infer that the first episode of delayed MTX elimination does not predict subsequent high MTX levels in later HDMTX courses. This is also supported by the study of Hempel et al., in which they showed that glomerular toxicity at the end of HDMTX can be completely reversed until the next HDMTX course [12].

The last finding was that the first HDMTX courses had a great incidence of liver and gastrointestinal toxicities followed by a sharp reduction of the incidence in later courses. These results may be explained by the plasma folate concentrations in HDMTX courses. Valik et al. [23] reported a severe encephalopathy occurred at the first HDMTX course but not the second course in a male with acute leukemia, where pretreatment plasma folate concentrations were low before the first HDMTX course and then 10-fold higher before the second course. In addition, Sterba et al. [24] showed the plasma folate concentrations increase significantly with increasing number of HDMTX courses in children with ALL and NHL, and they suggested that the increasing folate baseline concentration could be caused by repetitive LV administration. Similar result was reported in osteosarcoma patients [25]. Consequently, low frequencies of gastrointestinal and liver toxicity in later HDMTX courses in our study may be explained by the difference of pretreatment folate levels according to HDMTX courses, although plasma folate levels were not available in our study. In contrast to the non-hematological toxicities, incidence of hematological toxicity showed few changes by the HDMTX courses and plasma MTX levels, suggesting that hematological toxicity was more affected by CPA and THP than HDMTX. This finding shows the need of prophylaxis and countermeasure for patients with neutropenia to prevent developing severe infections throughout the HDMTX courses.

In this study we employed a 24-hr infusion of HDMTX. However, recent studies have shown the efficacy of 4-hr infusion of HDMTX for childhood B-NHL. Woessmann et al. [26] compared the 4-hr infusion and 24-hr infusion of HDMTX in the NHL-BFM95 study and concluded that a 4-hr infusion is not inferior to, but less toxic than, a 24-hr infusion for low- and intermediate-risk patients. In addition, Cairo et al. [27] have reported that a 4-hr infusion of HDMTX resulted in a favorable outcome for high-risk BNHL patients in the FAB/LMB96 study. Consequently, 4-hr infusion of HDMTX should be considered in our next studies.

In summary, we did not find evidence for relation between plasma MTX levels and MTX-related toxicities except nephrotoxicity. This suggests that when high blood MTX levels are associated with nephrotoxicity, the occurrence of other developing toxicities should be taken into consideration. In addition, the first HDMTX administration was associated with a great incidence of gastrointestinal and liver toxicities followed by a reduction of the incidence in later courses. Hence, these findings suggest that the first episode of severe non-hematological toxicity does not predict the recurrence of severe toxicities in later courses.

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REFERENCES

1. Reiter A, Schrappe M, Tiemann M, Ludwig WD, Yakisan E, Zimmermann M, Woessmann W, Mann G, Chott A, Ebell W, Klingebiel T, Graf N, Kremens B, Muller-Weihrich S, Oluss HJ, Zintl F, Henze G, Riehm H. Improved treatment results in childhood B-cell neoplasms with tailored intensification of therapy: Reports of the Berlin–Frankfurt–Münster Group trial NHL–BFM 90. Blood 1999;94:3294–3306.
2. Patte C, Auperin A, Michon J, Behrendt H, Leverger G, Frappaz D, Lutz P, Coze C, Perel Y, Rahael M, Terriel-Lacombe MJ. The Société Française d'Oncologie Pédiatrique LMB89 protocol: Highly effective multiagent chemotherapy tailored to the tumor burden and response in 561 unselected children with B-cell lymphomas and L3 leukemia. Blood 2001;97:3370–3379.
3. Patte C, Auperin A, Gerrard M, Michon J, Pinkerton R, Sposto R, Weston C, Raphael M, Perkins SL, McCarthy K, Cairo MS. Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-Hodgkin lymphoma in children and adolescents. It is possible to reduce treatment for the early responding patients. Blood 2007;109:2773–2780.
4. Tsurusawa M, Mori T, Kikuchi A, Mitsui T, Sunami S, Kobayashi R, Takimoto T, Saito A, Watanabe T, Fujimoto J, Nakazawa A, Ohshima K, Horibe K. Improved treatment results of children with B-cell non-Hodgkin lymphoma: A report from the Japanese Pediatric Leukemia/Lymphoma Study Group B-NHL03 Study. Pediatr Blood Cancer 2014;61:1215–1221.
5. Iversen O, Iversen U, Ziegler J, Bluming A. Cell kinetics in Burkitt lymphoma. Eur J Cancer 1974;10:155–163.
6. Murphy SB, Melvin SL, Mauer AM. Correlation of tumor cell kinetics studies with surface marker results in childhood non-Hodgkin's lymphoma. Cancer Res 1979;39:1534–1538.
7. Reiter A, Schrappe M, Ludwig WD, Lampert F, Harbott F, Henze G, Niemeyer CM, Gadner H, Muller-Weihrich S, Ritter J, Odenwald E, Riehm H. Favorable outcome of B-cell acute lymphoblastic leukemia in childhood: A report of three consecutive studies of the BFM group. Blood 1992;80:2471–2478.
8. Tsurusawa M, Aoyama M, Saeki K, Fujimoto T. Cell cycle kinetics in childhood acute leukemia studied with in vitro bromodeoxyuridine labeling Ki67-reactivity, and flow cytometry. Leukemia 1995;11:1921–1925.
9. Evans WE, Pratt CB, Taylor RH, Barker LF, Crom WR. Pharmacokinetic monitoring of high-dose methotrexate. Early recognition of high-risk patients. Cancer Chemother Pharmacol 1979;3:161–166.
10. Stoller RG, Hande KR, Jacobs SA, Rosenberg SA, Chabner BA. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N Engl J Med 1977;297:630–634.
11. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13–22.
12. Hempel L, Misselwitz J, Fleck C, Kentouche K, Leder C, Appenroth D, Rost M, Zintl F. Influence of high-dose methotrexate (HD-MTX) on glomerular and tubular kidney function. Med Pediatr Oncol 2003;40:348–354.
13. Kodidela S, Chandra PS, Dubashi B. Pharmacogenetics of methotrexate in acute lymphoblastic leukemia: Why still at the bench level. Eur J Clin Pharmacol 2014;70:253–260.
14. Kishi S, Cheng C, French D, Pei D, Das S, Cook EH, Hijiya N, Rizzari C, Rosner GL, Frudakis T, Pui CH, Evans WE, Relling MV. Ancestry and pharmacogenetics of antileukemic dug toxicity. Blood 2007;109:4151–4157.
15. Schmiegelow K. Advances in individual prediction of methotrexate toxicity: A review. Br J Haematol 2009;146:489–503.
16. May J, Carson KR, Butler S, Liu W, Bartlett N, Wagner-Johnston ND. High incidence of methotorexate associated renal toxicity in patients with lymphoma: A retrospective analysis. Leuk Lymphoma 2014;55:1345–1349.
17. Mary V, Fairclough D, Ayers D, Crom WR, Rodman JH, Pui CH, Evans WE. Patients characteristics associated with high-risk methotrexate concentrations and toxicity. J Clin Oncol 1994;12:1667–1672.
18. Rau T, Erney B, Gores R, Eschenhagen T, Beck J, Langer T. High-dose methotrexate in pediatric acute lymphoblastic leukemia: Impact of ABCC2 polymorphism on plasma concentrations. Clin Pharmacol 2006;80:468–476.
19. Ramsey LB, Panetta JC, Smith C, yang W, Fan Y, Winick NJ, Matin PL, Cheng C, Devidas M, Pui CH, Evans WE, Hunger SP, Loh M, Relling MV. Genome-wide study of methotrexate clearance replicates SLCO1B1. Blood 2012;121:898–904.
20. Noha M, Sahar M, Manal A, EEN Ebid. Study of the pharmacokinetic and pharmacogenetic contribution of the toxicity of high-dose methotrexate in children with acute lymphoblastic leukemia. Med Oncol 2012;29:2053–2062.
21. Joannon P, Oviedo I, Cambell M, Tordeccila J. High-dose methotrexate therapy of childhood acute lymphoblastic leukemias: Lack of relation between serum methotrexate concentration and creatinine clearance. Pediatr Blood Cancer 2004;43:17–22.
22. Mao J, Zhang L, Shen H, Tang Y, Song H, Zhao F, Xu W. Creatinine clearance rate and serum concentration are related to delayed methotrexate elimination in children with lymphoblastic malignancies. Neoplasma 2014;61:77–82.
23. Valik D, Sterba J, Bajciova V, Demlova R. Severe encephalopathy induced by the first but not the second course of high-dose methotrexate mirrored by plasma homocysteine elevations and preceded by extreme differences in pretreatment plasma folate. Oncology 2005;69:269–272.
24. Sterba J, Dusek L, Demlova R, Valik D. Pretreatment plasma folate modulates the pharmacodynamics effect of high-dose methotrexate in children with acute lymphoblastic leukemia and non-Hodgkin lymphoma: 'folate overrescue' concept revisited. Clin Chem 2006;52:692–700.
25. Holmboe L, Andersen AM, Morkid L, Slordal L, Hall KS. High dose methotrexate chemotherapy: Pharmacokinetics, folate and toxicity in osteosarcoma patients. Br J Clin Pharmacol 2011;73:106–114.
26. Woessmann W1, Seidemann K, Mann G, Zimmermann M, Burkhardt B, Oschlies I, Ludwig WD, Klingebiel T, Graf N, Gruhn B, Juergens H, Niggli, F, Parwaresch R, Gadner H, Riehm H, Schrrape M, Reiter R. The impact of the methotrexate administration schedule and dose in the treatment of children and adolescents with B-cell neoplasms: A report of the BFM Group Study NHL-BFM95. Blood 2005;105:948–958.
27. Cairo MS, Gerrard M, Sposto R, Auperin A, Pinkerton R, Michon J, Weston C, Gerrard M, Perkins SL, Raphael M, McCarthy K, Patte C. Results of a randomized international study of high-risk central nervous system B non-Hodgkin lymphoma and B acute lymphoblastic leukemia in children and adolescents. Blood 2007;109:2736–2743.

Haematopoietic stem cell transplantation for relapsed or refractory anaplastic large cell lymphoma: a study of children and adolescents in Japan

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Summary

To evaluate haematopoietic stem cell transplantation (HSCT) in children and adolescents, we reviewed the records of 47 patients who were ≤ 18 years, had relapsed or refractory anaplastic large cell lymphoma, and received HSCT between 1990 and 2010. At HSCT, complete remission (CR) was less common in allogeneic HSCT recipients ($n = 24$) than in autologous HSCT recipients ($n = 23$) ($P = 0.01$). The autologous and allogeneic HSCT groups differed in terms of 5-year event-free survival (EFS) (38% vs. 50%, $P = 0.63$), cumulative incidence of progress or relapse (49% vs. 28%, $P = 0.25$), and treatment-related mortality (12% vs. 25%, $P = 0.40$). However, these differences were not significant. Patients with non-CR at autologous HSCT had a significantly lower EFS rate (14% vs. 48%, $P = 0.03$). Conversely, although those with non-CR at allogeneic HSCT had a lower EFS rate, this was not significant (44% vs. 63%, $P = 0.26$). Reduced-intensity conditioning regimens were used for three of the 16 allogeneic HSCTs received by patients with non-CR. These three patients achieved CR, surviving 32–65 months after HSCT. These results demonstrated that allogeneic HSCT might be a treatment option for patients who do not achieve CR through conventional chemotherapy.

Keywords: anaplastic large cell lymphoma, children, adolescents, haematopoietic stem cell transplantation, reduced-intensity conditioning.

Anaplastic large cell lymphoma (ALCL) is rare in children, accounting for 10–15% of childhood non-Hodgkin lymphoma cases (Murphy, 1994). The event-free survival (EFS) rate is 65–75% in children and adolescents receiving a first-line strategy based on short-pulse chemotherapy over a period of 3–6 months (Brugières et al, 1998, 2009a; Seidemann et al, 2001; Le Deley et al, 2010). Accordingly, the relapse rate is approximately 30% in most study series. The treatment of relapsed and refractory ALCL remains a matter of debate. Patients with relapsed ALCL have a 30–60% chance of survival under current treatment strategies, which include high-dose chemotherapy with haematopoietic stem cell transplantation (HSCT) and long-term treatment with vinblastine (Brugières et al, 2000, 2009b; Williams et al, 2002; Mori et al, 2006; Woessmann et al, 2006; Stockklausner et al, 2008; Gross et al, 2010). In contrast, patients who experience ALCL progression during first-line chemotherapy have extremely poor outcomes (Woessmann et al, 2006) and autologous or allogeneic HSCT is required as the most appropriate therapy.

Some evidence is available regarding the roles of autologous and allogeneic HSCT in paediatric ALCL. However, data are limited to several HSCT case series and case reports. In particular, few reports have been published regarding allogeneic HSCT for paediatric ALCL. We previously reported a retrospective analysis of 26 paediatric patients with recurrent ALCL in Japan (Mori et al, 2006). In that study, only three of the eight patients who received autologous HSCT while in their second complete remission (CR) survived without further relapse. In contrast, all six patients who received allogeneic HSCT while in their second CR survived without further relapse. However, our previous study included too few patients for us to discuss the efficacy of HSCT for relapsed or refractory childhood ALCL.

