Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukaemia. *Science*, **306**, 269–271.

Winter, S.S., Jiang, Z., Khawaja, H.M., Griffin, T., Devidas, M., Asselin, B.L. & Larson, R.S. (2007) Identification of genomic classifiers that distinguish induction failure in T-lineage acute lymphoblastic leukaemia: a report from the Children's Oncology Group. *Blood*, **110**, 1429–1438.

Zhu, Y.M., Zhao, W.L., Fu, J.F., Shi, J.Y., Pan, Q., Hu, J., Gao, X.D., Chen, B., Li, J.M., Xiong, S.M., Gu, L.J., Tang, J.Y., Liang, H., Jiang, H., Xue, Y.Q., Shen, Z.X., Chen, Z. & Chen, S.J. (2006) NOTCH1 mutations in T cell acute lymphoblastic leukaemia: prognostic significance and implication in multifactorial leukemogenesis. *Clinical Cancer Research*, **12**, 3043–3049.

Supporting information

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

Fig S1. Treatment plan for JACLS ALL T-97 protocol.

Fig S2. Newly identified *FBW7* mutations in T-ALL patients.

Fig S3. Kaplan–Meier estimate of (A) event-free survival and (B) overall survival of T-ALL patients with or without *NOTCH1* mutation.

Fig S4. Kaplan–Meier estimate of (A) event-free survival and (B) overall survival of T-ALL patients with or without *FBW7* mutation.

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Retrospective Analysis of Relapsed or Primary Refractory Childhood Lymphoblastic Lymphoma in Japan

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Background and Procedure. To assess the clinical course with response to second-line treatment and to evaluate the role of hematopoietic stem cell transplantation (SCT) in children with relapsed or primary refractory lymphoblastic lymphoma (LBL), we analyzed data of 48 patients with relapsed/primary refractory diseases among 260 LBL patients identified in a national survey of 1996–2004. ***Results.*** Twenty-six patients achieved second complete remission; 9 achieved partial remission. Of 13 patients who showed progression despite first and second line therapy, only one patient was alive on the second relapse after unrelated cord blood transplantation. Among 40 relapsed patients, the median time between initial diagnosis and relapse was 12.5 months (range 3–56 months). The sites of relapse were isolated BM (n = 9), primary local site with BM (9), primary local site (6), isolated CNS (4), local site with mediastinum (4), primary local site with other site (4), and others (4). Of all 48 patients, 3 were alive after chemotherapy alone. Of the 33 patients, 14 were alive after high dose chemotherapy (HDC)/SCT. With a 27.5-month median follow up period, the 3-year OS rate was 43.2 ± 7.4% (estimate ± SE). Univariate analysis identified two features (relapse within 12 months, T cell phenotype) as significant variables that predicted poor survival. Multivariate analysis showed novel statistically significant variables including relapse within 12 months from initial diagnosis (Hazard ratio 3.60) and absence of HDC/SCT (2.64). ***Conclusion.*** Outcomes of patients with relapsed/primary refractory LBL were poor, but HDC/SCT for these patients was associated with good results. Pediatr Blood Cancer 2009;52:591–595. © 2009 Wiley-Liss, Inc.

Key words: children; lymphoblastic lymphoma; recurrence; refractory

INTRODUCTION

Malignant lymphoma is the fourth most frequent of all Japanese childhood cancers. It represents 5% of all new cases. Lymphoblastic lymphoma (LBL) is a major histology of childhood NHL, accounting for about 30%. Excellent outcomes for children with LBL have been reported with protocols closely modeled on therapy designed for acute lymphoblastic leukemia (ALL) [1]. However, 20–40% of patients develop relapse or primary refractory disease. They have poor prognoses [2,3]. The clinical courses and outcomes of these relapsed or primary refractory LBL of children have not been well documented [2,4].

To determine the response to second-line treatment and the outcomes of children with a relapsed or primary refractory LBL and to evaluate the role of high dose chemotherapy and stem cell transplantation (HDC/SCT) in these patients, we performed a retrospective nationwide analysis of LBL patients in Japan.

PATIENTS AND METHODS

Among 260 patients with LBL registered in a national survey during 1996–2004, 48 patients (18.5%) from 39 institutions with primary refractory or relapsed diseases were found, including 8 primary refractory diseases and 40 relapses. Their medical records were reviewed. Relapse was defined as appearance of new lesions, re-growth of original masses and obvious enlargement of the mediastinal mass as revealed by imaging study with pathological examination in principle, and appearance of tumor cells in bone marrow and cerebrospinal fluid. Among 40 relapsed patients, 25 were confirmed relapse by histological/cytological examinations, 9 were defined with only clinical courses and imaging studies, and the rest of 6 were unknown about precise information. Among five patients recurred with mediastinal masses, four were confirmed by histological/cytological study, and one was determined by only imaging studies. Clinical data including treatment and follow-up information were gathered from a review of relapsed or primary refractory patient charts through the Japanese Pediatric Leukemia Lymphoma Study Group (JPLSG). The JPLSG comprises four children's hematology/oncology study groups: Japan Association of Childhood Leukemia Study, Tokyo Children's Cancer Study Group, Japan Children's Cancer and Leukemia Study Group, and Kyushu-Yamaguchi Children's Cancer Study Group. First line treatments differed among groups. The most frequently used treatment regimens were based on the framework of the LSA2-L2 protocol or the BFM group strategy [5,6]. After 4–6 weeks of ALL-therapy-like induction, some courses of consolidation and intensification were done for first line therapy followed by maintenance consisting of multi-agent block therapy or oral 6-MP with weekly MTX. Actual drugs and dose during consolidation, intensification and maintenance varied among groups. Total durations of therapy were of two types: 18 and 24 months.

Second line treatment also varied. Among 41 patients for whom descriptions of second line chemotherapy regimen were available, 11 received their own first line protocol similar to high risk ALL

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

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induction, 12 received therapy of other high risk ALL induction, 5 received AML type therapy, 3 underwent an ifosfamide, carboplatin, etoposide (ICE) [7] regimen, 6 received myeloablative stem cell transplantation as a re-induction therapy, and 4 received other therapies. Because of the lack of uniformity in the therapeutic regimens for refractory or recurrent cases, we mainly examined these patients' characteristics with the prognostic significance of the variables on overall survival.

Using Kaplan–Meier estimates, curves were calculated for the probability of overall survival together with standard error (SE). Univariate analyses of the association of various clinical factors were done with overall survival. The curves were compared using a double-sided log rank test. $P < 0.05$ at both sides was considered significant. The overall survival (OS) rate was calculated from the time of initial diagnosis to death. Progression free survival (PFS) was calculated from the time of relapse or refractory phase to disease progression. Multivariate analyses were performed using the Cox proportional-hazard model. Variables with P-values ≤ 0.1 in prior univariate testing were included.

RESULTS

Table I portrays representative characteristics of primary refractory or relapsed patients. Male patients were 66.7%, which is similar to the 70% males among all NHL patients. Of the patients, 81% showed greater than clinical stage III at initial diagnosis. Among 48 patients, 2 eventually revealed an NK type immunophenotype after initiation of first line LBL type therapy. Both achieved complete remission (CR) with first line therapy, but recurred. One was refractory to second line therapy: the patient received unrelated cord blood transplantation (UCBSCT) and died of graft failure. Another patient achieved partial remission (PR) with second line therapy, received allogeneic bone marrow transplantation (BMT), and entered into continuous CR.

Sites of relapse were the primary local site (12.5%), and the primary site with another site (35.4%) (Table II). Of 40 relapsed patients, 33 exhibited recurrence during first line chemotherapy and 7 after it (3–56 months after diagnosis, median 12.5 months). The patients' clinical courses and outcomes are shown in Figures 1 and 2. Of all primary refractory/relapsed patients, 26 patients achieved CR; and 9 patients achieved PR after second line chemotherapy. Among 13 patients who progressed in spite of first and second line chemotherapy, 1 patient was alive at the analysis on second relapse after UCBSCT, 8 patients died of therapy related toxicity, and 4 died of disease progression. Among the eight primary refractory patients, only one patient who had CNS local disease was alive after chemotherapy with radiation without HDC/SCT. HDC/SCT was done for five patients. Two patients were alive: one survived for 50 months after auto BMT for local mediastinal disease; the other was PR for 5 months after UCBSCT. Among 40 relapsed patients, 2 were alive under chemotherapy alone and gained CR after second line chemotherapy, 1 was alive for 55 months after BM relapse, and 1 was alive for 57 months with radiation after CNS local disease. Among 28 patients who had HDC/SCT after relapse, 12 patients were alive: 7 had had advanced disease and 5 had had local disease.

TABLE I. Patient Characteristics Initial Diagnosis (n = 48)

Age at diagnosis (years), median (range)	9 (1–15)
Male sex (%)	32 (66.7%)
Stage (Murphy's classification)	
I	2
II	7
III	26
IV	13
Histological Immunophenotype	
Precursor B	9
Precursor T	32
Others (not determined 4, NK 2,T, B mix 1)	7

TABLE II. Site of Relapse

Primary site only	6
BM	9
CNS	4
BM and CNS	1
Mediastinum	1
Others	2
Primary site + α*	17

BM denotes bone marrow; CNS, central nervous system; *+ α BM, 9; Mediastinum, 4; CNS, 1; Others, 3.

With a median follow-up period of 27.5 months, the 3-year OS rate was 43.2 ± 7.4% (estimate ± SE) (Fig. 3). Univariate analysis identified two features that were significant (Table III) as variables that were predictive of OS: relapse within 12 months and T cell phenotype. The presence of HDC/SCT was not significant. Regarding the total duration of first line therapy, we found no significant difference between 18 months and 24 months ($P = 0.90$). The 3-year progression free survival rate was 37.0 ± 7.3%. Univariate analysis for PFS with the same variables for OS showed a significant difference only in the presence of HDC/SCT (3-year PFS 36.9 ± 9.1% vs. 21.4 ± 11.0, $P = 0.03$).

The OS rates for 25 patients who underwent HDC/SCT during CR or PR, and for 8 patients who received chemotherapy without HDC/SCT after achieving CR or PR were 61.5 ± 10.3% and 37.5 ± 17.1%, respectively; they were not significantly different ($P = 0.06$). Regarding patients who underwent HDC/SCT during CR or PR, 6 among 19 patients who underwent allogeneic SCT had relapsed; 4 among 6 patients who had undergone autologous SCT had relapsed. Of those 19 allogeneic SCT recipients, 10 survived without further progression (median 22 months after transplantation), although only 2 of 6 autologous recipients survived (median 40 months). Regarding transplantation-related toxicity, three allogeneic recipients died of toxicity, although none had died with autologous transplantation. Among all transplanted patients, the median times to transplantation from the refractory/relapse phase were 5 months for allogeneic (n = 26) and 4 for autologous (n = 7); BM involvement appeared respectively in 10 cases and 1. The OS rates between these were, respectively 54.0 ± 10.4% and 28.6 ± 17.1%. No significant difference was observed ($P = 0.42$) although a higher OS rate was observed in the allogeneic group. The donor type, whether related or unrelated, also showed no significant difference ($P = 0.86$) among allogeneic transplantation cases. Regarding the transplant preparative regimen, except for one patient who could not undergo the myeloablative regimen, all preparative regimens were myeloablative. Additive chemotherapy varied among patients, for example (ara-C, ara-C + VP-16, VP16 + CY, ara-C + VP-16 + CY, CY + TT, BUS + L-PAM, L-PAM + FDA); no significant difference was found between TBI (n = 22) and non-TBI regimens (9) ($P = 0.73$).

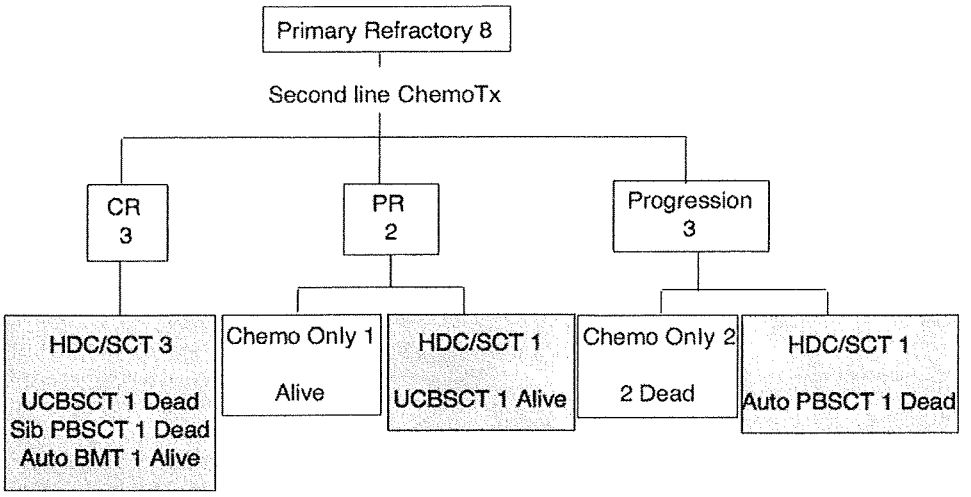

Fig. 1. Clinical courses and final outcomes of primary refractory patients. Tx denotes therapy; CR, complete remission; PR, partial remission; HDC/SCT, high dose chemotherapy and stem cell transplantation; BMT, bone marrow transplantation; Sib, sibling; PBSCT, peripheral blood stem cell transplantation; UCBSCT, unrelated cord blood transplantation.

