$). The probability of relapse for patients after SCT was as low as 17%. The lower incidence of relapse may indicate a GVL effect against NK-lineage tumors.

Acute leukemia relapses after allogeneic SCT has a high mortality rate. DLI can exert a GVL effect in the treatment of the molecular, cytogenetic, and chronic-phase relapses of CML with a remission induction rate as high as 80% [1–3]. When DLI is used to treat other types of leukemia, far lower response rates have been observed [1,2,4–8].

However, Huang et al. [18] reported the efficacy of DLI in patients who had a leukemic relapse after haploidentical SCT. Twenty patients received DLI at a median of 177 days after SCT. Eight patients survived in CR for a median of 1,118 days. The 2-year probability of leukemia-free survival was 40%. These data suggest that DLI is therapeutically effective in the haploidentical setting.

Two studies of patients pre- and post-SCT from an HLA-haploidentical donor describe the loss of HLA expression occurring in leukemia cells following relapse [19,20]. It was speculated in their reports and by others in the context of haploidentical transplantation that selective pressure mediated by donor T cells led to the loss of HLA haplotype in AML relapse. In our patient's case, his leukemic cells without mismatched HLA expression might have been predisposed to selective expansion through in vivo escape from immune surveillance by alloreactive T cells. Because the isolated

	A allele		B allele		Cw allele		DRB1 allele	
Patient	02:01	02:07	40:02	46:01	03:03	01:02	15:01	08:03
Donor	02:01	02:01	40:02	48:01	03:03	08:03	15:01	15:01
Blasts	02:01	-	40:02	-	03:03	-	15:01	-

Fig. 2. Loss of unshared human leukocyte antigen in enriched relapsing leukemic cells. Donors and recipient are KIR-epitope matched at the HLA-C locus.

blasts were not investigated for HLA expression at the relapse, we could not determine the time when the leukemic cells had lost their HLA. Therefore, we cannot rule out the possibility that other immune mediators such as NK cells might play a role especially in the prevention of rapid growth of the blasts that had lost the HLA.

Although our patient had a leukemic relapse with loss of mismatched HLA cell surface expression, our report demonstrates that a strong GVL effect of haploidentical SCT by directly targeting mismatched HLAs might also be expected in patients with MNKL. Novel therapeutic tools are needed for targeted or prophylaxis treatment of these peculiar variants of post-transplantation relapse.

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OPEN

Assessment of Corticosteroid-induced Osteonecrosis in Children Undergoing Chemotherapy for Acute Lymphoblastic Leukemia: A Report From the Japanese Childhood Cancer and Leukemia Study Group

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Summary: Steroid-induced osteonecrosis (ON) is a challenging complication encountered during modern chemotherapy for childhood acute lymphoblastic leukemia (ALL). We retrospectively assessed the incidence of ON and its risk factors in a total of 1095 patients enrolled in 3 consecutive Japanese Children's Cancer and Leukemia Study Group ALL studies (ALL941 [1994 to 2000], n = 464; ALL2000 [2000 to 2004], n = 305; and ALL2004 [2004 to 2010], n = 326). ON was diagnosed in 16 patients, of whom 15 were symptomatic. The cumulative incidence of ON was 0.76% in ALL941, 0.35% in ALL2000, and 3.6% in ALL2004. The incidence of ON in ALL941/2000, in which only prednisolone was administered as a steroid, was significantly lower than that in ALL2004, in which dexamethasone was used as a partial substitute for prednisolone ($P < 0.01$). In ALL2004, sex and age were significantly correlated with the incidence of ON (1.3% in boys vs. 6.7% in girls, $P = 0.0132$; 0.42% for age < 10 y vs. 15.6% for age ≥ 10 y, $P < 0.0001$), suggesting that girls aged 10 years and above are at a greater risk of ON onset. These results indicate that the risk of ON should be considered when administering dexamethasone as part of ALL protocol treatment in girls aged 10 years and above.

