adverse effects. Further trials are needed to assess whether methotrexate can be safely omitted from a short intensive treatment similar to the ALCL99 regimen for some subgroups of patients.

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Appendix

The following groups participated in this study: European Intergroup for Childhood Non-Hodgkin Lymphoma; Société Française de Lutte Contre les Cancers et Leucémies de l'Enfant; Associazione Italiana di Ematologia ed Oncologia Pediatrica; United Kingdom Children's Cancer and Leukaemia Group; Japanese Pediatric Leukemia/Lymphoma Study Group; Polish Pediatric Leukemia/Lymphoma Study Group; Austria–Berlin-Frankfurt-Muenster Group; Dutch Childhood Oncology Group; Belgian Society of Paediatric Haematology and Oncology; Nordic Society for Pediatric Hematology and Oncology; and Berlin-Frankfurt-Muenster Group.

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ORIGINAL ARTICLE

Outcome of bone marrow transplantation from HLA-identical sibling donor in children with hematological malignancies using methotrexate alone as prophylaxis for graft-versus-host disease

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Abstract Most previous studies of graft-versus-host disease (GVHD) prophylaxis with methotrexate (MTX) alone in patients undergoing HLA-identical sibling donor bone marrow transplantation were performed in adults. With this background, we attempted to analyze the incidence and risk factors of GVHD in bone marrow transplantation (BMT) from an HLA-identical sibling donor in children with hematological malignancies using MTX alone as a prophylaxis for GVHD. Ninety-four patients received MTX by intravenous bolus injection, with a dose of 15 mg/m^2 on day +1, followed by 10 mg/m^2 on days +3, +6, and +11, and then weekly until day +60. The probability of developing grade II–IV acute GVHD and chronic GVHD was 19.1 and 31.8%, respectively. Age at transplantation and a female donor to male recipient were identified as risk factors for chronic GVHD in multivariate analysis, but no factors were identified for acute GVHD. The cumulative incidence of transplant-related mortality during the first 100 days was 9.6%. Disease-free survival at 5 years for standard- and high-risk patients was 82.1 and 39.5%, respectively. These results suggest that GVHD prophylaxis with MTX alone is safe and effective in young children under 10 years old at transplantation and in a setting other than female donor to male recipient.

Keywords HLA-identical sibling donor · GVHD prophylaxis · Methotrexate alone

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1 Introduction

Allogeneic bone marrow transplantation (BMT) is an effective treatment for patients with hematologic malignancies, bone marrow failure syndromes, and congenital disorders of the lymphohematopoietic system. The transplant outcome depends on the severity of complications such as graft failure, infection, graft-versus-host disease (GVHD), organ damage, and the disease stage GVHD is a major complication of allogeneic BMT that results in significant morbidity and mortality. It occurs, despite prophylaxis, in 30–50% of patients undergoing transplantation from HLA-identical sibling donors [1] and in 50–80% of patients with transplants from HLA-matched unrelated donors [2]. Previous studies have shown that the combination of cyclosporine-A (CyA) and four doses of methotrexate (MTX) is more effective than either agent alone in the prevention of GVHD [1]. Thus, a regimen including CyA or FK506 plus short-term MTX (sMTX) was established in adults, even for unrelated donors [3, 4]. Although several investigators have reported data from multicenter randomized clinical trials to evaluate the effectiveness of GVHD prophylaxis regimens in adults,

few data are available for pediatric patients, who usually show a lower incidence and less severe GVHD than adult patients. Ringden et al. [1] reported that the probability of developing acute GVHD did not differ between single or combined prophylaxis regimens in a pediatric population, and Locatelli et al. [5] reported that the incidence of GVHD in childhood was low compared to that in adults. Furthermore, Bacigalupo et al. [6] demonstrated in a randomized trial involving adults that GVHD prophylaxis with low-dose CyA (1 mg/kg per day) decreases the risk of relapse more than a higher dose (5 mg/kg per day), possibly because of a graft-versus-leukemia (GVL) effect. However, there is still a lack of data on pediatric patients, who usually show a different incidence and severity of GVHD than adult patients. Locatelli et al. confirmed that the use of low-dose CyA (1 mg/kg per day) led to a more favorable survival rate than regular-dose CyA (3 mg/kg per day) as a single prophylactic agent in pediatric patients [7]. However, in their report, almost all patients showed standard features, including acute leukemia in first or second complete remission (CR).

Herein, we report the effectiveness of MTX as a single agent for GVHD prophylaxis in 94 pediatric patients with hematological malignancies who underwent BMT from HLA-identical sibling donors including high-risk features. We also retrospectively analyzed the risk factors and incidence of GVHD.

2 Patients and methods

2.1 Patient characteristics

Ninety-four patients, aged 1–15 (median: 8 years old) received transplantations from HLA-identical sibling donors at the Japanese Red Cross Nagoya First Hospital between 1984 and 2000. The clinical characteristics of the patients are shown in Table 1.

All patients received MTX alone for GVHD prophylaxis. Patients were classified as having standard- or high-risk disease based on previously described criteria [8, 9]. Briefly, patients were categorized as standard-risk cases if they had acute lymphoblastic leukemia (ALL) in first or second complete remission (CR), acute myelogeneous leukemia (AML) in first CR, chronic myelogenous leukemia (CML) in the first chronic phase (CP), or malignant lymphoma in first CR. The other 38 patients, including those who received a second transplantation (five cases), were categorized as high-risk cases. Chromosomal abnormalities classified as standard risk included ALL with translocations of 9;22 (three cases) and 11q23 (three cases), as well as AML with translocations 8;21 (six cases) and 15;17 (two cases). ALL patients with 9;22 (four cases) and 11q23 (two

Table 1 Patient and donor characteristics

Patients	$n = 94$		%
Sex	Female	43	45.7
	Male	51	54.3
Age, median (range)		8 (1–15)	
	<10	56	59.6
	≥10	38	40.4
Disease	ALL	42	44.7
	CR1–2	27	
	CR3–5	3	
	Relapse	12	
	AML	35	37.2
	CR1	23	
	CR2	5	
	Relapse	7	
	AUL	4	4.3
	CR1	2	
	Relapse	2	
	CML	3	3.2
	CP1	2	
	BP	1	
	ML	5	5.3
	CR1	3	
	Relapse	2	
	MDS	5	5.3
Risk[a]	Standard risk	56	59.6
	High risk	38	40.4
Time at SCT	First	89	94.5
	Second	5	5.5
Conditioning	TBI	30	32
	Non-TBI	64	68
Post-BMT growth factor	None	43	45.7
	G-CSF	51	54.3
Donors	Age	9 (1–21)	
Donor sex	Female	45	47.9
	Male	49	52.1
Donor/patient sex	F to F	24	25.5
	F to M	21	22.3
	M to F	19	20.2
	M to M	30	31.9
ABO blood group	Compatible	63	67
	Minor mismatch	10	10.6
	Major mismatch	12	12.8
	Major and minor mismatch	9	9.6

ALL acute lymphoblastic leukemia, *CR* complete remission, *AML* acute myelogeneous leukemia, *AUL* acute unclassified leukemia, *CML* chronic myelocytic leukemia, *CP* chronic phase, *BP* blastic phase, *ML* malignant lymphoma, *MDS* myelodysplastic syndrome, *SCT* stem cell transplantation, *TBI* total body irradiation[a] standard risk; ALL CR1 or –2, AML CR1, AUL CR1, ML CR1, CML CP1, high risk; others

[a] Standard risk; ALL CR1 or –2, AML CR1, AUL CR1, ML CR1, CML CP1, high risk; others

cases) were included as high-risk patients because they received BMT at relapse. As of December 2005, the median follow-up duration was 161 (66–249) months. HLA typing of the donors and recipients was performed by serology. Previous chemotherapy regimens varied because the patients were treated at their referring institutions.

2.2 Pretransplant preparative regimens

The conditioning regimens are described in Table 1. Thirty-two patients received a preparative regimen consisting of busulfan (4 mg/kg per day × 4 days) and melphalan (LPAM) (180–210 mg/m^2), and 32 patients received busulfan (4 mg/kg per day × 2 days) in addition to LPAM + TBI (12–13.2 Gy). Thirty patients received other TBI-based regimens, such as cytarabine (CA) (4–6 g/m^2 per day × 2 days)/cyclophosphamide (CY) (60 mg/kg per day × 2 days)/TBI, CY/TBI, thiotepa (TEPA) (800 mg/m^2)/TBI, TEPA/CY/TBI, LPAM/TBI, and VP-16 (60 mg/kg per day)/LPAM/TBI.

2.3 Prophylaxis and treatment of GVHD

All patients received MTX alone as GVHD prophylaxis. MTX was scheduled to be given intravenously as a bolus injection at a dose of 15 mg/m^2 on day +1, followed by 10 mg/m^2 on days +3, +6, and +11, and then weekly until day +60, shorter than the Seattle protocol [10]. Folinic acid was given at 3 mg orally in divided doses on the next day of MTX injection to prevent mucositis caused by MTX. When patients developed acute GVHD of grade II or more, and extensive-type chronic GVHD, steroid therapy was started. If patients showed no improvement, CyA was added, according to the physician's assessment.

Acute GVHD was evaluated on an individual basis according to the standard criteria by Glucksberg [10]. Chronic GVHD was assessed as either limited or extensive, based on clinical and/or histological findings, as described by Glucksberg and Shulman, respectively [10, 11]. Mucositis and liver dysfunction were graded using the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Interstitial pneumonia was diagnosed based on the clinical condition and computed tomography. If patients developed mucosal toxicity, liver/renal dysfunction, and interstitial pneumonia, the dose of MTX was withheld, at the physician's discretion.

