Table V. Major toxicities associated with MLL9601 protocol.

Category	Toxicity	No. of cases
Induction therapy		
Infectious	Neutropenia with fever	4
	Sepsis (*Streptococcus viridans*), sepsis (unknown cause)	2
	Central venous catheter infection	1
	Liver/spleen abscess (*C. albicans*)	1
	Rota virus infection	1
	Cutaneous abscess	1
	Bronchitis	1
	Perirectal abscess	1
Other	Diarrhoea/vomiting	5
	Liver dysfunction	4
	Gastrointestinal haemorrhage	1
	Hypertension	1
Postremission therapy		
Infectious	Neutropenia with fever	8
	Sepsis (*alpha-Streptococcus*), bacteraemia (*K. pneumoniae*), Sepsis (*Streptococcus viridans*), sepsis (unknown cause)	5
	Urinary tract infection	1
	Protein-losing enteropathy, malabsorption	1
	Bronchiolitis (RSV infection)	1
	Pneumonia (*alpha-Streptococcus*)	1
Other	Liver dysfunction	10
	Diarrhoea/vomiting	8
	Disseminated intravascular coagulation	2
	Acute renal failure/renal tubular acidosis	1
	Body weight loss	1

Each case represents a single episode in one patient. RSV, respiratory syncytial virus.

overly time consuming and FISH results correlate well with those obtained by standard Southern analysis (Martinez-Climent *et al*, 1995; Mathew *et al*, 1999). This strategy enabled the patients to be divided into MLL^+ and MLL^- subgroups on d 14 after the start of treatment and to select risk-appropriate therapy for each cohort: multiagent chemotherapy with high-dose cytarabine, high-dose methotrexate and anthracyclines followed by HSCT for MLL^+ patients, and a standard protocol of intensive chemotherapy, with emphasis an antimetabolites, for those without this high-risk feature. The MLL^+ infants selected by this method consisted mainly of younger girls with higher leucocyte counts, lower platelet counts and an increased frequency of CNS involvement, in agreement with other reports (Reaman *et al*, 1985; Greaves, 1996; Ross & Robinson, 1997). Haemorrhagic conditions and splenomegaly were also frequent presenting features of this subgroup. These unfavourable characteristics mirrored the more primitive state of leukaemic cells in the MLL^+ subgroup, defined by the lack of CD10 expression and co-expression of myeloid antigens (CD15 and Cdw65) (Chen *et al*, 1993; Rubnitz *et al*, 1996).

In previous studies of infant ALL not classified by *MLL* gene status, EFS rates were estimated to range from 25% to 43% (Ishii *et al*, 1991; Chessells *et al*, 1994; Ferster *et al*, 1994; Hilden *et al*, 1995; Bucsky *et al*, 1998; Isoyama *et al*, 1999; Nishimura *et al*, 1999). In those series in which the *MLL* gene rearrangement status was known but therapy was not tailored to this feature, the EFS rates have ranged from 10% to 20% (Heerema *et al*, 1994; Pui *et al*, 1994; Cimino *et al*, 1995; Hilden *et al*, 1995; Taki *et al*, 1996; Reaman *et al*, 1999). Our result, 34·7% ± 7·6% at 3 years, suggests an improved outcome with consistent use of high-dose cytarabine and anthracyclines, and with HSCT in cases with an HLA-matched donor. However, this conclusion must remain tentative because of the marked heterogeneity among infant ALL study populations and methods of determining *MLL* gene status, and because additional relapses may occur with extended follow-up.

In most studies that evaluated prognostic factors in infants with ALL, there were significant inter-relationships among variables such as *MLL* gene rearrangement, lack of CD10 expression, hyperleucocytosis and age < 6 months (Heerema *et al*, 1994; Pui *et al*, 1994; Cimino *et al*, 1995; Hilden *et al*, 1995; Taki *et al*, 1996; Reaman *et al*, 1999). Thus, continued study is needed to ascertain the independent strength of these factors in the context of well-defined treatment programmes. Our Cox multivariate analysis of complete data on a large set of potentially important clinical and biological features demonstrated independent adverse prognostic significance for age < 6 months, in agreement with many authors (Heerema *et al*, 1994; Pui *et al*, 1994; Cimino *et al*, 1995; Hilden *et al*, 1995; Taki *et al*, 1996; Reaman *et al*, 1999), but in contrast to others (Behm *et al*,

1996). Overt CNS disease at presentation was also a major predictor of a worse outcome in *MLL*$^+$ patients. Of eight infants with this complication, seven relapsed within 18 months, despite the intensive use of triple intrathecal chemotherapy.