Key Words: acute lymphoblastic leukemia, osteonecrosis, corticosteroid, dexamethasone

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Recent advances in treatment strategies for childhood acute lymphoblastic leukemia (ALL) have improved the overall survival rate by 80% to 90%.[1–3] Enhanced chemotherapeutic agents, refined risk classification criteria, and improved supportive care have contributed to these high cure rates, but significant toxicity remains a major risk factor that causes long-term morbidity and decreased quality of life. Osteonecrosis (ON) has been increasingly documented in pediatric ALL and presents a challenging complication during modern chemotherapy.[4–7] ON can result in joint dysfunction and subsequent impairments in activities of daily living among long-term survivors.[8,9] Well-known risk factors for ON include age above 10 years, female sex, and use of dexamethasone (DEX).[5,7] Although the precise pathophysiology of ON remains unknown, corticosteroid administration has been shown to induce ischemia, upregulate apoptosis of osteoblasts and osteocytes, and prolong osteoclast lifespans.[10]

Most previous studies regarding ON in children with ALL have been limited to European and North American study groups, as there is little data concerning Japanese or Asian patients. Therefore, the aim of the present study was to assess the incidence, risk factors, and morbidity of corticosteroid-induced ON in ALL studies conducted by the Japanese Childhood Cancer and Leukemia Study Group (JCCLSG). We retrospectively analyzed the data of 1095 patients enrolled in 3 consecutive ALL studies (ALL941, ALL2000, and ALL2004) conducted by the JCCLSG. Prednisolone (PSL) was used as the primary corticosteroid in all studies, with DEX acting as a partial substitute for PSL in ALL2004. ON patients were practically identified by symptoms and ON was confirmed with imaging studies in all patients.

MATERIALS AND METHODS

Patients and Treatment

ALL941, ALL2000, and ALL2004 were conducted between 1994 and 2000, 2000 and 2004, and 2004 and 2010, respectively. The therapies on these studies were risk adjusted but not randomized. Patients enrolled in ALL941 and ALL2000 were aged 1 to 15 years, whereas those enrolled in ALL2004 were aged 1 to 18 years. All participants were newly diagnosed with B-precursor ALL or T-cell ALL, and those with a mature B-cell phenotype and Philadelphia chromosome-positive ALL were excluded. All studies were conducted across 18 hospitals that were members of the JCCLSG. The study protocol was approved by the institutional review board of each study,

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and written informed consent was provided by the patients or their legal guardians before treatment.

The treatment protocols adopted in ALL941 and ALL2000 were reported previously.[11,12] The patients were stratified according to leukocyte count and age at the time of diagnosis into standard-risk (SR), high-risk (HR), and high-high-risk (HHR) groups. The ALL941 and ALL2000 study protocols were almost identical except for the addition of doxorubicin administration to patients with a leukocyte count $<10{,}000/\mu L$ and age below 5 years in ALL2000. Treatment schedules and adopted drugs are briefly described in the supplement (see Supplemental Digital Content 1, Table 1,http://links.lww.com/JPHO/A55).

The ALL2004 treatment protocols are described in Figure 1 and Table 1. Previous risk classification criteria were modified according to the National Cancer Institute criteria,[13] resulting in a shift from HR to SR in 6- to 9-year-old patients with leukocyte counts of 5000 to 10,000 cells/μL. After a 7-day PSL regimen, induction therapy in the SR and HR groups was almost identical to that in previous studies.[11,12] In the HHR group, cyclophosphamide was added on day 8. After achieving complete remission, all risk groups received the same intensification therapy (Int-1). At week 15, SR patients with MRD levels $<10^{-3}$ received further intensification therapy (Int-2) that was followed by maintenance therapy (M-1 and M-2) until week 110. In the HR and HHR groups, patients with MRD levels $<10^{-3}$ at week 15 received 2 cycles of reinduction/intensification therapy (Rc1/Int-2 and Rc1/Int-3 in HR, Rc2/Int-4 and Rc2/Int-5 in HHR group) that was followed by the same maintenance therapy (M-3 and M-4) until week 165. Patients with MRD levels $\geq 10^{-3}$ at week 12 in the SR and HR/HHR groups were assigned to salvage arms 1 and 2, respectively. In the salvage regimen, patients received intensification therapy comprising 2 cycles of Rc-2/etoposide + cytarabine + L-asparaginase. For CNS prophylaxis, SR and HR patients received extended TIT injections beginning from day 1. When IT was combined with a high dose of methotrexate, only cytarabine and hydrocortisone were injected (double intrathecal [DIT] injection). TIT injections were repeated every 6 weeks in the first year, every 8 weeks in the second year, and every 12 weeks in the third year. The HHR group patients included in salvage arm 2 received 18 Gy of CRT in addition to 6 and 7 doses of TIT injections until week 22 and 32 of therapy, respectively.

Cumulative doses of the corticosteroids administered in ALL941/2000 and ALL2004 are listed in Table 2.

Identification of ON Patients

Because the significance of this therapy-related toxicity had not been fully appreciated until the early 2000s, case report form in the 3 studies did not request data regarding ON. Thus, cases with ON were collected by the questionnaire specified for ON to the investigators of JCCLSG. Most of the ON patients were identified based on clinical symptoms such as bone pain and further confirmed with diagnostic imaging studies (x-ray/magnetic resonance imaging [MRI]) by the each institutional radiologists, except one who was asymptomatic and diagnosed by imaging studies at the discretion of primary physician.