2.4 Engraftment

Engraftment of neutrophils and platelets was defined as the first of three consecutive days with an absolute neutrophil count (ANC) >0.5 × 10^9/l and unsupported platelet count >50 × 10^9/l.

2.5 Statistical analysis

Acute and chronic GVHD, overall survival, disease-free survival (DFS), rate of relapse of malignant diseases, and transplant-related mortality (TRM) were assessed using the cumulative incidence and Kaplan–Meier product limit estimates. Significance between patient populations was tested using the log-rank test. In DFS analysis, both relapse and death in remission due to any cause were considered events, whereas, in relapse rate analysis, only disease relapse was considered as failure. In TRM analysis, all deaths not due to disease relapse were considered events. Risk factors of acute and chronic GVHD were analyzed using Cox proportional hazard analysis. Children showing sustained donor engraftment and surviving for more than 21 days and more than 100 days after the transplant were assessable for the occurrence and severity of acute and chronic GVHD, respectively. Factors that appeared to be predictive of developing grade II–IV acute GVHD and chronic GVHD in univariate analysis ($P < 0.10$) were considered for inclusion in multivariate Cox regression models. The likelihood ratio test was used to determine whether variables should be added or dropped from the multivariate model. The STATA package (STATACORP LP, College Station, TX, USA) was used for data analysis.

3 Results

3.1 Engraftment

The median amount of infused marrow-nucleated cell dose was 4.0 × 10^8/kg (range: 0.98–7.2 × 10^8/kg), and 92 patients (98%) showed neutrophil engraftment at a median of 17 days (range: 10–40), and 67 patients (71%) exhibited platelet engraftment at a median of 35 days. In patients receiving granulocyte colony-stimulating factor (G-CSF) after BMT, neutrophil engraftment was confirmed at 15 days, and that without G-CSF was confirmed at 20 days ($P < 0.01$). Three patients died before neutrophil engraftment of hepatic veno-occlusive disease (VOD) or invasive fungal infection with bacterial pneumonia, and 28 patients died prior to platelet engraftment.

3.2 Acute GVHD

In 91 evaluable patients, 30 (33%) developed grade I–IV acute GVHD. The cumulative incidence of grades II–IV and III–IV acute GVHD was 19.8 and 11%, respectively (Fig. 1). For 18 patients who developed acute GVHD (grade ≥II), MTX was replaced with prednisolone for the treatment of acute GVHD. CyA was added in 11 patients to treat GVHD, and ATG (anti-thymocyte globulin) was

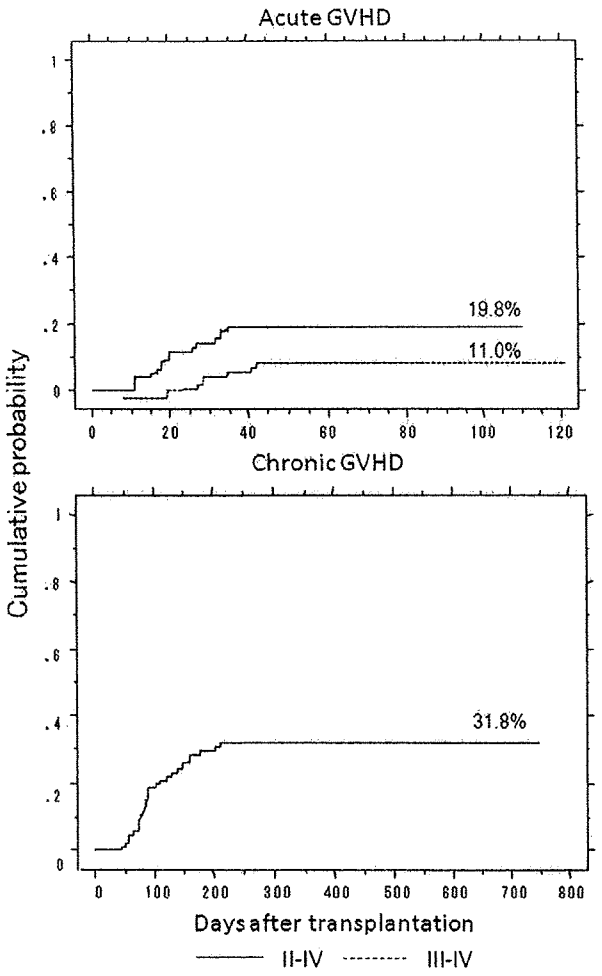

Fig. 1 Cumulative incidence of acute and chronic GVHD. *Upper* and *lower panels* show the cumulative incidence of acute and chronic GVHD, respectively

Table 2 Univariate analysis of potential risk factors for chronic GVHD

Factor	RR	95% CI	*P* value
Sex			
Male	1.00		
Female	1.12	0.53–2.38	0.77
Patient age (years)			
<10	1.00		
≥10	2.45	1.13–5.24	0.02
Risk			
Standard risk	1.00		
High risk	1.71	0.80–3.66	0.17
Conditioning			
TBI +	1.00		
TBI −	0.94	0.43–2.05	0.88
Busulfan +	1.00		
Busulfan −	0.86	0.38–1.97	0.73
Donors age			
<10	1.00		
≥10	1.14	0.54–2.43	0.73
Donor sex			
Male	1.00		0.26
Female	1.55	0.73–3.32	0.02
Donor/patient sex			
M to M	1.00		
F to F	1.80	0.48–6.70	0.38
M to F	2.99	0.90–9.93	0.07
F to M	3.87	1.21–12.34	0.02
ABO blood group			
Compatible	1.00		
Mismatch	1.12	0.51–2.45	0.77

RR indicates relative risk, *CI* confidence interval

given to four patients. Although the data are not shown, no risk factors for the development of grade II–IV acute GVHD were identified in univariate analysis.

3.3 Chronic GVHD

Although GVHD at 100 days or later after transplantation is defined as chronic GVHD by the classical criteria [10], typical clinical and histological features of chronic GVHD could occur as early as 40 days post-transplantation. In our study, 27 of 85 assessable patients (31.8%) developed limited (seven patients) or extensive (20 patients) chronic GVHD (Fig. 1), and ten of 27 patients with cGVHD stopped receiving MTX. Sixteen of 27 patients developed cGVHD before 100 days after transplantation, including 11 patients diagnosed by histological examination and five patients with diagnostic signs based on the National Institutes of Health (NIH) consensus criteria [12]. On univariate analysis, an older patient age (≥10 years old) and female donor to male recipient were significantly associated with the risk of developing chronic GVHD (Table 2). Even in multivariate analysis, these two factors were identified as significant risk factors for chronic GVHD, and female donor to male recipient was the most significant predictive factor in different pairs of sex combinations (Table 3).

3.4 Compliance and toxicity of MTX administration

Twenty-three patients stopped receiving MTX by day +60, with a median of day +25 (range: 1–46), and a median of six doses (range: 1–9). The reasons for MTX discontinuation were the treatment of acute GVHD (nine patients), liver dysfunction (ten patients, including six patients with VOD and four patients with abnormal liver function test (grade 3 NCI-CTC)), two with respiratory failure, and two early deaths with severe infection. No patients stopped

Table 3 Multivariate analysis of potential risk factors for chronic GVHD

Factor	RR	95% CI	*P* value
Patient age (years)			<0.001
<10	1.00		
≥10	3.09	1.40–6.84	
Donor/patient sex			<0.001
M to M	1.00		
F to F	1.55	0.42–5.80	0.51
M to F	3.32	0.99–11.08	0.05
F to M	4.80	1.48–15.57	<0.001

receiving MTX because of grade IV mucositis of NCI-CTC. For these patients who stopped receiving MTX before day +60, prednisolone was started. The risk factors for MTX discontinuation were acute GVHD (≥grade 2) and second stem cell transplantation (SCT) (data not shown). Eighteen of 23 patients who stopped receiving MTX survived for more than 100 days after transplantation, and ten of 18 patients developed chronic GVHD. Thirteen patients (14.8%) developed interstitial pneumonia, and five of 13 patients died of respiratory failure (two cases) or other reasons (three cases).

3.5 Relapse and survival

The relapse rate for all patients was 22%, with a median of 5.73 months (range: 0.87–137). The relapse rates of standard-risk (SR) and high-risk (HR) patients were 11.6 and 36.8%, respectively, which were significant ($P = 0.002$) (Fig. 2).

The rate of transplant-related mortality (TRM) was 7.1 and 27.5% in SR and HR patients, respectively ($P = 0.01$).

Causes of death are listed in Table 4. Relapse was the most frequent cause of death. After relapse, respiratory failure (e.g., interstitial pneumonia, bronchiolitis obliterans) was the major cause of death. The probability of transplant-related mortality was 14.4% for all patients, and that of early (<100 days) TRM was 9.6%. The risk of transplant-related mortality was significantly greater in HR patients (TRM: 27.5%, early TRM: 18.4%) compared to SR patients (all TRM: 7.1%, early TRM: 3.6%) ($P = 0.01$). Disease-free survival (DFS) for all patients was 64.9% at 5 years, and was significantly higher in SR (82.1%) compared to HR (39.5%) patients ($P = 0.001$) (Fig. 2).