Based on results of the univariate study (Table III), multivariate analysis was done with an adjustment for relapse within 12 months (vs. beyond), absence of HDC/SCT (vs. presence), Stage III/IV (vs. I/II), cell phenotype (T cell vs. B), and age at diagnosis (10 years and older vs. younger than 10). Histological immunophenotype lost its significance ($P = 0.25$) as a prognostic factor, while relapse within 12 months and absence of HDC/SCT were found to be statistically significant (Table III).

DISCUSSION

Although the prognosis of patients with LBL has been greatly improved, relapsed or primary refractory disease remains difficult to cure. According to the Children's Cancer Group's 5912 study, the survival rate was 33% at 2 years after relapse for 68 patients with non-Hodgkin lymphoma, including 26 LBL [3]. Nationwide studies performed in Austria (one patient survived among four relapsed/progressed LBL) and Germany (4 patients survived among 29 progressive T-LBL) also show poor prognosis of refractory/relapsed LBL [4,8]. In our present study, 3-year OS 43.2% was not satisfactory. Therapy for these patients remains poorly defined. Results of some studies suggest some roles of HDC/SCT for these patients [4,8,9]. Results of the present study show a significant hazard ratio for OS in absence of HDC/SCT. Although some selection bias might affect this retrospective study, it is interesting that it identified the possibility of a benefit of HDC/SCT for relapsed LBL. For acute lymphoblastic leukemia, which shares some characteristics with LBL, HDC/SCT reportedly results in longer

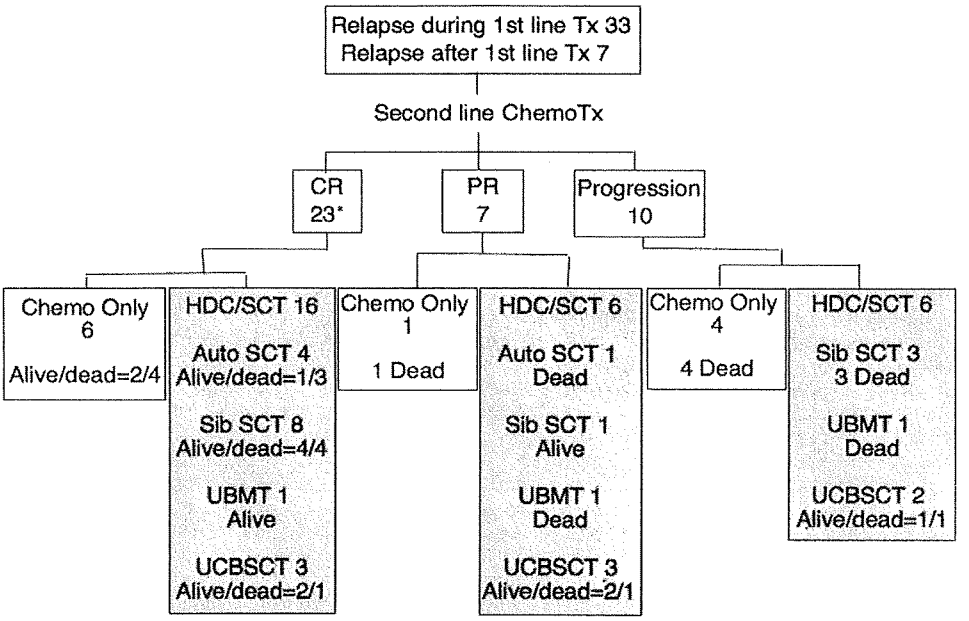

Fig. 2. Clinical courses and final outcomes of recurrent patients. *This includes a patient with unknown subsequent therapy.

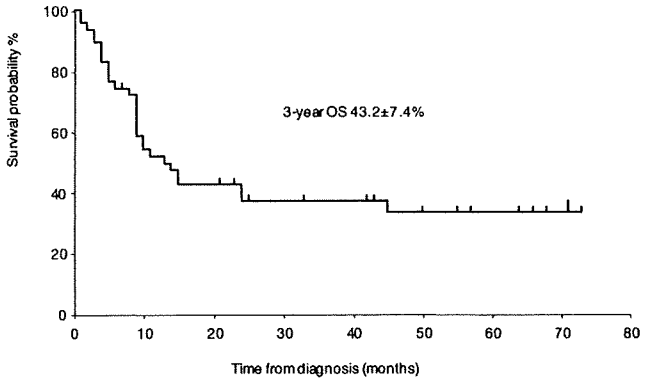

Fig. 3. Kaplan–Meier estimate of overall survival. The median follow-up period was 27.5 months.

leukemia-free survival when compared to outcomes of chemotherapy, especially for patients with poor prognoses [10,11]. However, a CCG1941 prospective study comparing chemotherapy versus HDC/SCT showed no significant advantage for HDC/SCT against chemotherapy for patients relapsing less than 12 months after completion of primary therapy [12]. The UK R1 study also showed that the related allograft was not significantly better than chemotherapy, although there was a moderate EFS benefit, especially in patients with a short first remission [13]. In our study, among 20 patients who relapsed within 12 months from initial diagnosis, 15 received HDC/SCT and 6 got CCR. The remaining 5 patients, all of whom had received chemotherapy, did not survive. Nevertheless, no significance for OS was found between these groups of patients ($P = 0.052$).

For relapsed LBL patients, it is unknown which of allogeneic or autologous is the better donor source. Two recent reports described that allogeneic transplants engendered fewer recurrences but had higher related mortality in LBL patients [14,15]. Burkhardt reported that HDC followed by allogeneic SCT might have beneficial effects in refractory/relapsed cases of T-lymphoblastic lymphoma [8]. Although our study showed no significant difference of survival rates between allogeneic and autologous groups among all transplanted-patients, allogeneic SCT resulted in fewer relapses and progressive diseases than autologous SCT (8 patients among 26 showed relapse or progression after allogeneic transplantation, while 4 among 7 showed relapse or progression after autologous transplantation). Recent progress in supportive therapy during the SCT phase and adaptation to reduced intensity stem cell transplants might reduce transplantation-related mortality and lead to better outcomes [16].

Among the eight patients with primary refractory disease, only three achieved CR under ICE regimen or AML type therapy. For these patients, HDC/SCT was not a suitable salvage therapy. Six patients received allogeneic HDC/SCT as salvage therapy. Among them, only one patient who was on second relapse at the time of this analysis remained alive; all other patients died of recurrence or regimen-related toxicity. Three patients who showed progression after both first and second line chemotherapy died within 6 months from the initial diagnosis. Among 10 patients who showed progression of the disease in spite of second line therapy after relapse, only one patient was alive after UCBSCT. These

TABLE III. Analysis of Variable Factors Against Overall Survival

Factor	3-year OS	*P* for OS*	Hazard ratio	Confidence interval	*P* for OS**
Time of relapse, months					
≤12 (20)	31.9 ± 10.7	0.03	3.60	1.41–9.22	0.007
>12 (20)	51.6 ± 11.7				
Immunophenotype					
T cell (32)	32.8 ± 8.6	0.03	2.45	0.52–11.47	0.25
B cell (9)	72.9 ± 16.5				
Stage					
III/IV (39)	32.1 ± 7.9	0.07	1.55	0.41–5.86	0.51
I/II (9)	88.9 ± 10.5				
Age at diagnosis, years					
≥10 (22)	34.0 ± 10.5	0.09	2.08	0.88–4.92	0.10
<10 (26)	50.9 ± 10.2				
HDC/SCT					
Absence (14)	28.6 ± 12.1	0.10	2.64	1.07–6.52	0.035
Presence (33)	47.2 ± 9.3				
BM+					
Absence (29)	49.5 ± 9.6	0.42	NA	NA	NA
Presence (19)	34.0 ± 11.2				
CNS+					
Absence (40)	44.8 ± 8.2	0.40	NA	NA	NA
Presence (8)	37.5 ± 17.1				
Sex					
Male (32)	39.2 ± 9.3	0.58	NA	NA	NA
Female (16)	50.0 ± 12.5				

BM+, BM involvement at refractory/relapsed phase; CNS+, CNS involvement at refractory/relapsed phase; NA, not applicable; Number of patients are enclosed in parentheses. *P value is calculated using a double-sided log rank test; **Hazard ratio and P value is calculated using a Cox proportional-hazard model.

expressly refractory patients require novel therapeutic agents and strategies.

Predictive factors of poor survival are important when selecting patients for a new experimental therapy. Our data show that the time to relapse has prognostic importance. A BFM group study of relapsed T-cell LBL (29 patients) showed that age, gender, and localization of relapse had no prognostic value, which were same findings of our study. In contrast, they reported that the time of relapse was not associated with the outcome [8].

The outcomes of patients with relapsed/ primary refractory LBL were not satisfactory. However, those who responded to second line chemotherapy showed a respectable chance of survival. For these patients, HDC/SCT was associated with good prognosis. The relative rarity of refractory LBL patients highlights the need for carefully designed clinical trials through multicenter/international collaboration to answer issues of efficient second line chemotherapy, prognostic factors for these refractory patients, and SCT efficacy.

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REFERENCES

1. Reiter A, Schrappe M, Ludwig W-D, et al. Intensive ALL-type therapy without local radiotherapy provides a 90% event-free survival for children with T-cell lymphoblastic lymphoma: BFM Group report. Blood 2000;15:416–421.
2. Sandlund JT, Downing JR, Crist WM. Non-Hodgkin's lymphoma in Childhood. N Engl J Med 1996;334:1238–1248.
3. Kobrinsky NL, Sposto R, Shah NR, et al. Outcomes of treatment of children and adolescents with recurrent non-Hodgkin's lymphoma and Hodgkin's disease with dexamethasone, etoposide, cisplatin, cytrabin, and l-asparaginase, maintenance chemotherapy, and transplantation: Children's Cancer Group study CCG-5912. J Clin Oncol 2001;19:2390–2396.
4. Attarbaschi A, Dworzak M, Steiner M, et al. Outcome of children with primary resistant or relapsed non-Hodgkin lymphoma and mature B-cell leukemia after intensive first line treatment: A population-based analysis of the Austrian Cooperative Study Group. Pediat Blood Cancer 2005;44:70–76.
5. Wollner N, Burchenal J, Lieberman P, et al. Non-Hodgkin's lymphoma in children. A comparative study of two modalities of therapy. Cancer 1976;37:123–134.
6. Reiter A, Schrappe M, Yakisan E, et al. NHL-BFM 90 therapy study in treatment of malignant non-Hodgkin's lymphomas in children and adolescents. Part 2. An intermediate term analysis of the non-B-NHL therapy group. BFM Study Group. Klin Padiatr 1994;206:234–241.
7. Fields KK, Zorsky PE, Hiemenz JW, et al. Ifosfamide, carboplatin, and etoposide: A new regimen with a broad spectrum of activity. J Clin Oncol 1994;12:544–552.
8. Burkhardt B, Reiter A, Lang P, et al. Relapse in pediatric patients with T-cell lymphoblastic lymphoma: Clinical characteristics and outcome in the BFM group. Pediat Blood Cancer 2006;46:842.
9. Sandlund I, Bowman I, Heslop H, et al. Intensive chemotherapy with hematopoietic stem-cell support for children with recurrent or refractory NHL. Cytotherapy 2002;4:253–258.
10. Dopfer R, Henze G, Bender-Gotze C, et al. Allogeneic bone marrow transplantation for childhood acute lymphoblastic leukemia in second remission after intensive primary and relapse therapy according to the BFM- and CoALL-protocols: Results of the German cooperative study. Blood 1991;78:2780–2784.
11. Borgmann A, Stackelberg Av, Hartmann R, et al. Unrelated donor stem cell transplantation compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission: A matched-pair analysis. Blood 2003;101:3835–3839.
12. Gaynon PS, Harris RE, Altman AJ, et al. Bone marrow transplantation versus prolonged intensive chemotherapy for children with acute lymphoblastic leukemia and an initial bone marrow relapse within 12 months of the completion of primary therapy: Children's Oncology Group Study CCG-1941. J Clin Oncol 2006; 24:3150–3156.
13. Harrison G, Richard S, Lawson S, et al. Comparison of allogeneic transplant versus chemotherapy for relapsed childhood acute lymphoblastic leukaemia in the MRC UKALL R1 trial. Ann Oncol 2000;11:999–1006.
14. Peniket AJ, Elvira MRd, Taghipour G, et al. An EBMT registry matched study of allogeneic stem cell transplants for lymphoma: Allogeneic transplantation is associated with a lower relapse rate but with a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transpl 2003;31:667–678.
15. Levine JE, Harris RE, Loberiza FR, Jr., et al. A comparison of allogeneic and autologous bone marrow transplantation for lymphoblastic lymphoma. Blood 2003;101:2476–2482.
16. Corradini P, Dodero A, Farina L, et al. Allogeneic stem cell transplantation following reduced-intensity conditioning can induce durable clinical and molecular remissions in relapsed lymphomas: Pre-transplant disease status and histotype heavily influence outcome. Leukemia 2007;21:2316–2323.