Statistical Analysis

Differences in the categorical variables of patient characteristics were analyzed using the χ^2 test. The cumulative incidence of ON during the study period was estimated using Kaplan-Meier analysis. The median follow-up periods were 147.4, 100.3, and 43.6 months for patients enrolled in ALL941, ALL2000, and ALL2004, respectively (median follow-up period, 78.7 mo for all patients). Differences in cumulative incidence between patient subsets were tested using the log-rank test.

RESULTS

A total of 1095 patients were enrolled in the present study (ALL941, n = 464; ALL2000, n = 305; and ALL2004, n = 326). Sixteen of 1095 patients developed ON during or after treatment, 4 (0.86%), 2 (0.65%), and 10 (3.1%) were in ALL941, ALL2000, and ALL2004, respectively. In patients with ON, the median age at diagnosis of ALL was 11.5 years (range, 5 to 16y) and the male to female ratio was 1:3. When patients were evaluated by risk classification, only 1 patient in the SR group and 15 patients in the HR/HHR groups developed ON.

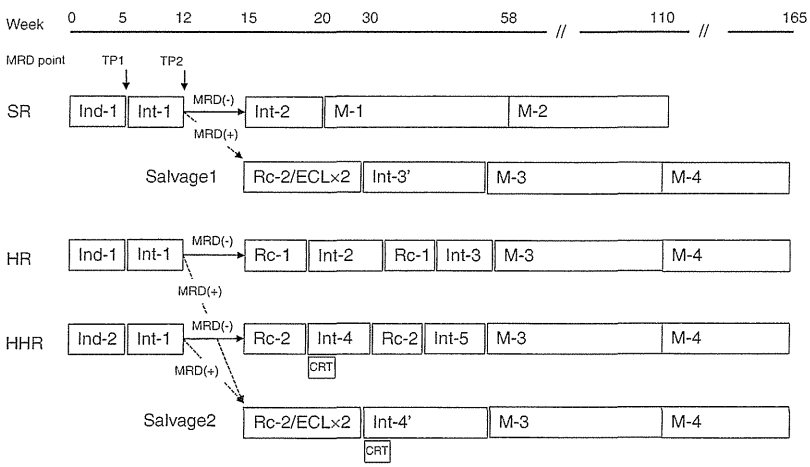

FIGURE 1. Treatment framework and minimal residual disease (MRD) stratification in the ALL2004 study. Patients with MRD levels $\geq 10^{-3}$ at week 12 received salvage therapy (dotted arrows), whereas the remainder continued to receive the initial risk-adapted therapy (solid arrows). Treatment schedules are shown in Table 1. CRT indicates cranial radiotherapy; HR, high-risk group; HHR, high-high-risk group; SR, standard-risk group.