In the present study, we sought to evaluate the efficacy of HSCT for relapsed or refractory ALCL in children and adolescents. We performed a further retrospective analysis of 47 patients who received autologous or allogeneic HSCT for relapsed or refractory ALCL between 1990 and 2010.

Patients and methods

Patients and transplantations

This study was approved by the institutional ethics committee of National Kyushu Cancer Centre. Data on patients who had undergone HSCT were collected from the registries belonging to the Transplant Registry Unified Management Program system of the Japan Society for Hematopoietic Cell Transplantation. The study included 47 patients who had a diagnosis of relapsed or refractory ALCL and received HSCT at age ≤18 years between March 1990 and September 2010. Twenty-three patients received autologous HSCT and 24 patients received allogeneic HSCT. Refractory disease was defined as progression

during fist-line treatment. Reduced-intensity conditioning (RIC) regimens were defined as (a) total body irradiation of ≤500 cGy as a single fraction or ≤800 cGy if fractionated, (b) <9 mg/kg of busulfan, (c) ≤180 mg/m² of melphalan, (d) <10 mg/kg of thiotepa, or (e) the BEAM regimen (carmustine, etoposide, cytarabine and melphalan), according to previous reports (Yaniv & Stein, 2008; Giralt et al, 2009; Ohta et al, 2010; Luger et al, 2012). All other conditioning regimens were defined as myeloablative conditioning (MAC) regimens.

Statistical analysis

Overall survival (OS), EFS, cumulative incidences of relapse and treatment-related mortality (TRM) were estimated using the Kaplan–Meier method. The Mann–Whitney U test, χ^2-test, and Fisher's exact test were used to assess differences in patient characteristics. The level of statistical significance was set at $P < 0.05$. All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Autologous HSCT

The patients' characteristics are shown in Table I. Twenty-three patients received autologous HSCT for relapsed or refractory disease as their first transplantation. The median follow-up duration for survivors after autologous HSCT was 154 (range: 9–224) months. The median age at HSCT was 15 (range: 7–18) years. Sixteen patients had achieved CR at HSCT and seven patients had residual disease. Bone marrow and peripheral blood were the stem cell sources in three and 20 patients, respectively. Engraftment was observed in 23 (100%) cases, occurring at a median of 12 d. The 5-year cumulative incidence of relapse was 49% ± 11% (Fig 1A). Treatment-related death occurred in three of the patients who received autologous HSCT and the 5-year cumulative incidence of TRM was 12% ± 9% (Fig 1B). Two of the three patients died of infectious complications and one patient died of multiple organ failure. The 5-year OS and EFS rates were 51% ± 11% and 38% ± 10%, respectively (Fig 2A, B). We observed 5-year EFS rates of 48% ± 13% and 14% ± 13% for patients with CR and non-CR, respectively, at autologous HSCT (Fig 3A), which constituted a significant difference ($P = 0.03$).

Allogeneic HSCT

Twenty-four patients received allogeneic HSCT for relapsed or refractory disease (Table I). The median follow-up duration for survivors after allogeneic HSCT was 68 (range: 32–212) months. The median age at HSCT was 13·5 (range: 3–18) years. Of the 24 patients, four had received previous autologous HSCT. Eight patients had achieved CR at HSCT and 16 patients had residual disease (Table I). The sources of stem cells were bone marrow in 13 patients, cord blood in

Table I. Characteristics of patients with relapsed or refractory ALCL according to the receipt of autologous or allogeneic HSCT.

	Autologous	Allogeneic	P
Patients (n)	23	24	
Age at HSCT (years)			
Median	15	13·5	0·27
Range	7–18	3–18	
Sex			
Male	17	21	0·24
Female	6	3	
Stage at diagnosis			
I	1	0	0·36
II	3	4	
III	11	6	
IV	4	8	
Unknown	4	6	
Disease status at HSCT			
CR2/CR≥3	14/2	5/3	0·01
Non-CR	7	16	
Conditioning			
TBI/TLI based	7/1	17/1	0·06
Non-TBI based	15	6	
Stem cell source			
BM	3	13	
PB	20	5	
CB	0	6	
Donor			
MRD	–	7	
MUD	–	2	
MMRD	–	6	
MMUD	–	7	
Unknown	–	2	

HSCT, haematopoietic stem cell transplantation; CR, complete remission; BM, bone marrow; CB, cord blood; PB, peripheral blood; MRD, matched related donor; MUD, matched unrelated donor; MMRD, mismatched related donor; MMUD, mismatched unrelated donor; TBI, total body irradiation; TLI, total lymphoid irradiation.

six patients and peripheral blood in five patients. Seven patients had human leucocyte antigen (HLA)-matched related donors, and two patients received stem cells from HLA-matched unrelated donors. Thirteen patients had HLA-mismatched donors. Engraftment was observed in 21 (88%) cases, occurring at a median of 17 d. Two patients died of infection and one died of disease progression before engraftment. The 5-year cumulative incidence of relapse was 28% ± 10% (Fig 1A). Treatment-related death occurred in five patients; four patients died of infectious complications and one patient died of acute graft-versus-host disease (GVHD). The 5-year cumulative incidence of TRM was 25% ± 10% (Fig 1B). Acute GVHD of any grade occurred in 13 patients, nine of whom had grade II–IV GVHD. The 5-year OS and EFS rates were 54% ± 10% and 50% ± 10%, respectively (Fig 2A, B). Seven of 24 patients had multiple relapses before their HSCT; the 5-year EFS rates among patients with and without multiple relapses were

43% ± 19% and 53% ± 12%, respectively (P = 0·67). We observed 5-year EFS rates of 63% ± 17% and 44% ± 12% among patients with CR and those with non-CR respectively, at allogeneic HSCT (Fig 3B), which did not constitute a significant difference (P = 0·13).

At HSCT, CR was less common among allogeneic HSCT recipients than it was among autologous HSCT recipients (P = 0·01). However, there were no significant differences between the autologous and allogeneic HSCT patients in terms of cumulative incidence of relapse (P = 0·25), cumulative incidence of TRM (P = 0·40), 5-year OS (P = 0·95) or 5-year EFS (P = 0·63).

RIC regimens

Of the 24 patients in the allogeneic group, four underwent allogeneic HSCT using RIC. Their outcomes are shown in Table II. One of the four patients died of bacterial infection and the other three patients survived in CR without relapse after allogeneic HSCT. Interestingly, none of these three patients were in CR at HSCT.

Discussion

Currently, the efficacy and toxicity of HSCT are poorly defined for childhood cases of relapsed or refractory ALCL. Evidence is especially lacking in regards to the efficacy and toxicity of allogeneic HSCT. The present study included 23 patients who underwent autologous HSCT and 24 patients who underwent allogeneic HSCT. Each of the patients was a child or adolescent who had relapsed or refractory ALCL and underwent HSCT in Japan. This report comprises the largest cohort concerning allogeneic HSCT for relapsed or refractory ALCL in childhood.

The Berlin-Frankfürt-Münster (BFM) cohort had efficacies of autologous HSCT (77% OS and 59% EFS among 39 children with relapsed ALCL) that lie at or above the upper range of previously reported series (Woessmann et al, 2011). In national case series from the United Kingdom and France, one of six and nine of 15 patients stayed in continuous CR (Brugières et al, 2000; Williams et al, 2002; Woessmann et al, 2011). The Center for International Blood and Marrow Transplant Research (CIBMTR) has reported another large series of autologous HSCTs that were performed for ALCL, noting an EFS of 35% in 24 patients (Gross et al, 2010). Previously, we have reported a retrospective analysis of relapsed ALCL, which included 26 patients in Japan (Mori et al, 2006). Three of the eight patients who underwent autologous HSCT survived in continuous CR. In the current study, the 5-year OS rate, EFS rate and cumulative incidence of relapse among the 23 patients who underwent autologous HSCT were 51%, 38% and 49%, respectively. These results are similar to the findings of a previous CIBMTR report (Gross et al, 2010). In a study of 64 adult and paediatric cases of autologous HSCT for ALCL, Fanin et al (1999) reported that disease status at HSCT
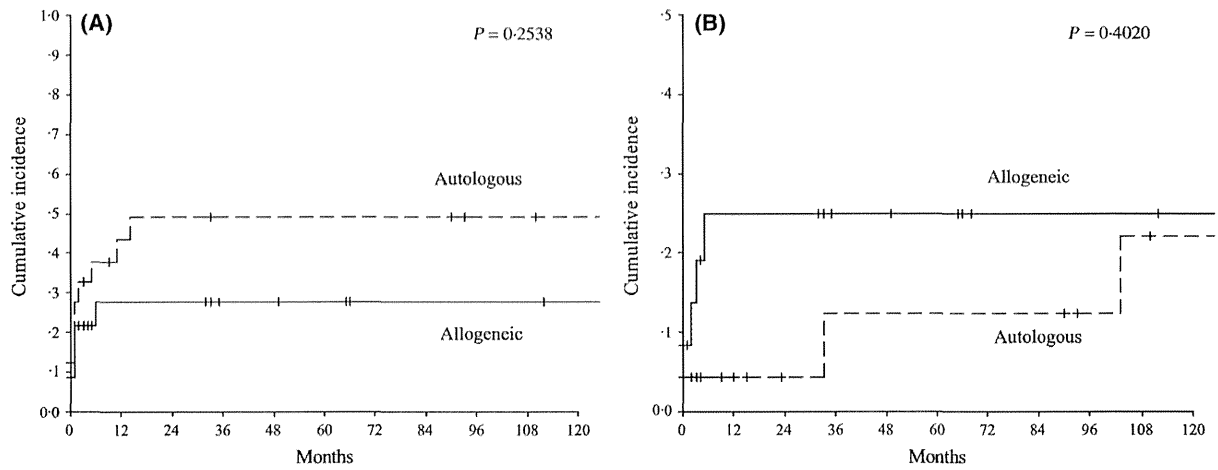
Fig 1. The cumulative incidence of relapse (A) and treatment-related mortality (B) according to autologous and allogeneic haematopoietic stem cell transplantation.
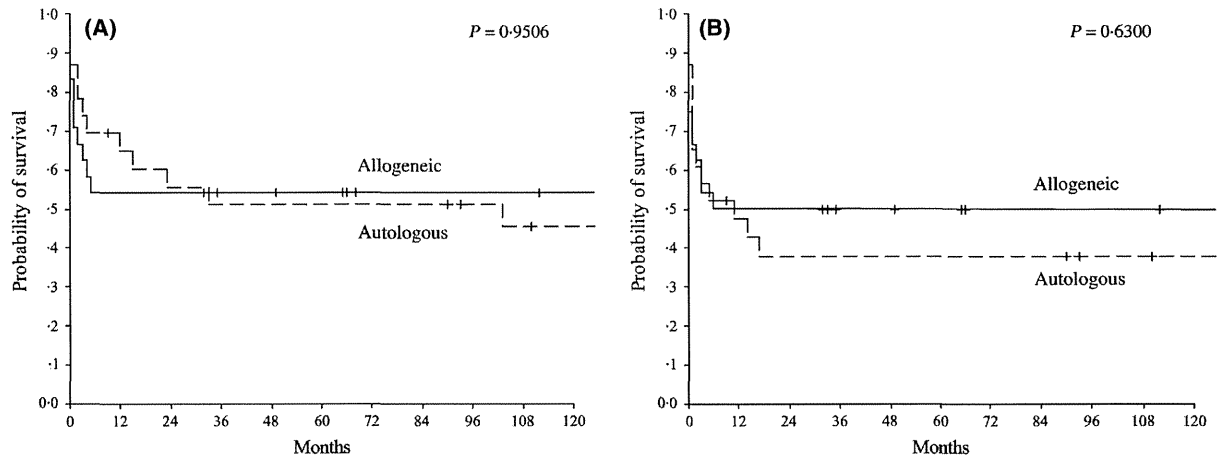

Fig 2. Overall survival (A) and event-free survival (B) according to autologous and allogeneic haematopoietic stem cell transplantation.
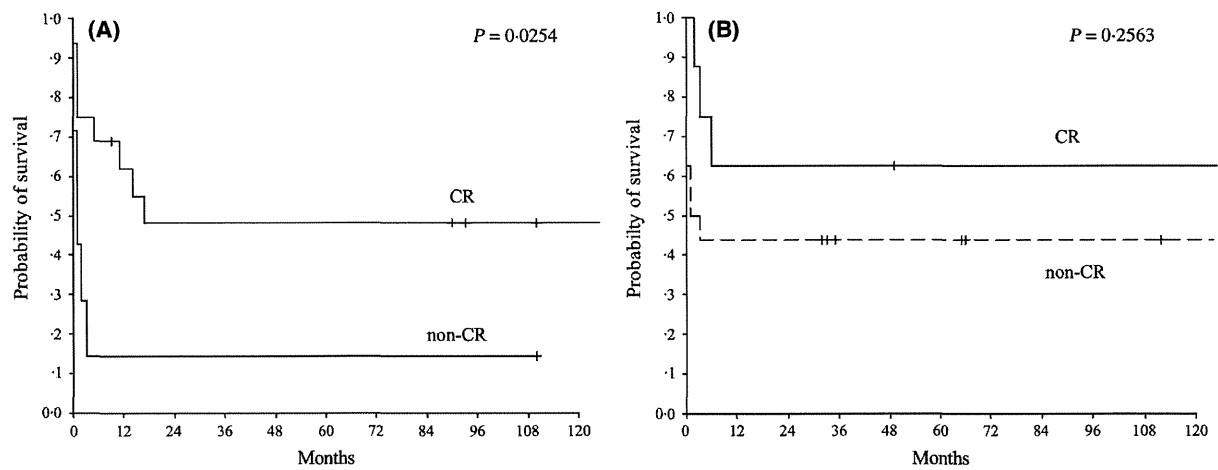

Fig 3. Event-free survival according to disease status at HSCT. (A) Autologous HSCT, (B) allogeneic HSCT. HSCT, haematopoietic stem cell transplantation; CR complete remission.