Stratifying the risk of disease, we analyzed the GVL effect with or without cGVHD. In fact, the relapse rate for SR patients with cGVHD was 6.7% compared with the 14.1% observed in patients without cGVHD ($P = 0.52$). For HR patients, the development of cGVHD was associated with a lower relapse rate (25%) than that of patients without cGVHD (47.4%), even though this was not significant ($P = 0.15$). In the same way, no significant

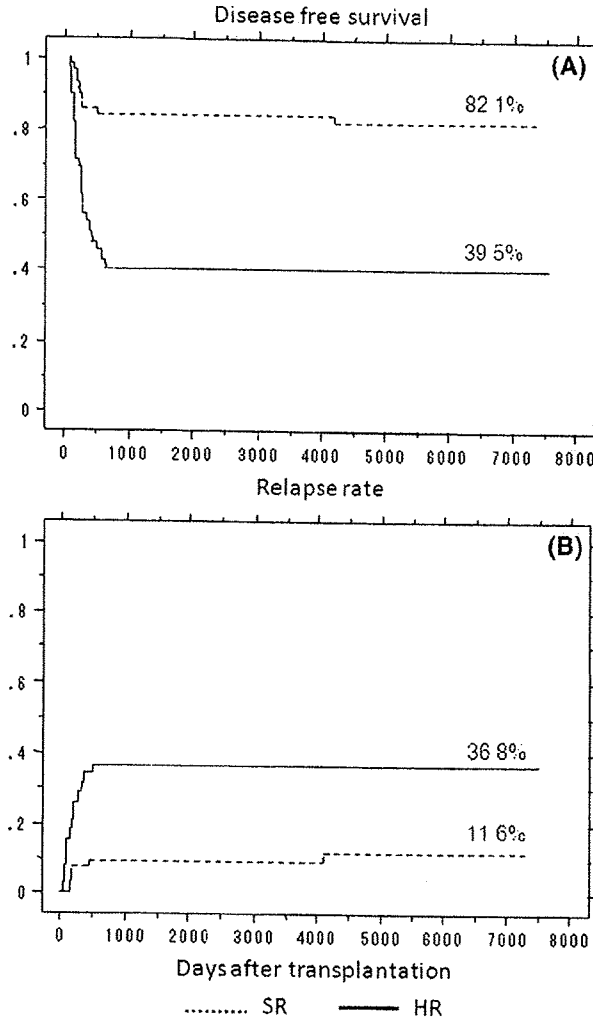

Fig. 2 a Disease-free survival. b Cumulative incidence of relapse. Standard-risk (*SR*) patients (*discontinuous line*), high-risk (*HR*) patients (*continuous line*)

Table 4 Cause of death ($n = 32$)

Cause	
Relapse	18
Rejection	1
Interstitial pneumonitis	2
Obstructive bronchiolitis	4
Infection	1
Acute GVHD	1
Veno occlusive disease	3
CNS toxicity	2

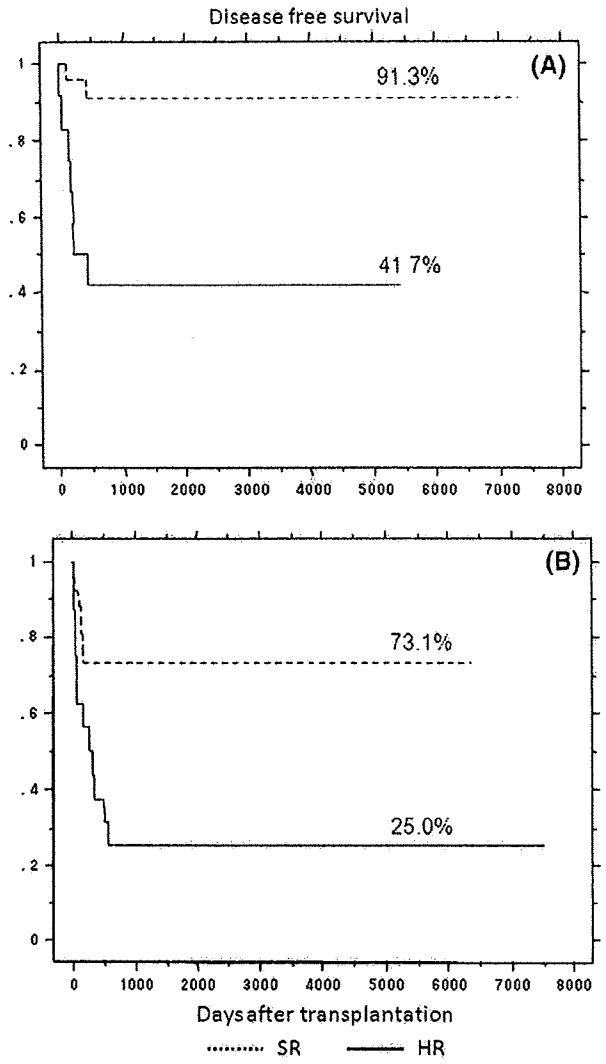

Fig. 3 Disease-free survival in patients with (a) acute myelogenous leukemia (*AML*) and (b) acute lymphoblastic leukemia (*ALL*). Standard-risk (*SR*) patients (*discontinuous line*), high-risk (HR) patients (*continuous line*)

difference was observed for DFS between patients with or without cGVHD (64.2 vs. 66.7%, respectively, P = NS). Meanwhile, stratifying the type of disease, DFS in AML patients was 91.3% in SR and 41.7% in HR patients, and the relapse rate was 4.3 and 41.7%, respectively. In ALL patients, DFS was 73.1% in SR and 25% in HR patients, and the relapse rate was 16 and 50%, respectively (Fig. 3).

4 Discussion

In this study, we analyzed the probability and risk factors of GVHD using MTX monotherapy as a prophylaxis in HLA-matched sibling bone marrow transplantation for patients with hematological malignancies. In previous studies, the incidence of GVHD using MTX as a prophylaxis was 48–53% for grade II–IV acute GVHD and 9–36% for chronic GVHD [1, 13]. In a randomized study of patients with leukemia, the incidence and severity of acute GVHD was lower in patients receiving CyA + MTX than in those with CyA monotherapy [14]. Furthermore, compared with MTX alone, CyA was associated with lower rates of interstitial pneumonia, treatment-related mortality, and treatment failure [1]. However, these studies were exclusively performed in adult populations, and few reports have described the incidence and severity of GVHD using MTX monotherapy as a prophylaxis in a pediatric population. Aschan et al. [15], demonstrated that MTX combined with CyA increases leukemic relapse compared to monotherapy, even though it decreases GVHD, and the GVL effect is supported by studies that improved leukemia-free survival in adults with AML who had acute or chronic GVHD [16]. Based on previous experience, the risk of GVHD in a pediatric population has been considered to be lower than that in adults, and an older patient age is a risk factor for the development of GVHD [17]. For the above reasons, a single agent could be sufficient for the prevention of GVHD in pediatric patients. Koga et al. [8] reported no significant difference in the incidence of acute GVHD (grades II–IV) or any type of chronic GVHD between patients who received MTX or CyA (28.3 vs. 44% for acute GVHD and 19 vs. 20% for chronic GVHD, respectively).

In this study, we reported the feasibility of GVHD prophylaxis with MTX alone in 94 pediatric patients with hematological malignancies. Although the incidence of chronic GVHD was comparable with previous studies, the incidence of acute GVHD using MTX alone as a prophylaxis was lower in our study. This reason could be due to the genetic homogeneity of Japanese [9]. The relapse rate was 11.6% in standard-risk and 36.8% in high-risk patients. In the standard-risk setting, this result was superior to other reports [6, 7, 13]. The survival rate of all patients was 64.9%, which is also comparable to previous reports [7, 18, 19]. Especially, in standard-risk patients with AML, the DFS rate was higher than in previous reports [19, 20]. Neudorf et al. [19] reported the results of allogeneic bone marrow transplantation for children with AML in first CR using MTX alone as GVHD prophylaxis. The patients received 4 × 4 mg/kg of busulfan and 50 mg/kg × 4 of cyclophosphamide as a conditioning regimen and MTX alone as GVHD prophylaxis until day 100. In their study, the incidence of chronic GVHD, overall survival, and DFS rates were 21, 67, and 57%, respectively. In our study, AML patients received MTX until day 60 as GVHD prophylaxis, and the incidence of chronic GVHD in our patients was relatively higher (31%), but the 91% DFS rate

and 4.3% relapse rate in SR patients were superior to those of previous reports. Similarly to what Matsuyama et al. reported previously, almost all of our patients received busulfan (4 mg/kg per day × 4 days) and melphalan (LPAM) (180–210 mg/m^2) as a conditioning regimen [21]. Probably, our results are dependent on the graft-versus-leukemia effect and eradication of leukemic cells by melphalan.

Based on karyotypic analysis at diagnosis, AML patients with translocations 8;21 and 15;17 are classified as having a favorable risk. Slovak et al. [22] observed superior overall survival after transplantation compared to chemotherapy among AML patients showing favorable chromosomal abnormalities. Conversely, Schlenk et al. [23] observed no difference between allogeneic stem cell transplantation (SCT) and intensive chemotherapy for this group of AML patients. Indeed, our current practice does not suggest that AML with an abnormal karyotype of t(8;21) and t(15;17) is an indication for sibling donor SCT in the first remission. However, in our study, among AML patients without these favorable abnormal karyotypes, DFS was 93% in standard-risk and 41.7% in high-risk patients (data not shown).

Although Horeowitz et al. [24] reported the direct antileukemic effect of MTX on relapse after transplantation for ALL, in our study, DFS for standard-risk ALL patients was not superior to that of AML patients. The reasons may be that, in our study, more AML patients received transplantation at first CR and the graft-versus-leukemia effect might occur more preferentially in AML patients [25].

Although one of the major toxicities of MTX is mucositis, it was not a reason for MTX cessation in this study. The major reason for its cessation was liver dysfunction because of GVHD or VOD, and predictive factors of MTX cessation were the development of acute GVHD (≥ grade 2) and second transplantation. Ringden et al. [1] reported that MTX was associated with increased rate of interstitial pneumonia, treatment-related mortality, and treatment failure, compared with CyA in adult patients. However, in our study, the incidence of interstitial pneumonia was 14.8%, being lower than in previous reports [1, 24].