TABLE 1. Drug Dosage and Schedule for ALL2004

	Regimen	Daily Dose	Administration	Days
Induction phase				
Ind-1	VCR	2 mg/m^2	IV	8, 15, 22, 29
	LASP	2000 U/m^2	IM	9-34 (3/wk)
	PSL*	60 mg/m^2	Oral	1-28
	DOX	25 mg/m^2	IV	8, 15, 22
Ind-2	Same as in Ind-1 except for CY (1200 mg/m^2)			8
Intensification phase				
Int-1				
AA	THP	20 mg/m^2	IV	1, 43
	VCR	2 mg/m^2	IV	1, 43
	PSL	120 mg/m^2	Oral	1-5, 43-47
	6MP	250 mg/m^2	Oral	1-5, 43-47
C	CY	400 mg/m^2	IV	15
	CA	50 mg/m^2 × 2	IV	15-18
	6MP	125 mg/m^2	Oral	15-19
BH	MTX	3000 mg/m^2	IV	29
Weekly LASP	LASP	6000 U/m^2	IM	1-50 (1/wk)
Int-2				
C + BH × 3 + A	VCR	2 mg/m^2	IV	43
	PSL	120 mg/m^2	Oral	43-47
	6MP	250 mg/m^2	Oral	43-47
Int-3				
CH + BH × 3	CY	500 mg/m^2	IV	1, 15
	CA	75 mg/m^2 × 2	IV	1-4, 15-18
	6MP	60 mg/m^2	Oral	1-28
AA + C + BH†				63-91
Weekly LASP†				63-98 (1/wk)
Int-4				
CHs	CY	500 mg/m^2	IV	1, 15
	CA	75 mg/m^2 × 2	IV	1-4, 15-18
	6MP	30 mg/m^2	Oral	1-28
AA + C + BI‡	MTX	500 mg/m^2	IV	63
Weekly LASP‡				35-70 (1/wk)
Int-5				
ECL + AA + C + BI	E	100 mg/m^2	IV	1-4
	CA	2000 mg/m^2 × 2	IV	1-4
	LASP	6000 U/m^2	IM	5
Weekly LASP				21-98 (1/wk)
Reinduction phase				
Rc-1	VCR	2 mg/m^2	IV	1, 8, 15, 22
	LASP	2000 U/m^2	IM	2-20 (3/wk)
	DEX*	10 mg/m^2	Oral	1-14
	DNR	25 mg/m^2	IV	1, 8, 15
Rc-2	VCR	2 mg/m^2	IV	1, 8, 15, 22
	LASP	6000 U/m^2	IM	9-20 (3/wk)
	DEX*	10 mg/m^2	Oral	1-14
	DNR	40 mg/m^2	IV	1, 8, 15
	CY	1200 mg/m^2	IV	1
Maintenance phase				
M-1 (C + B + A)	MTX	225 mg/m^2	IV	15
	LASP	2000 U/m^2	IM	15
M-2 (B + As)	VCR	2 mg/m^2	IV	15
	PSL	80 mg/m^2	Oral	15-19
M-3 (AA-C-B)				1-29
M-4 (Bs + As)	MTX	225 mg/m^2	IV	1
CNS prophylaxis				
TIT	MTX	12 mg/m^2	IT	
	CA	30 mg/m^2		
	HDC	50 mg/m^2		
DIT	Same as TIT except for methotrexate			

*PSL and DEX were tapered off (PSL; 30 mg/m^2 for 3 d and 15 mg/m^2 for 4 d, DEX; 5 mg/m^2 for 3 d and 2.5 mg/m^2 for 4 d).
†Repeat 2 cycles in Int-3′ for salvage 1.
‡Repeat 3 cycles in Int-4′ for salvage 2.
6MP indicates 6-mercaptopurine; CA, cytarabine; CY, cyclophosphamide; DEX, dexamethasone; DNR, daunorubicin; DOX, doxorubicin; E, etoposide; HDC, hydrocortisone; LASP, L-asparaginase; MTX, methotrexate; PSL, prednisolon; THP, pirarubicin; VCR, vincristine.

TABLE 2. Cumulative Dose of Corticosteroid in Trials ALL941/2000 and ALL2004

	ALL941/2000		ALL2004	
	PSL (mg/m²)	DEX (mg/m²)	PSL (mg/m²)	DEX (mg/m²)
Induction	1830	—	1830	—
Reinduction				
SR	—	—	—	—
HR/HHR	1830	—	—	330
Intensification				
SR	600	—	1200	—
HR/HHR	1200	—	2400	—
Maintenance				
SR	18,000	—	9200	—
HR/HHR	15,000	—	12,200	—
Total*				
SR	20,430		12,230	
HR/HHR	19,860		18,575	

*Calculated in PSL equivalents (1 mg of DEX = 6.5 mg of PSL).
DEX indicates dexamethasone; HHR, high-high risk; HR, high risk; PSL, prednisolone; SR, standard risk.

Comparisons of the characteristics of patients with and without ON are presented in Table 3, which shows a predominance of females aged 10 years and above, treatment with ALL2004, and high risk ($P < 0.01$) in patients with ON. Notably, 9 of the 12 female and 3 of the 4 male patients with ON were aged 10 years and above, the latter was marginally significant ($P = 0.044$). ON was diagnosed at median treatment weeks 56.5 (range, 32 to 264) and 66 (range, 37 to 120) in ALL941/2000 and ALL2004, respectively. The median cumulative corticosteroid doses at the time of ON onset were as follows: PSL, 5700 mg/m² (range, 3480 to 13,880 mg/m²) in ALL941/2000 and PSL, 6030 mg/m² (range, 3480 to 13,800 mg/m²) and DEX, 330 mg/m² (range, 240 to 330 mg/m²) in ALL2004. As described in Table 2, SR patients in ALL2004 originally did not receive DEX, and despite the cumulative dose of PSL far exceeded the median doses for patients with ON at onset, none of them eventually developed ON. To obtain total PSL equivalents, DEX was multiplied by a conversion factor of 6.5[14]; therefore, a relatively higher steroid dose