Table II. Details and outcomes of patients treated with reduced intensity conditioning and allogeneic HSCT.

Patients	Status at HSCT	Age at HSCT (years)	Donor	Stem cell source	Conditioning regimen	GVHD prophylaxis	aGVHD (Grade)	Extensive cGVHD	Outcome	Follow-up (months)
1	PR	3	UD	CB	TLI 2 Gy, Flu, Mel	Tac, MTX	III	–	CR	32
2	PR	9	UD	CB	Flu, Mel	Tac, MTX	II	–	CR	65
3	CR	18	UD	BM	Flu, Mel, ATG	Tac, MTX	0	NA	TRM	5
4	PR	16	UD	BM	Bu, Flu	Tac, MTX	III	+	CR	33

HSCT, haematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; UD, unrelated donor; BM, bone marrow; CB, cord blood; TLI, total lymphoid irradiation; Bu, busulfan; Flu, fludarabine; Mel, melphalan; ATG, antithymocyte globulin; GVHD, graft-*versus*-host disease; Tac, tacrolimus; MTX, methotrexate; aGVHD, acute GVHD; cGVHD, chronic GVHD; TRM, treatment-related mortality; NA, not applicable.

had predictive value for OS and EFS. In the current study, the EFS of the patients with CR at autologous HSCT was significantly higher than that of the patients with non-CR at autologous HSCT. Brugières *et al* (2000) reported that an interval of <12 months between diagnosis and relapse was associated with a higher risk of failure for the treatment of relapsed ALCL, including autologous HSCT. However, our cohort did not provide sufficient data to compare the risk of failure with the interval between diagnosis and relapse.

The role of allogeneic HSCT has not been defined for cases of childhood ALCL. The currently available evidence is limited to a few reports. The BFM group reported a series of 20 paediatric patients who underwent allogeneic HSCT for relapsed or refractory ALCL, finding a 75% 3-year EFS (Woessmann *et al*, 2006). Twelve of the patients in this study were in CR at HSCT. The CIBMTR has reported another large series of allogeneic HSCTs that were performed for ALCL, observing an EFS of 46% for 12 relapsed or refractory patients (Gross *et al*, 2010). Giulino-Roth *et al* (2013) also reported the cases of 13 paediatric patients with ALCL, eight of whom underwent autologous HSCT and five of whom underwent allogeneic HSCT. The OS and disease-free survival rates were 83% and 77%, respectively. Although our previous study noted that all six patients who underwent allogeneic HSCT during their second CR survived without further relapse (Mori *et al*, 2006), 5-year OS and EFS rates were limited to 54% and 50% in the present study. Patients who underwent allogeneic HSCT while in CR accounted for only eight of the 24 cases. Indeed, the rate of CR at HSCT was lower in the current study than in previous reports of allogeneic HSCT. In the present study, we found no significant difference in EFS according to disease status (CR or non-CR) at allogeneic HSCT. However, the low CR rate at allogeneic HSCT might be associated with the survival rate in the current study, which was lower than the rates noted in previous reports.

In the present study, we observed a 25% TRM rate among patients who underwent allogeneic HSCT for relapsed and refractory disease. Although the cumulative incidence of TRM for allogeneic HSCT was higher than that for autologous HSCT, the difference was not significant (P = 0·40) (Fig 1B). Several investigations have shown that RIC followed by allogeneic HSCT has the potential to reduce

TRM and long-term toxicity in cases of malignant and non-malignant diseases (Carella *et al*, 2000; Dreger *et al*, 2003; Jacobsohn *et al*, 2004; Bradley *et al*, 2007). The BFM cohort of allogeneic HSCTs included one case in which an RIC regimen was administered to a patient with ALCL. The RIC regimen comprised total lymphoid irradiation (2 Gy), fludarabine and melphalan (Brugières *et al*, 2000). Another case in which an RIC regimen [thraco-abdominal irradiation (2 Gy), fludarabine and melphalan] was used has also been reported (Ohta *et al*, 2010). Both of these patients survived in continuous CR following allogeneic HSCT. In the present study, four patients received an RIC regimen followed by allogeneic HSCT. Of these four patients, three were in non-CR at allogeneic HSCT, yet survived in CR for 32–65 months without relapse after HSCT. These results suggest that RIC for relapsed or refractory ALCL may be useful in cases involving allogeneic HSCT, regardless of disease status. However, there are only a few reports of allogeneic HSCT using an RIC regimen for paediatric ALCL. Further evaluations of the efficacy of RIC are necessary and should include larger numbers of patients and a prospective design.

The treatment of relapsed or refractory ALCL remains a matter of debate. Recent studies have reported the efficacies of second-line treatments for relapsed or refractory ALCL, including vinblastine monotherapy, brentuximab vedotin and crizotinib. Brugières *et al* (2009b) studied 36 paediatric patients treated with weekly vinblastine for relapsed or refractory ALCL, finding that this treatment was highly efficacious, with a CR rate of 83%. Furthermore, the 5-year EFS rate was 30%, at which time all but two of the patients had stopped vinblastine for more than 2 years. In adults, a phase II trial of brentuximab vedotin was conducted in patients with relapsed or refractory systemic ALCL. Fifty of 58 patients (86%) achieved an objective response, including 33 patients (57%) in CR (Pro *et al*, 2012). The Children's Oncology Group reported a phase I study of crizotinib for paediatric patients with refractory ALCL, finding that seven of nine children acheived CR following crizotinib monotherapy (Mossé *et al*, 2013). Autologous and allogeneic HSCTs are associated with high rates of toxicities and TRM. Consequently, it will be necessary to speculate about the selection of second-line treatments for relapsed or refractory ALCL in children and adolescents.

In conclusion, both autologous and allogeneic HSCT can offer the prospect of durable disease-free survival for relapsed and refractory ALCL in childhood and adolescence. Patients with CR at the time of autologous HSCT had significantly greater EFS than patients with non-CR at the time of autologous HSCT. Our results suggest that allogeneic HSCT might provide a better outcome for patients who are resistant to chemotherapy after relapse, and those with non-CR at the time of HSCT. Furthermore, an RIC regimen followed by allogeneic HSCT might even be useful for these patients. However, the small number of patients in our cohort prevented us from investigating the efficacy of allogeneic HSCT with an RIC regimen. In the new era of molecular target drugs, the best candidates for autologous and allogeneic HSCT remain to be clarified by further analyses and prospective studies of relapsed or refractory ALCL in childhood and adolescence.

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Author contributions

R Kobayashi, T Mori and R Fukano designed the research study; M Chin, H Goto, Y Takahashi, J Hara, YD Park, M Inoue, Y Koga, J Inagaki, H Sakamaki, S Adachi, K Kawa, K Kato and R Suzuki collected the data; R Fukano analysed the data and wrote the paper. All authors reviewed the manuscript.

Conflict of interest

There are no conflicts of interest to declare.

References

Bradley, M.B., Satwani, P., Baldinger, L., Morris, E., van de Ven, C., Del Toro, G., Garvin, J., George, D., Bhatia, M., Roman, E., Baxter-Lowe, L.A., Schwartz, J., Qualter, E., Hawks, R., Wolownik, K., Foley, S., Militano, O., Leclere, J., Cheung, Y.K. & Cairo, M.S. (2007) Reduced intensity allogeneic umbilical cord blood transplantation in children and adolescent recipients with malignant and non-malignant diseases. *Bone Marrow Transplantation*, 40, 621–631.

Brugières, L., Deley, M.C., Pacquement, H., Meguerian-Bedoyan, Z., Terrier-Lacombe, M.J., Robert, A., Pondarré, C., Leverger, G., Devalck, C., Rodary, C., Delsol, G. & Hartmann, O. (1998) CD30(+) anaplastic large-cell lymphoma in children: analysis of 82 patients enrolled in two consecutive studies of the French Society of Pediatric Oncology. *Blood*, 92, 3591–3598.

Brugières, L., Quartier, P., Le Deley, M.C., Pacquement, H., Perel, Y., Bergeron, C., Schmitt, C., Landmann, J., Patte, C., Terrier-Lacombe, M.J., Delsol, G. & Hartmann, O. (2000) Relapses of childhood anaplastic large-cell lymphoma: treatment results in a series of 41 children—A report from the French Society of Pediatric Oncology. *Annals of Oncology*, 11, 53–58.

Brugières, L., Le Deley, M.C., Rosolen, A., Williams, D., Horibe, K., Wrobel, G., Mann, G., Zsiros, J., Uyttebroeck, A., Marky, I., Lamant, L. & Reiter, A. (2009a) Impact of the methotrexate administration dose on the need for intrathecal treatment in children and adolescents with anaplastic large-cell lymphoma: results of a randomized trial of the EICNHL Group. *Journal of Clinical Oncology*, 27, 897–903.

Brugières, L., Pacquement, H., Le Deley, M.C., Leverger, G., Lutz, P., Paillard, C., Baruchel, A., Frappaz, D., Nelken, B., Lamant, L. & Patte, C. (2009b) Single-drug vinblastine as salvage treatment for refractory or relapsed anaplastic

large-cell lymphoma: a report from the French Society of Pediatric Oncology. *Journal of Clinical Oncology*, 27, 5056–5061.

Carella, A.M., Cavaliere, M., Lerma, E., Ferrara, R., Tedeschi, L., Romanelli, A., Vinci, M., Pinotti, G., Lambelet, P., Loni, C., Verdiani, S., De Stefano, F., Valbonesi, M. & Corsetti, M.T. (2000) Autografting followed by nonmyeloablative immunosuppressive chemotherapy and allogeneic peripheral-blood hematopoietic stem-cell transplantation as treatment of resistant Hodgkin's disease and non-Hodgkin's lymphoma. *Journal of Clinical Oncology*, 18, 3918–3924.

Dreger, P., Brand, R., Hansz, J., Milligan, D., Corradini, P., Finke, J., Deliliers, G.L., Martino, R., Russell, N., Van Biezen, A., Michallet, M. & Niederwieser, D. (2003) Treatment-related mortality and graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. *Leukemia*, 17, 841–848.

Fanin, R., Ruiz de Elvira, M.C., Sperotto, A., Baccarani, M. & Goldstone, A. (1999) Autologous stem cell transplantation for T and null cell CD30-positive anaplastic large cell lymphoma: analysis of 64 adult and paediatric cases reported to the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplantation*, 23, 437–442.

Giralt, S., Ballen, K., Rizzo, D., Bacigalupo, A., Horowitz, M., Pasquini, M. & Sandmaier, B.; Reduced-intensity conditioning regimen workshop: defining the dose spectrum. (2009) Report of a workshop convened by the center for international blood and marrow transplant research. *Biology of Blood and Marrow Transplantation*, 15, 367–369.

Giulino-Roth, L., Ricafort, R., Kernan, N.A., Small, T.N., Trippett, T.M., Steinherz, P.G., Prockop, S.E., Scaradavou, A., Chiu, M., O'Reilly, R.J. & Boulad, F. (2013) Ten-year follow-up of pediatric patients with non-Hodgkin lymphoma trea-

ted with allogeneic or autologous stem cell transplantation. *Pediatric Blood & Cancer*, 60, 2018–2024.

Gross, T.G., Hale, G.A., He, W., Camitta, B.M., Sanders, J.E., Cairo, M.S., Hayashi, R.J., Termuhlen, A.M., Zhang, M.J., Davies, S.M. & Eapen, M. (2010) Hematopoietic stem cell transplantation for refractory or recurrent non-Hodgkin lymphoma in children and adolescents. *Biology of Blood and Marrow Transplantation*, 16, 223–230.

Jacobsohn, D.A., Duerst, R., Tse, W. & Kletzel, M. (2004) Reduced intensity haemopoietic stem-cell transplantation for treatment of non-malignant diseases in children. *Lancet*, 364, 156–162.

Le Deley, M.C., Rosolen, A., Williams, D.M., Horibe, K., Wrobel, G., Attarbaschi, A., Zsiros, J., Uyttebroeck, A., Marky, I.M., Lamant, L., Woessmann, W., Pillon, M., Hobson, R., Mauguen, A., Reiter, A. & Brugières, L. (2010) Vinblastine in children and adolescents with highrisk anaplastic large-cell lymphoma: results of the randomized ALCL99-vinblastine trial. *Journal of Clinical Oncology*, 28, 3987–3993.