Leukemia (2010) 24, 383–396

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EDUCATIONAL REPORT

Long-term results of Tokyo Children's Cancer Study Group trials for childhood acute lymphoblastic leukemia, 1984–1999

M Tsuchida[1], A Ohara[2], A Manabe[3], M Kumagai[4], H Shimada[5], A Kikuchi[6], T Mori[4], M Saito[7], M Akiyama[8], T Fukushima[9], K Koike[1], M Shiobara[10], C Ogawa[3], T Kanazawa[11], Y Noguchi[12], S Oota[13], Y Okimoto[14], H Yabe[15], M Kajiwara[16], D Tomizawa[16], K Ko[17], K Sugita[18], T Kaneko[19], M Maeda[20], T Inukai[21], H Goto[22], H Takahashi[23], K Isoyama[24], Y Hayashi[25], R Hosoya[3] and R Hanada[17] on behalf of Tokyo Children's Cancer Study Group, Tokyo, Japan

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We report the long-term results of Tokyo Children's Cancer Study Group's studies L84-11, L89-12, L92-13, and L95-14 for 1846 children with acute lymphoblastic leukemia, which were conducted between 1984 and 1999. The value of event-free survival (EFS) ± s.e. was 67.2 ± 2.2% at 10 years in L84-11, which was not improved in the following two studies, and eventually improved to 75.0 ± 1.8% at 10 years in L95-14 study. The lower EFS of the L89-12 reflected a high rate of induction failure because of infection and delayed remission in very high-risk patients. The L92-13 study was characterized by short maintenance therapy; it resulted in poor EFS, particularly in the standard-risk (SR) group and boys. Females did significantly better than males in EFS in the early three studies. The gender difference was not significant in overall survival, partly because >60% of the males survived after the testicular relapse. Randomized studies in the former three protocols revealed that intermediate- or high-dose methotrexate therapy significantly reduced the testicular relapse rate. In the L95-14 study, gender difference disappeared in EFS. Contrary to the results of larger-scale studies, the randomized control study in the L95-14 reconfirmed with updated data that dexamethasone 8 mg/m^2 had no advantage over prednisolone 60 mg/m^2 in the SR and intermediate-risk groups. Prophylactic cranial irradiation was assigned to 100, 80, 44, and 44% of the patients in the studies, respectively. Isolated central nervous system relapse rates decreased to <2% in the last two trials. Secondary brain tumors developed in 12 patients at 8–22 years after cranial irradiation. Improvement of the remission induction rates and the complete omission of irradiation are currently main objectives in our studies.
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Keywords: acute lymphoblastic leukemia; children; long-term results; cranial irradiation; secondary malignancy

Introduction

We present here the long-term results of four studies for childhood acute lymphoblastic leukemia (ALL) of Tokyo Children's Cancer Study Group (TCCSG) conducted between 1984 and 1999.

Treatment protocol for SR and IR of the L84-11 study[1,2] was based on the early St Jude's total therapy.[3] ALL-BFM 81[4] protocol was modified and introduced to extremely high-risk group regimen for the first time. The protocols of the following three studies L89-12,[1,5] L92-13,[1,6] and L95-14,[7] were designed on the basis of the ALL-BFM framework. All the four protocols contained trials to reduce the number of patients who received irradiation, as had been reported in other studies.[8,9] The second point of analysis was on a gender difference[10–12] with respect to long-term event-free survival (EFS) and overall survival (OS). Randomized studies were mostly designed to test whether or not intermediate-dose methotrexate (ID-MTX) and high-dose methotrexate (HD-MTX) could replace the cranial irradiation. It is needed to describe the further long-term outcome of the patients who were treated in L92-13 study, which was characterized by very short maintenance therapy. We published the discordant results on the randomized comparison between dexamethasone and prednisolone in 2005, which was updated in this analysis.[7]

Materials and methods

Total of 1846 newly diagnosed patients with ALL aged 1–15 years entered into the four studies—that is L84-11 (n=484),

Table 1 Event-free survival, overall survival, and CNS relapse of TCCSG studies L84-11, L89-12, L92-13, and L95-14

Study	*Year*	*Number of patients*	*Complete remission rate (corrected)*[a]	*Event-free survival ± s.e.%*			*Overall survival ± s.e.%*			*Isolated and any CNS relapse rate ± s.e.% 10 year*
				5 years	*10 years*	*15 years*	*5 years*	*10 years*	*15 year*	
L84-11	1984–1989	484	97.3 (98.6)%	71.2 ± 2.1	67.2 ± 2.2	66.3 ± 2.2	80.7 ± 1.8	74.3 ± 2.0	73.5 ± 2.1	4.1 ± 1.0 5.5 ± 1.1
L89-12	1989–1992	418	92.8 (95.7)%	67.2 ± 2.4	64.4 ± 2.4	62.3 ± 2.6	77.7 (2.1)	73.5 ± 2.2	71.9 ± 2.2	3.7 ± 1.1 5.4 ± 1.3
L92-13	1992–1995	347	96.5 (97.7)%	63.7 ± 2.7	60.1 ± 2.7	57.7 ± 2.9	80.4 (2.1)	77.9 ± 2.2	77.4 ± 2.4	1.0 ± 0.6 2.6 ± 1.0
L95-14	1995–1999	597	95.0 (97.4)%	76.8 ± 1.8	75.0 ± 1.8	—	84.9 (1.5)	82.0 ± 1.6	—	1.7 ± 0.6 2.8 ± 0.7

Abbreviations: CNS, central nervous system; s.e., standard error; TCCSG, Tokyo Children's Cancer Study Group.
[a]Corrected remission (rate %): patients who achieved delayed remission were included in remission, and censored patients during the induction phase were excluded from the total.

L89-12 ($n=418$), L92-13 ($n=347$), and L95-14 ($N=597$)—as shown in Table 1. Diagnoses were made based on morphology, immunophenotype, and cytogenetics in each institution; the ALL committee evaluated these results for eligibility. Patients aged 1–6 years presented with a leukocyte count $<20\times10^9$/l and B-precursor phenotype were classified into the standard-risk (SR) group in all the studies. Definitions of the intermediate-risk (IR) and high-risk (HR) or extremely high-risk groups varied across the four studies. Nonetheless, HR patients were mostly defined as having one of the following: initial leukocyte count $\geqslant100\times10^9$/l, age of $\geqslant10$ years, leukocyte count $\geqslant50\times10^9$/l; Philadelphia chromosome (Ph) or BCR–ABL fusion gene product positive, 11q23 chromosome translocation or MLL gene rearrangements, and T-ALL with otherwise IR-risk factors. The reminder of the SR and HR patients was assigned to the IR group. Analysis of the outcome was based on the risk classification of the NCI/Rome criteria.[13]

Leukemic-cell karyotype was obtained from 20 to 30% of the patients in the first three studies. The DNA index was measured by flow cytometry.

Infants were excluded from these studies, and their treatment results were already published elsewhere.[14–16]

Treatment

The precise regimens of L84-11,[2] L89-12,[5] L92-13,[6] and L95-14[7] studies were available in earlier publications. Table 2 provides a summary of regimens in each study.

L84-11 study (1984–1989). Both the SR and HR groups were randomized at early intensification into two arms—that is S1 and S2, and H1 and H2, respectively. In the S2 and H2 arms, the patients received three courses of ID-MTX (500 mg/m^2) with a single dose of leucovorin rescue (12 mg/m^2) at 48 h, in conjunction with double-drug intrathecal injections (DIT) before cranial irradiation. In the S1and H1 arms, 18 Gy of cranial irradiation with five doses of triple-drug intrathecal injections (TIT) were administered without ID-MTX.

The DIT consisted of methotrexate (MTX) 15 mg/m^2 $\leqslant$15 mg and hydrocortisone 30 mg/m^2 $\leqslant$30 mg, respectively. The TIT consisted of DIT and cytosine arabinoside (CA) 30 mg/m^2 $\leqslant$30 mg.

L89-12 study (1989–1992). The regimen was based on the BFM backbone in all three risk groups. There was a week of prophase treatment with prednisolone alone to evaluate initial steroid response, as BFM group described.[17] The main objective was to determine whether cranial irradiation was essential to the treatment of SR patients or not. To do so, the SR patients were randomly assigned to the SR0 and SR18 arms, and patients in the SR0 arm were given three courses of HD-MTX (3 g/m^2) with three DIT without cranial irradiation. The doses of intrathecal injection were reduced from those of the earlier study, changing to age-adjusted calculation. The patients assigned to the SR18 arm received 18 Gy of cranial irradiation and three doses of TIT. The randomization ratio in SR arms changed from 1:1 to 2:1 in the last half period, so that there were 83 patients enrolled in SR0 arm and 64 in SR18 arm. The HR group was treated with a single arm of BFM-style therapy for 2 years, modified with an insertion of HD-MTX (3 g/m^2, two courses) between the induction (Ia) and early intensification and cranial irradiation (Ib). Four courses of multiple-drug intensifications were given during the first year followed by 1-year maintenance therapy.

L92-13 study (1992–1994). A major objective was to evaluate 1-year therapy in all risk groups. The length of the maintenance therapy was kept to a minimum of 6 months in the SR group and 3 months in each of the IR and HR groups. All three risk regimens had BFM-type structures. This protocol was characterized by the use of intermediate-dose cytosine arabinoside (ID-CA, 500 mg/m^2/day for 4 days) and high-dose cytosine arabinoside (HD-CA, 1 or 2 g/m^2/day for 4 days) in the early intensification and in the re-intensification phases.

The SR regimen had two courses of HD-MTX (3 g/m^2) and two DITs. The early intensification phases were complete before week 28; 24 weeks were left for the continuous therapy. IR group was randomized either to IR18 arm with 18-Gy cranial irradiation, or to IR0 arm with two courses of HD-MTX (3 g/m^2/day) without cranial irradiation. All patients of the HR group were given 2 weekly courses of HD-CA (2 g/m^2, six doses for 3 days) and mitoxantrone (2 days) after remission induction.

L95-14 study (1994–1999). SR and IR groups were randomized into prednisolone arm (PSL) and dexamethasone arm (DEX) not only in the induction, but also in re-induction phase and three courses of late intensification for SR and two courses for IR. During remission induction, prednisolone (60 mg/m^2) or dexamethasone (8 mg/m^2) was given for 4 weeks and tapered. In the re-induction and intensification courses, prednisolone (40 mg/m^2) or dexamethasone (6 mg/m^2) were given for 2 weeks in each arm. For patients presenting with leukocyte count $\geqslant150\times10^9$/l and aged 7 years or older (assigned to allo-stem-cell transplantation (SCT) group), allogeneic bone marrow transplantation from HLA-matched family donor, if any, and autologous blood or marrow SCT or chemotherapy could be elected. For patients presented with