Unlike previous authors (Heerema *et al*, 1994; Pui *et al*, 1994; Cimino *et al*, 1995; Hilden *et al*, 1995; Taki *et al*, 1996; Reaman *et al*, 1999), we did not find any adverse prognostic significance associated with t(4;11)(q21;q23) compared with other 11q23 chromosome abnormalities or normal karyotypes. This discrepancy may reflect the lack of cytogenetic data on as many as 60% of patients in earlier studies (Heerema *et al*, 1994; Reaman *et al*, 1999) or perhaps a disproportionately high number of infants < 6 months of age in some series (Heerema *et al*, 1994; Pui *et al*, 1994; Cimino *et al*, 1995; Hilden *et al*, 1995; Taki *et al*, 1996; Reaman *et al*, 1999), creating a bias in favour of the t(4;11). Firm conclusions regarding the prognostic significance of specific *MLL* rearrangements and their partner genes will require detailed analysis of larger numbers of cases and more uniform laboratory assessments among study groups. Finally, the initial response to therapy, whether evaluated by the degree of leukaemic cytoreduction in the bone marrow by d 14 of induction therapy or by the reduction in peripheral blood lymphoblasts following a 7 d course of prednisone, has emerged as a powerful independent predictor of outcome in infant ALL (Dordelman *et al*, 1999; Reaman *et al*, 1999). Although data to address the resistance of leukaemic cells to induction therapy were not prospectively collected in the present study, the timing of relapses in the *MLL*$^+$ subgroup (Fig 3A) would suggest that the intrinsic resistance of the leukaemic blast cells to chemotherapy played a major role in the poor prognosis of these infants and may outweigh the influence exerted by either CNS involvement or age < 6 months.

Nineteen of the 42 infants with *MLL* gene rearrangement underwent allogeneic HSCT during first CR. This procedure has been attempted by others as primary treatment for infants with ALL (Emminger *et al*, 1992; Pirich *et al*, 1999; Marco *et al*, 2000), but its precise impact on prognosis has not been established because of the relatively small number of patients with documented *MLL*$^+$ blast cells. Comparison of outcomes obtained with HSCT and chemotherapy alone in our study is difficult because of the high early relapse rate among infants with adverse prognostic features at diagnosis (age < 6 months, CNS involvement, or both), which limited the number of increased-risk patients who were eligible to undergo HSCT in first CR. Such analysis was further complicated by the limited number of transplants attempted, and the variability among donors and conditioning regimens. Nonetheless, there are no compelling data in the medical literature to support a primary role for HSCT in infants with *MLL*$^+$ ALL (Emminger *et al*, 1992; Pirich *et al*, 1999; Marco *et al*, 2000). Whether this procedure would offer a prognostic advantage in patients < 6 month of age or with initial CNS involvement is an intriguing possibility that warrants further investigation.

Most of the relapses in the *MLL*$^+$ group were scheduled to undergo HSCT, raising questions about the timing of transplantation. For some patients, HSCT may offer a greater chance for cure if it is scheduled immediately after remission induction therapy or perhaps during this phase of treatment.

The prognosis for infants without an 11q23/*MLL* gene rearrangement is unclear. In several reports, the estimated EFS rates for this subgroup range from 42% to 80% (Chen *et al*, 1993; Rubnitz *et al*, 1994; Silverman *et al*, 1997). Despite the relatively small patient sample and limited follow-up, our estimate of 92·3% at 3 years post diagnosis appears to represent a substantial improvement over previous experience, suggesting that careful segregation of *MLL*$^-$ from *MLL*$^+$ cases, followed by standard intensive chemotherapy for high-risk childhood B-lineage ALL, is an effective strategy of management for infant leukaemias lacking a rearranged *MLL* gene. In conclusion, our data demonstrate the value of early molecular detection of *MLL* gene rearrangements, allowing the selection of appropriate risk-directed therapies that may improve prognosis in infant ALL overall. Novel therapeutic initiatives are needed to overcome the problem of early drug resistance in the subgroup of patients with rearranged *MLL* genes and either initial CNS involvement or age < 6 months, or both.

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APPENDIX

The investigators who registered patients with ALL in the Japan Infant Leukaemia Study Group were as follows:

Asahikawa Medical College (H. Higashi); Chiba University School of Medicine (Y. Noguchi, T. Sato); Daisan Hospital, Jikei University School of Medicine (Y. Takano); Ehime University School of Medicine (K Ohtoh, Y. Ishida); Gunma Children's Medical Center (T. Shitara); Hamamatsu University School of Medicine (T. Hongo); Hamanomachi Hospital (E. Ishii); Hirosaki University School of Medicine (J. Kitazawa, E. Itoh); Hyogo Prefectural Children's Hospital (H. Misu); Ibaraki Children's Medical Center (M. Tsuchida); Japanese Red Cross Himeji Hospital (M. Miyoshi); Japanese Red Cross Kitami Hospital (M. Hasegawa); Japanese Red Cross Nagoya First Hospital (K. Kato, T. Matsuyama); Japanese Red Cross Nagoya Second Hospital (M. Ishii); Japanese Red Cross Ohtsu Hospital (T. Takimoto); Juntendo University School of Medicine (M. Saito, H. Fujita); Kanagawa Children's Medical Center (K. Koh, Y. Toyoda); Kansai Medical University (U. Kohdera); Kawasaki Medical School (S. Ogita); Keio University School of Medicine (A. Kinoshita); Kobe City General Hospital (T. Tsutsui, M. Kubota); Kobe University School of Medicine (Y. Kosaka); Kurashiki Central Hospital (I. Kawamura); Kurume University School of Medicine (H. Inada, H. Eguchi); Kyoto Prefectural University of Medicine (S. Hibi); Kyoto University School of Medicine (Y. Akiyama); Matsudo City Hospital (I. Komori); Miyazaki Medical College (Y. Horii, H. Kuroda); Nagoya University School of Medicine (K. Horibe, S. Kojima); Nara Medical University School of Medicine (Y.-D. Park); National Children's Hospital (M. Kumagai, Y. Tsunematsu); National Kyushu Cancer Center (Y. Nagatoshi, J. Okamura); National Tochigi Hospital (S. Tanitsu, T. Ishii); Nihon University Itabashi Hospital (H. Mugishima, M. Chen); Nippon Medical School (M. Maeda); Ohta Nishinouchi Hospital (M. Hiwatari); Okayama University Faculty of Medicine (M. Oda); Osaka Medical Center and Research Institute for Maternal and Child Health (T. Okamura, K. Kawa); Osaka University Faculty of Medicine (J. Hara); Sapporo Medical University (T. Oda, T. Kudo); Sinshu University School of Medicine (K. Sakashita, K. Koike); Showa University Fujigaoka Hospital (K. Isoyama); Yokohama City University School of Medicine (K. Ikuta); Teikyo University Mizonokuchi Hospital (A. Ishiguro); The University of Tokyo, Faculty of Medicine (H. Ohnishi, Y. Hayashi); Tohoku University, School of Medicine (M. Imaizumi); University of Occupational and Environmental Health (J. Kamizono); Yamaguchi University School of Medicine (K. Kawasaki, K. Ayukawa).