Luger, S.M., Ringdén, O., Zhang, M.J., Pérez, W.S., Bishop, M.R., Bornhauser, M., Bredeson, C.N., Cairo, M.S., Copelan, E.A., Gale, R.P., Giralt, S.A., Gulbas, Z., Gupta, V., Hale, G.A., Lazarus, H.M., Lewis, V.A., Lill, M.C., McCarthy, P.L., Weisdorf, D.J. & Pulsipher, M.A. (2012) Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. *Bone Marrow Transplantation*, 47, 203–211.

Mori, T., Takimoto, T., Katano, N., Kikuchi, A., Tabuchi, K., Kobayashi, R., Ayukawa, H., Kumagai, M.A., Horibe, K. & Tsurusawa, M. (2006) Recurrent childhood anaplastic large cell lymphoma: a retrospective analysis of registered cases in Japan. *British Journal of Haematology*, 132, 594–597.

Mossé, Y.P., Lim, M.S., Voss, S.D., Wilner, K., Ruffner, K., Laliberte, J., Rolland, D., Balis, F.M., Maris, J.M., Weigel, B.J., Ingle, A.M., Ahern, C., Adamson, P.C. & Blaney, S.M. (2013) Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. *Lancet Oncology*, **14**, 472–480.

Murphy, S.B. (1994) Paediatric lymphomas: recent advances and commentary on Ki-1-positive anaplastic large-cell lymphomas of childhood. *Annals of Oncology*, **5**, 31–33.

Ohta, H., Kusuki, S., Yoshida, H., Sato, E., Hashii, Y. & Ozono, K. (2010) Allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning for a child with recurrent anaplastic large cell lymphoma. *International Journal of Hematology*, **92**, 190–193.

Pro, B., Advani, R., Brice, P., Bartlett, N.L., Rosenblatt, J.D., Illidge, T., Matous, J., Ramchandren, R., Fanale, M., Connors, J.M., Yang, Y., Sievers, E.L., Kennedy, D.A. & Shustov, A. (2012) Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. *Journal of Clinical Oncology*, **30**, 2190–2196.

Seidemann, K., Tiemann, M., Schrappe, M., Yakisan, E., Simonitsch, I., Janka-Schaub, G., Dörffel, W., Zimmermann, M., Mann, G., Gadner, H., Parwaresch, R., Riehm, H. & Reiter, A. (2001) Short-pulse B-non-Hodgkin lymphoma-type chemotherapy is efficacious treatment for pediatric anaplastic large cell lymphoma: a report of the Berlin-Frankfurt- Munster Group Trial NHL-BFM 90. *Blood*, **97**, 3699–3706.

Stockklausner, C., Behnisch, W., Mechtersheimer, G., Möller, P. & Kulozik, A.E. (2008) Long-term remission of children with relapsed and secondary anaplastic large cell non-Hodgkin lymphoma (ALCL) following treatment with pulsed dexamethasone and low dose etoposide. *Paediatric Blood & Cancer*, **50**, 126–129.

Williams, D.M., Hobson, R., Imeson, J., Gerrard, M., McCarthy, K. & Pinkerton, C.R. (2002) Anaplastic large cell lymphoma in childhood: analysis of 72 patients treated on The United Kingdom Children's Cancer Study Group chemotherapy regimens. *British Journal of Haematology*, **117**, 812–820.

Woessmann, W., Peters, C., Lenhard, M., Burkhardt, B., Sykora, K.W., Dilloo, D., Kremens, B., Lang, P., Führer, M., Kühne, T., Parwaresch, R., Ebell, W. & Reiter, A. (2006) Allogeneic haematopoietic stem cell transplantation in relapsed or refractory anaplastic large cell lymphoma of children and adolescents: a Berlin- Frankfurt-Munster group report. *British Journal of Haematology*, **133**, 176–182.

Woessmann, W., Zimmermann, M., Lenhard, M., Burkhardt, B., Rossig, C., Kremens, B., Lang, P., Attarbaschi, A., Mann, G., Oschlies, I., Klapper, W. & Reiter, A. (2011) Relapsed or refractory anaplastic large-cell lymphoma in children and adolescents after Berlin-Frankfurt-Muenster (BFM)-type first-line therapy: a BFM-group study. *Journal of Clinical Oncology*, **29**, 3065–3071.

Yaniv, I. & Stein, J. (2008) Reduced-intensity conditioning in children: a reappraisal in 2008. *Bone Marrow Transplantation*, **41** (Suppl 2), S18–S22.

Improved Treatment Results of Children With B-Cell Non-Hodgkin Lymphoma: A Report From the Japanese Pediatric Leukemia/Lymphoma Study Group B-NHL03 Study

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Background. Previous Japanese studies of childhood B-cell non-Hodgkin lymphoma (B-NHL) have shown a favorable outcome, though the study size was too small to effectively assess the efficacy and safety of treatment for childhood B-NHL. **Procedure.** We performed a nation-wide prospective B-NHL03 study to assess the efficacy and safety of short-pulse intensive chemotherapy for children with B-NHL. They were stratified into four treatment groups according to disease stage, tumor resectability and bone marrow/CNS involvement: Group 1 with all resected stage I/II, Group 2 with non-resected stage I/II, Group 3 with stage III & CNS-negative stage IV, and Group 4 with CNS-positive stage IV & Burkitt leukemia. Treatment duration was 2 courses for Group 1, 4 courses for Group 2, and 6 courses for Groups 3 and 4, respectively. CNS irradiation was omitted in all patients. **Results.** The follow-up time ranged from 0.8 to 88 months, with a median of being 45 months. For 321 patients analyzed in this study, overall survival and event-free survival (EFS) at 4 years was 92.7% and 87.4%, respectively. The 4-year EFS according to treatment group were 94% for Group 1 (n = 17), 98% for Group 2 (n = 103), 84% for Group 3 (n = 111), and 78% for Group 4 (n = 90). There was no significant difference in outcome by histology. Therapy-related death occurred in three patients in remission. **Conclusions.** Our nationwide large-scale study resulted in a cure rate above 90% with <1% toxic death in childhood B-NHL. Pediatr Blood Cancer 2014;61:1215–1221. © 2014 Wiley Periodicals, Inc.

Key words: B-NHL03; childhood; JPLSG; non-Hodgkin lymphoma

INTRODUCTION

Childhood B-cell non-Hodgkin Lymphoma (B-NHL) consists mainly of two histological subtypes, namely Burkitt lymphoma (BL), which includes Burkitt leukemia (B-ALL), and diffuse large B-cell lymphoma (DLBCL). The cure rate of childhood BL has been markedly improved over the past 30 years, and long-term event-free survival (EFS) of patients has reached to approximately 90%. This is largely due to prospective studies of European and North American groups that developed a short intensive chemotherapy regimen, including a high-dose methotrexate (HDMTX), an intermediate dose of cyclophosphamide (CPA), and anthracyclines [1–6]. Although DLBCL is a distinct disease entity from BL, the treatment is the same as that for patients with Burkitt histology, and excellent outcome has been reported [1–6]. Previously most clinical experiences of childhood B-NHL were reported by European and North American study groups, and there were few data on Japanese or Asian patients with B-NHL. In the1990s, we conducted group-wide trials for childhood B-NHL [7–10]: Horibe et al. showed a 4-year EFS with 70% for 57 patients (BL 31, B-ALL 17, DLBCL 9) [8], Kikuchi et al. showed a 6-year EFS with 82% for 91 patients (BL 45, B-ALL 9, DLBCL 26, others 11) [10], and Tsurusawa et al. showed a 7-year EFS with 93% for 30 patients with DLBCL [9]. In addition, Lee et al. has recently shown a 5-year EFS with 95% for 61 patients (BL 46, DLBCL 15) [11]. However, the treatment duration of these studies was relatively long and the number of patients was small compared to the European and North American studies [1–6].

Here, we report on the results of the nation-wide large prospective study for children with B-NHL. The primary object was to evaluate the efficacy and safety of short-pulse intensive chemotherapy regimen designed by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG).

PATIENTS AND METHODS

Study Design and Diagnostic Criteria

The B-NHL03 study was a prospective nonrandomized trial that investigated the efficacy and safety of short-pulse intensive chemotherapy in childhood B-NHL. The chief aim was to improve the outcomes of patients enrolled in the B-NHL03 study to the level of those of European and North American studies.

The diagnosis of B-NHL was based on histopathology, immunocytochemistry, and cytogenetics. All histopathological specimens were first classified by the institutional pathologist and finally each of them were reviewed by a group of seven pathologists of a central pathological review committee according to WHO classification, that is, BL or Burkitt-like lymphoma (BLL), DLBCL, mediastinal large B-cell lymphoma (MLBCL), and mature B-cell neoplasm, NOS (not otherwise specified) [12]. A mature B-cell phenotype was primarily defined as positive for C20 and/(or) CD79a and negative for CD3 and terminal deoxynucleotidyl transferase. When an immunophenotype study was not available, specific translocations t(8;14)(q24;q32), t(2;8)(p11;q24), t(8;22)(q24;q11) at cytogenetic analysis were included. CNS involvement was diagnosed by the presence of one or more of the following: any blasts with FAB L3 morphology in CSF, isolated intracerebral mass, or intra-spinal extension. The clinical stage was defined by Murphy's classification [13].

Treatments

The treatment outline is shown in Figure 1 and chemotherapy regimens are shown in Table I. They were stratified into four treatment groups according to disease stage, tumor resectability and bone marrow/CNS involvement: Group 1 with all resected stage I/II, Group 2 with non-resected stage I/II, Group 3 with stage III & CNS-negative stage IV, and Group 4 with CNS-positive stage IV & B-ALL. All groups except Group 1 received a pre-phase therapy of prednisolone (PSL), vincristine (VCR), CPA and it (intrathecal) MTX to reduce tumor volume. As shown in Figure 1, Group 1 received two courses (1A × 2), Group 2 received 4 courses (2A × 2 + 2B × 2), Group 3 received 6 courses (3A × 4 + 3B × 2), and Group 4 received 6 courses (4A1 × 2 + 4A2 × 2 + 4B × 2), respectively. No patients received prophylactic cranial irradiation. Patients with CNS involvements received HDMTX ($5 g/m^2$) plus an extended it regimen (14 times), but no therapeutic cranial irradiation. The schedule of HDMTX administration was identical
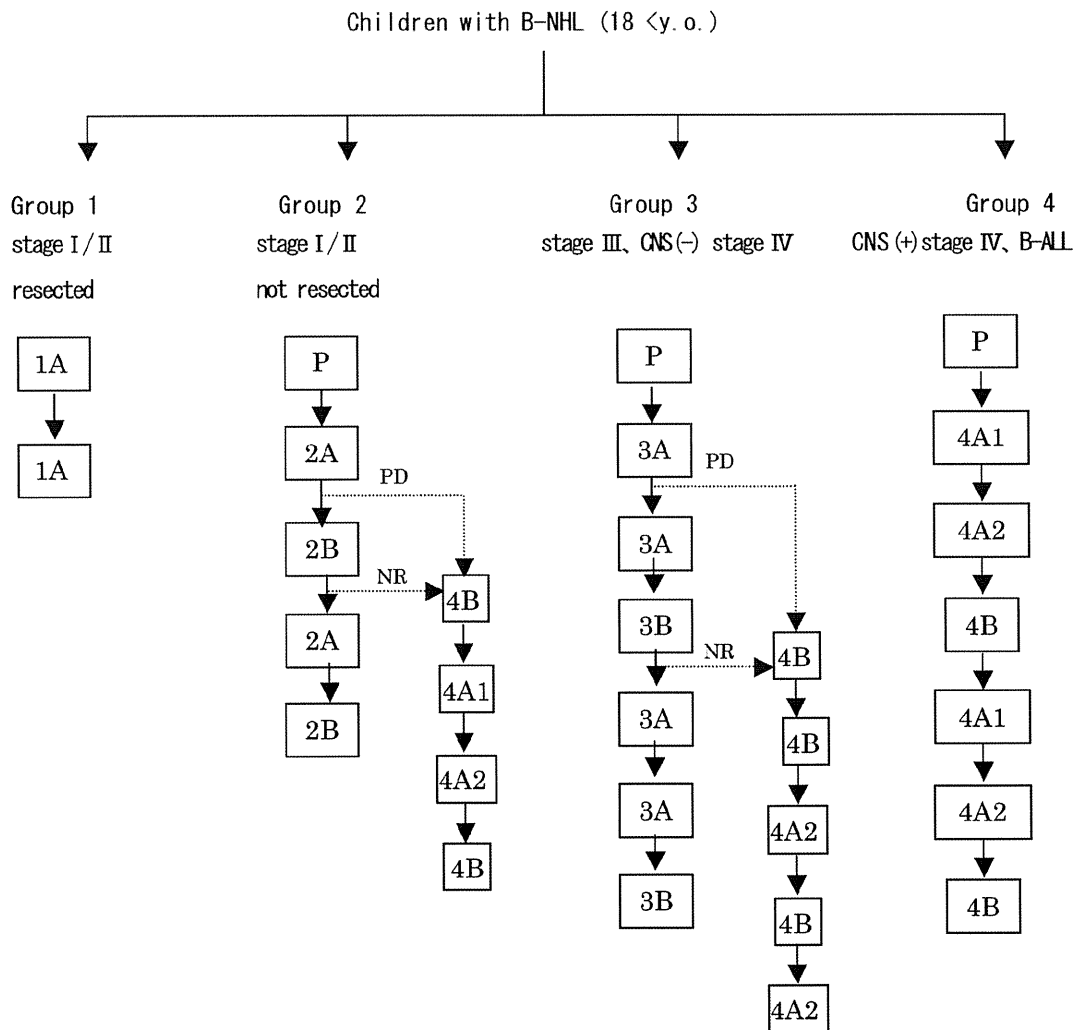
Fig. 1. Treatment framework of the B-NHL03 study. Patients were stratified into four treatment groups according to disease stage, tumor resectability, and BM/CNS involvement. All groups except Group 1 received pre-phase therapy. Group 1 received two courses of chemotherapy, Group 2 received 4 courses, Groups 3 and 4 received 6 courses, respectively. When patients in Group 2 or 3 did not achieve CR or CRu during the first 2 or 3 courses, they received salvage therapy consisting of 4B and 4A1/2 courses.