In the search for predictive factors of GVHD development, patient age and female donor to male recipient were found to be significant for the development of chronic GVHD, but no risk factors for acute GVHD were identified. Neudorf et al. [19] demonstrated that children older than 10 years are at a higher risk for developing severe acute GVHD, and others reported that age at transplantation and female donor to male recipient were risk factors for chronic GVHD in adult and pediatric populations [26]. Although Kollman et al. [27] demonstrated that donor age was a significant risk factor for GVHD, we did not document donor age as a risk factor of GVHD. Although the data are not shown, patient age and female donor to male recipient were also significant risk factors for extensive chronic GVHD. In this study, the association of acute and chronic GVHD with a reduced risk of relapse was not documented, along with the association with overall survival, for patients with each high- or standard-risk malignancy. In the future, in addition to MTX, calcineurin inhibitors should be considered for patients undergoing bone marrow transplantation from an HLA-identical sibling in the setting of patients aged over 10 years old and a female donor to male recipient.

In this study, we reported the results of BMT from HLA-identical sibling donors in 94 pediatric patients with hematological malignancies using MTX alone as GVHD prophylaxis, and the relapse rate, OS, and DFS were found to be favorable compared to previous reports. In conclusion, we consider that the use of MTX alone is feasible to prevent severe acute GVHD and may reduce the risk of leukemia recurrence, possibly because of an enhanced GVL effect in the pediatric population, although the incidence of chronic GVHD was comparable to previous reports. In the future, a randomized control study should be considered to document the availability of MTX alone as GVHD prophylaxis in pediatric patients with hematological malignancies.

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Clinical features and outcome of *MLL* gene rearranged acute lymphoblastic leukemia in infants with additional chromosomal abnormalities other than 11q23 translocation

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Abstract

The treatment outcome for infant acute lymphoblastic leukemia (ALL) with positive *MLL* gene rearrangements remains poor. We analyzed whether additional chromosomal abnormalities (ACA) other than 11q23 translocation could affect the disease behavior and its prognosis.

Eighteen of seventy-four patients with infant acute lymphoblastic leukemia showed ACA, including three-way translocations in four, other novel translocations in four, and complex structural chromosomal changes in four. Only age less than 6 months and positive central nervous system leukemia were significant prognostic factors by multivariate analysis. However, overall survival rates were worse in patients with ACA compared to those with non-ACA. Genetic alterations induced by additional chromosomal changes may be associated with disease progression and poorer overall survival rates in infants with *MLL*-rearranged ALL.

Keywords: Acute lymphoblastic leukemia; Infants; *MLL* gene rearrangements; Additional chromosomal abnormalities; Prognostic factor

Abbreviations: ALL, acute lymphoblastic leukemia; *MLL, mixed lineage leukemia*; MLL-R, *MLL* gene rearranged; FISH, fluorescence in situ hybridization; ACA, additional chromosomal abnormalities other than 11q23 translocation; EFS, event-free survival; OS, overall survival; SEs, standard errors; CIs, confidence intervals.

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1. Introduction

Efforts in clinical trials to improve the outcome for infants with acute lymphoblastic leukemia (ALL), one of the subtypes of childhood ALL with poor outcome, enabled overall survival rates of 40% or higher [1–3]. However, outcomes for infants with positive *mixed lineage leukemia* (*MLL*) gene rearrangements, found in 70–80% of infant ALL cases studied with molecular techniques, remain poor, despite the use of intensive multiagent chemotherapy in combination with hematopoietic stem cell transplantation [1,4,5]. Multivariate analyses on recently conducted large-scale clinical studies have revealed several risk factors among infants with ALL, including a rearranged *MLL* gene, younger age (<3 or 6 months), very high white blood cell count (≥300,000/μL), and poor response to initial prednisone therapy [2,3]. Among these factors, presence of *MLL* gene rearrangement is the most important, significantly correlated with both the adverse clinical features and the poor prognosis that is characteristic of this distinct subtype of childhood ALL [4].

The *MLL* gene is disrupted by 11q23 translocation and fuses to more than 55 different partner genes; mainly, *AF4/FEL* in 4q21, *AF9* in 9p22, *ENL* in 19p13, *AF6* in 6q27 and *ELL* in 19p13.1 [6,7]. The partner genes encode nuclear proteins with transcriptional activities or proteins with dimerization/oligomerization motifs, suggesting that the impaired transcriptional activity by the fusion with *MLL* gene could be associated with leukemogenesis in infant leukemia [8]. In addition to these translocations, partial duplication or deletion of the 11q23 locus disrupts the function of the *MLL* gene [9]. In fact, several previous studies demonstrated that different types of *MLL* gene rearrangements, especially the presence of t(4;11)(q21;q23), the most common *MLL* gene translocation in infant ALL, confer a poor outcome in infants [10–13]. However, we have demonstrated that different 11q23 translocations are not associated with inferior prognosis in *MLL* positive infant ALL [4,5].

Although the rearranged *MLL* gene plays an essential role in leukemogenesis of infant ALL, it is still obscure whether rearrangement of the *MLL* gene is sufficient for leukemic transformation. The murine knock-in model of t(9;11)(p22;q23) (*MLL-AF9*) required a long period to the onset of leukemia [14]. It has been known that some cases harbor additional chromosomal abnormalities other than 11q23 or complex chromosomal changes in *MLL* positive ALL infants [15,16]. Thus, it is possible that several unknown genes located in these chromosomal changes are disrupted, and are associated with leukemogenesis or progression of the disease. Recently, Moorman et al. has reported that no prognostic effect of additional chromosomal abnormalities was observed in a cohort of infants and children with ALL and 11q23 abnormalities in a large collaborative retrospective study [17]. On the other hand, to further improve the outcome of this subset of ALL, it is necessary to identify appropriate prognostic factors for additional risk stratification along with an improvement in anti-leukemic therapy. We therefore conducted a study investigating the prognostic relevance of complex chromosomal abnormalities in infants with ALL and a *MLL* gene rearrangement treated with Japanese MLL96 and MLL98 protocols.

2. Materials and methods

2.1. Patients

Between December 1995 and December 2001, 102 consecutive infants with ALL, younger than 12 months, were registered and treated with two protocols, designated MLL96 (55 patients) and MLL98 (47 patients). Five other patients were also treated with MLL98 protocol without registration in the study. Prior to treatment, each patient was evaluated with respect to the characteristics of their leukemic cells, including immunophenotype, cytogenetics, and *MLL* gene rearrangement. Among the enrolled patients, 86 were identified as *MLL* gene-rearranged (MLL-R). The details of the therapeutic regimens used in the MLL96 and MLL98 studies are described elsewhere [4,5]; briefly, all the 86 patients in the MLL-R group were assigned to receive induction therapy and three courses of postremission intensification therapy followed by allogeneic hematopoietic stem cell transplantation in first remission if a suitable donor was available [1,4,5]. Written informed consent, provided according to the Declaration of Helsinki, was obtained from the guardians of the patients, with institutional review board approval of the study enrollment.

2.2. Cytogenetics

The *MLL* gene status in each patient was determined by Southern blot analysis and/or fluorescence *in situ* hybridization (FISH) as previously published [4]. Two genomic probes were used to detect *MLL* gene rearrangement by FISH analysis: the S1363 probe located in the 5′ region of the *MLL* gene, including *MLL* exon 1, and the LB140 probe in the 3′ region of the *MLL* gene (kindly provided by Dr. Misao Ohki, National Cancer Institute, Japan). BAC clone 216H7 (Research Genetics, Huntsville, AL), which is located on 4q21 and covers introns 3 and 4 of the *AF4* gene, was used for the detection of a *MLL-AF4* fusion gene in combination with the S1363 and LB140 cosmid probes. The karyotypes of leukemic cells were determined by cytogenetic analysis performed by a G-banding technique, also as previously described [4]. Briefly, mononuclear cells were separated from the bone marrow or peripheral blood. After 24 h of incubation without external stimulation, the samples were fixed in Carnoy's fixative solution (3:1 methanol and acetic acid). Slides for cytogenetic analysis were prepared using the trypsin-G banding technique. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature (ISCN2005) [18].

2.3. Classification

Among the 86 MLL-R infants, only the patients with complete karyotypic data were included in the current analysis ($n = 83$).

Nine patients with normal karyotype were excluded from the study, because these patients had *MLL* gene rearrangements that were not detected by conventional cytogenetics. The remaining 74 were therefore classified into two subgroups: "ACA group", comprising those with additional chromosomal abnormalities other than 11q23 translocation, and "non-ACA group", comprising patients with sole 11q23 translocation with *MLL* gene rearrangements. Three-way 11q23 translocations and simple or complex structural chromosomal changes other than 11q23 abnormalities were also included in the additional chromosomal abnormalities (ACA) group, because several genetic changes in addition to *MLL* could be involved in these cases, as described in previous reports [16,17].

2.4. Statistical analysis

The analysis of treatment outcome was updated on 30 September 2007. Event-free survival (EFS) and Overall survival (OS) rates were estimated by the method of Kaplan–Meier and standard errors (SEs) with the Greenwood formula, and then were compared with the log-rank test. Confidence intervals (CIs) were computed with a 95% confidence level. The clinical and biologic features of patients in the two different subgroups were compared with χ^2 tests for homogencity. A Cox regression model was used for the multivariate analysis. *P*-values, when cited, are two sided, with a value of 0.05 or less taken to indicate statistical significance.