TABLE 3. Comparison of Patient Characteristics Between With and Without ON

	Patients With ON (%)	Patients Without ON (%)	P (χ^2)
All	16	1079	
Sex			< 0.01
Male	4 (25)	606 (56)	
Female	12 (75)	473 (44)	
Age (y)			< 0.01
Male			0.044
1-5	1 (6)	345 (32)	
6-9	0	124 (11)	
>10	3 (19)	137 (13)	
Female			< 0.01
1-5	0	271 (25)	
6-9	3 (19)	102 (10)	
>10	9 (56)	100 (9)	
WBC			
< 10,000	6 (38)	571 (53)	
10,000-100,000	9 (56)	398 (37)	
> 100,000	1 (6)	110 (10)	
Immunophenotype			
BCP	9 (56)	739 (68)	
T	3 (19)	99 (9)	
Others	3 (19)	158 (15)	
NK	1 (6)	90 (8)	
Treatment			0.015
ALL941	4 (25)	460 (43)	
ALL2000	2 (12)	303 (28)	
ALL2004	10 (63)	316 (29)	
Risk			< 0.01
SR	1 (6)	629 (58)	
HR/HHR	15 (94)	450 (42)	

BCP indicates B-cell precursor; HHR, high-high risk; HR, high risk; NK, not known; ON, osteonecrosis; SR, standard risk; WBC, white blood cell count.

was used in ALL2004 compared with that used in ALL941/2000.

The cumulative 5-year incidence of ON was 0.76% (SE, 0.43%), 0.35% (SE, 0.35%), and 3.6% (SE, 1.1%) in ALL941, ALL2000, and ALL2004, respectively (Fig. 2), with a significant difference between ALL2004 and ALL941/2000 ($P < 0.01$). To assess the contribution of sex and age to ON incidence in patients receiving DEX-containing protocols, the cumulative incidence of ON was estimated in ALL2004 (Figs. 3A, B). Both sex and age were significantly associated with the 5-year ON incidence rate ($P < 0.01$), whereas female sex and age 10 years and above were HR factors for ON. The cumulative 5-year incidence of ON for girls over 10 years of age was 25.6% (SE, 8.4%), which was extremely higher than the rest of patients in ALL2004 ($P < 0.0001$) (Fig. 3C).

The characteristics of the 16 patients who eventually developed ON are listed in Table 4. All patients showed typical imaging findings on MRI except 1 (case 941-3) who underwent only x-ray that showed bilateral flattened femoral head. The most commonly affected joints and bones were the hip joint (44%), the knee joint (25%), and the femur (13%). Three patients (19%) exhibited multiple lesions. Nine (56%) continued to receive the planned steroid therapy despite the diagnosis of ON, whereas the doses were decreased or withdrawn in 7 (44%). ON management varied for each patient depending on the physician discretion. Most patients (75%) received supportive care only and were advised to avoid lifting heavy weights (grade 2 according to Common Terminology Criteria for Adverse Event version 4.0). Three patients (19%) underwent surgical intervention (grade 3) and 1 was treated with oral bisphosphonates (grade 2). With the median follow-up times of 33 months (range, 4 to 194), the clinical outcomes of ON were as follows: 12 with amelioration of ON and 3 with stable disease, except 1 who suffered a relapse of leukemia.

DISCUSSION

In the 3 most recent JCCLSG ALL studies, we found that a significant number of patients developed ON during or after treatment. ALL2004 study was conducted to evaluate the efficacy of DEX usage as a corticosteroid in the context of intensification of reinduction phase, comparing with the preceding 2 studies wherein PSL was the only corticosteroid adopted. This strategy also enabled us to compare the DEX toxicity with that of PSL. The results clearly demonstrated the higher incidence of ON in

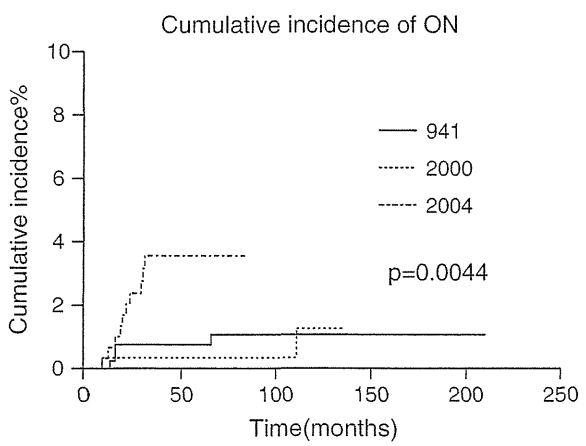

FIGURE 2. The cumulative incidence of osteonecrosis (ON) in the 3 Japanese Childhood Cancer and Leukemia Study Group studies on acute lymphoblastic leukemia (ALL). ALL941: 0.76%, SE, 0.43%; ALL2000: 0.35%, SE, 0.35%; ALL2004: 3.6%, SE, 1.1%.