TABLE I. B-NHL03 Treatment Schedules

Regimen	Administration	Daily dose	Days
Pre-phase			
Prednisolone	Orally	30 mg and 60 mg/m^2	Days 1–3 and 4–7
Vincristine	IV	1 mg/m^2	Day 3
Cyclophosphamide	IV	150 mg/m^2	Days 4–6
Methotrexate	TIT	12 mg/m^2	Day 1, (4)[a]
Hydrocortisone	TIT	25 mg/m^2	Day 1, (4)[a]
Cytarabine	TIT	30 mg/m^2	Day (4)[a]
Regimen 1A			
Prednisolone	Orally	60 mg/m^2	Days 1–5
Methotrexate	IV	1 g/m^2	Day 1
Vincristine	IV	1.5 mg/m^2	Day 2
Cyclophosphamide	IV	250 g/m^2 × 2	Days 2–4
THP-adriamycin	IV	30 mg/m^2	Days 3, 4
Methotrexate	DIT	12 mg/m^2	Day 1
Hydrocortisone	DIT	25 mg/m^2	Day 1
Regimen 2A			
Same as 1A except for dexamethasone	Orally	10 mg/m^2	Days 1–7
Methotrexate	IV 24 hours with LV rescue	3 g/m^2	Day 1
Regimen 3A			
Same as 2A except for *t.i.t* at day 1			
Regimen 4A1			
Same as 3A except for methotrexate	IV 24 hours with LV rescue	5 g/m^2	Day 1
Methotrexate	TIT	12 mg/m^2	Day 1, (5),[a] 8
Hydrocortisone	TIT	25 mg/m^2	Day 1, (5),[a] 8
Cytarabine	TIT	30 mg/m^2	Day 1, (5),[a] 8
Regimen 4A2			
Same as 4A1 except for cyclophosphamide	IV	1 g/m^2	Days 4, 5
Regimen 2B			
Methotrexate	IV 6 hours	500 mg/m^2	Day 1
Cytarabine	cIV	150 mg/m^2	Days 1–5
Methotrexate	DIT	12 mg/m^2	Day 1
Hydrocortisone	DIT	25 mg/m^2	Day 1
Regimen 3B			
Same as 2B except for TIT at day 1, and cytarabine	cIV	150 mg/m^2	Days 1–6
Etoposide	IV	100 mg/m^2 × 2	Days 3–5
Regimen 4B			
Same as 3B except for without methotrexate,	Orally	10 mg/m^2	Days 1–7
DIT at day 1 and TIT at day 8, and dexamethasone			
Cytarabine	IV	2 g/m^2 × 2	Days 2–4
Etoposide	IV	150 mg/m^2	Days 2–5
Vincristine	IV	1.5 mg/m^2	Day 1

LV, leucovorin; IV, intravenous; cIV, continuous intravenous; DIT, double intrathecal; TIT, triple intrathecal. [a]For CNS positive patients.

to that of the B-NHL960 study [9]: HDMTX was administered for the first 24 hours, and 12 hours later, leucovorin (LV) 15 mg/m^2 was given orally every 6 hours, for a total of seven doses [9]. Blood MTX concentration was measured 24, 48, and 72 hours after the MTX administration. When patients showed delayed MTX clearance ($\geq 0.2\,\mu$M after 72 hours), LV rescue was continued until MTX concentration level decreased to less than 0.2 μM.

Induction failure (IF) was defined as patients who did not achieve complete remission (CR) or unconfirmed remission (CRu) until the last evaluation time (before the second course of 2A in Group 2, before the third course of 3A in Group 3, before the second course of 4A1 in Group 4). When patients in Group 2 or 3 were evaluated to have progressive disease or no response during the first 2 or 3 courses, they received salvage therapy consisting of regimens 4B and 4A1/2. The cumulative dose of cytotoxic drugs for treatment groups was as follows: CPA 3 g/m^2, THP 120 mg/m^2 for Group 1;

CPA 3.45 g/m^2, THP 120 mg/m^2 for Group2; CPA 6.45 g/m^2, THP 240 mg/m^2, VP16 0.6 g/m^2 for Group 3; CPA 7.45 g/m^2, THP 240 mg/m^2, VP16 1.2 g/m^2 for Group 4.

Statistical Analysis

Final statistical analyses were performed based on data obtained in June 2012. Overall survival (OS) was defined as the time between diagnosis and death from any causes, and EFS was defined as the time to first events defined as an occurrence of induction failure, relapse at any site, death from any causes, or second malignant neoplasm. For patients who did not experience an event, EFS was defined as the time to the last follow-up. Survival curves were prepared using the Kaplan–Meier method and standard errors (SEs) with the Greenwood formula. The significance of differences in survival outcomes was determined by means of the log-rank test.

STATA[®] statistical analysis software (version 11.0; StataCorp LP, College Station, TX) was used for all computations.

RESULTS

Patients

The protocol was conducted in 112 hospitals of the JPLSG after approval by each institution's review board, and written informed consent was provided by patients or legal guardians before treatment. Between November 2004 and January 2011, 346 cases of newly diagnosed B-NHL were enrolled in this study. Of these, 25 cases were excluded: 14 due to ineligible pathology, 8 for late enrollment, 2 for ineligible clinical stage, and 1 for prior chemotherapy. A total of 321 cases of four treatment groups were analyzed (Fig. 2).

Patient characteristic are shown in Table II. There were few protocol deviations: 10 patients in the Group 3/4 skipped or postponed HDMTX therapy in the A course, 5 because of retention of ascites or pleural effusion, 2 because of renal dysfunction, 2 due to septic infection, and one for stomatitis.

EFS and OS

The follow-up time ranged from 0.8 to 88 months, with a median 47 months. For the 321 patients analyzed in this study, 4-year OS was 92.7% ± 1.4% and 4-year EFS was 87.3% ± 1.8% (Fig. 3A). There was no significant difference in outcome by gender (4-year EFS, male 87.5% ± 2.2% vs. female 87.0% ± 3.8%, P=0.864). The 4-year OS and EFS according to treatment subgroup were 100% and 94.1% ± 5.7% for Group 1, 100% and 98.6% ± 1.4% for Group 2, 93.6% ± 2.3% and 83.6% ± 3.5% for Group 3, and 82.1% ± 4.1% and 77.8% ± 4.4% for Group 4 (Fig. 3B). The 4-year OS and EFS according to clinical stage were 100% and 97.7% ± 2.3% for stage I, 100% and 97.8% ± 2.0% for stage II, 92.0% ± 2.9% and 82.9% ± 4.0% for stage III, 84.6% ± 5.8% and 71.8% ± 7.2% for stage IV. The 4-year OS and EFS of B-ALL were 86.2% ± 4.0% and 83.6% ± 4.3%. The 4-year EFS by histology was 86.1% ± 2.6% for BL/BLL, 87.3% ± 3.5% for DLBCL, 92.1% ± 4.3% for others, and 100% for MLBCL (P=0.717) (Fig. 3C). When we analyzed the outcome of patients who had BM or CNS disease, the 4-year EFS was 83.8% ± 4.3% for patients (n = 74) with BM involvement only (BM+/CNS−), 60.0% ± 1.5%
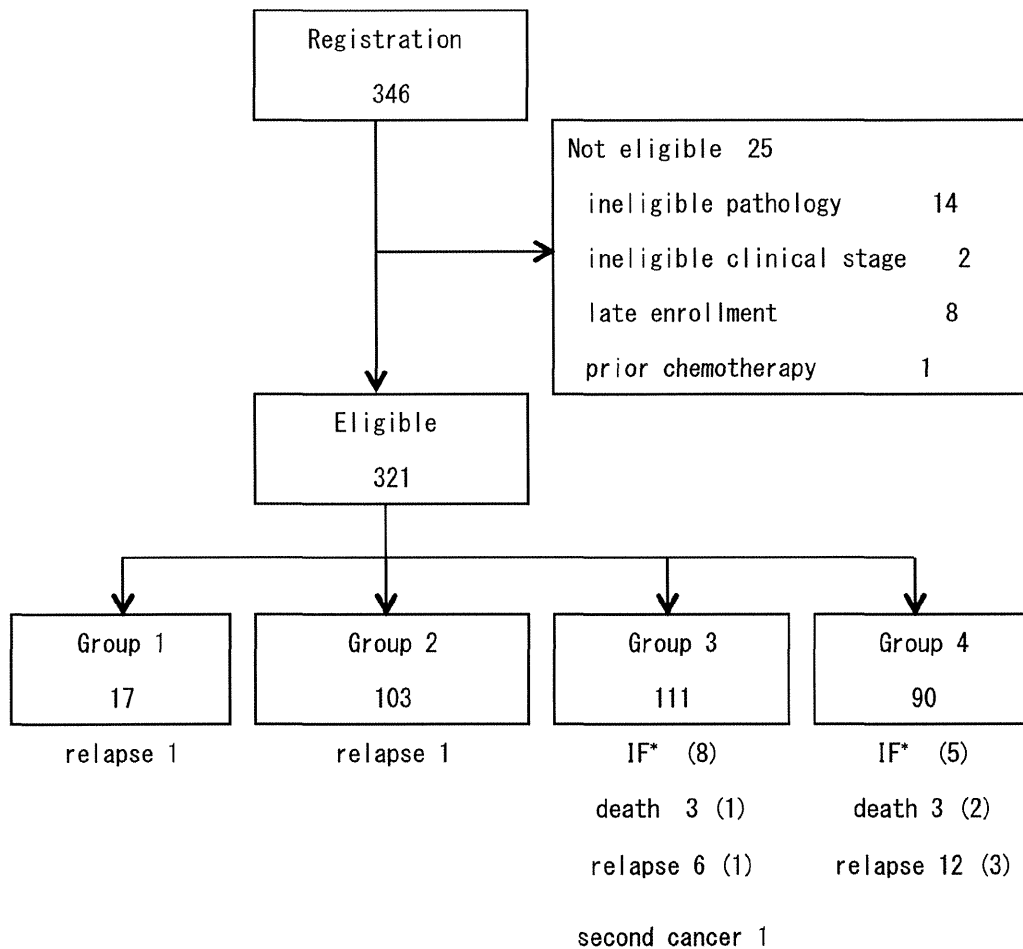

Fig. 2. Patient flow chart and events according to the treatment group. There were 40 events which consisted of each one in Group 1 and 2, 18 in Group 3, and 20 in Group 4. Number in parentheses indicates events occurred during protocol chemotherapy. Number in parenthesis indicates events occurred during protocol chemotherapy. *IF, induction failure defined as patients did not achieve complete remission or unconfirmed remission at the last evaluation time in group 3/4.

TABLE II. Patients Characteristics

Therapy groups	G1	G2	G3	G4	Total (%)
No. of patients	17	103	111	90	321
Sex					
Male	12	72	90	71	245 (76)
Female	5	31	21	19	76 (24)
Age					
0–4	2	12	18	16	48 (15)
5–9	3	45	42	39	129 (40)
10–14	8	42	42	27	119 (37)
15–	4	4	9	8	25 (8)
Histology					
BL/BLL/B-ALL	5	33	62	80	180 (56)
DLBCL	12	58	26	5	101 (31.4)
MLBCL	0	0	2	0	2 (0.6)
Others	0	12	21	5	38 (12)
Primary sites					
Thorax	5	30	7	1	43
Head & neck	5	39	12	2	58
Peripheral lymph nodes	0	3	3	0	6
Abdomen	7	29	75	11	122
Mediastinum	0	0	8	0	8
B-ALL	0	0	0	73	73
CNS	0	0	0	2	2
Other tumor site	0	2	5	0	7
Not specified	0	0	1	1	2
BM involvement	0	0	22	80	102 (32)
CNS involvement	0	0	0	38	38 (12)

BL, Burkitt lymphoma; BLL, Burkitt-like lymphoma; B-ALL, Burkitt leukemia; DLBCL, diffuse large B-cell lymphoma, MLBCL, mediastinal large.

for patients (n = 10) with CNS involvement only (BM−, CNS+), and 75.0% ± 8.2% for patients (n = 28) with BM and CNS involvements (BM+/CNS+), (P = 0.102) (Fig. 3D). Outcome by treatment response to initial A courses were as follows: The 4-year OS and EFS for patients who achieved CR (n = 236) or CRu (n = 54) at the last evaluation time were 95.7% ± 1.6% and 93.5% ± 1.6%, and 96.1% ± 2.7% and 86.9% ± 4.6%, respectively, while the 4-year OS and EFS for patients (n = 13) who did not achieve CR/CRu was 69.2% ± 12.8% and 15.4% ± 10.1% (P < 0.001), respectively.