Table 1
Eighteen *MLL* rearranged ALL infants with additional chromosomal abnormalities

Patient #	Karyotype	Sex	Age (month)	WBC, $\times 10^6$/L	CNS[a]	HSCT in CR1	Outcome
1	46,XX,add(11)(q25) [6]/46,XX [11]	F	4	193.8	–	No	BM relapse. DOD (2nd relapse) after UBMT
2	46,XY,t(4;11)(q21;q23),t(2;4)(q31;q32) [20] 46,XY,t(4;11)(q21;q23),t(2;4)(q31;q32) (2qter → 2q31::4q32 → 4q21::11q23 → cen → 11pter)	M	3	169.9	–	No	BM relapse. TRD after BMT
3[b]	46,XX[18].ish ins(4;11)(q21;q23.3q23.3)(RP11-216H7+, MLL5′+; MLL5′−,MLL3′+)[10]	F	2	953.0	+	No	BM relapse. DOD (2nd relapse) after UCBT
4	46,XX,t(4;11;15)(q21;q23;q22) [9]/46,XX [1]	F	0	121.6	+	RBMT	Death in CCR (TRD)
5	46,XX,add(1)(q32),der(2)t(2;4)(p1?;q21),add(4)(q21), del(11)(q?),add(16)(p11) [20]	F	8	7.7	–	No	CCR
6[b]	46,XX[20]. ish ins(4;11)(q21;q23.3q23.3)(RP11-216H7+, MLL5′+,MLL3′+; MLL5′-,MLL3′-)	F	2	500.0	–	RBMT	CCR
7	48,XX,+X,t(4;11)(q21;q23),+der(4)t(4;11)(q21;q23) [20]	F	0	421.5	–	No	BM relapse. DOD
8	46,XY,der(9)t(9;11)(p22;q13),add(11)(q13) [20]	M	1	473.5	+	No	Induction failure. TRD after RBMT
9	46,XY,t(4;11;5)(q21;q23;p11) [20]	M	3	1000.0	–	UCBT	Death in CCR (TRD after 2nd UCBT because of rejection)
10	46,XX,t(2;9)(p10;q10),add(7)(p22),add(9)(p13), add(11)(p11) [20]	F	7	1.7	–	UCBT	CCR
11	46,XX,t(4;11;9)(q21;q23;q22) [20]	F	9	250.7	+	UCBT	Death in CCR (TRD)
12	46,XX,add(4)(q11) [4]/46,XX [6]	F	5	12.1	+	ABMT	Relapse. TRD after UCBT
13	46,XY, t(6;11)(p10;q10),add(11)(q23) [20]	M	5	NA	NA	No	CNS relapse. TRD after RBMT
14	48,XY,+X,add(2)(p21),del(2)(p?),+6,der(7)add(7)(p11) add(7)(q32),del(11)(q?),add(12)(q13),-17,-17,add(19)(p13), +der(?)t(?;17)(?;q21),+mar1 [20]	M	2	25.6	+	No	DOD before initial therapy
15[b]	46,XY[20].ish ins(10;11)(p12;q23.3q23.3) (MLL5′+,MLL3′+;MLL5′, MLL3′−)	M	2	537.0	–	No	BM relapse. CCR after UBMT in CR2
16	47XX,t(4;11)(q21;q23),+?i(8)(q10) [20]	F	5	59.0	NA	No	BM relapse. DOD
17	47XX,+5,t(9;11)(p22;q23) [5]/46.XX [2]	F	3	22.8	–	UCBT	CCR
18[b]	46,XX,t(4;11)(q21;q23)[20].ish t(4;11;21)(q21;q23;q22) (216H7+; 216H7+,MLL5′+,MLL3′-; MLL3′+)	F	0	198.2	–	No	Induction failure. CCR after UBMT

F, female; M, male; WBC, white blood cell: BM, bone marrow; CNS, central nervous system; CR1, first complete remission; CCR, continuous complete remission: ABMT, autologous bone marrow transplantation; RBMT, related donor bone marrow transplantation; UCBT, unrelated cord blood transplantation; UBMT, unrelated bone marrow transplantation; DOD, death of disease; TRD, treatment-related death; NA, data not available.

[a] CNS disease was diagnosed if more than five leukemic cells/μl were found in cerebrospinal fluid.

[b] FISH analysis has proven complex chromosomal abnormality in these patients. Cloning of the breakpoint regions revealed that patient #6 had 46,XX, ins(4;11)(4pter → 4q21::11q24.1 → 11q23.3(MLL3′)::11q23.3 → 11q23.3(MLL5′)::4q21 → 4qter;11pter → 11q23.3:: 11q24.1 → 11qter), and patient #15 had 46,XY, ins(10;11)(10pter → 10p12::11q23.3 → 11q23.3(MLL3′)::11q23.3 → 11q23.3(MLL5′)::10p12 → 10qter;11pter → 11q23.3:: 11q23.3 → 11qter).

3. Results

Among the 74 eligible infants, 18 (24.3%) were classified as the ACA group, as shown in Table 1. Four patients (patients #4, #9, #11, and #18) had three-way 11q23 translocation. Other novel translocations were also observed in four patients: t(2;4)(q31;q32) in patient #2, t(9;11)(p22;q13) in patient #8, t(2;9)(p10;q10) in patient #10, and t(6;11)(p10;q10) in patient #13. FISH analysis confirmed complex structural chromosomal changes in four patients including insertion of 4q21 fragment to 11q23 locus and *vice versa* resulting in *MLL-AF4* fusion gene (patients #3, #6, and #18) or insertion of 10p12 to 11q23 locus resulting in *MLL-AF10* fusion gene (patient #15). Other frequent chromosomal changes were +X in two patients, involvement in chromosome 4 in two, chromosome 5 in two, chromosome 7 in two, and chromosome 11 except 11q23 in four.

The clinical and biologic findings were compared between the ACA and non-ACA groups, including age at disease onset, sex, initial white blood cell (WBC) count, central nervous system (CNS) involvement, and type of 11q23 translocation. As shown in Table 2, the frequency of sole t(4;11)(q21;q23) was significantly higher in the non-ACA group than in the ACA group. The frequency of positive central nervous system leukemia or young age at onset also tended to be higher in the ACA group than the non-ACA group, although the difference was not statistically significant.

Among the 18 patients in the ACA group, a total of 14 events were observed: one leukemic death before initiating therapy (patient #14); two induction failure (patients #8, and #18); eight relapses (patients #1, #2, #3, #7, #12, #13, #15, and #16); three treatment-related deaths (patients #4, #9, and #11). Only four patients in this group survived without any evidence of disease (patients #5, #6, #10, and #17) (Table 1).

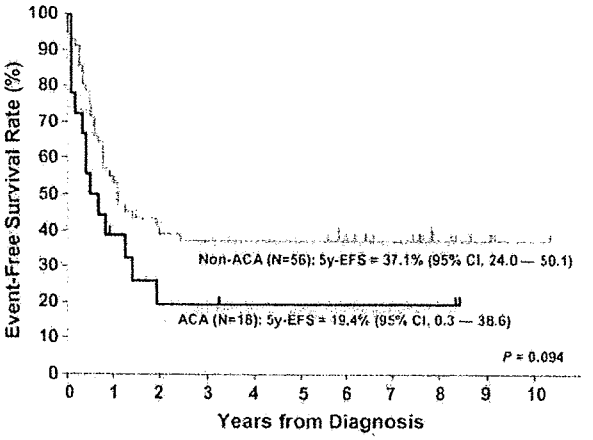

Fig. 1. Event-free survival estimates for 74 infants with ALL and *MLL* gene rearrangements in the MLL96 and MLL98 studies; a comparison between patients with additional chromosomal abnormalities and patients with sole 11q23 abnormality excluding normal karyotype with *MLL* gene rearrangements. Median follow-up period: 78 months (range, 8–124 months).

The EFS and OS rates were also compared between two groups. The 5-year EFS rate in the ACA group tended to be worse than that in the non-ACA group, without a statistically significant difference between two groups (Fig. 1). The 5-year OS in the ACA group was significantly worse than that in the non-ACA group; 26.7% (95% CI, 4.7–48.8%) *vs.* 52.1%

Table 2
Comparison in clinical and laboratory findings between the ACA and non-ACA groups

	Total number of Pt. (%)	ACA group number of Pt. (%)	Non-ACA group number of Pt. (%)	*P*-value[a]
Total number of patients	74	18	56	
Age, month				0.136
<3	21 (28.4)	8 (44.4)	13 (23.2)	
≥ 3, <6	29 (39.2)	7 (38.9)	22 (39.3)	
≥ 6	24 (32.4)	3 (16.7)	21 (37.5)	
Sex				0.650
Male	28 (37.8)	6 (33.3)	22 (39.3)	
Female	46 (62.2)	12 (66.7)	34 (60.7)	
WBC count, $\times 10^9$/L				0.599
<100	23 (31.1)	6 (33.3)	17 (30.3)	
≥ 100, <300	29 (39.2)	5 (27.8)	24 (42.9)	
>300	21 (28.4)	6 (33.3)	15 (26.8)	
NA	1 (1.3)	1 (5.6)	0 (0.0)	
CNS disease[b]				0.131
Positive	16 (21.6)	6 (33.3)	10 (17.9)	
Negative	52 (70.3)	10 (55.6)	42 (75.0)	
Unknown	6 (8.1)	2 (11.1)	4 (7.1)	
Karyotype				0.012
t(4;11)(q21;q23)	47 (63.5)	7 (38.9)	40 (71.4)	
Other 11q23	27 (36.5)	11 (61.1)	16 (28.6)	

ACA, additional chromosomal abnormalities other than 11q23 translocation; Pt., patients; WBC, white blood cell; CNS, central nervous system; NA, data not available.