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Outcome after Relapse in Childhood Acute Lymphoblastic Leukemia

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Abstract

Among 157 children with acute lymphoblastic leukemia (ALL) who experienced relapse at 54 institutes participating in the Japan Association of Childhood Leukemia Study, we analyzed the outcomes after relapse in 103 and 30 eligible cases with bone marrow (BM) and central nervous system (CNS) relapse, respectively. Reinduction rates in BM and CNS relapse cases were 72.3% and 83.3%, respectively. High reinduction rates were observed in B-precursor (B-pre) phenotype ALL in both relapse groups and in late (more than 24 months from onset) BM-relapse patients. After BM relapse, the overall 5-year survival rate was superior in the allogeneic stem cell transplantation (SCT) group compared to the non-SCT group (41.9% ± 8.2% versus 13.6% ± 6.5%, $P < .0001$). In contrast, the 4-year overall survival rate was not significantly different between the SCT (allogeneic plus autologous) and non-SCT groups after CNS relapse (26.8% ± 14.2% versus 61.9% ± 12.3%, $P = .252$). The late BM-relapse patients showed a significantly higher survival rate than did early-relapse patients, and survival rates were similar between the allogeneic and autologous group when the patients underwent SCT during a second complete remission. Moreover, B-pre ALL patients classified in the standard-risk group according to National Cancer Institute/Rome's criteria at onset had a good prognosis after allogeneic SCT. Improving the cure rate in relapsed ALL patients requires more intensive reinduction therapy and efforts to succeed with SCT in early BM-relapse patients as well as the establishment of a treatment strategy including indications of SCT for CNS-relapse patients. *Int J Hematol.* 2002;76:61-68.
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Key words: Acute lymphoblastic leukemia; Relapse; Stem cell transplantation; Children

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common hematological malignancy in childhood. The development of systematic treatments by group studies has improved the survival rate in pediatric ALL cases up to 75% [1,2]. However, a cure remains difficult once patients have a relapse [3]. Because the cure rates in relapsed patients are different according to the site of relapse or duration of first remission, it remains unclear which treatment after relapse should be chosen, stem cell transplantation (SCT) or chemotherapy. Therefore, the strategy for treatment after relapse differs among institutes. The establishment of suitable treatments for relapsed patients is important to improve overall survival in childhood cases of ALL. Since April 1997, small

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Table 1
Summary of Relapsed Patients*

	Bone Marrow Relapse	Central Nervous System Relapse	Extramedullary Relapse
No. of patients	117	32	8
Male:female	75:42	22:10	8:0
Isolated	95	30	8
Combined	22	2	0
B-precursor	102	25	7
Ph1	4	1	0
T/AUL/Mix	11	6	1

*Ph1 indicates Philadelphia chromosome; T, T-cell acute lymphoblastic leukemia; AUL, acute undifferentiated leukemia; Mix, mixed lineage leukemia.

ALL study groups in the Hokkaido, Tokai, Kinki, Chugoku, and Shikoku areas have been united as the Japan Association Childhood Leukemia Study (JACLS) group and used in a protocol study for newly diagnosed ALL patients. To design a study for relapsed ALL patients, we analyzed retrospectively the actual outcome of relapsed ALL patients at 54 institutes participating in the JACLS.

2. Patients and Methods

2.1. Patients

Between January 1991 and March 1997, 739 patients with a diagnosis of ALL at 54 institutes participating in the JACLS met the following criteria: (1) age at onset older than 1 year and younger than 16 years, and (2) non–B-cell ALL. All patients received chemotherapy according to the protocols of each institute. Excluding relapse after SCT, 157 patients had experienced relapse as of December 31, 1998: 117 patients with bone marrow (BM) relapses (including 22 of combined BM with extramedullary relapses), 30 with isolated central nervous system (CNS) relapses, 2 with CNS combined with other extramedullary relapses, and 8 with isolated extramedullary relapses other than CNS (Table 1). Information about the treatment and outcome after relapse up to March 1, 1999, was available in 103 cases with BM relapse and 30 cases with isolated CNS relapse.