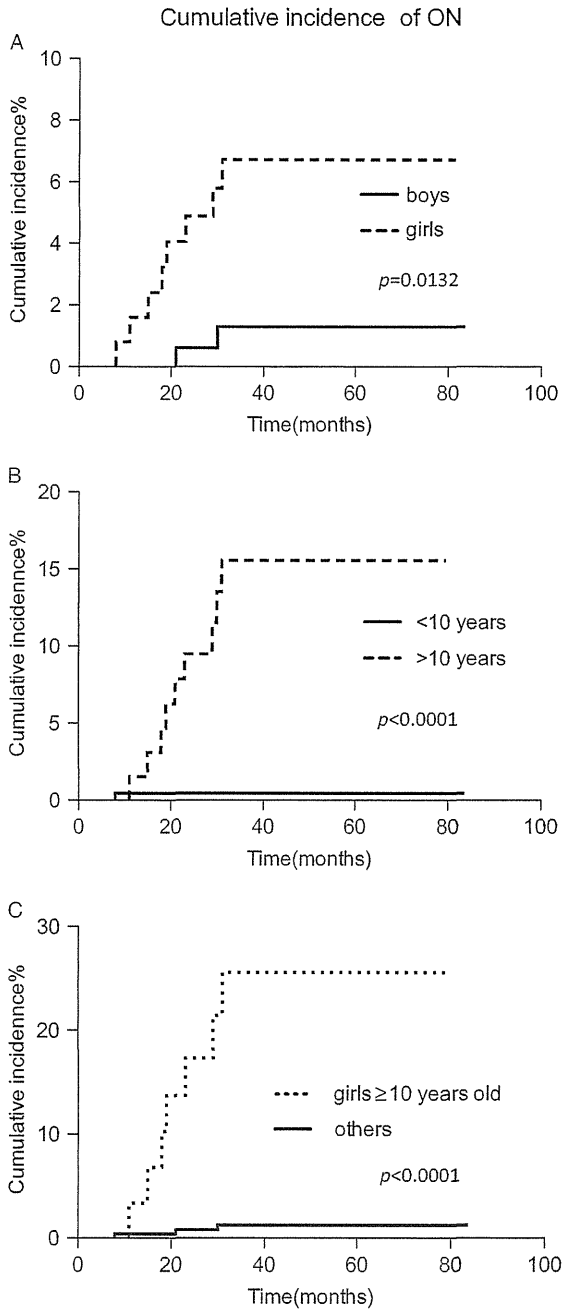

FIGURE 3. The cumulative incidence of osteonecrosis (ON) in ALL2004 according to sex (a), age (b), and combined (c). A, Boys (n=2/190): 1.3%, SE, 0.9%; girls (n=8/136): 6.7%, SE, 2.3%. B, Age below 10 years (n=1/249): 0.42%, SE, 0.42%; age 10 years and above (n=9/77): 15.6%, SE, 4.8%. C, Girls 10 years and above (n=7/33): 25.6%, SE, 8.42%; others (n=3/293): 1.19%, SE, 0.68%.

TABLE 4. Affected Joints and Clinical Course of Patients With ON

Cases	Affected Lesion	Corticosteroids	Management	Outcome
941-1	Right hip	Reduced	Avoidance of weight-bearing	Improve
2	Bilateral hips	Continue	Prohibit hard exercise	Stable
3	Bilateral hips	Continue	Avoidance of weight-bearing	Improve
4	Bilateral hips	Reduced	Avoidance of weight-bearing	Improve
2000-1	Left hip	Continue	Bracing	Improve
2	Bilateral knees, right talus	Withhold	Observation	Improve
2004-1	Bilateral hips	Withhold	Avoidance of weight-bearing	Stable
2	Bilateral hips	Withhold	Observation	Improve
3	Bilateral knees	Continue	Surgery	Improve
4	Left femur	Continue	Avoidance of weight-bearing	Improve
5	Right femur	Continue	Avoidance of weight-bearing	Improve
6	Bilateral hips and knees	Continue	Observation	ALL relapse
7	Right knee	Continue	Observation	Improve
8	Right knee	Withhold	Surgery	Improve
9	Bilateral hips and knees	Reduced	Bisphosphonate	Stable
10	Bilateral knees	Continue	Surgery	Improve

ALL indicates acute lymphoblastic leukemia; ON, osteonecrosis.