Treatment Failure Events

Forty patients experienced an event and 25 have died (Fig. 2). The cause of death was tumor progression in 14, infection in 7, stem cell transplantation-related death in 3, and pulmonary bleeding in 1. The 40 events consisted of 13 induction failures, 6 deaths, 20 relapses, and one second cancer. Of the 13 patients (6 in Group 3 and 7 in Group 4) who failed the initial treatment, 4 patients in Group 3 received salvage therapy and achieved CRu. At the time of the last analysis, 8 patients (4 in Group 3 and 4 in Group 4) were alive without tumor. Death in remission occurred in 3/321 (1%) patients: two died of infection and one died of pulmonary bleeding. The longest duration before relapse from the start of therapy was 38.9 months in DLBCL and 13.6 months in Burkitt histology. Relapse sites were 10 in local, 6 in BM, 2 in BM+CNS, one in local + CNS, and one in CNS. All CNS relapse occurred in patients with BL, but not with DLBCL. Thus, isolated CNS failure was only one among 38 patients with CNS involvement. Of the 20 relapsed

patients, 11 died and 9 survived without tumor. A second cancer occurred among the patients who failed the initial treatment: a 12-year-old male with BL developed a secondary malignancy with acute myeloid leukemia (FAB M5) 17 months after the initial diagnosis.

Toxicity

Acute toxicity of treatment courses (A and B) was evaluated by the scale of NCI-CTC version 2.0., and rates of acute toxicity Grade 3 among patients in Groups 2, 3, and 4 are shown in Supplemental Table I. Anemia and neutropenia were the most frequent hematological toxicities with grade III or IV in all groups. In particular, grade IV neutropenia occurred in almost all patients (>98%) during A courses. In nonhematologic toxicity, infection was the single most frequent occurring with grade III or IV at least once in 70% of patients although the rate of grade IV infection was very small (<1%). Stomatitis and hepatotoxicity were also frequent, occurring with grade III or IV at least once in 20–35% and 24–38% of patients, respectively. The rate of renal toxicity grade III was very low. Leukoencephalopathy was reported in two patients of Group 3, and their MRI findings disappeared within 2 months without neurological symptoms. The overall incidence of renal insufficiency associated with tumor lysis syndrome was 2 out of 96 (2%) in Group 4, and these required assisted renal support with continuous hemodiafiltration.

DISCUSSION

During the last two decades, the survival outcome of children with B-NHL has been markedly improved through consecutive
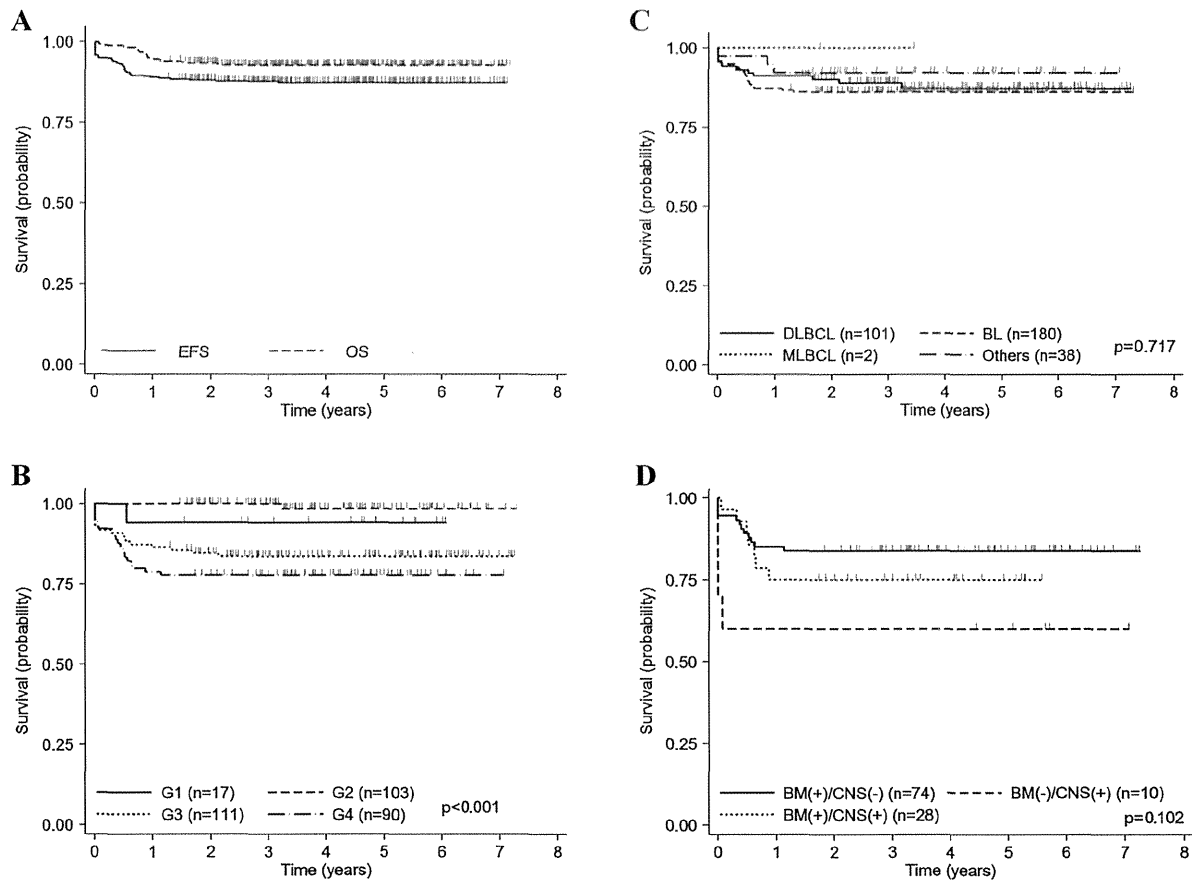
Fig. 3. Kaplan–Meier curves for OS and EFS of all patients (**A**). Kaplan–Meier curves for EFS according to treatment group (**B**), histology (**C**), and BM/CNS involvement (**D**).

clinical trials in large study groups, and the cure rate of childhood B-NHL has reached 90% [1–6]. In the present study, we showed an excellent survival outcome with 4-year OS 93% in children with B-NHL. In our study, the 4-year EFS 84% of Group 3 patients was considerably lower than the 4-year EFS 90% of intermediate risk group in the FAB/LMB96 study [5] or the 6-year EFS 88% of stage III patients in the BFM90 study [2], whereas, the 4-year EFS 78% of Group 4 patients compared favorably with the 4-year EFS 79% of high-risk group in the FAB/LMB96 study [5] and the 6-year EFS 74% of stage IV/B-ALL patients in the BFM90 study [2]. This outcome was obtained via the short-intensive chemotherapy regimen based on COPAD (CPM, VCR, PSL, and ADR) regimen plus the HDMTX of the lymphomas malin B (LMB) studies [3]. We omitted cranial irradiation for all patients, because recent studies have suggested the possibility of deleting radiotherapy in treating CNS diseases as well as CNS prophylaxis [2,3,5,9]. However, having no experience in administrating 8 g/m² HDMTX, we employed 5 g/m² HDMTX over 24 hour-infusion and not the 8 g/m² HDMTX over 4 hour-infusion in the LMB protocols for treating patients with CNS disease [3,5]. The treatment result for CNS disease was satisfactory, because CNS failure was only one of 38 patients with primary CNS disease in the present study.

This suggests that the 5 g/m² HDMTX over 24 hour-infusion is equally as effective to the CNS-positive disease as the aforementioned 8 g/m² HDMTX over 4 hours infusion, and reinforces the

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possibility that CNS irradiation could be omitted without jeopardizing the outcome of patients with CNS disease by using systemic and it MTX therapy [3,5,9].

The treatment of DLBCL as well as BL was another important focus of our study, because the incidence of DLBCL in childhood B-NHL is relatively more frequent than that of Western countries: the number of DLBCL was almost similar to that of BL (excluding B-ALL) in the present study and our recent national survey for childhood hematological malignancies has shown that the ratio of DLBCL to BL was 0.79 [14]. In our study, according to the strategy that DLBCL was treated by short-pulse chemotherapy as well as BL [15], we followed the same protocol, and achieved a favorable outcome of 4-year EFS with 87% for DLBCL which was not inferior to that of BL. This outcome can be partly explained by shared biological features, that is, that more than half of childhood DLBCL has the molecular subtypes of BL [16].

Several factors associated with poor outcome in the high-risk group in childhood B-NHL have been reported. Cairo et al. has shown a significantly inferior outcome (4-year EFS 61% ± 6%) of the subgroup of children with combined BM and CNS involvement at diagnosis as compared with children with BM or CNS only [5]. However, our results in Group 4 showed that the outcome (4-year EFS 75% ± 8%) of this subgroup with BM+/CNS+ was not significantly inferior than that of the subgroup with BM+ (83% ± 4%) or CNS+ (60% ± 1%). Failure to initial therapy is

also known to be a strong, unfavorable prognostic factor. Past studies in LMB 89/96 have shown that non-responders to pre-phase therapy (COP regimen) suffer a significantly inferior outcome as compared with responders or incomplete responders [3,5]. In our study, an appropriate evaluation of tumor regression just after pre-phase therapy was difficult for many patients, such that we compared the outcome according to response at the final evaluation time after two or three courses of therapy. These results showed that 4-year EFS of patients who did not achieve CR/CRu was only 15% ± 10%, which was as dismal as the outcome of poor-responders to COP regimen in the FAB/LMB 96 study [5]. To rescue the poor-responders in our study, we employed salvage therapy with high-dose Ara-C and VP16 to patients who did not achieve remission after 2 or 3 courses of therapy in Group 2 or 3, as in the BFM90 or FAB96 study [2,4]. As a result, 4 of 6 patients in Group 3 received salvage therapy and survived without tumor. This response rate was similar to that of FAB96 study, in which 10 out of 16 patients who received the second phase treatment intensification after the consolidation phase were alive. Thus, our results reconfirmed the efficacy of the salvage therapy.

Management of acute toxicity by short-pulse intensive chemotherapy is essential to successfully carry out the treatment protocol for childhood B-NHL. In our study, grade IV neutropenia occurred in almost all patients, but the rate of grade IV infection was quite low. Consequently, therapy-related death was less than 1% in all patients, and 2.1% in Group 4 patients. These results show the safety and feasibility of our treatment protocol. Anthracycline cardiotoxicity and secondary malignancy by alkylating agents are serious late events in pediatric cancer treatment [17,18]. To reduce the risk of cardiotoxicity, we employed THP-adriamycin (pirarubicin) instead of ADR. Pirarubicin is a derivative of ADR with reportedly less cardiotoxicity in adults [19–24]. Recently, we have reported that no significant cardiac dysfunction was detected in long-term survivors of children with acute lymphoblastic leukemia who received THP treatment [25–27]. In the present study, there were no patients with cardiac insufficiency or cardiac myopathy during the 7-year observation period. These results suggest that late-onset cardiotoxicity induced by pirarubicin is uncommon in childhood lymphoid malignancies, at least up to the cumulative dose of 240 mg/m². In our study, there was one male with a second cancer with acute myeloid leukemia, although the correlation between his second cancer and the protocol treatment is uncertain because he was resistant to the pre-phase followed by arbitrary treatment.

As shown above, chemotherapy-related toxicity of our protocol treatment was within acceptable range. However, a 6-course treatment for Group 3 seemed to be more intensive as compared with a 4-course treatment for intermediate risk group in the FAB96 study [4]. In order to reduce the total dose of cytotoxic drugs without impairing the survival outcome, new approaches including targeted monoclonal antibody therapy in combination with chemotherapy [28,29], are needed for children with an advanced or resistant disease in coming studies.

In conclusion, our nationwide study resulted in a cure rate above 90% with <1% toxic death in childhood B-NHL.

ACKNOWLEDGMENTS

REFERENCES

1. Reiter A, Schrappe M, Parwaresch R, et al. Non-Hodgkin's lymphomas of childhood and adolescence: Results of a treatment stratified for biologic subtypes and stage. A report of the Berlin Frankfurt-Münster Group. J Clin Oncol 1995;13:359–372.

2. Reiter A, Schrappe M, Tiemann M, et al. Improved treatment results in childhood B-cell neoplasms with tailored intensification of therapy: Reports of the Berlin–Frankfurt–Münster Group trial NHL–BFM 90. Blood 1999;94:3294–3306.

3. Patte C, Auperin A, Michon J, et al. The Société Française d'Oncologie Pédiatrique LMB89 protocol: Highly effective multiagent chemotherapy tailored to the tumor burden and response in 561 unselected children with B-cell lymphomas and L3 leukemia. Blood 2001;97:3370–3379.