2.2. Treatment after Relapse

All relapsed patients were treated according to the strategy of the institute involved. Sixty-three patients with BM relapse and 14 patients with CNS relapse underwent SCT after relapse (SCT group). The remaining 40 patients with BM relapse and 16 patients with CNS relapse were treated by chemotherapy alone or chemotherapy combined with radiotherapy (non-SCT group). In the BM-relapse group, SCT was allogeneic in 49 patients (allo-SCT group) and autologous in 14 patients (auto-SCT group). Allo-SCT consisted of 23 HLA-matched related donor (MRD) SCTs, 20 HLA-matched unrelated donor (MUD) SCTs, and 6 HLA-mismatched related (MMRD) SCTs. Of the MMRD SCTs, 4 were transplantations of selected $CD34^+$ cells (CD34-SCT) because the donors had 2 or 3 HLA loci that were different from those of their recipients. Two patients who underwent SCT from 1-locus mismatched family members were included in the MRD-SCT group in the following analyses. The source of stem cells in auto-SCT was BM and peripheral blood in each of the 7 patients. In CNS relapse, 9 SCTs were allogeneic (5 related donor SCTs and 4 unrelated donor SCTs), and 5 were autologous.

2.3. Methods of Analysis

In these 133 cases, we estimated the reinduction and survival rates in each group of relapsed patients classified by sites of relapse, duration from onset (<24 months and ≥24 months), phenotype of leukemia (B-precursor [B-pre] and non–B-pre ALL), initial risk assessment in B-precursor ALL, and treatments after relapse (allo-SCT, auto-SCT, or non-SCT). Initial risk assessments were defined as standard risk (SR) and high risk (HR) according to the National Cancer Institute/Rome criteria, which define patients 1 to 9 years of age with white blood cells counts of less than 50,000/μL at diagnosis as SR and the rest as HR [4]. In addition, we analyzed the outcome of the patients who received SCT after relapse and compared stem cell sources and status at SCT. The survival rates were calculated as overall survival (OS) rates after relapse in SCT and non-SCT groups, because some patients who did not achieve a second complete remission (CR2) or had a second relapse before receiving SCT were rescued by SCT and the cause of all deaths but one in the non-SCT group was leukemia. Event-free survival (EFS) rates of the patients who achieved CR2 and were treated by chemotherapy or SCT were calculated. Moreover, EFS rates after SCT were calculated in comparing stem cell sources or initial risk assessments.

Kaplan-Meier methods were used for the estimation of survival after relapse, and statistical analysis was performed by a log-rank test. The statistical difference of the reinduction rates was analyzed by a chi-square test.

3. Results

3.1. BM-Relapse Patients

3.1.1. Relapse Site, Duration from Onset, and Phenotype

Of 103 patients with BM relapse, 87 relapsed in BM alone, and 16 relapsed simultaneously in BM and extramedullary sites (Table 2). B-pre ALL was diagnosed in 92 patients. In 9

Table 2
Patient Characteristics*

	Bone Marrow Relapse Group				Central Nervous System Relapse Group		
	Allo-SCT Group	Auto-SCT Group	Non-SCT Group	*P*	SCT Group	Non-SCT Group	*P*
No. of patients	49	14	40		14	16	
Male:female	40:23	7:7	25:15	.64	10:4	10:6	.6
Type of leukemia				.68			.19
B-precursor	45	13	34		13	12	
Standard risk	31	8	22		8	8	
High risk	14†	5	12†		5†	4	
T/AUL	4	1	6		1	4	
Duration from onset				.06			.6
<24 mo	21	5	26		10	12	
≥24 mo	28	9	14		4	4	
Achieved CR2				<.001			.022
Yes	38	14	21		14	11	
No	11	0	19		0	5	

*Allo indicates allogeneic; SCT, stem cell transplantation; auto, autologous; T, T-cell acute lymphoblastic leukemia; AUL, acute undifferentiated leukemia; CR2, second complete remission.

†Including each case with Philadelphia chromosome–positive acute lymphoblastic leukemia.

patients, T-cell ALL (T-ALL) was diagnosed, and in 2 patients acute undifferentiated leukemia (AUL) was diagnosed. In the B-pre ALL group, 2 patients had Philadelphia chromosome–positive ($Ph1^+$) ALL. Twenty-one patients in the allo-SCT group, 5 in the auto-SCT group, and 26 in the non-SCT group relapsed within 24 months of onset (early relapse). The remaining 51 patients relapsed after 24 months (late relapse). Seventy-seven percent of patients in the allo-SCT group and all auto-SCT group patients achieved CR2, whereas half the patients in the non-SCT group obtained CR2. The sex ratio and proportion of phenotypes among the 3 groups did not differ, but the non-SCT group had a significantly higher proportion of non-CR2 patients ($P < .001$) and tended to have a higher proportion of early-relapse patients ($P = .06$) than did the SCT group.

3.1.2. Reinduction Rate

A total of 101 patients received reinduction chemotherapy, and 73 achieved CR2 (Table 3). In 2 cases, the parents refused aggressive reinduction therapy. In the early-relapse group, the reinduction rate was significantly lower than that of the late-relapse group (51.9% versus 90.2%, $P < .001$). B-pre ALL had a significantly higher reinduction rate than T-ALL and AUL (77.8% versus 27.3%, $P < .001$). Among patients with B-pre ALL, those at HR at onset showed a 70.0% reinduction rate, which was slightly lower than that of SR patients (81.6%), but this difference was not significant.