Table 2 Treatment protocols of the four studies

Studies	*TCCSG risk*	*Number*	*Therapy period (years)*	*Cranial irradiation***		*Remission induction*	*Early intensification*	*CNS prophylaxis*	*Reindution*	*Intensification*	*Continuation*
L84-11	SR	194	3.5	100%	100%	P V5 Asp	**Randomized S1:CRX18/ itMHC(5) vs S2:IDMTX(3)/ itMH(3)**	**S1:none vs S2:CRX18/itMHC(5)**	Dex V2/itMH..q16m(7) Dex V2 IDMTX/itMH..q12w(4)—2.5–3.5 years		MTX+6mp (throughout)
	HR	244	3.5		100%	P V5 Asp Cy	**Randomized H1:CRX24/ itMHC(5) vs H2:IDMTX(3)/ itMH(3)**	**H1:none vs H2:CRX24/itMHC(5)**	Dex V2 D2, Dex V2 Cy, Dex B Acr, Dex V2 Asp, Dex V2 MTX(iv)—first, second year Cy(4), HDCA(4), IDMTX/itMHC(4)—third year		MTX+6mp (throughout)
	HEX	48	2.5		100%	P V4 Asp D3 Cy1 itMHC(3)	Cy1 CA(4x4) 6mp	CRX24/itMHC(5)	Dex V4 Ad4 Asp, Cy B(8) itMH(2)	itMHC(8), PV2(4)	MTX+6mp
L89-12	SR	142	2	80%	44%	P V4 Asp T2 itMHC(1)	Vp CA(4x3) 6mp itMHC(3)	**Randomized HDMTX/ itMH(3) vs CRX18/itMHC(3)**	Dex V3 Asp T(3)	Vp4 B4 6mp/itMH(2)	MTX+6mp (1.5 years)
	IR	100	2		100%	P V4 Asp T3 itMHC(1)	CRX18 itMH(3)	Cy1 CA(4x4) 6mp itMHC(3)	Dex V3 Asp T4	P Vp4 B4 Acr(2), P Vp4 Cy4 Aap(2)	MTX+6mp (1 year)
	HR	146	2		100%	P V4 Asp T3 itMHC(1-2)	HDMTX(2)/itMH(2)	CRX18 /itMHC(3) Cy2 CA(4x4) 6mp	Dex V3 Asp T4	mP HDCA Asp Mit(2), P Vp4 B4 Acr(2), P Vp4 Cy4 Asp(2)	MTX+6mp (1 year)
L92-13	SR	124	1	44%	0%	P V4 Asp T2 itMH(1)	Mit CA(4x4) 6mp	HDMTX/itMH(2)	P V3 Asp T2	IDCA0.5gx4/Mit(2)	MTX+6mp (6 months)
	IR	122	1		47%	P V4 Asp T3 itMH(1)	Cy1 CA(4x4) 6mp itMHC(3)	**Randomized HDMTX(2)/ itMH(2) vs CRX18/itMH (3)**	P V3 Asp T2	HDCA1gx4/Mit(2) it(MH), Vp B Asp(2)	MTX+6mp (3–4 months)
	HR	101	1		100%	P V4 Asp T3 itMHC(2-3)	HDCA2gx6/Mit (2) itMH(2)	CRX18/itMHC(3) Cy1 CA(4x4) 6mp	P V3 Asp T(2)	HDCA2gx4/Mit/ it(MH)(2), Vp B Asp(2)	MTX(iv)q4W+ 6mp (3–4 months)
L95-14	SR	231	2	44%	0%	**Randomized* P vs Dex** and V5 Asp T2 itMH(2)	Cy1 CA(5x3) 6mp itMHC(3)	HDMTX/itMH(3)	**P vs Dex*** V3 Asp T3	Cy1 CA(2x5) 6mp(1), **P vs Dex*** V3 Asp(3) IDMTX(no CF)/itMH(3)	MTX(iv)+6mp, MTX+6mp (1 year+)
	IR	129	2		18%	**Randomized* P vs Dex** and V5 Asp T2 Cy1 itMH(2)	Cy1 CA(5x3) 6mp itMHC(3)	**Randomized HDMTX/itMH(3) vs CRX18/itMH(3)** Asp MTX+6mp	**P vs Dex*** V3 Asp T3	Cy1CA(2x5) 6mp(1), **P vs Dex*** V3 Asp T3(2) HDCA/Asp(1), IDMTX(no CF)(2), Cy1 CA(2x5) 6mp(1)	MTX(iv)q2W+ 6mp, MTX+6mp (1 year+)
	HR	237	2		100%	P V5 Asp D4 Cy2/ itMH(2-3)	HDCA2gx4/Asp(2)/itMH(2)	CRX18/itMHC(3) Cy1 CA(5x3) 6mp(1)	Dex V4 Ad4 Asp(1), P V3 Asp Ad2(2)	HDCA2gx8/itMH(2) IDMTX(no CF)(2), Cy1 CA(2x5) 6mp(1)	MTX+6mp (1 year)

Abbreviations: CNS, central nervous system; HEX, extremely high risk; HR, high risk; IR, intermediate risk; SR, standard risk; TCCSG, Tokyo Children's Cancer Study Group.
Acr, aclarubicin; Ad, doxorubicin; Asp, L-asparaginase; B, behenoyl cytosine arabinoside; CA, cytosine arabinoside; CRX18, cranial irradiation 18 Gy; Cy, cytoxan; D, daunorubicin; Dex, dexamethasone (8 mg/m^2 in induction 6 mg/m^2 consolidation of dex arm); HDCA, high-dose cytosine arabinoside (1–2 g/m^2); HDMTX, high-dose methotrexate (3 g/m^2); IDCA, intermediate-dose cytosine arabinoside (500 mg/m^2); itMH, double intrathecal injection of methotrexate and hydrocortisone; itMHC, triple intrathecal injection of methotrexate, cytosine arabinoside, and hydrocortisone; IDMTX, intermediate-dose methotrexate (500 mg/m^2); Mit, mitoxantrone; mP, methy-prednisolone; MTX, oral methotrexate; MTX(iv), intravenous MTX (75 mg/m^2); (noCF), no leucovorine rescue; P, prednisolone (*60 mg/m^2 in induction 40 mg/m^2 consolidation of P arm); T, THP-adriamycin (pirarubicin); V, vincristine; Vp, etoposide; 6mp, oral 6 mercapropurine.
Number after drug-dose, (Numer), repeat. Randomizations were written with bold letters. Randomized*, initially randomized for whole course. **Proportion of the patients who were initially assigned to cranial irradiation arm; actual proportion was lower than the assigned.

leukocyte count $\geqslant 100 \times 10^9$/l, or 10 years old or older with leukocyte count $\geqslant 50 \times 10^9$/l (assigned to auto-SCT group), autologous blood or marrow SCT or chemotherapy could be elected. Each institute declared the choice in advance of the study initiation.

Statistical analysis

The duration of EFS was defined as the time from the initiation of therapy to the date of failure (that is any relapse, death, or diagnosis of secondary malignancy) or to the date when patients were confirmed to be in remission and alive. Patients who did not achieve complete remission at the end of the initial induction phase or who died before the confirmation of remission were considered to have failed at day 0, even if they entered remission later with a second course or through additional treatment.. The probability of EFS and s.e. was estimated by the Kaplan–Meier method (Greenwood), and differences were tested by the log-rank test. Analysis was performed with the intent to treat. 'Any central nervous system (CNS) relapse' include both 'isolated CNS relapse' and CNS relapse combined with other sites. Probability of cumulative CNS relapse was estimated by inversed Kaplan–Meier method, which involves subtraction of Kaplan–Meier products from 100%. Only patients who had CNS relapse were failure, and all the others were censored. Cumulative probability of any secondary malignancy was calculated using the same method. Patients who received modified treatment were censored at that point in time. The patients who did not enter complete remission or had died during induction were treated as at the date of the beginning of treatment. Patients who were confirmed as remaining in first remission and alive, or who were lost of follow-up, were censored for EFS analysis; all those who were alive with or without disease were censored in OS analysis at the date of last contact.

Follow-up was updated in 2008. The proportions of patients whose data of the last 5 years were available were 144 of 357 (40.3%) in L84-11 study, 197 of 306 (64.3%) in L89-12, 220 of 266 (82.7%) in L92-13, and 449 of 489 (91.8%) in L95-14.

Results

Probability of EFS, OS, and cumulative CNS relapse rate of each study are shown in Tables 1 and 3. There was no improvement in EFS during the first three studies. The OS of L92-13 improved,

Table 3 Summary of the study results

Studies	*L84-11*	*L89-12*	*L92-13*	*L95-14*
Number of eligible patients (B+T)	484	418	347	597
Number of B/T	420/32	375/43	315/32	539/58
Average age (B/T) year	5.7/8.8	5.9/8.2	5.8/7.7	5.9/7.7
Average WBC (B/T)	20.1/108.0	31.6/137.5	38.4/146.1	30.6/167.0
Number of censored early	0	1 (0.2%)	2 (0.6%)	9 (1.5%)[a]
Death during induction	3 (0.6%)	12 (2.9%)[b]	5 (1.4%)	10 (1.7%)[c]
Failure of initial remission	11 (2.3%)[d]	17 (4.1%)[e]	5 (1.4%)	11 (1.8%)[f]
Complete remission (rate)	470 (97.1%)	388 (92.8%)	335 (96.0%)	567 (95.0%)
Corrected remission (rate)[g]	477 (98.6%)	399 (95.7%)	337 (97.7%)	573 (97.4%)
Death in first remission	19 (3.9%)	7 (1.7%)	6 (1.7%)	22 (3.7%)[h]
Number of censored in first remission	13 (2.7%)	13 (3.1%)[i]	31 (8.9%)[j]	21 (3.5%)[k]
Number of patients at event free	308 (63.6%)	256 (61.2%)	180 (55.3%)	428 (71.7%)
Number of relapse after remission	123 (26.1%)	104 (26.9%)	112 (33.4%)	92 (16.7%)
Site of relapse: total	123 (100%)	104 (100%)	112 (100%)	92 (100%)
Isolated bone marrow (BM)	72 (58.5%)	70 (67.3%)	87 (78.4%)	68 (73.9%)
Isolated CNS	17 (13.8%)	13 (12.5%)	3 (2.7%)	10 (10.9%)
Isolated testis	19 (15.4%)	6 (5.8%)	9 (78.4%)	7 (7.6%)
BM+CNS	6 (4.9%)	4 (3.8%)	3 (7.2%)	5 (5.4%)
BM+testis	7 (5.7%)	7 (6.7%)	6 (3.6%	1 (1.1%)
CNS+testis	1 (0.8%)	1 (0.9%)	0	0 (0%)
Other sites	1 (0.8%)	3 (2.9%)	3 (2.7%)	1 (1.1%)
Secondary AML/MDS	0/1	3/1	0/0	2/1
Brain tumor/Other	5/1[l]	4	2	1
Any BM	85 (69.1%)	81 (77.9%)	97 (87.4%)	74 (80.4%)
Any CNS	24 (19.5%)	18 (17.3%)	6 (5.4%)	15 (16.3%)
Any testis	27 (22.0%)	14 (13.5%)	15 (13.3%)	8 (8.7%)
Any testis/males	27 (10.3%)	14 (5.8%)	15 (8.5%)	8 (2.4%)

Abbreviations: AML, acute myeloid leukemia; CNS, central nervous system; MDS, myelodysplastic syndrome; SCT, stem-cell transplantation; WBC, white blood cells.
[a]Four patients assigned in dexamethasone arm dropped off, one in prednisolone arm, and four in HR risk group dropped off.
[b]Marrow suppression and infection.
[c]Five deaths in dexamethasone arm, two deaths in prednisolone arm, three deaths in HR risk.
[d]7/11 entered into remission in the following phase.
[e]11/17 patients entered remission in the following phase.
[f]All 11 failures in HR risk group; 3 Ph+ALL, 4 chromosomal translocations, 6/11 entered into remission in the following phase.
[g]Corrected remission (rate %): patients who achieved delayed remission were included in remission, and censored patients during the induction phase were excluded from the total.
[h]18/22 deaths in HR risk group, 5 related with transplants.
[i]7/13 patients underwent SCT in CR1.
[j]26/31 patients underwent SCT in CR1.
[k]9/21 patients underwent SCT in CR1.
[l]Olfactory neuroblastoma.

compared with these of the earlier two studies. The L95-14 study achieved internationally acceptable level of EFS and OS (log-rank $P<0.0001$). The cumulative 'any CNS relapse' rate decreased from 5.5% (any CNS) in the L84-11 study to 2.8% in the L95-14 study.

Twelve treatment-related brain tumors developed in patients who had received cranial irradiation in the four studies—that is 5, 4, 2, and 1 patient, respectively. They developed in six males and six females. No brain tumor occurred in the non-irradiated patients. The tumors developed between 8 and 22 years after cranial irradiation, seven in the 18-Gy irradiated group and five in the 24-Gy irradiated group. The probability of cumulative incidence (±s.e.) of brain tumors was 1.9±0.6% at 15 years and 2.8±0.9% at 20 years among the 1234 irradiated patients. Secondary acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS) developed in eight patients—that is 0/1, 3/1, 0, and 2/1 in each study. Two of them (L89-12) were confirmed to have 11q23 chromosome abnormality. Seven of the eight patients were female, whereas brain tumors developed evenly in terms of gender. AML/MDS occurred only in the irradiated patients without exception. The probability of cumulative incidence ± s.e. of AML/MDS among irradiated patients was 0.57±0.25% at 3 years and 1.1±0.4% at 10 years.

Cerebrovasucular lesions such as Moyamoya disease developed after radiation in the TCCSG studies and published elsewhere.[18] Neurocognitive evaluation study was not carried out as a group.

Protocol-specific treatment result

L84-11 study. For 484 patients enrolled, EFS±s.e. and OS±s.e. were 66.3±2.2 and 73.5±2.1% at 15 years, respectively. There were 357 long-term survivors, and their median follow-up period was 16.6 years. Among survivors, seven had serious neurological sequelae, such as paraparesis or leukoencephalopathy, which developed most probably because of cranial irradiation and concentrated use of five TITs at body-surface-adjusted dose setting. Probability of cumulative incidence of brain tumors in L84-11 was 1.2±0.7% at 15 years (Tables 3 and 4; Figure 1).

Males fared significantly worse than females in terms of EFS (Table 4; $P=0.006$), but not in terms of OS ($P=0.205$). Isolated or combined testicular relapses developed in 27 out of 261 males (10.3%) and they comprised 22% of all relapses.

As a result of the randomized comparison in SR, the EFS±s.e. rates of the S1 and S2 arms were 68.5±4.8 and 81.0±4.1%, respectively, at 15 years (log-rank test, $P=0.071$). The probabilities of cumulative incidence ±s.e. of any testicular relapse were 24.3±6.7% in S1 arm and 4.7±3.3% in S2 arm (log-rank $P=0.015$).