4. Patte C, Auperin A, Gerrard M, et al. Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-Hodgkin lymphoma in children and adolescents. It is possible to reduce treatment for the early responding patients. Blood 2007;109:2773–2780.

5. Cairo MS, Gerrard M, Sposto R, et al. Results of a randomized international study of high-risk central nervous system B non-Hodgkin lymphoma and B acute lymphoblastic leukemia in children and adolescents. Blood 2007;109:2736–2743.

6. Gerrard M, Cairo MS, Weston C, et al. Excellent survival following two courses of COPAD chemotherapy in children and adolescents with resected localized B-cell non-Hodgkin's lymphoma: Results of the FAB/LMB 96 international study. Br J Haematol 2008;141:840–847.

7. Shimizu H, Kikuchi M, Takaue Y, et al. Improved treatment results of non-Hodgkin's lymphoma in children: A report from the Children's Cancer and Leukemia Study Group of Japan. Int J Hematol 1995;61:85–96.

8. Horibe K, Akiyama Y, Kobayashi M, et al. Treatment outcome of AT-B88 regimen for B-cell non-Hodgkin's lymphoma and surface immunoglobulin-positive acute lymphoblastic leukemia in children. Int J Hematol 1997;66:89–98.

9. Tsurusawa M, Taga T, Horikoshi Y, et al. Favourable outcomes in children with diffuse large B-cell lymphoma treated by a short-term ALL-like regimen: A report on the NHL960 study from the Japanese Childhood Cancer and Leukemia Study Group. Leuk Lymphoma 2008;49:734–739.

10. Kikuchi A, Mori T, Fujimoto J, et al. Outcome of childhood B-cell non-Hodgkin lymphoma and B-cell acute lymphoblastic leukemia treated with the Tokyo Children's Cancer Study Group NHL B9604 protocol. Leuk Lymphoma 2008;49:757–762.

11. Lee SH, Yoo KH, Sung KW, et al. Should children with non-Hodgkin lymphoma be treated with different protocols according to histopathologic subtype? Pediatr Blood Cancer 2013;60:1842–1847.

12. Jaffe ES, Harris NL, Stein H, et al, editors. WHO classification of tumors, pathology and genetics of tumors of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001.

13. Murphy SB. Classification, staging and results of treatment of childhood NHL, dissimilarities from lymphoma in adults. Seminars in Oncology 1980;7:332–339.

14. Horibe K, Saito AM, Takimoto T, et al. Incidence and survival rates of hematological malignancies in Japanese children and adolescents (2006-2010): Based on registry data from the Japanese Society of Pediatric Hematology. Int J hematol 2013;98:74–88.

15. Reiter A, Klapper W. Recent advances in the understanding and management of diffuse large B-cell lymphoma in children. Br J Haematol 2008;142:329–347.

16. Klapper W, Szczepanowski M, Burkhardt B, et al. Molecular profiling of pediatric mature B-cell lymphoma treated in population-based prospective clinical trials. Blood 2008;112:1374–1381.

17. Sorensen K, Levitt GA, Bull C, et al. Late anthracycline cardiotoxicity after childhood cancer: A prospective longitudinal study. Cancer 2003;97:1991–1998.

18. Davies SM. Subsequent malignant neoplasms in survivors of childhood cancer: Childhood Cancer Survivor Study (CCSS) studies. Pediatric Blood Cancer 2007;48:727–730.

19. Umezawa H, Takahashi Y, Kinoshita M, et al. Tetrahydropyranyl derivatives of daunomycin and adriamycin. J Antibiot 1979;32:1082–1084.

20. Takagi T, Sakai C, Oguro M. Combination chemotherapy with pirarubicin (THP), cyclophosphamide, vincristine, and prednisolone (VEP-THP therapy) in the treatment of non-Hodgkins lymphoma. Oncology 1990;47:25–28.

21. Niitsu N, Umeda M. Biweekly THP-COPBLM (pirarubicin, cyclophosphamide, vincristine, prednisone, bleomycin and procarbazine) regimen combined with granulocyte colony-stimulating factor (G-CSF) for intermediate- and high-grade non-Hodgkins's lymphoma. Leukemia 1998;12:1457–1460.

22. Niitsu N, Umeda M. Response and adverse drug reactions to combination chemotherapy in elderly patients with aggressive non-Hodgkin's lymphoma: Comparison of CHOP, COP-BLAM, COP-BLAM III, and THP-COPBLM. Eur J Haematol 1999;63:337–344.

23. Tsurumi H, Yamada T, Sawada M, et al. Biweekly CHOP or THP-COP regimens in the treatment of newly diagnosed aggressive non-Hodgkin's lymphoma. A comparison of doxorubicin and pirarubicin: A randomized phase II study. J Cancer Res Clin Oncol 2004;130:107–113.

24. Zhai L, Guo C, Cao Y, et al. Long-term results of pirarubicin versus doxorubicin in combination chemotherapy for aggressive non-Hodgkin's lymphoma: Single center, 15-year experience. Int J Hematol 2010;91:78–86.

25. Tsurusawa M, Shimomura Y, Asami K, et al. Long-term results of the Japanese Childhood Cancer and Leukemia Study Group studies 811, 874 and 911 on childhood acute lymphoblastic leukemia. Leukemia 2010;24:335–344.

26. Yamaji K, Okamoto T, Yokota S, et al. Minimal residual disease-based augmented therapy in childhood acute lymphoblastic leukemia: A report from the Japanese Childhood Cancer and Leukemia Study Group Study. Pediatr Blood Cancer 2010;55:1287–1295.

27. Shimomura Y, Baba R, Watanabe A, et al. Japanese Childhood Cancer and Leukemia Study Group (JCCLSG). Assessment of late cardiotoxicity of pirarubicin (THP) in children with acute lymphoblastic leukemia. Pediatr Blood Cancer 2011;57:461–466.

28. Meinhardt A, Burkhardt B, Zimmermann M, et al. Phase II window study on rituximab in newly diagnosed pediatric mature B-cell non-Hodgkin's lymphoma and Burkitt leukemia. J Clin Oncol 2010;28:3115–3121.

29. Barth MJ, Goldman S, Smith L, et al. Rituximab pharmacokinetics in children and adolescents with de novo intermediate and advanced mature B-cell lymphoma/leukaemia: A Children's Oncology Group report. Br J Haematol 2013;162:678–683.

臨床研究

小児未分化大細胞型リンパ腫に対する
ランダム化国際臨床試験 ALCL99-R1 国内登録例の解析

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欧州と日本の共同研究による ALCL99-R1 は，小児未分化大細胞型リンパ腫（anaplastic large cell lympho-ma, ALCL）を対象とした最大規模の国際臨床試験である。試験治療の国内における有効性と安全性を示すことを目的として，国内登録例の集計を行い国際臨床試験の結果と比較した。国際臨床試験登録 352 例，国内登録 44 例の臨床的特徴は類似し，観察期間中央値，2 年無イベント生存率，生存率はそれぞれ，3.8 年，74％，93％，および 3.5 年，81％，96％であった。メトトレキサートの投与量・時間，および髄注の有無による無イベント生存率，化学療法コース毎の毒性発症割合を比較した無作為割付け試験の結果は，国内登録，国際臨床試験で同様であった。小児 ALCL に対する標準治療に位置づけられる ALCL99-R1 の，国内における有効性と安全性は国際臨床試験の結果と同様と考えられた。国際臨床試験は頻度の低い小児リンパ腫に対する治療開発に貢献する方法と考えられる。（臨床血液 55（5）：526～533, 2014）

緒　言

未分化大細胞型リンパ腫（anaplastic large cell lym-phoma, ALCL）は，小児非ホジキンリンパ腫（non-Hodgkin lymphoma, NHL）の 10～20％の頻度[1,2]，国内における発症頻度は年間 15～25 例程度の稀な疾患である。疾患概念が提唱された 1980 年代後半以降，欧米の研究グループによりさまざまな小規模臨床試験が行われ，いずれも 60～75％の無イベント生存率が報告された[3-8]。日本における同時期の後方視的集計も同様の転帰を報告している[9]。

欧 州 の 国 際 研 究 グ ル ー プ EICNHL（European Intergroup for Childhood Non-Hodgkin lymphoma）は，BFM（Berlin-Frankfurt-Münster）グ ル ー プ[3,4]，SFOP（Socié té　　Française　　d'Oncologie　　Pédiatrique）[5]，UKCCSG（United　Kingdom　Children's　Cancer　Study Group）[6]による小児 ALCL を対象とした臨床試験登録の後方視的合同解析を行い[10]，最も良好な成績が報告された NHL-BFM90 の化学療法[3,4]を治療骨格とし，縦隔病変，皮膚病変，臓器病変（肺病変・肝浸潤・脾浸潤）のいずれかを伴う例を高リスク群に設定した大規模国際臨床試験 ALCL99 を計画した。

ALCL99 は，(1) 6 コースの化学療法の各コースにおいて髄注とメトトレキサート（methotrexate, MTX）1 g/m²/24 時間投与（42 時間からロイコボリン救済）を行う MTX1 群と，同各コースで髄注を行わず MTX 3 g/m²/3 時間投与（24 時間からロイコボリン救済）を行う MTX3 群間の無イベント生存率の比較を目的とした無作為割り付け試験（ALCL99-R1）[11]，(2) 高リスク群において，6 コースの化学療法終了後に，総治療期間 1 年まで週に 1 回のビンブラスチン投与を継続する維持療法の有無による無イベント生存率の比較を目的とした第 2 の無作為割り付け試験（ALCL99-R2）[12]，およびすべての小児 ALCL 例を対象とした登録・観察研究により構成された。1999 年 11 月に欧州における登録が開始され，2002 年 6 月に日本小児白血病リンパ腫研究グループ（Japanese Pediatric Leukemia/Lymphoma Study Group,

JPLSG）における登録が開始された。

　日本および欧州の 12 か国の参加による ALCL99-R1 は 2005 年 12 月に，ALCL99-R2 は 2006 年 12 月に登録を終了し，観察期間を経て，研究成果は既に論文発表されている[11, 12]。ALCL99-R1 の成果[11] の概要を以下に記す。解析対象例（352 例）の観察期間中央値は 3.8 年，MTX1 群（175 例），MTX3 群（177 例）の 2 年無イベント生存率はそれぞれ 73.6％，74.5％，2 年生存率はそれぞれ 90.1％，94.9％で，いずれも両群間に有意な差を認めなかった。中枢神経再発は 2 例で，いずれも MTX1 群であった。両群計 2,050 コースの化学療法後の毒性発現比率が比較され，MTX1 群において，grade 4 の血液毒性，grade 3～4 の口内炎などの発現比率が有意に高値であった。

　本研究では，ALCL99-R1 試験治療の国内における有効性と安全性を示すことを目的として，国内登録例の集計を行い国際臨床試験の結果と比較した。国際臨床試験において検証された事象について，少数の国内登録例において同様の傾向を確認することにより，本試験治療を国内における標準治療に位置づけることが可能になると考えられる。

方　　法

　国際臨床試験 ALCL99-R1 は，22 歳未満の病理組織診断された ALCL 例を対象とし，孤立した皮膚病変のみの例，中枢神経浸潤を伴う例，完全切除された stage I 例などは除外された。試験参加施設の倫理審査委員会による研究計画の承認，および対象患者あるいは代諾者からインフォームドコンセントが得られている。治療開始前の身体所見，血液・尿・骨髄・脳脊髄液検査，画像検査などは既に報告された方法[11] により評価された。化学療法レジメンを Table 1 に示す。5 日間のプレフェーズの後に無作為割り付けが行われ，引き続きコース A，コース B が 21 日の間隔で交互に合計 6 コース行われ

Table 1　Chemotherapy drug, dose, and schedule in each course of ALCL99

Course and Drug	Dose and Schedule
Prephase	
Dexamethasone	5 mg/m² on days 1 and 2; 10 mg/m² on days 3 to 5
Cyclophosphamide	200 mg/m² on days 1 and 2
Triple intrathecal injection	Day 1
Course A	
Dexamethasone	10 mg/m² on days 1 to 5
Methotrexate	Random assignment* on day 1
Ifosphamide	800 mg/m² on days 1 to 5
Cytarabine	150 mg/m² ×2 on days 4 and 5
Etoposide	100 mg/m² on days 4 and 5
Course B	
Dexamethasone	10 mg/m² on days 1 to 5
Methotrexate	Random assignment* on day 1
Cyclophosphamide	200 mg/m² on days 1 to 5
Doxorubicin	25 mg/m² on days 4 and 5

All patients received a 5-days prephase followed by six alternating courses （A and B） administered every 21 days.
* Arm MTX1 included methotrexate 1 g/m² in 24-hour infusion with triple intrathecal injection at day 1 and leucovorin rescue （15 mg/m²） at 42, 48, and 54 hours. Arm MTX3 included methotrexate 3 g/m² in 3-hour infusion with no intrathecal injection and leucovorin rescue （15 mg/m² every 6 hours） starting at 24 hours and ending when the methotrexate level was ＜0.15 μm/l.
Additionally, high-risk patients could enter the second randomized trial before the first course B, which randomly assigned patients to receive or not receive a vinblastine injection （6 mg/m²） during the five latter courses and then weekly for a total duration of treatment of 1 year.