3.1.3. OS and EFS Rates

The 5-year OS rate after relapse for all BM-relapse patients was 32.9% ± 5.4%. Of 40 patients in the non-SCT group, only 6 remained in CR from 11 to 74 months after relapse. The remaining patients but one died, and their 5-year OS rate was only 13.6% ± 6.5%. The 5-year OS rates were 41.9% ± 8.2% in the allo-SCT group and 59.8% ± 14.0% in the auto-SCT group, which were significantly superior to the rates of the non-SCT group ($P < .0001$, Figure 1). All surviving patients in the SCT group remained in CR. The EFS rate of the non-SCT patients who achieved CR2 was also significantly inferior to that of the patients who underwent allo-SCT in CR2 (21.8% ± 10.1% versus 58.3% ± 9.3%, $P = .001$). In the allo-SCT and non-SCT groups, the 5-year OS rates of early-relapse patients were significantly inferior to those of the late-relapse patients

Table 3
Reinduction Rates*

	Bone Marrow Relapse Group			Central Nervous System Relapse Group		
	No. of Patients	CR2+ (%)	*P*	No. of Patients	CR2+ (%)	*P*
Type of leukemia						
B-precursor ALL	90	70 (77.8)		25	23 (92.0)	
Standard risk	60	49 (81.6)		14	14 (100.0)	
High risk	30	21 (70.0)	.0004	11	9 (81.8)	.0016
T/AUL	11	3 (27.3)		5	2 (40.0)	
Duration of onset			<.0001			.7
<24 mo	50	27 (51.9)		22	18 (81.8)	
≥24 mo	51	46 (90.2)		8	7 (87.5)	

*CR2 indicates second complete remission; ALL, acute lymphoblastic leukemia; T, T-cell acute lymphoblastic leukemia; AUL, acute undifferentiated leukemia.

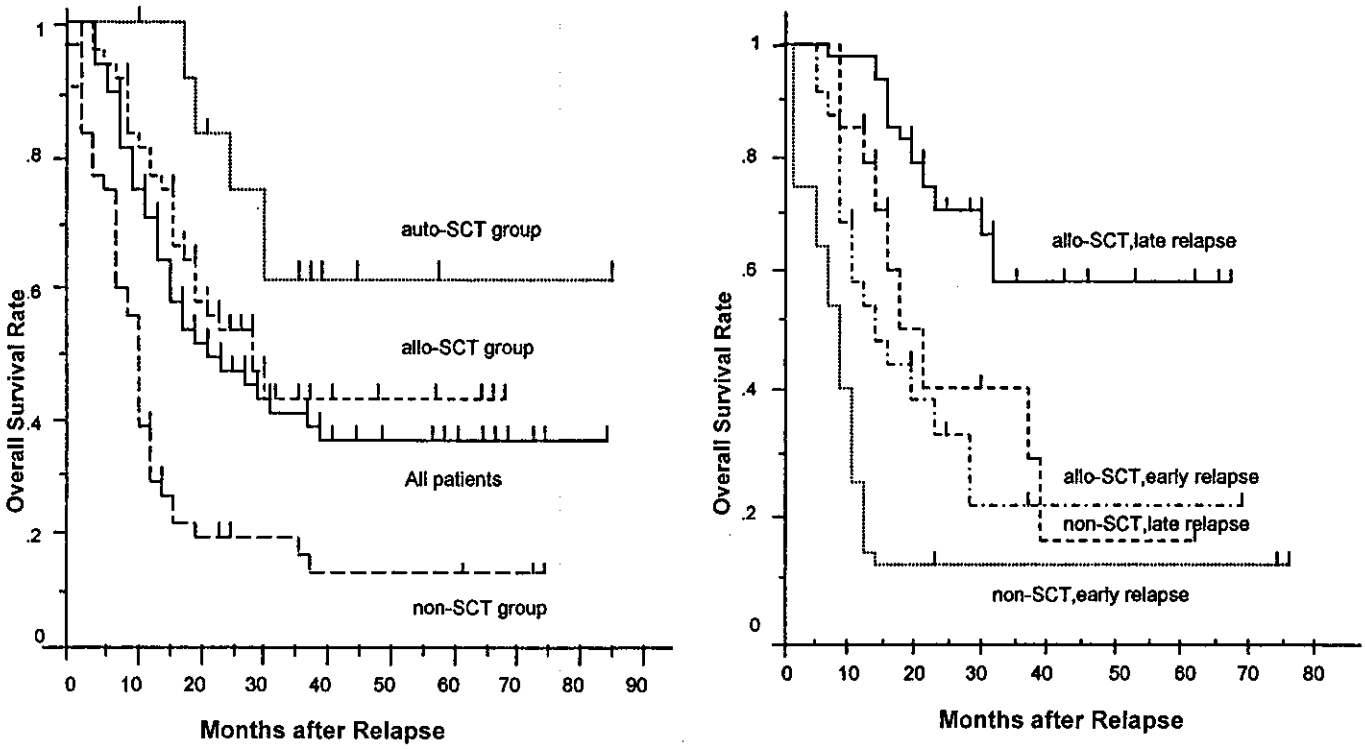

Figure 1. Kaplan-Meier analyses of the overall survival (OS) rates in bone marrow–relapse patients. Left, The OS curves of all patients; patients underwent allogeneic stem cell transplantation (allo-SCT group) or autologous stem cell transplantation (auto-SCT group) or did not undergo transplantation (non-SCT group). The OS rates of the allo- and auto-SCT groups are significantly superior to that of the non-SCT group. Right, The survival curves of early-relapse and late-relapse patients in the allo-SCT and non-SCT groups. The survival rates of late-relapse patients are significantly superior to those of early-relapse patients in both the allo-SCT and the non-SCT groups ($P < .005$ in the SCT group and $P < .01$ in the non-SCT group).