ALL2004, indicating DEX exposure was the risk for ON in ALL chemotherapy.

The overall incidence of ON was 1.5% (16/1095), which was comparable with that in a previous study by the Japan Association of Childhood Leukemia Study (JACLS) (2.4%, Hiroki H, Yasushi I, Teruaki H, Makoto Y, Megumi O, Tooru K, Shinichiro N, Junichi H, Keizo H, Keiko Y, and Tatsutoshi N; unpublished data). In studies from Europe and the United states, the ON incidence was highly variable (1% to 2% up to 9%) and dependent on patient characteristics and treatment intensity.[5–7] Furthermore, the detection methods of ON have significantly affected the incidence. Recent report from St Jude Total XV study showed that 17.6% of patients had the symptomatic ON, whereas the asymptomatic ON was detected in > 50% of patients by the prospective screening with MRI test.[15] With regard to the effects of race, the incidence of ON is reportedly higher in whites than in patients of African descent.[7] Although it remains unclear whether the Asian race is related to an increased risk of ON, our results showed that the incidence of ON in Japanese children seemed to be comparable with that in European and American children. However, it should be taken into account the limitation of the present assessment: the possible missing of asymptomatic cases and the diagnosis partly depending on physician's discretion.

In this study, female sex, age 10 years and above, and the use of DEX as a corticosteroid were significant risk factors for ON. Of the 33 female patients aged over 10 years who received DEX, 7 developed ON (cumulative incidence, 25.6%). This was the extremely higher incidence of ON comparing with the rest of patients. Although females were found to be at a higher risk of developing ON in the Children's Cancer Study Group (CCG) and Italian studies,[5,7] there was no such correlation in studies performed in the UK and Germany and at the Dana Farber Cancer Institute (Boston, MA).[6,16,17] In addition, a Japanese study conducted by the JACLS failed to show a significant female predominance (male to female ratio, 7:9). Therefore, the effects of sex on ON pathogenesis remain unclear.

A significant contribution of age to ON onset has been robustly documented by most retrospective and prospective studies.[5–7,9,16,18–21] Among children aged 10 years and above, those aged 16 to 20 years were at the highest risk of ON. The eligible patient age was 1 to 15 years in ALL941/2000 and 1 to 18 years in ALL2004; therefore, we may have underestimated the incidence of ON. Further monitoring is necessary when ALL treatment protocols designed for children are extended to adolescence and young adulthood.

The potential effect of DEX on ALL is 6.5 times that of PSL, resulting in an increase in the use of DEX for ALL treatment. Because DEX is more toxic to bone tissues,[14,22] a higher incidence of ON has been a major concern in the design of treatment protocols. In ALL2004, DEX was incorporated only in the reinduction phase because an increased incidence of ON and mortality was reported with the use of DEX in the induction phase.[23] Nonetheless, our data revealed a higher cumulative incidence of ON associated with DEX administration; this finding was comparable with the results of the Dana Farber Consortium study DFCI 00-01, wherein DEX was used in postremission intensification therapy and/or in the maintenance phase.[24] Although the total corticosteroid dose (analyzed as PSL equivalents) at therapy completion were slightly lower in ALL2004 than in ALL941/2000 (Table 2), ON was most frequent in patients who had received only DEX in the HR group in ALL2004. These results suggest that DEX administration at any dose (as PSL equivalents) and in any treatment phase affects the incidence of ON. A recent report from the CCG found that DEX administration could influence the risk of ON[21] and that alternate-week DEX administration during delayed intensification therapy decreased ON incidence compared with continuous DEX. In our ALL2004 protocol, DEX was administered continuously for 2 weeks, and it would have been beneficial to modify the DEX schedule from continuous administration to alternate-week administration.

Recently, biological and genetical basis for ON development has been extensively investigated. Children's Oncology Group tested 12 polymorphisms of candidate genes and identified children with *PAI-1* GA/AA genotypes were significantly associated with ON.[25] Another study from St Jude Children's Research Hospital showed polymorphisms of *ACP1* were associated with risk of

symptomatic ON as well as with lower serum albumin and higher cholesterol levels.[15] These results suggest that some patients are prone to develop ON and individualized therapy should be needed in the future ALL studies.

In the present report, cases with ON were retrospectively collected by the questionnaire, and most of the ON patients were identified by symptoms and confirmed with imaging studies (x-ray/MRI) without central review. Despite such limitations, the clinical features of all 16 ON patients in our study were virtually comparable with those of patients in previous studies.[6,7,16] Weight-bearing joints were commonly affected, whereas asymptomatic lesions might have been overlooked.[15] Once ON is confirmed, the physician must decide whether steroids should be withheld or continued, considering that no consensus guideline is available thus far. Most of our patients were prescribed a planned dose of steroids without compromising functional outcomes after ON development. We believe that it may not be necessary to withhold steroids at the risk of leukemia relapse.