L89-12 study. For the 418 patients enrolled, the EFS±s.e. and OS rate were 62.3±2.6 and 71.9±2.2% at 1 year, respectively. Probability of cumulative isolated CNS and any

Table 4 Treatment results according to presenting features in non-infant patients treated in study L84-11

Factors	*Number of patients*	*Event-free survival ± s.e.%*				*Overall survival ± s.e.%*			
		5 years	*10 years*	*15 years*	*log-rank P-value*	*5 years*	*10 years*	*15 years*	*log-rank P-value*
Non-T lineage									
NCI standard	314	72.8±2.5	69.4±2.6	68.5±2.7	0.074	83.4±2.1	77.6±2.4	77.2±2.4	0.012
NCI high	106	67.6±4.7	61.0±4.9	59.0±5.1		73.6±4.4	66.1±4.8	64.8±5.0	
T-lineage									
NCI standard	9	55.6±16.6	44.4±16.6	44.4±16.6	0.636	66.7±15.7	55.6±16.6	41.7±17.3	0.487
NCI high	23	60.9±10.1	60.9±10.1	60.9±10.1		65.2±9.9	65.2±9.9	65.2±9.9	
Sex									
Male	261	66.4±3.0	61.3±3.2	60.8±3.1	0.006	80.1±2.5	72.1±2.8	71.1±2.9	0.205
Female	222	78.1±3.0	74.5±3.0	73.1±3.1		81.5±2.6	76.9±2.9	76.4±2.9	
Age at diagnosis (years)									
1–9	392	72.6±2.3	69.2±2.4	68.5±2.4	0.068	82.7±1.9	76.5±2.2	75.9±2.2	0.007
⩾10	91	65.0±5.2	58.7±5.3	56.8±5.5		72.0±4.8	64.7±5.1	63.2±5.2	
WBC × 10^9/l									
<10k	265	76.5±2.6	73.1±2.8	71.9±2.9	0.0131	86.4±2.1	80.9±2.5	80.4±2.5	0.002
10–49k	159	64.6±3.9	59.7±4.0	5.9±4.0		75.8±3.4	67.5±3.8	66.0±3.9	
50–99k	31	63.5±8.8	56.0±9.2	56.0±9.2		70.0±8.3	58.4±9.3	58.4±9.3	
⩾100k	28	67.9±8.8	67.9±8.8	67.9±8.8		67.3±9.0	67.3±9.0	67.3±9.0	
Cell lineage									
Non-T	420	71.5±2.2	67.3±2.3	66.3±2.4	0.121	81.0±1.9	74.7±2.2	74.1±2.2	0.038
T	32	59.4±8.7	55.9±8.8	55.9±8.8		65.6±8.4	62.2±8.6	58.5±8.1	
TCCSG risk arms									
S1	102	74.4±4.4	69.9±4.7	68.5±4.8	0.071	91.0±2.9	83.1±3.8	79.6±5.1	0.227
S2	93	85.7±3.7	81.0±4.1	79.1±4.5		94.5±2.5	87.3±3.6	87.3±3.6	
H1	129	69.8±4.1	67.2±4.2	66.0±4.3	0.131	77.7±3.7	73.4±4.0	71.4±4.1	0.046
H2	113	62.7±4.6	57.5±4.8	57.5±4.8		70.9±4.3	61.9±4.7	61.9±4.7	
S1 testis	49	21.8±6.4	24.3±6.7	24.3±6.7	0.009				
S2 testis	50	2.3±2.3	4.7±3.3	4.7±3.3					

Abbreviations: NCI, National Cancer Institute risk group; s.e., standard error; TCCSG, Tokyo Children's Cancer Study Group, WBC, white blood cells. Testis: probability of cumulative any testicular relapse rate in males.

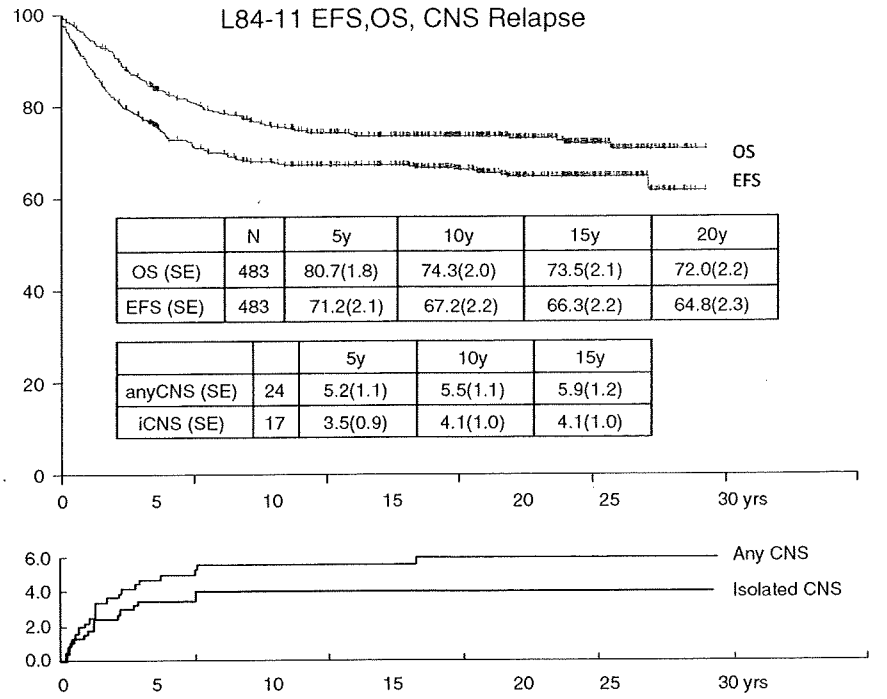


Figure 1 EFS, OS, and cumulative incidence of isolated or any CNS relapses in L84-11 study.

CNS relapse rates were 3.7 ± 1.1 and 5.4 ± 1.3% at 15 years, respectively. Of the 306 surviving patients, the median survival period was 14.6 years. Secondary neoplasms consisted of four brain tumors, three AML, and one MDS. Remission induction rate was 92.8%, which was the lowest of the four studies (Table 3). Twelve patients (2.9%) died during or after the remission induction course, between days 10 and 82. The major cause of death was prolonged marrow suppression and infection. Of 17 patients (4.1%) failed to enter remission at the end of induction, six patients (1.4%) died within 4–24 months; one Ph positive ALL, and four with leukocyte count $>145 \times 10^9$/l. The other 11 patients entered remission in the following phase; five patients with leukocyte counts $>100 \times 10^9$/l, seven Ph positive ALL. The corrected remission rate was 95.7% when the patients who entered into delayed remission were included in remission and those who were dropped off during induction were excluded from the total number. Pirarubicin used for induction at a dosage of 30 mg/m^2 (two or three doses) was amended to 20 mg/m^2 in October 1990. Nine out of 12 deaths occurred before the amendment. Testicular relapse was significantly fewer in incidence in SR0 (HD-MTX) arm than the SR18 arm ($P=0.018$; Tables 3, 5; Figure 2).

L92-13 study. EFS ± s.e. and OS ± s.e. for 347 eligible patients enrolled were 60.1 ± 2.7 and 77.9 ± 2.2% at 10 years, respectively. Cumulative rate of isolated CNS relapse was 1.0 ± 1.0 at 10 years, which might be underestimated by high bone marrow relapse rate. The median follow-up period was 13.0 years for the 271 (78.1%) patients remaining alive, including 64 patients who experienced relapse. Twenty-one HR patients underwent hematopoietic SCT at first remission (treated as censored), and 18 were alive in CR (Tables 3, 6; Figure 3).

Brain tumors occurred in two patients. No myeloid leukemia or MDS developed. The rate of remission induction was 96.0%.

Seven of 26 relapses among 62 males in SR group relapsed very late at 5–13 years of the initial therapy, whereas females stopped recurring at 5 years. Overall, the EFS in males was 47.5 ± 4.3% at 15 years, which was significantly lower than that in females (68.0 ± 3.8%, $P=0.0003$). Males were, however, more efficiently salvaged. The OS of males was 75.8 ± 3.3% and that of females 80.3 ± 3.1% ($P=0.731$; Table 6). Ten of 14 patients with isolated or combined testicular survived. After relapse, 51 patients survived out of 84 who had undergone hematopoietic SCT (actual survival 60.7%). Of 25 who had been treated with chemotherapy, 15 survived after relapse (60%). The OS rate of 77.4 ± 2.4% eventually exceeded the preceding two studies.

L95-14 study. L95-14 study achieved 5-year EFS ± s.e 75.0 ± 1.8% and the OS ± s.e. 82.0 ± 1.6%, at 10 years' follow-up. For the 489 patients who remained alive, the median follow-up period was 10.0 years. The remission induction rate after the initial course was 95.0%. The corrected remission induction rate was 97.5% when nine patients who were off during induction were excluded and six patients who entered into remission in the following phase were included. The cumulative isolated CNS relapse rate was 1.7 ± 0.6% and 'any CNS relapse' rates was 2.8 ± 0.7% for all patients, and the latter level was 4.3 ± 1.4% in the HR. One brain tumor occurred at 8.3 years, two AML, and one MDS all were diagnosed between 1.5 and 5.2 years of therapy (Tables 3, 7; Figures 4).

The results of randomized control study was updated and showed again no advantage of DEX arm over PSL arm in SR and IR groups[7] (Tables 2, 7). Three extramedullary relapses occurred in the DEX arm, whereas eight developed in the PSL arm.

Hematopoietic SCTs, either allogeneic or autologous blood and marrow source, were elected by institutional intention to

Table 5 Treatment results according to presenting features in non-infant patients treated in study L89-12

Factors	*Number of patients*	*Event-free survival ± s.e.%*				*Overall survival ± s.e.%*			
		5 years	*10 years*	*15 years*	*log-rank P-value*	*5 years*	*10 years*	*15 years*	*log-rank P-value*
Non-T lineage									
NCI standard	314	72.8 ± 2.5	69.4 ± 2.6	68.5 ± 2.7	0.074	83.4 ± 2.1	77.6 ± 2.4	77.2 ± 2.4	0.012
NCI high	106	67.6 ± 4.7	61.0 ± 4.9	59.0 ± 5.1		73.6 ± 4.4	66.1 ± 4.8	64.8 ± 5.0	
T-lineage									
NCI standard	11	70.1 ± 14.7	70.1 ± 14.7	70.1 ± 14.7	0.169	70.1 ± 14.7	70.1 ± 14.7	70.1 ± 14.7	0.369
NCI high	32	51.9 ± 9.0	51.9 ± 9.0	43.3 ± 10.9		55.3 ± 8.9	55.3 ± 8.9	55.3 ± 8.9	
Sex									
Male	240	62.1 ± 3.2	59.8 ± 3.3	57.8 ± 3.4	0.044	76.3 ± 2.8	72.2 ± 3.5	71.1 ± 3.0	0.564
Female	178	74.1 ± 3.4	70.8 ± 3.5	68.3 ± 3.7		79.6 ± 3.1	75.2 ± 3.3	73.0 ± 3.5	
Age at diagnosis (years)									
1–9	320	70.8 ± 2.6	68.0 ± 2.7	66.6 ± 2.7	0.0002	81.8 ± 2.2	78.3 ± 2.4	77.5 ± 2.4	<0.0001
⩾10	97	54.3 ± 5.3	51.6 ± 5.4	46.2 ± 5.7		64.2 ± 4.9	57.5 ± 5.1	53.0 ± 5.4	
WBC × 10^9/l									
<10k	203	75.5 ± 3.1	70.7 ± 3.4	67.8 ± 3.5	<0.0001	88.1 ± 2.3	83.5 ± 2.7	81.5 ± 3.0	<0.0001
10–49k	133	67.7 ± 4.1	66.0 ± 4.2	66.0 ± 4.2		77.5 ± 3.7	73.5 ± 3.9	72.7 ± 3.9	
50–99k	31	47.1 ± 9.1	43.5 ± 9.1	43.5 ± 9.1		61.2 ± 8.7	54.8 ± 8.9	51.4 ± 9.0	
⩾100k	50	44.4 ± 7.2	44.4 ± 7.2	40.0 ± 7.7		46.7 ± 7.2	44.6 ± 7.2	44.6 ± 7.2	
Cell lineage									
Non-T	374	68.3 ± 2.5	65.2 ± 2.6	63.3 ± 2.6	0.053	79.8 ± 2.1	75.0 ± 2.3	73.3 ± 2.4	0.009
T	43	57.1 ± 7.7	50.7 ± 9.1	50.7 ± 9.1		59.1 ± 7.7	59.1 ± 7.7	59.1 ± 7.7	
CNS status									
CNS blast +	12	42.9 ± 15.7	42.9 ± 15.7	42.9 ± 15.7	0.132	56.3 ± 14.8	46.9 ± 15.0	46.9 ± 15.0	0.033
CNS blast−	406	68.1 ± 2.4	65.0 ± 2.4	62.8 ± 2.5		78.3 ± 2.1	74.2 ± 2.2	72.6 ± 2.3	
TCCSG SR arms									
SR0	83	75.4 ± 4.9	72.7 ± 5.1	72.7 ± 5.1	0.399	90.6 ± 3.4	89.2 ± 3.6	87.7 ± 3.9	0.148
SR18	64	71.5 ± 5.7	66.5 ± 6.0	66.5 ± 6.0		85.8 ± 4.4	80.9 ± 5.0	78.1 ± 5.5	
SR0 CNS	83	5.4 ± 2.6	—	—	0.999				
SR18 CNS	64	5.2 ± 2.9	—	—					
SR0 testis	83	3.3 ± 3.3	—	—	0.018				
SR18 testis	64	19.4 ± 7.1	22.9 ± 7.6	—					