(19.5% ± 10.6% versus 57.1% ± 11.1% in the SCT group, $P = .001$; 11.1% ± 6.0% versus 13.2% ± 11.9% in the non-SCT group, $P = .04$) (Figure 1).

3.1.4. Outcomes of Patients in the SCT Group

Tables 4 and 5 show the outcomes of patients who underwent SCT. Only 1 of 5 patients with the T/AUL phenotype remained in CR at 30 months after MUD-SCT. The 5-year EFS rate after allo-SCT for all patients with B-pre ALL was 41.7% ± 8.5%. (Figure 2). Comparing initial risk assessment, 19 of 31 SR patients and 3 of 14 HR patients remained in CR. The EFS rate of SR patients was significantly superior to that of HR patients (55.8% ± 10.2% versus 9.9% ± 9.2%, $P = .002$). Concerning the kinds of SCT, 23 of 45 patients with HLA-matched allo-SCT and 8 of 14 patients with auto-SCT remained in CR from 2 to 54 months after SCT. None of the CD34-SCT group survived. Among early-relapse patients with B-pre ALL, only 1 of 5 in the auto-SCT group and 5 of 21 in the allo-SCT group remained in CR. About half of the patients with early relapse underwent SCT in non-CR status. However, among patients who underwent SCT in CR2, the EFS rate for early-relapse patients was inferior to that of late-relapse patients (44.4% ± 16.6% versus 73.8% ± 9.4%, $P = .002$). Among late-relapse patients, most underwent SCT in CR2, and there was no significant difference in EFS rate among the MRD-, the MUD-, and the auto-SCT groups (Figure 2).

3.2. Isolated CNS-Relapse Patients

3.2.1. Duration from Onset, Phenotype, and Treatment

Thirty patients had an initial relapse at the site of CNS (Table 2). Five patients had the T-phenotype, and the remainder had the B-pre phenotype. Sixteen patients were treated with radiotherapy and/or chemotherapy after relapse. Early-relapse patients numbered 10 in the SCT group and 12 in the non-SCT group. Thirteen of 25 patients with B-pre ALL and 1 of 5 patients with T-ALL underwent SCT, 5 from related and 4 from unrelated donors. The other 5 patients received auto-SCT. Between the SCT and non-SCT group, the sex, phenotype, and duration from onset to relapse did not differ. All patients in the SCT group achieved CR2, whereas only 5 of 16 patients in the non-SCT group did not achieve CR2 ($P = .02$).

3.2.2. Reinduction Rate

All but 2 patients with B-pre ALL and only 2 patients with T-ALL achieved CR2 after reinduction therapy (Table 3). The reinduction rates were 92.0% and 40.0% in

Table 4
Treatment and Outcome after Relapse*

	Allo-SCT Group		Auto-SCT Group		Non-SCT Group	
	No.	CCR	No.	CCR	No.	CCR
BM-relapse group						
Type of leukemia						
B-precursor ALL	45	22	13	8	34	6
Standard risk	33	19	8	5	22	5
High risk	12	3	5	3	12	1
T/AUL	4	1	1	0	6	0
Duration from onset						
<24 mo	21	5	5	1	26	2
≥24 mo	28	17	9	7	14	4
CNS-relapse group						
Type of leukemia						
B-precursor ALL	8	2	5	1	12	10
Standard risk	4	1	4	1	8	8
High risk	4	1	1	0	4	2
T/AUL	1	0	0	0	4	0
Duration from onset						
<24 mo	6	2	4	1	12	7
≥24 mo	2	1	1	0	4	3

*Allo indicates allogeneic; SCT, stem cell transplantation; auto, autologous; CCR, continuous complete remission; BM, bone marrow; ALL, acute lymphoblastic leukemia; T, T-cell acute lymphoblastic leukemia; AUL, acute undifferentiated leukemia; CNS, central nervous system.

B-pre and T-ALL, respectively ($P < .01$). In B-pre ALL, the reinduction rate was 100% in SR patients and 81.8% in HR patients, but this difference was not significant.

3.2.3. OS Rate

Tables 4 and 5 show the outcome for CNS-relapse patients. Fourteen of 30 patients remained in CR from 14 to 69 months after relapse. In the SCT group, only 4 patients remained in CR, and the rest died. The 4-year OS rate after relapse in the SCT group was 26.8% ± 14.2%. One of the patients who remained in CR underwent auto-SCT in CR2 but experienced subsequent BM relapse and then was rescued by allo-SCT. In the non-SCT group, 10 of 16 patients remained in CR. All 10 patients had the B-pre phenotype, and 6 patients had not received prior prophylactic cranial radiation before relapse. The 4-year OS rate in the non-SCT group was 61.9% ± 12.3%, which was not significantly different from that of the SCT group ($P = .252$). The duration of first remission did not influence the prognosis of CNS-relapse patients. Ten of 22 patients in the early-relapse group and 3 of 8 patients in the late-relapse group remained in CR.