Bisphosphonates, which are structurally similar to pyrophosphates, inhibit osteoclast activity and bone turnover, thus exerting beneficial effects on bone mineralization.[26] Alendronate, a third-generation bisphosphonate, is reportedly effective in the prevention of femoral head collapse in ON patients.[27] Wiernikowski et al[28] showed that alendronate-induced changes in bone mineral metabolism/homeostasis benefited bone mineralization in children with ALL or non-Hodgkin lymphoma with steroid-induced osteopenia. Another bisphosphonate, pamidronate, was shown to be effective in the management of pain and motor function recovery in symptomatic ON occurring in children with ALL.[29] In the present study, alendronate was administered to 1 patient with symptomatic ON of the bilateral hip and knee joints; this resulted in no further deterioration of functional outcome and no treatment-induced side effects. However, further studies are required to clarify the potential benefits of concomitant bisphosphonate and steroid use for ON treatment.

In summary, the overall incidence of ON was 1.5% in the JCCLSG ALL studies, which was comparable with that reported in previous studies conducted in the Unites States and Europe. The known risk factors of age above 10 years, female sex, and DEX use were all significantly associated with an increase in the cumulative incidence of ON. In our future studies, we are intending to routinely screen for ON development with MRI test, especially those incorporating DEX in the treatment protocol. Although an ON management regimen remains to be established, steroids should not be withheld at the risk of ALL relapse.

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Peripheral blood progenitor cell collection by two programs for autologous and allogeneic transplantation

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BACKGROUND: In the Spectra apheresis instrument (Terumo BCT), both manual (Spectra-MNC) and automated (Spectra-Auto) programs have been widely used to collect peripheral blood progenitor cells (PBPCs). However, direct comparison of these programs remains extremely limited.

STUDY DESIGN AND METHODS: We investigated 188 collections and products from autologous (patient) and allogeneic (donor) subjects and analyzed a subset of 89 allogeneic collections and products. Twenty-nine subjects who received apheresis for 2 consecutive days using both programs were also evaluated with a paired crossover comparison.

RESULTS: The two programs processed similar volumes, but run time was longer with Spectra-Auto. Yield and efficiency of CD34+ cell collection were similar between these programs in the whole cohort, although white blood cell (WBC) and mononuclear cell (MNC) yields were higher with Spectra-MNC. In the allogeneic cohort, yield and efficiency of WBC collection were greater in Spectra-MNC. However, collected WBCs, MNCs, and CD34+ cells were similar between these programs in paired comparison. Regardless of program, preapheresis peripheral WBC, MNC, and CD34+ cell counts correlated with the number of cells collected. In contrast, preapheresis WBC counts in the whole cohort were negatively correlated with collection efficiencies of CD34+ cells in Spectra-MNC but not Spectra-Auto. The products collected using Spectra-MNC contained more contaminating platelets (PLTs) than Spectra-Auto, with a corresponding reduction in postdonation circulating PLTs.

CONCLUSION: Spectra-MNC and Spectra-Auto showed distinct features that should be considered on a case-by-case basis. Similar investigations should be undertaken as new collection platforms are introduced.

Autologous (auto-) and allogeneic (allo-) peripheral blood progenitor cell transplantation (PBPCT) has been widely used to restore hematopoiesis after high-dose chemotherapy and/or total body irradiation.[1,2] Compared to marrow cell collection, PBPC collection imposes risks related to either apheresis or administration of granulocyte–colony-stimulating factor (G-CSF).[2-5] Residual dimethyl sulfoxide (DMSO) and contaminating granulocytes in PBPC products are associated with adverse infusion reactions.[6-9] Moreover, additional donor immune cells are implicated in graft-versus-host disease in allohematopoietic cell transplantations.[10-12] Hence, efficient collection of PBPCs with fewer apheresis risks and less contamination in the products is crucial for the safety of donors and patients.

We previously reported a crossover study in which an automated program of the Amicus (Baxter Healthcare, Deerfield, IL) and a manual program of the Spectra (Spectra-MNC, Software Version 4.7, Terumo BCT, Lakewood, CO) collected similar numbers of CD34+ cells

ABBREVIATIONS: CE = collection efficiency; PBPCT = peripheral blood progenitor cell transplantation; TBV(s) = total blood volume(s).

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