[a] Comparison between two different groups.

[b] CNS disease was diagnosed if more than five leukemic cells/μL were found in cerebrospinal fluid.

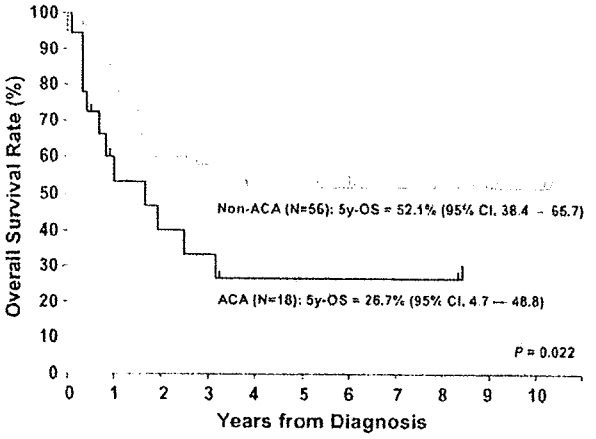

Fig. 2. Overall survival estimates for 74 infants with ALL and *MLL* gene rearrangements in the MLL96 and MLL98 studies; a comparison between patients with additional chromosomal abnormalities and patients with sole 11q23 abnormality excluding normal karyotype with *MLL* gene rearrangements. Median follow-up period: 78 months (range, 8–124 months).

Table 3
Multivariate analysis of prognostic factors in *MLL* rearranged ALL infants

	Parameter estimates	Risk ratio (95% CI)	*P*-value
Age, less than 6 months	0.724	2.063 (1.026–4.146)	0.041
Additional chromosomal abnormalities	0.418	1.519 (0.771–2.993)	0.226
t(4;11)(q21;q23)	0.345	1.413 (0.744–2.683)	0.290
WBC ≥ 300,000/uL	0.387	1.473 (0.771–2.812)	0.239
CNS leukemia	1.166	3.209 (1.497–6.881)	0.002
Registered in the MLL98 study	0.387	1.472 (0.756–2.865)	0.254

CI, confidence intervals; WBC, white blood cell; CNS, central nervous system.

(95% CI, 38.4–65.7%) ($P = 0.022$) (Fig. 2). In a multivariate analysis, only age at diagnosis (younger than 6 months) and positive central nervous system leukemia were significant prognostic factors for poor outcome in this study (Table 3).

4. Discussion

This study demonstrated that complex chromosomal abnormalities were associated with poor outcome in infant ALL with *MLL* gene rearrangements. The previous study described by Moorman et al. showed different findings, in that no prognostic effect of additional chromosomal abnormalities was observed in infants and children with ALL and 11q23 abnormalities [17]. However, it is difficult to simply compare between the study by Moorman et al. and ours as follows. First, Moorman et al. collected data from several cooperative study groups, which comprise different treatment cohorts. Secondly, accurate analyses of karyotypes and *MLL* gene rearrangements were not performed in all patients. Thirdly, the EFS rate in this previous study was too low to evaluate the effect of the additional chromosomal abnormalities in infants with *MLL*-rearranged ALL.

Moorman et al. stated that the frequency of additional chromosomal abnormalities depends on the different 11q23 translocations: high frequency of +X in t(4;11) and t(11;19), involvements in chromosomes 6, 9, and 12 in del(11)(q23) and other 11q23 [17]. In our study, several novel translocations were observed: t(2;4)(q31;q32), t(9;11)(p22;q13), t(2;9)(p10;q10), and t(6;11)(p10;q10). Other frequent chromosomal changes were +X and involvements of chromosomes 4, 7, and 11. In our study, a three-way 11q23 translocation was observed in four patients: t(4;11;15), t(4;11;5), t(4;11;9), and t(4; 11; 21). Different three-way translocations have been also detected in several other reports [15,17,19]. Complex structural chromosomal changes were observed in four patients, including insertion of a 4q21 fragment to the 11q23 locus or insertion of 10p12 to the 11q23 locus in our study. Kowarz et al. described ten patients with three-way translocation or complex structural chromosomal changes in MLL-AF4$^+$/AF4-MLL$^-$ ALL [16]. These findings indicate that complex chromosomal changes in leukemic cells disrupt several genes owing to the "cut and paste" recombination mechanism [16].

Recently, the functions of the partner genes fused to *MLL* gene located in 11q23 locus have been clarified: *AF4* at 4q21, *AF9* at 9p22, *ENL* at 19p133, *ELL* at 19p13.1, *AFX* at Xq13, and *AF6q21* at 6q21 are all transcription factors; *CBP* at 16p13 is a transcriptional coactivator; *AF1q* at 1q21 is a growth factor; and *AF17* at 17q21 is a dimerization protein [7]. In addition, several known genetic changes, such as *p53*, *p16*, and *RAS* mutations, are present in some cases in addition to *MLL* gene rearrangement, which might indicate the essential role of additional genetic changes in combination with *MLL* gene translocation in leukemogenesis [20]. Disruption of the Ikaros gene is also detected as an additional alteration in infant ALL [21]. Table 4 summarizes the genes at the breakpoint region of complex chromosomal abnormalities observed in our study, which have been reported only in hematologic malignancies, such as leukemia or lymphoma [22–29]. The function of each gene varies: *PMS1* at 2q31 and *FANCG* at 9p13 are a mismatch or DNA repair gene [23,27]; *Pax5* also located at 9p13, a differentiation factor of B-cells; and *HOXD13* also located at 2q13, a homeobox gene [24,28]. *PML* at 15q22, usually observed as *PMR-RARα* in acute promyelocytic leukemia with t(15;17), and *E2A* at 19p13, usually observed as *E2A-PBX1* in pre-B ALL with t(1;19), are both transcription factors [34,37]. Other genes such as *CHIC2* at 4q11 is associated with exocytosis, *SYK* and *NR4A3* at 9q22 are a tyrosine kinase and membrane receptor, respectively [29], and *CCND1* (*BCL1*) at 11q13 is associated with cell cycle [31]. Thus, if these genes are functionally disrupted after chromosomal changes, this could promote leukemogenesis.

In our study, the overall survival was significantly worse in the ACA group than that in the non-ACA group, but ACA was

Table 4
Breakpoint of chromosomes and possible located genes

Breakpoint	Located genes	Function	Associated translocation	Associated disease	Reference
1q32			t(1;13)(q32;q14)	Diffuse large B-cell lymphoma	[22]
2q31	*PMS1*	Mismatch repair gene	t(2;12)(q31;p13)	Non-Hodgkin lymphoma, MDS	[23]
	HOXD13	Homeobox gene	t(2;11)(q31;p15)	Therapy-related AML	[24]
4q11	*CHIC2*	Exocytosis	t(4;12)(411;p13)	AML	[25]
7p11			dic(7;9)(p11-13;p11)	Pre-B ALL	[26]
9p13	*FANCG*	DNA repair	t(2;9)(p11;p13)	Pre-B ALL	[27]
	Pax5	B-cell differentiation	t(7;9)(q11;p13)	B-ALL	[28]
9q22	*SYK*	Tyrosine kinase	t(5;9)(q33;q22)	Peripheral T-cell lymphoma	[29]
	NR4A3	Membrane receptor	t(9;12)(q22;p12)	MDS	[29]
11p11			t(11;14)(p11;q32)	Splenic marginal-zone B-cell lymphoma	[30]
11q13	*CCND1 (BCL1)*	Cell cycle control	t(11;14)(q13;q32)	Mantle cell lymphoma, others	[31]
	MYBOV (Cyclin D)			Multiple myeloma	[32]
15q22	*PML*	Transcription factor	t(5;15)(q33;q22)	CML	[33]
			t(15;17)(q22;q21)	APL	[34]
16p11			t(3;16)(q27;p11)	Diffuse large B-cell lymphoma	[35]
			t(16;21)(p11;q22)	AML	[36]
19p13	*E2A*	Transcription factor	t(1;19)(q23;p13)	Pre-B ALL	[37]
			t(2;19)(p11;p13)	AML	[38]
	LYL1	Transcription factor	t(7;19)(q34;p13)	T-ALL	[39]
			t(17;19)(q22;p13)	ALL	[37]

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia.

not a significant factor in the multivariate analysis. However, given that young age and central nervous system leukemia are significant prognostic factors by multivariate analysis, it is likely that the poor survival outcome seen in the ACA group is associated with the combination of young age, positive central nervous system leukemia and ACA. Since another report showed no effect of additional chromosomal changes in *MLL* positive infant ALL [17], an analysis of the data from a greater number of patients treated with identical treatment protocols is underway to address this issue. In our study, the genes affected by the chromosomal changes varied among the patients, and the function of each gene was different. However, it can be postulated that some genetic alterations induced by additional chromosomal changes might be associated with leukemogenesis and disease progression in *MLL* positive infant ALL.

5. Conflict of interest

All the authors do not have any commercial or other associations that might pose a conflict of interest.

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Contributions. H. Tauchi, D. Tomizawa and E. Ishii contributed to the analysis and interpretation of data, writing the article. M. Eguchi, M. Eguchi-Ishimae, N. Kinukawa and Y. Hayashi contributed to the analysis and interpretation of data. M. Hirayama and N. Miyamura contributed to the data collection and analysis. K. Koh and K. Horibe contributed to the study conception, revising and approving the final version of the article.