4. Discussion

Survival after relapse in children with ALL is strongly influenced by the site of relapse and the duration of first CR [3,5]. The Children's Cancer Group study reported a high substantial survival rate after relapse in isolated or combined BM-relapse patients with a first CR longer than 36 months and in isolated extramedullary-relapse patients irrespective of the duration of first CR [5]. The duration of first CR that

Table 5
Outcomes of Relapsed Patients Who Underwent SCT*

	Bone Marrow Relapse Group				Central Nervous System Relapse Group			
	CR2 at SCT		Non-CR2 at SCT		CR2 at SCT		Non-CR2 at SCT	
	No.	CCR	No.	CCR	No.	CCR	No.	CCR
Kinds of SCT								
MRD	14	11	11	1	2	0	2	1
MUD	15	8	5	3	2	1	1	1
CD34	2	0	2	0	1	0	0	0
Autologous	11	7	3	1	3	1†	2	0
Duration from onset								
<24 mo	12	4	14	2	5	2†	5	1
≥24 mo	30	22	7	3	3	0	1	1

*SCT indicates stem cell transplantation; CR2, second complete remission; CCR, continuous complete remission; MRD, HLA-matched related donor; MUD, HLA-matched unrelated donor; CD34, CD34⁺ cells transplantation.

†Including 1 patient who underwent autologous bone marrow transplantation at CR2 and was rescued by allogeneic bone marrow transplantation after relapse.

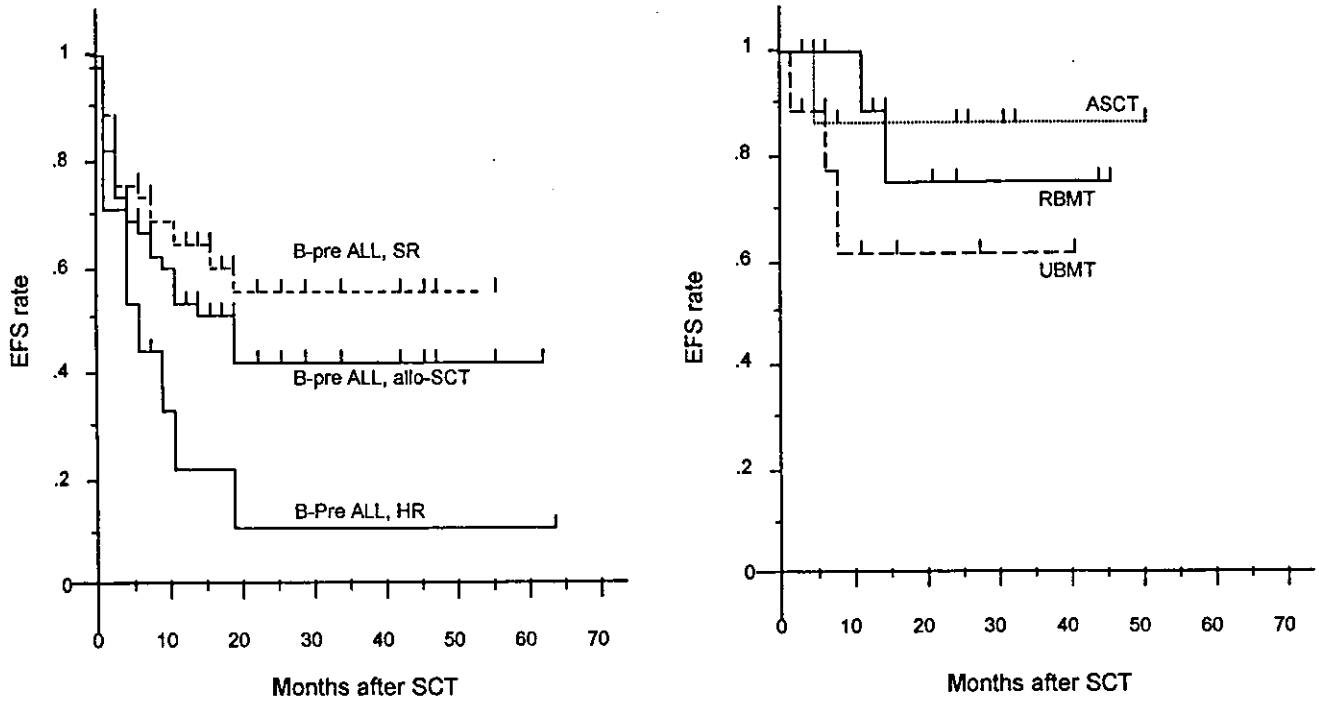

Figure 2. Kaplan-Meier analyses of the event-free survival (EFS) rates in the patients with B-precursor (B-pre) acute lymphoblastic leukemia (ALL) who underwent stem cell transplantation (SCT). Left, The EFS curves of patients who underwent allogeneic SCT (allo-SCT). The EFS rate of the patients with standard risk (SR) is significantly superior to that of high-risk (HR) patients. Right, The EFS rate in late-relapse patients who underwent SCT while in a second complete remission. The difference in the EFS rates is not significant among autologous SCT (ASCT), related bone marrow transplantation (RBMT), and unrelated bone marrow transplantation (UBMT).