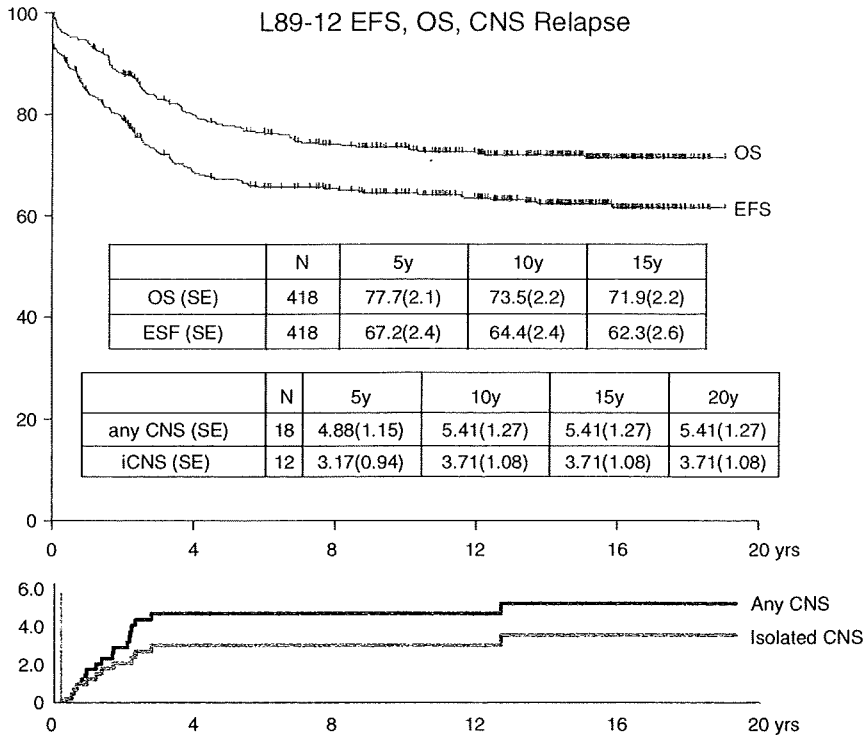
Abbreviations: CNS, central nervous system; NCI, National Cancer Institute risk group; s.e., standard error; SR, standard risk; TCCSG, Tokyo Children's Cancer Study Group; WBC, white blood cells.
CNS: probability of cumulative any CNS relapse rate.
Testis: probability of cumulative any testicular relapse rate.

treat decision in advance and executed for 61 (37 allo-SCT and 24 auto-SCT) of 126 patients who assigned to SCT (59 allo-SCT and 67 auto-SCT), among which 44 (actual rate72.1%) were alive without relapse. Of the 65 patients who assigned to SCT group, but elected chemotherapy, 30 (46, 2%) patients were alive; 29 were in first remission.

Treatment results according to presenting features

Well-documented prognostic factors were analyzed in each of the four studies (Tables 4–7). Infants were not included in these studies. Patients with B-precursor ALL and T-ALL were analyzed separately in each of the four studies, according to the NCI / Rome criteria. Age and leukocyte count at diagnosis were still independently strong prognostic factors.

Patients with T-ALL had poor prognosis. This was more evident in terms of OS (Tables 2–5). Clearly, patients with T-ALL could not be easily salvaged after relapse. Females fared significantly better than males in terms of EFS at 10 years by 13.2 points (L84-11, $P=0.006$), 11.0 points (L89-12, $P=0.044$), 15.6 points (L92-13, $P=0.003$), and –2.8 points (L95-14, males fared better, $P=0.519$), respectively (Table 3). 'Any testicular relapse' rate was 10.3, 5.8, 8.5, and 2.4% of all the males in the four studies, respectively (Table 3). The cumulative incidence of testicular relapse was significantly lower in ID-MTX or HD-MTX arms in randomized trials of the L84-11 SR, L89-12 IR, and L92-13 IR, as has been described.[19] The gender difference in EFS correlated well with the incidence of testicular relapse. Approximately 60% of the patients with any testicular relapse survived and contributed to the recovery of male OS to the same level as females. CNS involvement at presentation had negative prognostic impact on EFS (Tables 4 and 5). In L95-14 study (Table 7), patients who presented with DNA index of 1.16–1.60 showed EFS 84.2 ± 3.5%, which was significantly higher than the EFS rate of 72.3 ± 2.2% among those with DNA index <1.16 ($P=0.005$).[20] DNA index 1.16–1.60 group of patients also fared better than those with DNA index over 1.6 (EFS of 50.0 ± 17.7%, $P=0.003$).The outcome of the patients with Ph chromosome was dismal. Hematopoietic SCT was only curative treatment strategy so far.[21]



	N	5y	10y	15y
OS (SE)	418	77.7(2.1)	73.5(2.2)	71.9(2.2)
ESF (SE)	418	67.2(2.4)	64.4(2.4)	62.3(2.6)

	N	5y	10y	15y	20y
any CNS (SE)	18	4.88(1.15)	5.41(1.27)	5.41(1.27)	5.41(1.27)
iCNS (SE)	12	3.17(0.94)	3.71(1.08)	3.71(1.08)	3.71(1.08)

Figure 2 EFS, OS, and cumulative incidence of isolated or any CNS relapses in L89-12 study.

Discussion

Nine years passed since the earlier issue was published in '*Leukemia* 2000.'[1] The 1423 survivors in the four studies are now 22.5 years old on an average, ranging from 11.6 to 39.8 years of age. Of 1233 patients who received cranial irradiation, 873 were surviving. Twelve secondary brain tumors developed very late, that is at 8–22 years after initial therapy including cranial irradiation in the four studies presented here. The development of the brain tumors seemed not to depend on the studies. Hijiya *et al.*[22] reported from the St Jude that the cumulative incidence of brain tumor except for meningioma was $3.00 \pm 0.59\%$ at 30 years. It was $2.8 \pm 0.9\%$ at 20 years in the four studies.

As for the secondary AML/MDS, the incidence was variable depending on the study. They developed only in the irradiated patients without exception. Regimens of L89-12 and L92-13 studies included etoposide, which is a topo-II inhibitor and was highly associated with the development of secondary MAL/MDS with 11q23 chromosome translocations.[23,24] Two cases were confirmed to be associated with chromosome 11q23 translocations in L89-12 study. It was noteworthy that seven out of eight secondary AML/MDS patients were female, whereas the brain tumors developed equally across genders. It was described that girls were more sensitive to anthracycline cardiac toxicity than boys.[25] In addition, cognitive impairment, short stature, and excessive weight were all more prevalent among females than males.[26] Females responded more to the chemotherapy and remained in higher EFS than that of males. All these facts may suggest that girls are more sensitive to anti-leukemic drugs, resulting in better outcome of ALL and developed more therapy-related secondary AML/MDS.

Schmiegelow recently reported from NOPHO studies that children with low thiopurine methyltransferase activity were at lower risk of relapse of ALL[27] and were at higher risk of developing secondary malignancy.[28] In the latter article, of 20 secondary malignancies, 16 AML/MDS occurred in 6 males and 10 females, although the author did not mention the gender difference.

We had not performed neurocognitive assessment as a group, but many studies showed the negative influence of the cranial irradiation on the neurocognitive function particularly for the young patients,[26] and other study described that normal neurological function was preserved when irradiation was omitted.[29]

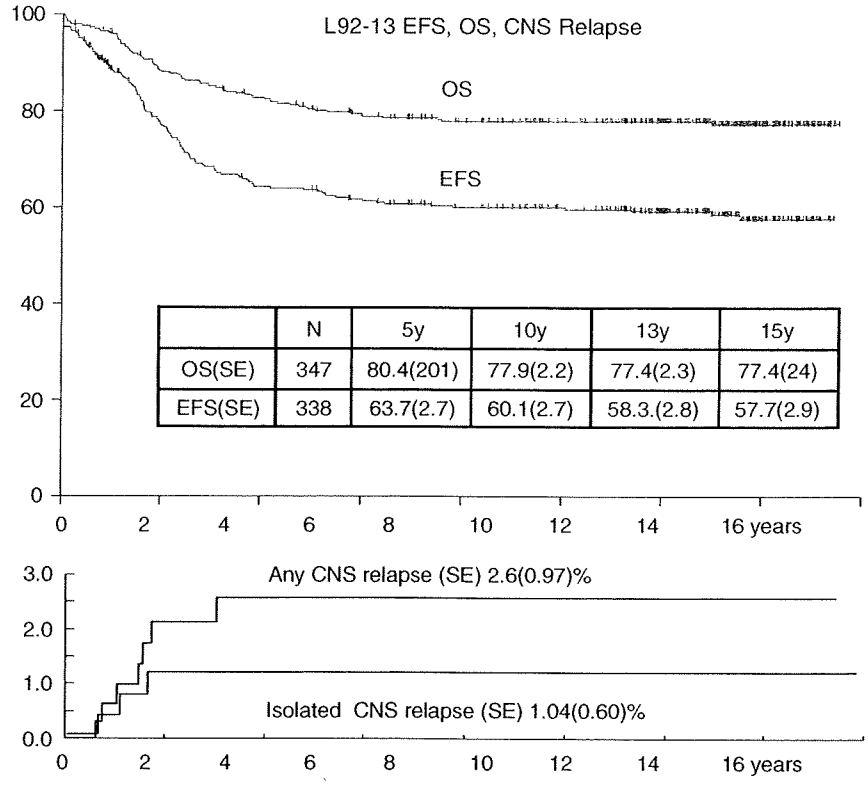
In the next study of TCCSG ALL L99-15, irradiated patients were limited to $<10\%$. In the currently active study, T-ALL and prednisolone poor responders were irradiated. The outcomes have already been reported on the protocols with no cranial irradiation from St Jude Children's Research Hospital,[30] EORTC,[31] Nordic countries,[32] and Netherlands.[33] To eliminate the cranial irradiation, the function of intrathecal injections would be expected. The 9–11 times intrathecal injections ended before 40 weeks in TCCSG protocols even when no cranial irradiation was administered. The proper number and timing of the extended intrathecal injections for patients at risk of CNS relapse such as hyper-leukocytosis and T-ALL remained to be determined in our future studies.

Gajjar *et al.*[34] express strong caution to traumatic lumber punctures as a risk factor of CNS relapse. The L89-12 and L92-13 studies had 1-week prophase of single therapy with oral prednisolone, and the initial intrathecal injection and cerebrospinal fluid examination was given on day 8.[5,35] The prednisolone prophase without spinal puncture might well have alleviated cerebrospinal fluid infiltration before the assessment. Consequently, initial ratio of patients with CNS-2 or CNS-3 was

Table 6 Treatment results according to presenting features in non-infant patients treated in study L92-13