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The Role of Hematopoietic Stem Cell Transplantation With Relapsed or Primary Refractory Childhood B-Cell Non-Hodgkin Lymphoma and Mature B-Cell Leukemia: A Retrospective Analysis of Enrolled Cases in Japan

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Background. There have been excellent treatment results for children with B-cell non-Hodgkin lymphoma (B-NHL) and mature B-cell leukemia (B-ALL) in the last few decades. However, a small subset of relapsed or refractory patients, after first-line therapy, still have a poor prognosis. ***Procedure.*** Thirty-three patients with relapsed or primary refractory B-NHL/B-ALL among 327 newly diagnosed patients between 1996 and 2004 were analyzed retrospectively. ***Results.*** After salvage therapy, 18 patients were chemotherapy-sensitive and 15 patients suffered from progression. Among 18 patients who had a chemotherapy-sensitive disease, 4 of 5 patients who underwent hematopoietic stem cell transplantation (HSCT) during remission survived without progression, while 3 of 12 patients who did not receive HSCT were alive without disease progression. Fifteen patients never sensitive to salvage therapy died. ***Conclusions.*** Patients with relapsed/primary refractory B-NHL/B-ALL have a poor prognosis with current treatment approaches, while the patients sensitive to salvage therapy have a respectable chance to achieve a sustained complete second remission with HSCT. Pediatr Blood Cancer 2008;51:188–192. © 2008 Wiley-Liss, Inc.

Key words: childhood; mature B-cell leukemia (B-ALL); non-Hodgkin lymphoma; refractory; relapsed; stem cell transplantation

INTRODUCTION

There have been excellent treatment results for children with B-cell non-Hodgkin lymphoma (B-NHL) and mature B-cell leukemia (B-ALL) in the last few decades along with the assignment of highly intensive and sequential chemotherapeutic regimens stratified according to risk [1–5]. However, patients with relapsed or refractory disease still have a poor prognosis, particularly in patients treated with intensive first-line therapy. And there are few reports on treatment in relapsed or refractory pediatric B-NHL/B-ALL. It is, therefore, very difficult to assess the role of megatherapy or other treatment. In this study, we summarized the results of 33 pediatric patients who had relapsed or primary resistant disease after first-line therapy with B-NHL/B-ALL enrolled in a national survey of Japan, and validate the availability of hematopoietic stem cell transplantation (HSCT) for these patients.

In Japan, there have been four study groups for pediatric hematological tumors; such as, the Japan Children's Cancer and Leukemia Study Group (JCCLSG), the Japan Association of Childhood Leukemia Study (JACLS), the Kyushu-Yamaguchi Children's Cancer Study Group (KYCCSG) and the Tokyo Children's Cancer Study Group (TCCSG). Treatment protocols of these groups for B-NHL modified French LMB89 [2] or German BFM90 [3] consist of short-duration, intensive, alkylating agent therapy (i.e., cyclophosphamide) coupled with other agents, such as intermediate- or high-dose methotrexate, vincristine, anthracyclines, etoposide and cytarabine. Result in survival rates of these collaborative groups with each chemotherapy regimens were 70–80% in stages III–IV.

PATIENTS AND METHODS

We analyzed the data on all children with relapsed/refractory B-NHL/B-ALL have been enrolled in four multicenter trials of childhood NHL. JCCLSG, JACLS, KYCCSG and TCCSG had enrolled 54 patients (JCCLSG NHL-960 study; 1996–2004), 125 patients (JACLS NHL-98 study; 1998–2002), 9 patients (KYCCSG NHL 96 study; 1996–2004) and 139 patients (TCCSG NHL 96 study; 1996–2001) respectively. The first-line treatments used in each study differed, however there were no considerable differences in therapeutic results. Of the 327 patients included in these series, 26 patients relapsed after achieving first complete remission (CR) and 7 patients did not achieve first CR. CR and partial remission (PR) were defined as previously described [6]. The medical records for these 33 patients were retrospectively collected from each study group. Details of salvage or second-line treatment are shown in Table I. NHL-B02 pilot regimen is now used for the patients with childhood B-NHL/B-ALL in Japan, and in other cases childhood ALL regimen of each group was used for relapsed B-NHL/B-ALL. Several patients were treated with regimens published in parts elsewhere [7–10]. Overall survival rate (OS) and progression-free survival rate (PFS) were estimated using the Kaplan–Meier method and data were compared by the log-rank test. The prognostic analysis was based on PFS. Multivariate Cox model was also fitted to adjust the potential effects of the baseline characteristics. Results were analyzed as of January 31, 2006.

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TABLE I. Salvage TherapyDose/Schedule

	Therapy dose/schedule
NHL-B02 pilot	HDMTX 5,000 mg/m^2 day 1 in 24 hr infusion + rescue
A	Dexamethasone 10 mg/m^2 (divided doses) days 1–7 then reduce over 4 days to 0
	Vincristine 1.5 mg/m^2 day 2 (maximum: 2.0 mg)
	Cyclophosphamide 1,000 mg/m^2 days 4, 5
	Pirarubicin 30 mg/m^2 days 4, 5 (maximum: 45 mg)
	IT MTX 3–12 mg/m^2 days 1, 8
	IT hydrocortisone 10–25 mg/m^2 days 1, 8
	IT cytarabine 6–30 mg/m^2 days 1, 8
B	Vincristine 1.5 mg/m^2 day 1 (maximum: 2.0 mg)
	Dexamethasone 10 mg/m^2 (divided doses) days 1–5 then reduce over 3 days to 0
	HD Ara-C 2,000 mg/m^2 every 12 hr days 2–4
	Etoposide 150 mg/m^2 days 2–5
	IT MTX 3–12 mg/m^2 days 1, 8
	IT hydrocortisone 10–25 mg/m^2 days 1, 8
	IT cytarabine 6–30 mg/m^2 days 8
JCCLSG NHL 960	HD Ara-C 2,000 mg/m^2 days 1–4
	Etoposide 200 mg/m^2 days 1–4
	IT MTX 7.5–15 mg/m^2 day 2
	IT hydrocortisone 30–50 mg/m^2 day 2
JACLS ALL-97	Vincristine 1.5 mg/m^2 days 1, 8, 15, 22, 29 (maximum: 2.0 mg)
HR	Dexamethasone 10 mg/m^2 days 1–7
	Pirarubicin 25 mg/m^2 days 2, 4
	Prednisone 40 mg/m^2 (divided doses) days 8–29 then reduce over 5 days to 0
	L-asparaginase 10,000 U/m^2 days 9, 11, 13, 16, 18, 20
	IT MTX 8–12 mg/m^2 days 1, 29
	IT hydrocortisone 15–25 mg/m^2 days 1, 29
	IT cytarabine 20–30 mg/m^2 days 1, 29
F	Mitoxantorone 8 mg/m^2 days 1–3
	Cytarabine 500 mg/m^2 days 1–3, 8–10
	Prednisone 40 mg/m^2 (divided doses) days 1–3, 8–10
	Etoposide 200 mg/m^2 days 8–10
	IT MTX 8–12 mg/m^2 day 1
	IT hydrocortisone 15–25 mg/m^2 day 1
	IT cytarabine 20–30 mg/m^2 day 1
TCCSG L99	Vincristine 1.5 mg/m^2 days 1, 8, 15, 22, 29 (maximum: 2.0 mg)
HEX	Cyclophosphamide 1,000 mg/m^2 days 1, 30
	Prednisone 60 mg/m^2 (divided doses) days 1–28 then reduce over 7 days to 0
	Pirarubicin 20 mg/m^2 days 2, 3, 31, 32
	Prednisone 60 mg/m^2 (divided doses) days 8–29 then reduce over 5 days to 0
	L-asparaginase 6,000 U/m^2 days 1, 3, 5, 7, 8, 10, 12, 14, 15, 17, 19, 21
	IT MTX 6–12.5 mg/m^2 days 8, 15, 22
	IT hydrocortisone 12–25 mg/m^2 days 8, 15, 22
	IT cytarabine 12–25 mg/m^2 days 8, 15, 29
Rituximab	Rituximab 375 mg/m^2 days 1, 8, 15, 22

HDMTX, high dose methotrexated; IT, intrathecal.

RESULTS

Characteristics of Patients

Twenty-three were males and 10 were females with a median age at onset of 13 years (range 1–16 years). Histological classification showed 20 Burkitt lymphoma/leukemia (BL), 12 diffuse large B-cell lymphoma (DLBCL) and one mature B-ALL not further classified. The diagnosis of B-NHL/B-ALL was based on histopathology and immunohistochemistry. From 24 of these 33 patients, the histopathological material was reviewed centrally by a reference laboratory utilized by the study [11]. Cytogenetic studies were performed in 14 patients and showed no abnormality in 5 patients, t(8;14) in 5 patients, and other abnormalities in 4. Murphy's stage was stage I or II in 3 cases and stage III or IV in 30 cases. Sites of relapse/progress included the primary sites in 23 and new sites in 10 cases. Fifteen had bone marrow (BM) involvement (include 6 cases with new BM lesion) and 6 had central nervous system (CNS) disease (include one case with new CNS lesion). Twenty-eight patients progressed or relapsed in the first 12 months from first diagnosis. Characteristics, details of treatment and follow-up of the patients with relapsed or refractory conditions are shown in the Tables II and III.