る。高リスク群においてはビンブラスチン投与を継続する維持療法の有無による無作為割り付け試験 ALCL99-R2 が併行された。治療効果，および毒性の判定は各化学療法コースの後に既に報告された方法[11]により行われた。毒性の判定は，小児を対象に修正された National Cancer Institute Common Toxicity Criteria, Version 2.0 により，grade 4 の血液毒性，および grade 3 または 4 の非血液毒性を重篤な毒性（severe toxicity）と分類した。無作為割り付け，およびデータ収集・管理は，フランス，ドイツ，イタリア，スウェーデン，日本に設置された地域データセンターで行われ，フランスに設置された中央データセンターで集計・解析された。プライマリーエンドポイントである無イベント生存率，および生存率，急性毒性発現比率の比較の算定は，既に報告された統計方法[11]により行われた。

日本の地域データセンター（国立病院機構名古屋医療センター内 JPLSG データセンター）により，国際臨床試験登録例として収集・管理され，中央データセンターに提出された国内登録例のデータを集計し，Brugières らにより報告された国際臨床試験の結果[11]と比較した。SPSS11.0 を用いて，生存率算定，生存曲線作成は Kaplan-Meier 法，生存曲線の比較は log-rank 法，登録例の治療開始前の評価の比較，および治療群間の毒性の発症比率の比較は χ^2 検定により行った。

結　果

Fig. 1 に，登録例の適格性，無作為割り付け結果を，国際臨床試験，国内登録例別に示す。48 例の ALCL99 国内登録例のうち，44 例が ALCL99-R1 無作為割り付け対象とされた（国際臨床試験では 487 例中 375 例）。対象外の 4 例中，2 例は孤立した皮膚病変のみの例，1 例は完全切除された stage I 例，1 例は登録の不備であった。無作為割り付け後に，病理組織診断の国際レビューにより ALCL 以外と診断され，解析対象外に分類された国内登録例はなかった（国際臨床試験では 375 例中 23 例が解析対象外に分類）。

Table 2 に，無作為割り付け対象例の治療開始前の評価結果を，国際臨床試験，国内登録例別に示す。国内登録例における男性の割合は 48%，診断時年齢の中央値は 10.3 歳（9 か月～16 歳 11 か月），St. Jude 病期分類による進行病期（stage III, IV）の割合は 82% で，国際臨床試験においては，それぞれ 59%，11.2 歳，74% であった。国内登録例における高リスク因子である縦隔病変，皮膚病変，臓器病変を伴う割合は，32%，23%，27% で，国際臨床試験においては，それぞれ 47%，19%，46% であった。

Table 3 に，無作為割り付け対象例の転帰を，国際臨床試験，国内登録例別に示す。国内登録例における観察期間の中央値は 42 か月（国際臨床試験 44 か月），イベント 11 件はすべて再発で，2 年無イベント生存率は 79.3%（国際臨床試験 74.1%），2 年生存率は 95.2%（国際臨床試験 92.5%）であった。国内登録例の 5 年無イベント生存率は 70.7%，5 年生存率は 92.0% であった。Fig. 2 に国内登録例の生存曲線を示す。国内登録例における MTX1 群，MTX3 群間で，2 年無イベント生存率，2 年生存率の差は明らかでなかった。
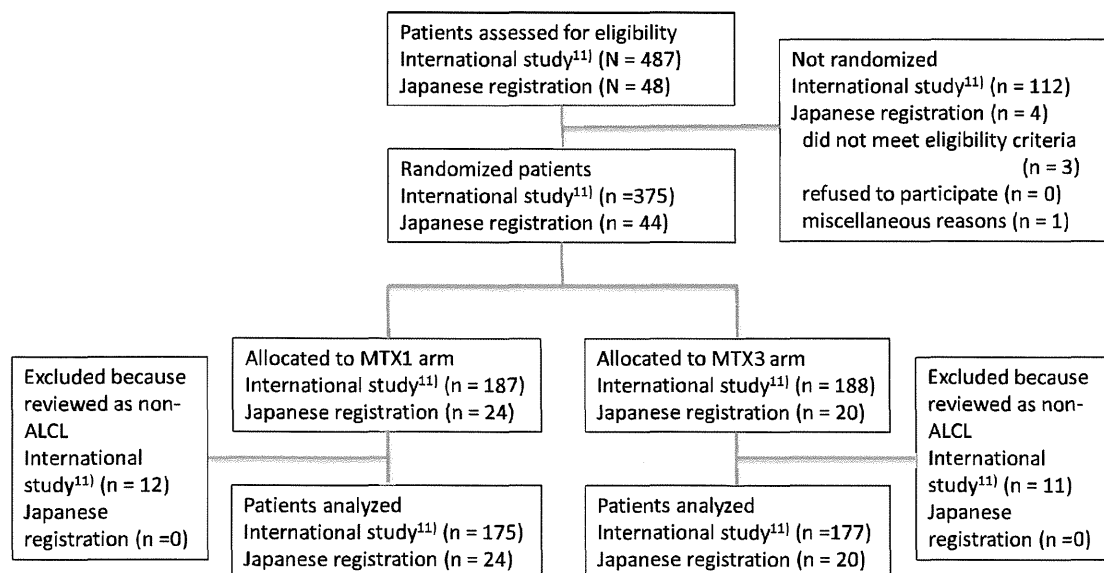

Fig. 1　Participant flow in ALCL99-R1 study. ALCL, anaplastic large cell lymphoma.

Table 2　Comparison of patient characteristics with Japanese registration

Total number of patients	International Study[11] 352	Japanese registration 44	P
Characteristics	No.　(%)	No.　(%)	
Male sex	221　(59%)	21　(48%)	0.053
Median age at diagnosis	11.2	10.3	—
"B" symptom	197　(56%)	27　(61%)	0.464
Site of disease			
Peripheral lymph node	308　(88%)	32　(73%)	0.008
Mediastinal involvement	167　(47%)	14　(32%)	0.050
Visceral involvement	162　(46%)	12　(27%)	0.018
Lung lesion	75　(21%)	5　(11%)	0.121
Liver involvement	49　(14%)	2　(5%)	0.080
Spleen involvement	64　(18%)	2　(5%)	0.022
Skin lesion	68　(19%)	10　(23%)	0.592
Soft tissue mass	55　(16%)	10　(23%)	0.230
Bone lesion	58/309　(19%)	12　(27%)	0.186
Bone marrow involvement	42　(12%)	4　(9%)	0.579
St. Jude stage III, IV	262　(74%)	36　(82%)	0.284

Table 3　Comparison of outcome and event with Japanese registration

Total number of patients	International Study[11] 352		Japanese registration 44	
Median follow-up time	44 months		42 months	
2-year EFS	74.1%		79.3%	
2-year OS	92.5%		95.2%	
	MTX1 arm n = 175	MTX3 arm n = 177	MTX1 arm n = 24	MTX3 arm n = 20
Complete remission	155 (89%)	154 (87%)	23　(96%)	17　(85%)
2-year EFS	73.6%	74.5%	74.5% P =	85.0% 0.6534
2-year OS	90.1%	94.9%	100% P =	90.0% 0.0735
Events	51	51	6	5
Progression	8	6	0	0
Toxic death as first event	1	3	0	0
Relapse	42	42	6	5
CNS relapse	2	0	0	0
Deaths	19	13	0	3

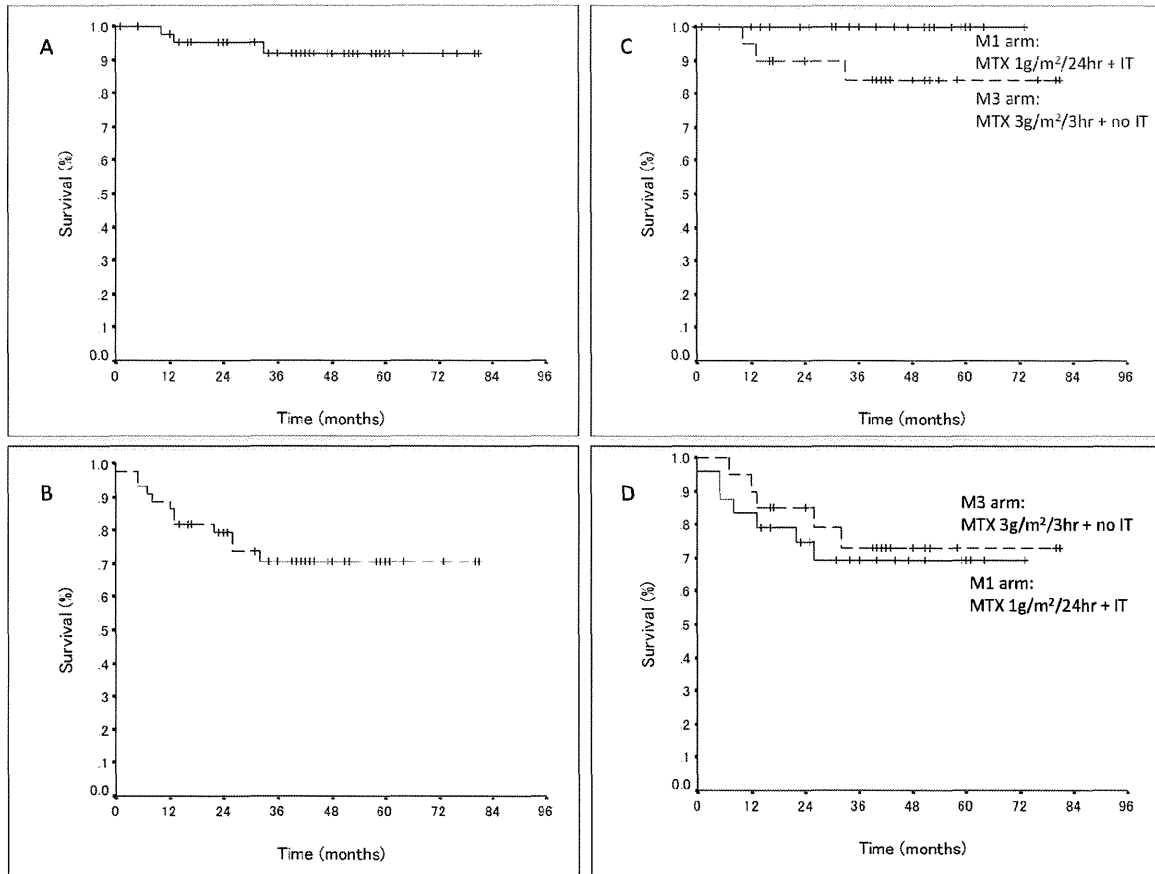OS, overall survival; EFS, event-free survival

Fig. 2　(A)　Overall survival, and　(B)　event-free survival of the patients registered in Japan.
　　　(C)　OS, and　(D)　EFS by treatment arm. MTX, methotrexate. IT, intrathecal injection.

Table 4 に，無作為割り付け対象例の試験群（MTX1，MTX3）間の化学療法コース後の毒性発症比率の比較を，国際臨床試験，国内登録例別に示す。国際臨床試験，国内登録例の両試験群それぞれ 1,025 コース，129 コースにおいて評価が行われた。国際臨床試験において，MTX1 群で毒性発症比率が統計学的に高値（P＜.05）と判定された，すべての grade のすべての毒性，重篤な毒性，grade 4 の血液毒性，すべての grade の感染症，grade 3～4 の口内炎は，国内登録例においても，MTX1 群で毒性発症比率が統計学的に高値と判定された。Grade 3～4 のその他の毒性の発症比率は，国際臨床試験において MTX1 群で統計学的に高値と判定されたが，国内登録例において有意差は認めなかった。Grade 3～4 の感染症，grade 3～4 の肝障害，grade 3～4 のその他のさまざまな毒性の発症比率は，国際臨床試験，国内登録例のいずれにおいても統計学的に差は認めなかった。

考　　察

ALCL99-R1 は，これまでに報告された小児 ALCL を対象とした最大規模の臨床試験であり，小児 ALCL に対する標準治療を提示する成果を示した。稀少な疾患である小児 ALCL に対する大規模臨床試験の成功には，EICNHL に参加する欧州各国，および日本による共同研究体制の構築と維持が寄与した。国内登録例の無作為割り付け率は 92％で，病理組織診断の国際レビューによる無作為割付け後除外症例を生じなかった。国際臨床試験において，無作為割り付け例 375 例中，27 例にプロトコール中止，変更が報告されているが，このうち国内登録例は 3 例であり，2 例は治療毒性による治療変更であった。国際臨床試験に参加した国内登録例は，欧州各国からの登録例と同等に試験プロトコールの遵守を果たしていると考えられた。

筆者らは，ALCL99 開始以前の国内例の後方視的集計により，小児 ALCL 例の病理組織像，臨床的特徴，転帰は，海外の報告と同様の傾向であることを報告した[9]。今回の ALCL99-R1 国内登録例の集計と国際臨床試験報告の比較により，国内小児 ALCL 例の性別，診断時年齢，治療開始前の病変，病期のみならず，同一の治療を