Factors	*Number of patients*	*Event-free survival ± s.e.%*				*Overall survival ± s.e.%*			
		5 years	*10 years*	*15 years*	*log-rank P-value*	*5 years*	*10 years*	*15 years*	*log-rank P-value*
Non-T lineage									
NCI standard	206	68.1 ± 3.3	64.0 ± 3.4	62.8 ± 3.4	0.01	88.7 ± 2.2	86.1 ± 2.4	86.1 ± 2.4	<0.0001
NCI high	108	56.5 ± 5.1	52.9 ± 5.1	52.9 ± 5.1		68.1 ± 4.5	64.9 ± 4.7	64.9 ± 4.7	
T-lineage									
NCI standard	7	83.3 ± 15.2	83.3 ± 15.2	83.3 ± 15.2	0.132	100	100	100	0.062
NCI high	25	50.8 ± 11.4	50.8 ± 11.4	50.8 ± 11.4		60.0 ± 9.8	60.0 ± 9.8	60.0 ± 9.8	
Sex									
Male	177	56.2 ± 3.9	52.4 ± 3.9	47.5 ± 4.9	0.003	80.5 ± 3.0	77.0 ± 3.0	75.8 ± 3.3	0.731
Female	170	71.3 ± 3.6	68.0 ± 3.7	68.0 ± 3.8		80.3 ± 3.0	80.3 ± 3.1	80.3 ± 3.2	
Age at diagnosis (years)									
1–9	264	66.4 ± 3.0	62.7 ± 3.1	59.7 ± 3.3	0.025	86.7 ± 2.1	84.7 ± 2.3	84.0 ± 2.4	<0.0001
⩾10	83	55.0 ± 5.8	51.7 ± 5.9	51.7 ± 5.10		67.7 ± 5.2	55.2 ± 5.3	55.2 ± 5.4	
WBC × 10^9/l									
<10k	164	65.9 ± 3.4	60.6 ± 3.9	59.9 ± 11.1	0.302	85.2 ± 2.8	82.7 ± 3.0	82.7 ± 3.1	0.008
10–49k	109	79.1 ± 4.0	64.5 ± 4.7	58.1 ± 5.4		81.5 ± 3.7	78.6 ± 4.0	77.1 ± 4.2	
50–99k	21	65.3 ± 10.6	59.9 ± 11.0	59.9 ± 11.1		76.2 ± 9.3	78.6 ± 4.0	77.1 ± 4.2	
⩾100k	50	53.9 ± 7.8	53.9 ± 7.8	53.9 ± 7.9		63.7 ± 6.8	63.7 ± 6.8	63.7 ± 6.8	
Cell lineage									
Non-T	315	64.1 ± 2.8%	60.3 ± 2.9%	57.6 ± 3.1%	0.779	81.6 ± 2.2%	78.9 ± 2.3%	78.2 ± 2.4%	0.177
T	32	58.5 ± 9.8%	58.5 ± 9.9%	58.5 ± 9.10%		68.7 ± 8.2%	68.7 ± 8.3%	68.7 ± 8.4%	
CNS status									
CNS-1	323	65.5 ± 2.8	61.7 ± 2.9	60.8 ± 2.9	0.525	80.9 ± 2.2	79.2 ± 2.4	78.5 ± 2.1	0.128
CNS-2	12	55.0 ± 15.0	55.0 ± 15.0	55.0 ± 15.0	0.076*	66.7 ± 13.6	58.3 ± 14.2	58.3 ± 14.2	
CNS-3	9	37.5 ± 17.1	37.5 ± 17.1	37.5 ± 17.1		88.9 ± 10.5	88.9 ± 10.5	88.9 ± 10.5	
DNA index or chromosome number (50–60 or others, others include cases not tested)									
1.16–1.60	25	68.0 ± 9.3	52.0 ± 10.0	52.0 ± 10.0	0.775	92.0 ± 5.4)	92.0 ± 5.4)	92.0 ± 5.4)	
Others	322	63.0 ± 2.8	60.5 ± 2.8	59.0 ± 2.9		78.5 ± 2.3)	76.9 ± 2.4)	76.3 ± 2.4)	
t(9;22) or BCR/ABL chimera message									
Present	12	16.7 ± 10.8	-	-	<0.0001	33.3 ± 13.6	33.3 ± 13.6	33.3 ± 13.6	<0.0001
Absent	335	64.6 ± 3.0	61.0 ± 2.8)	60.2 ± 2.8		82.1 ± 2.1	79.6 ± 2.2	79.0 ± 2.3	
TCCSG arms									
SR	123	65.9 ± 4.3	59.9 ± 4.5	56.3 ± 4.6		88.3 ± 2.9	84.9 ± 3.3	83.5 ± 3.5	
IR0	71	61.0 ± 5.9	58.0 ± 6.0	58.0 ± 6.0	0.942	87.1 ± 4.0	87.1 ± 4.0	87.1 ± 4.0	0.021
IR18	50	64.0 ± 6.8	60.0 ± 6.9	60.0 ± 6.9		74.0 ± 6.2	69.9 ± 6.5	69.9 ± 6.5	
IR0 testis	37	7.8 ± 5.5	7.8 ± 5.6	7.8 ± 5.7	0.053				
IR18 testis	22	26.4 ± 10.2	26.4 ± 10.3	26.4 ± 10.4					

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; NCI, National Cancer Institute risk group; s.e., standard error; SR, standard risk; TCCSG, Tokyo Children's Cancer Study Group; WBC, white blood cells.
IR0: the arm without cranial irradiation.
IR18: the arm with cranial irradiation.
Testis: probability of cumulative any testicular rate in males.
*CSF-1 vs CSF2 + 3.



	N	5y	10y	13y	15y
OS(SE)	347	80.4(201)	77.9(2.2)	77.4(2.3)	77.4(24)
EFS(SE)	338	63.7(2.7)	60.1(2.7)	58.3.(2.8)	57.7(2.9)

Figure 3 EFS, OS, and cumulative incidence of isolated or any CNS relapses in L92-13 study.

lower on day 8 in our studies than that on day 1 of other studies. It has been shown that the day 8 puncture did not increase CNS relapse.[5] The initial day 8 lumbar puncture is a safe method to avoid inadvertent introduction of leukemic blasts into the cerebrospinal fluid.

The duration of the maintenance therapy had been shortened step by step from 4 years in L81-10 study, 3 years for SR in L84-11 study, and 1.5 years for SR and 1 year for HR in L89-12 study without increasing relapses. The ID-MTX in S2 arm of L84-11 study efficiently reduced relapse after off therapy, whereas the control arm showed clusters of relapse starting at the point of off therapy. These results developed a hypothesis that an addition of a new intensified treatment on early phase might make it possible to shorten the duration of therapy further without sacrificing overall outcome. Randomized study could not be realized because a control arm was difficult to set. For the intensification of early therapy, ID-CA and HD-CA and mitoxantrone were administered in all risk groups. As a result, the relapse increased in both SR and HR groups. The short maintenance therapy affected more negatively on the lower-risk patients and males than on the higher risk and females (Table 6). EFS of HR patients was almost equivalent to that of SR. The early intensification might be more effective in HR than SR as CCG reported.[36] Randomized comparison of length in maintenance therapy for 18 months vs 24 months came to conclusion in ALL-BFM 81[4] and 83[37] studies, and ALL-BFM 86[38] study was amended to extend all the maintenance from 18 to 24 months. The appropriate length of maintenance therapy must be essential, particularly for the lower-risk patients and males. The duration between 18 months and 24 months were needed in the protocols of BFM-type structure. The boys had a higher risk of late relapse without sufficient maintenance therapy.

In 95-14, the randomized study in SR and IR compared between prednisolone (60 mg/m^2 at induction and 40 mg/m^2 at intensifications) and dexamethasone (8 mg/m^2 at induction and 6 mg/m^2 at intensifications) resulted in no significant difference in EFS rate.[7] Analysis with updated data on this comparison resulted in the same conclusion. Our results did not fully accord with those of other larger-scale studies. The results of CCG-1922 study[39] showed significantly better outcome in SR patients treated with dexamethasone at 6 mg/m^2 than prednisolone 40 mg/m^2. In UK Medical Research Council ALL97 trial,[40] dexamethasone given at 6.5 mg/m^2 and prednisolone given at 40 mg/m^2 were compared, and the dexamethasone arm showed better outcome. A conclusive result is anticipated in the trials with higher dose of dexamethasone at 10 mg/m^2 along with the evaluation of side effects.

In conclusion, analysis of long-term follow-up results brought us invaluable suggestions to consider for our future studies. Girls may generally be more drug sensitive than boys and they could be cured with shorter maintenance therapy than boys; at the same time, they may be at higher risk of secondary AML/MDS. The testicular relapse and lower EFS in boys were almost resolved in L95-14. TCCSG currently limited the indication of cranial irradiation to $<10\%$ of the patients. To avoid the secondary malignancy and neurological sequelae, it is of primary importance to omit the cranial irradiation and the etoposide completely as a primary therapy. Safe and effective induction and immediately given intensification, as well as appropriate length of maintenance therapy, are still major subjects to study. We seriously realized that an establishment of firm long-term follow-up system is mandatory to evaluate the ultimate result of the protocols.

Table 7 Treatment results according to presenting features in non-infant patients treated in study L95-14

Factors	*Number of patients*	*Event-free survival ± s.e.%*				*Overall survival ± s.e.%*			
		5 years	*10 years*	*13 years*	*log-rank value*	*5 years*	*10 years*	*13 years*	*log-rank P value*
Non-T lineage									
NCI standard	373	82.7 ± 2.0	81.3 ± 2.1	80.5. ± 2.2	<0.0001	92.5 ± 1.3	90.6 ± 1.5	88.9 ± 2.0	<0.0001
NCI high	183	67.4 ± 3.6	64.4 ± 3.7	64.4 ± 3.7		72.8 ± 3.3	68.5 ± 3.6	67.3 ± 3.7	
T-lineage									
NCI standard	8	87.5(11.7)	87.5(11.7)	87.5(11.7)	0.2676	100	100	100	0.095
NCI high	50	66.9 ± 6.8	66.9 ± 6.8	66.9 ± 6.8		70.0 ± 6.5	68.0 ± 6.6	68.0 ± 6.6	
Sex									
Male	340	78.5 ± 2.6	76.5 ± 2.7	76.5 ± 2.7	0.519	86.1 ± 2.1	84.4 ± 2.3	82.9 ± 2.7	0.211
Female	257	75.4 ± 2.4	73.7 ± 2.5	72.9 ± 2.6		84.0 ± 2.0	80.1 ± 2.2	78.7 ± 2.4	
Age at diagnosis (years)									
1–9	460	79.1 ± 1.9	77.6 ± 2.0	77.0 ± 2.1	0.002	88.6 ± 1.5	85.7 ± 1.7	83.8 ± 2.0	<0.0001
⩾10	134	68.6 ± 4.1	65.6 ± 4.3	65.6 ± 4.3		72.4 ± 3.9	69.2 ± 4.1	69.2 ± 4.1	
WBC × 10^9/l									
<10k	306	79.1 ± 2.3	77.2 ± 2.4	75.7 ± 2.6	<0.0001	91.1 ± 1.6	88.0 ± 1.9	86.52 ± 2.4	<0.0001
10–49k	160	74.8 ± 3.4	74.1 ± 3.4	74.1 ± 3.4		86.7 ± 2.7	85.3 ± 2.8	85.3 ± 2.8	
50–99k	58	56.9 ± 6.5	56.9 ± 6.5	56.9 ± 6.5		70.7 ± 6.0	65.8 ± 6.6	62.3 ± 7.1	
⩾100k	70	57.7 ± 6.0	55.6 ± 6.1	55.6 ± 6.1		65.4 ± 5.7	65.4 ± 5.7	65.4 ± 5.7	
Cell lineage									
Non-T	539	77.5 ± 1.8	75.5 ± 1.9	75.3 ± 2.0	0.159	86.1 ± 1.5	83.4 ± 1.7	81.4 ± 1.9	0.021
T	58	69.7 ± 6.2	69.7 ± 6.2	69.7 ± 6.2		73.9 ± 5.8	72.1 ± 5.9	72.1 ± 5.9	
CNS status									
0	378	85.6 ± 1.8	82.3 ± 2.0	81.8 ± 2.1	0.962	77.9 ± 2.0	77.9 ± 2.0	77.9 ± 2.0	0.514
1–4	183	85.1 ± 2.6	83.9 ± 2.7	80.2 ± 3		77.5 ± 3.0	74.7 ± 3.0	74.7 ± 3.0	
5–	20	90.0 ± 6.7	77.9 ± 9.9	77.9 ± 9.9		65.8 ± 11.0	65.8 ± 11.0	65.8 ± 11.0	
DNA index									
<1.16	464	74.3 ± 2.1	72.9 ± 2.1	72.3 ± 2.2	0.005*	82.5 ± 1.8	79.2 ± 2.9	78.2 ± 2.0	0.001*
1.16–1.60	124	87.5 ± 3.0	84.2 ± 3.5	84.2 ± 3.5	0.003**	94.3 ± 2.1	92.7 ± 2.4	92.7 ± 2.4	0.005**
>1.60	9	50.0 ± 17.7	50.0 ± 17.7	50.0 ± 17.7		77.8 ± 13.9	77.8 ± 13.9	77.8 ± 13.9	
t(9;22) or BCR/ABL chimera message									
Present	24	26.4 ± 9.7	26.4 ± 9.7	26.4 ± 9.7	<0.0001	41.7 ± 10.1	31.3 ± 9.9	25,9 ± 9.7	<0.0001
Absent	573	78.7 ± 1.7	76.9 ± 1.8	76.4 ± 1.9		86.8 ± 1.4	84.1 ± 15.7	83.9 ± 1.8	
t(1;19) or E2A/PBX1 chimera message									
Present	26	70.2 ± 9.5	70.2 ± 9.5	70.2 ± 9.5	0.449	73.0 ± 8.7	73.0 ± 8.7	73.0 ± 8.7	0.182
Absent	568	77.1 ± 1.8	75.1 ± 1.9	74,7 ± 1.9		85.5 ± 1.5	82.4 ± 1.6	80.8 ± 1.9	
11q23 or MLL rearrangement									
Present	5	75.0 ± 21.5	75.0 ± 21.5	75.0 ± 21.5	0.962	80.0 ± 17.9	80.0 ± 17.9	80.0 ± 17.9	0.879
Absent	589	76.8 ± 1.8	74.9 ± 1.8	74.5 ± 1.9		85.0 ± 1.5	82.0 ± 1.6	80.5 ± 1.8	
TCCSG SR+HR arm									
Dexamethasone	179	82.15 ± 2.9	80.5 ± 3.1	80.5 ± 3.1	0.5178	91.5 ± 2.1	89.1 ± 2.4	88.1 ± 2.6	0.190
Prednisolone	180	85.6 ± 2.7	83.5 ± 2.9	81.9 ± 3.2		95.0 ± 1.6	93.2 ± 1.9	90.2 ± 3.5	

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; NCI, National Cancer Institute risk group; s.e., standard error; SR, standard risk; TCCSG, Tokyo Children's Cancer Study Group; WBC, white blood cells.
*<1.16 vs 1.16-1.60, **1.16-1.60 vs>1.60.