Patient Outcome After First Relapse/Progress

After a median follow-up period of 48 months, the 4-year OS and PFS rates for these patients were 20.8 ± 8.2% and 20.5 ± 7.2%,

TABLE II. Characteristics, Treatment, and Outcome of the Patients With Relapsed B-NHL

Patient number	Stage	Time to relapse (months)	Histology	Site of relapse	Treatment of relapse (Table I)	Outcome
1	I	67	DLBCL	Abdomen	CHOP [7] + Rit + operation	Alive
2	III	18	DLBCL	Bone + spleen	NHL-B02 pilot: A, B + Rit + RT	Alive
3	III	35	DLBCL	Primary site (abdomen) + Neck	NHL-B02 pilot: A, B	Alive
4	III	25	BL	Primary site (neck)	NHL-B02 pilot: A, B + Rit + auto PBSCT	Alive
5	III	23	DLBCL	Primary site (abdomen + neck)	JACLS ALL97 HR + auto PBSCT	Alive
6	IV	5	BL	Primary site (abdomen + CNS)	JCCLSG NHL 960	Alive
7	III	12	DLBCL	Primary site (mediastinum)	NHL-B02 pilot: A, B	Died
8	IV	6	BL	Primary site (BM)	CA 100 mg/m^2 + VP 100 mg/m^2 days 1–3	Died
9	IV	5	BL	Primary site (BM)	ICE [8] + Rit + CBT	Died
10	IV	7	BL	BM	CBT	Died
11	IV	3	BL	Primary site (abdomen + CNS)	ALL-REZ BFM 90[9] + RT	Died
12	III	4	BL	BM	Palliatve	Died
13	IV	6	BL	Primary site (BM)	Not available	Died
14	III	4	DLBCL	Primary site (mediastinum + abdomen)	ICE [8] + Rit + related PBSCT	Died
15	IV	2	BL	Primary site (bone + BM)	HD-CA + VP + VCR + Dex + RT + related BMT	Died
16	III	8	DLBCL	CNS	Related PBSCT	Died
17	IV	8	B-ALL	Primary site (CNS) + BM	JACLS ALL97 F + related BMT	Died
18	III	7	BL	Primary site (mediastinum + abdomen)	ESHAP [10] + Rit + related PBSCT	Died
19	IV	6	BL	Primary site (BM)	TCCSG L99 HEX	Died
20	IV	5	BL	Primary site (BM)	TCCSG L99 HEX + related PBSCT	Died
21	III	5	BL	BM + abdomen	VP + VCR + PSL	Died
22	IV	4	BL	Primary site (neck + BM)	NHL-BFM 90[3]	Died
23	II	7	DLBCL	Neck	TCCSG L99 HEX	Died
24	III	6	BL	Primary site (abdomen) + BM	RT	Died
25	IV	5	BL	Primary site (BM)	TCCSG L99 HEX + related PBSCT	Died
26	III	4	BL	BM	Not available	Died

DLBCL, diffuse large B-cell lymphoma; BL, Burkitt lymphoma; CNS, central nervous system; BM, bone marrow; Rit, Rituximab; RT, radiation therapy; PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; BMT, bone marrow transplantation; CA, cytarabine; VP, etoposide; HD-CA, high dose cytarabine; VCR, vincristine; Dex, dexamethasone; PSL, prednisolone.

respectively. Nine of 33 patients are alive and 24 patients died. Twenty-one patients died of their primary disease, and 3 patients died of therapy-related toxicity. Outcomes according to the kinetics of response to therapy are depicted in Figure 1. All of 15 cases never reaching CR or PR died after salvage therapy with or without HSCT. Ten cases achieved CR and 8 cases achieved PR.

HSCT and Outcome

Among the patients achieving CR or PR, 4 of 5 patients who underwent HSCT and 3 of the 12 patients who did not receive HSCT were alive without disease progression. The other one patient who underwent HSCT with progression died of lymphoma (Fig. 1).

TABLE III. Characteristics, Treatment, and Outcome of the Patients With Primary Refractory B-NHL

Patient number	Stage	Histology	Site of progress	Treatment of progress	Outcome
27	II	DLBCL	Abdomen	Continuation of 1st-line treatment	Alive in CR
28	IV	DLBCL	CNS	Continuation of 1st-line treatment + auto PBSCT	Alive in PR
29	IV	BL	BM	Continuation of 1st-line treatment + related BMT	Alive in CR
30	IV	BL	BM + head + abdomen	Continuation of 1st-line treatment	Died
31	III	DLBCL	Abdomen	ICE[8] – Rituximab	Died
32	III	BL	Abdomen	Not available	Died
33	IV	DLBCL	Bone + CNS + abdomen	Continuation of 1st-line treatment	Died

CR, complete remission; PR, partial remission.

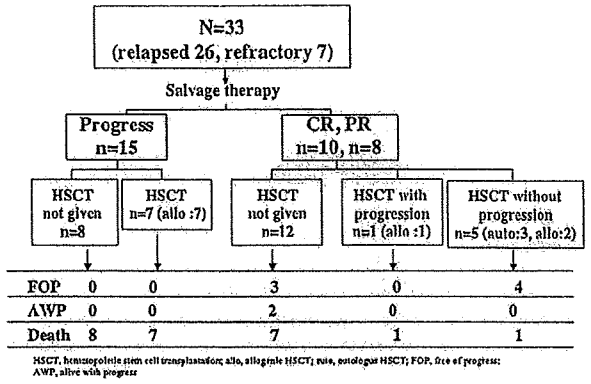

Fig. 1. Outcome in patients with relapsed/refractory B-NHL/B-ALL.

Details of treatment and outcome of the patients with HSCT are shown in Table IV. Disease status at the time of HSCT had an influence on prognosis, however, neither high-dose chemotherapy (HDC) regimens nor kind of graft related to. There were three survivors without disease progression who were not given HSCT. Two of the survivors had stage I or II at initial diagnosis and achieved second remissions after short intensive courses of chemotherapy. Another patient had stage IV DLBCL and relapsed at 18 months after diagnosis, he received a second-line treatment consisting of an intensive chemotherapy according to the NHL-B02 pilot regimen. The patient achieved a CR, however he could not receive HSCT because of contracting by Aspergillus pneumonia. Rituximab and local radiotherapy were successful and he continues in remission 38 months from diagnosis.

Prognostic Factors

In the multivariate analysis, response to salvage therapy was the only significant prognostic factor. PFS was worse among patients with poor response to salvage therapy as compared to the other ($P = 0.037$). On the contrary the PFS was not associated with histologic type, time to relapse, BM or CNS involvement.

DISCUSSION

Outcome of children with B-cell NHL/B-ALL has dramatically improved, while, for relapsed or primary resistant patients, the chance of cure with currently available therapy is low [12,13]. Also in this analysis, the 4-year OS and PFS rates for these patients is about 20%. None of the 15 patients who never reached CR or PR after salvage therapy was alive whereas, 9 of 18 children undergoing salvage therapy in CR or PR were alive. In our series, various retrieval chemotherapy regimens were used, making it difficult to make efficacy comparisons; however, the results of this are in line with previous reports [14,15] showing that chemoresistance is associated with a very poor outcome.

For the patients with second CR or PR, HSCT seems to be an effective strategy, as shown that 4 of 5 patients who received HSCT after having achieved a second CR or PR without progress were PFS, while only 3 of 12 not given HSCT were alive without disease. However, there was no theoretic influence of HDC regimens and previous reports observed in a small group of pediatric patients [16,17], so the optimum conditioning regimen in children is under discussion [18,19]. Previous reports [15,20,21] showed that the major determinant of survival was the remission status of patients before HDC, neither HDC regimens nor type of graft, and our results showed similar findings. In our study, 7 cases received chemotherapy combined with rituximab but there was no significant contribution to their response rate (data not shown).

Another finding from this analysis of factors contributing to PFS reveals that response to salvage therapy was the only significant prognostic factor. It appears important to focus on the salvage therapy. The schedule of a salvage therapy should be tailored to the known features of the tumor (e.g., cell resistance) and be selected of drugs for use nonoverlapping first-line therapy.

In summary, this study demonstrates that the prognosis for patients with relapsed/refractory childhood B-NHL/B-ALL was poor. However, for the patients sensitive to salvage therapy, HSCT seems to be an effective strategy.

TABLE IV. Details of the 13 Patients Treated With HSCT

Patient number	Status before HDC	HDC regimen	Graft (match of HLA)	HSCT	Outcome
4	CR	BU + L-PAM	Autologous	PBSCT	Alive in CR
5	CR	CY + VP + TBI	Autologous	PBSCT	Alive in CR
9	Progress	CY + TEPA + TBI	Unrelated (4/6)	CBT	Died of lymphoma
10	Progress	L-PAM + CA + TBI	Unrelated (6/6)	CBT	Died of HDC
14	Progress	Flu + ATG + L-PAM + TBI	Related (4/6)	PBSCT	Died of lymphoma
15	Progress	BU + L-PAM	Unrelated (6/6)	BMT	Died of HDC
16	Progress	CY + VP + CBDCA + MCNU	Allogeneic (not available)	PBSCT	Died of lymphoma
17	CR	Not available	Sibling (6/6)	BMT	Died of lymphoma
18	Progress	VP + TBI	Sibling (6/6)	PBSCT	Died of lymphoma
20	Progress	Flu + ALG + L-PAM + IDA	Related (4/6)	PBSCT	Died of lymphoma
25	Progress	Not available	Sibling (6/6)	PBSCT	Died of lymphoma
28	PR	TEPA + L-PAM	Autologous	PBSCT	Alive in PR
29	PR	CY + TBI	Sibling (6/6)	BMT	Alive in CR

HDC, high-dose chemotherapy; BU, busulfan; L-PAM, melphalan; CY, cyclophosphamide; VP, etoposide; TBI, total body irradiation; TEPA, thiotepa; CA, cytarabine; Flu, fludarabine; ATG, anti-thymocyte globuline; CBDCA, carboplatin; MCNU, ranimustine; ALG, anti-lymphocyte globuline; IDA, idarubicin hydrochloride: PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; BMT, bone marrow transplantation.