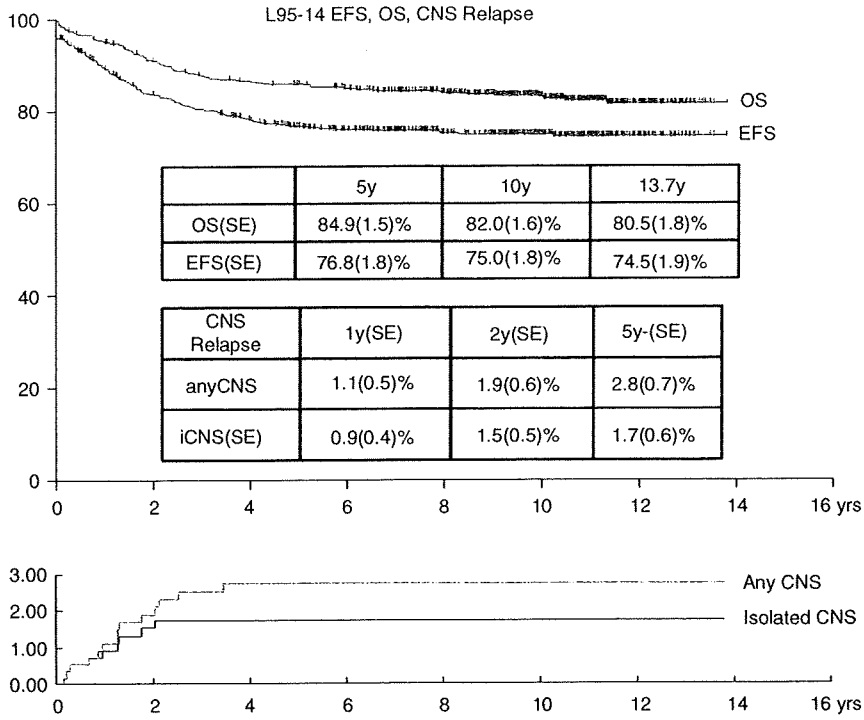


Figure 4 EFS, survival, and cumulative incidence of isolated or any CNS relapses in L95-14 study.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Tsuchida M, Ikuta K, Hanada R, Saito T, Isoyama K, Sugita K *et al.* Long-term follow-up of childhood acute lymphoblastic leukemia in Tokyo Children's Cancer Study Group 1981–1995. *Leukemia* 2000; **14**: 2295–2306.
2 Tsuchida M, Akatsuka J, Bessho F, Chihara H, Hayashi Y, Hoshi Y *et al.* Treatment of acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group—preliminary results of L84-11 protocol. *Acta Pediatr Jpn* 1991; **33**: 522–532.
3 Aur RJA, Simone JV, Verzosa MS, Hutsu HO, Barker LF, Pinkel DP *et al.* Childhood acute lymphocytic leukemia. *Cancer* 1978; **42**: 2133–2134.
4 Schrappe M, Beck J, Brandeis WE, Feickert HJ, Gadner H, Graf N *et al.* Treatment of acute lymphoblastic leukemia in childhood and adolescence: results of the multicenter therapy study ALL-BFM81. *Klin Padiatr* 1987; **199**: 133–150.
5 Manabe A, Tsuchida M, Hanada R, Ikuta K, Toyoda Y, Okimoto Y *et al.* Delay of the diagnostic lumbar puncture and intrathecal chemotherapy in children with acute lymphoblastic leukemia who undergo routine corticosteroid testing: Tokyo Children's Cancer Study Group study L89-12. *J Clin Oncol* 2001; **19**: 3182–3187.
6 Toyoda Y, Manabe A, Tsuchida M, Hanada R, Ikuta K, Okimoto Y, *et al.* for the Acute Lymphoblastic Leukemia Committee of the Tokyo Children's Cancer Study Group. Six months of maintenance chemotherapy after intensified treatment for acute lymphoblastic leukemia of childhood. *J Clin Oncol* 2000; **18**: 1508–1515.
7 Igarashi S, Manabe A, Ohara A, Kumagai M, Saito T, Okimoto Y *et al.* No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. *J Clin Oncol* 2005; **23**: 6489–6498.
8 Conter V, Aricò M, Valsecchi MG, Rizzari C, Testi AM, Messina C *et al.* Extended intrathecal methotrexate may replace cranial irradiation for prevention of CNS relapse in children with intermediate-risk acute lymphoblastic leukemia treated with Berlin-Frankfurt-Münster-based intensive chemotherapy. The Associazione Italiana di Ematologia ed Oncologia Pediatrica. *J Clin Oncol* 1995; **13**: 2497–2502.
9 Schrappe M, Reiter A, Ludwig WD, Harbott J, Zimmermann M, Hiddemann W *et al.* Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. *Blood* 2000; **95**: 3310–3322.
10 Pui CH, Boyett JM, Relling MV, Harrison PL, Rivera GK, Behm FG *et al.* Sex differences in prognosis for children with acute lymphoblastic leukemia. *J Clin Oncol* 1999; **17**: 818–824.
11 Shuster JJ, Wacker P, Pullen J, Humbert J, Land VJ, Mahoney Jr DH *et al.* Significance of sex in childhood B-precursor acute lymphoblastic leukemia: a Pediatric Oncology Group Study. *J Clin Oncol* 1998; **16**: 2854–2863.
12 Chessells JM, Richards SM, Bailey CC, Lilleyman JS, Eden OB. Gender and treatment outcome in childhood lymphoblastic leukaemia: report from the MRC UKALL trials. *Br J Haematol* 1995; **89**: 364–372.
13 Smith M, Arthur D, Camitta B, Carroll AJ, Crist W, Gaynon P *et al.* Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. *J Clin Oncol* 1996; **14**: 18–24.
14 Ishii E, Okamura J, Tsuchida M, Kobayashi M, Akiyama Y, Nakahata T *et al.* Infant leukemia in Japan: clinical and biological analysis of 48 cases. *Med Pediatr Oncol* 1991; **19**: 28–32.
15 Isoyama K, Okawa H, Hayashi Y, Hanada R, Okimoto Y, Maeda M *et al.* Clinical and biological aspects of acute lymphoblastic leukemia in 62 infants: retrospective analysis of the Tokyo Children's Cancer Study Group. *Pediatr Int* 1999; **41**: 477–483.

16 Kosaka Y, Koh K, Kinukawa N, Wakazono Y, Isoyama K, Oda T *et al.* Infant acute lymphoblastic leukemia with MLL gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation. *Blood* 2004; **104**: 3527–3534.
17 Riehm H, Schrappe M. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. *Blood* 1999; **94**: 1209–1217.
18 Kikuchi A, Maeda M, Hanada R, Okimoto Y, Ishimoto K, Kaneko T *et al.* Tokyo Children's Cancer Study Group (TCCSG). Moyamoya syndrome following childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2007; **48**: 268–272.
19 Dördelmann M, Reiter A, Zimmermann M, Fengler R, Henze G, Riehm H *et al.* Intermediate dose methotrexate is as effective as high dose methotrexate in preventing isolated testicular relapse in childhood acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 1998; **20**: 444–450.
20 Aricò M, Valsecchi MG, Rizzari C, Barisone E, Biondi A, Casale F *et al.* Long-term results of the AIEOP-ALL-95 Trial for Childhood Acute Lymphoblastic Leukemia: insight on the prognostic value of DNA index in the framework of Berlin-Frankfurt-Muenster based chemotherapy. *J Clin Oncol* 2008; **26**: 283–289.
21 Mori T, Manabe A, Tsuchida M, Hanada R, Yabe H, Ohara A *et al.* Allogeneic bone marrow transplantation in first remission rescues children with Philadelphia chromosome-positive acute lymphoblastic leukemia: Tokyo Children's Cancer Study Group (TCCSG) studies L89-12 and L92-13. *Med Pediatr Oncol* 2001; **37**: 426–431.
22 Hijiya N, Hudson M, Lensing S, Zacher M, Onciu M, Behm FG *et al.* Cumulative incidence of secondary neoplasms as the first event after treatment of childhood acute lymphoblastic leukemia increases over 30 years. *JAMA* 2007; **297**: 1207–1215.
23 Pui CH, Ribeiro RC, Hancock ML, Rivera GK, Evans WE, Raimondi SC *et al.* Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphoblastic leukemia. *N Engl J Med* 1991; **325**: 1682–1687.
24 Pui C-H, Relling MV, Rivera GK, Hancock ML, Raimondi SC, Heslop HE *et al.* Epipodophyllotoxin-related acute myeloid leukemia—a study of 35 cases. *Leukemia* 1995; **9**: 1990–1996.
25 Silber JH, Jakachi RI, Larsen RL, Goldwein JW, Barber G. Forecasting cardiac function after anthracyclines in childhood: the role of dose, age, gender. In: Bricker JT, Green DM and D'Angio GJ (eds). *Cardiac Toxicity After Treatment for Childhood Cancer.* Wiley-Liss: New York, 1994, pp 95–102.
26 Waber DP, Urion DK, Tarbell NJ, Niemeyer C, Gelber R, Sallan SE. Late effects of central nervous system treatment of acute lymphoblastic leukemia in childhood are sex-dependent. *Dev Med Child Neurol* 1990; **32**: 238–248.
27 Schmiegelow K, Forestier E, Kristinsson J, Soderhall S, Vettenranta K, Weinshilboun R, *et al.*, on behalf of the Nordic Society of Pediatric Haematology and Oncology (NOPHO). Thiopurine methyltransferase activity is related to the risk of relapse of children of acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. *Leukemia* 2009; **23**: 557–564.
28 Schmiegelow K, Al-Modhwahi I, Andersen MK, Behrendtz M, Forestier E, Hasle H, *et al.*, Nordic Society for Paediatric Haematology and Oncology. Methotrexate/6-mercaptopurine maintenance therapy influences the risk of a second malignant neoplasm after childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. *Blood* 2009; **113**: 6077–6084.
29 Krappmann P, Paulides M, Stöhr W, Ittner E, Plattig B, Nickel P *et al.* Almost normal cognitive function in patients during therapy for childhood acute lymphoblastic leukemia without cranial irradiation according to ALL-BFM 95 and COALL 06-97 protocols: results of an Austrian-German multicenter longitudinal study and implications for follow-up. *Pediatr Hematol Oncol* 2007; **24**: 101–109.
30 Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC *et al.* Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med* 2009; **360**: 2730–2741.
31 Vilmer E, Suciu S, Ferster A, Bertrand Y, Cavé H, Thyss A *et al.* Long-term results of three randomized trials (58831, 58832, 58881) in childhood acute lymphoblastic leukemia: a CLCG-EORTC report. Children Leukemia Cooperative Group. *Leukemia* 2000; **14**: 2257–2266.
32 Gustafsson G, Schmiegelow K, Forestier E, Clausen N, Glomstein A, Jonmundsson G *et al.* Improving outcome through two decades in childhood ALL in the Nordic countries: the impact of high-dose methotrexate in the reduction of CNS irradiation. Nordic Society of Pediatric Haematology and Oncology (NOPHO). *Leukemia* 2000; **14**: 2267–2275.
33 Kamps WA, Bökkerink JP, Hählen K, Hermans J, Riehm H, Gadner H *et al.* Intensive treatment of children with acute lymphoblastic leukemia according to ALL-BFM-86 without cranial radiotherapy: results of Dutch Childhood Leukemia Study Group Protocol ALL-7 (1988–1991). *Blood* 1999; **94**: 1226–1236.
34 Gajjar A, Harrison PL, Sandlund JT, Rivera GK, Ribeirio RC, Rubnitz JE *et al.* Traumatic lumbar puncture at diagnosis adversely affects outcome in childhood acute lymphoblastic leukemia. *Blood* 2000; **96**: 3381–3384.
35 Manabe A, Ohara A, Hasegawa D, Koh K, Saito T, Kiyokawa N *et al.* Significance of the complete clearance of peripheral blasts after 7 days of prednisolone treatment in children with acute lymphoblastic leukemia: the Tokyo Children's Cancer Study Group Study L99-15. *Haematologica* 2008; **93**: 1155–1160.
36 Seibel NL, Steinherz PG, Sather HN, Nachman JB, Delaat C, Ettinger LJ *et al.* Early post-induction intensification therapy improves survival for children and adolescents with high-risk acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Blood* 2008; **111**: 2548–2555.
37 Riehm H, Reiter A, Schrappe M, Berthold F, Dopfer R, Gerein V *et al.* Corticosteroid-dependent reduction of leukocyte count in blood as a prognostic factor in acute lymphoblastic leukemia in childhood (therapy study ALL-BFM 83). *Klin Padiatr* 1987; **199**: 151–160.
38 Reiter A, Schrappe M, Ludwig WD, Hiddemann W, Sauter S, Henze G *et al.* Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. *Blood* 1994; **84**: 3122–3133.
39 Bostrom BC, Sensel MR, Sather HN, Gaynon PS, La MK, Johnston K *et al.*, Children's Cancer Group. Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. *Blood* 2003; **101**: 3809–3817.
40 Mitchell CD, Richards SM, Kinsey SE, Lilleyman J, Vora A, Eden TO. Medical Research Council Childhood Leukaemia Working Party. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL97 randomized trial. *Br J Haematol* 2005; **129**: 734–745.

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