influenced survival varied among previous reports, as 18, 24, 30, and 36 months from onset [5-8]. This study included few patients who relapsed after 36 months. Therefore, we compared only 2 groups who relapsed within 24 and after 24 months. In the BM-relapse group, a significant difference in the survival rate was seen between these 2 groups regardless of the treatment after relapse. The phenotype also predicts the prognosis after relapse. In the Berlin-Frankfurt-Münster (BFM) study, no T-ALL patients who relapsed within 18 months of onset survived beyond 15 months after relapse [6]. The BFM group also showed that a positive Ph1 chromosome is an independent risk factor in relapsed ALL patients [9]. Similar to these findings, all patients with T-ALL and $Ph1^+$ ALL in the present study suffered from subsequent BM relapse and died. The $Ph1^+$ ALL patients numbered only 2 in the present study, because relapsed patients after SCT were excluded.

In BM relapse, several studies on chemotherapy versus SCT for patients in CR2 have been reported [10-15]. The survival rate of patients who undergo allo-SCT after relapse is generally superior to that of patients treated with chemotherapy [12-15]. The efficacy of allo-SCT is significant in early BM-relapse patients. EFS rates in the patients who relapsed within 24 or 36 months after onset and were treated with chemotherapy alone were reported to range from only 10% to 22%, whereas the EFS rates in the SCT group were 35% to 56%. However, the efficacy of SCT remains unclear in late-relapse patients. It was reported that intensive chemotherapy resulted in a 24% to 65% EFS rate in patients who relapsed more than 6 months after discontinuation of the therapy [5-8,16,17]. In the present study, the 5-year OS rate of late-relapse (after 24 months from onset) patients in the non-SCT group was only 13.2% and significantly inferior to that of the allo-SCT group (57.1%). This disparity might have been due to the following factors. First, the late-relapse patients in the present study included patients who relapsed during therapy because of the various lengths of the initial chemotherapy. Second, the chemotherapy that patients received after relapse also varied. Moreover, because this is a retrospective study, there might be a selection bias between the SCT group and the chemotherapy group. Recently, it was reported that the diversity of the source of stem cells influenced the outcome of SCT [18]. However, in the present study, the EFS rates of 3 kinds of SCTs (matched related, matched unrelated, and autologous) were similar in the late-relapse patients. This finding is in consistent with previous observations [19-21]. These findings indicate that auto-SCT is one therapeutic option for late-relapse patients. Further randomized studies may be required to clarify the best treatment for such patients.

In early BM-relapse patients, the prognosis is poor despite SCT after relapse. In the present study, 16 early-relapse patients with B-pre ALL underwent allo-SCT, and only 4 remained in CR. However, 4 of 6 patients who underwent SCT in CR2 remained in CR. The causes of failure were relapse and transplantation-related mortality for each of the

4 patients. These findings suggest that intensive chemotherapy that sustains the status of CR2 until SCT, less toxic preparative conditioning regimens for SCT, and alternative therapies such as immunotherapy after SCT may be required to improve the outcome of early-relapse patients.

This study showed that the outcome after allo-SCT from HLA-matched donors was also influenced by initial risk assessment in B-pre ALL. In patients who underwent BMT from either MRD or MUD, SR patients had a significantly higher survival rate than HR patients. In 12 HR patients, only 3 remained in CR, and 2 of them were late-relapse patients. Other studies also demonstrated that the outcome of SCT differed with the initial leukocyte counts [10-15]. Taking these findings together leads to the conclusion that it is very difficult to rescue HR patients with B-pre ALL if they experience BM relapse early after onset. Recent studies showed that detection of minimal residual disease during the early phase of first treatment is a good marker for predicting a subsequent relapse [22,23]. Therefore, it may be better that HR patients undergo SCT during first CR based on minimal residual disease.

Intensive CNS-directed treatment including high-dose methotrexate and triple intrathecal therapy reduced the rate of CNS relapse to only a few percent among ALL patients [24,25]. Moreover, CNS-relapse patients who had not received prior prophylactic cranial irradiation showed a higher survival rate after relapse than those who had received prior irradiation [5]. For the treatment of isolated CNS relapse, it has been reported that systemic chemotherapy and cranial irradiation with triple intrathecal therapy improved survival [26,27]. In isolated CNS relapse, the role of SCT remains unclear. Indeed, only 3 of 13 patients who underwent SCT in the present study remained in CR. The causes of failure were transplantation-related mortality and relapse. In SR patients, 8 of 9 patients treated by systemic and intrathecal chemotherapy and/or irradiation survived. This finding suggests that systemic and CNS-directed therapy may be adequate to cure SR patients who suffer an isolated CNS relapse. For HR patients with B-pre ALL and non–B-pre ALL, further study is required to determine which treatment is feasible for the first isolated CNS relapse.

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Appendix: Participating Principal Investigators in the JACLS as of March 1, 1999

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Nagoya City University Medical School	Makoto Yazaki
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Minimal Residual Disease in Early Phase of Chemotherapy Reflects Poor Outcome in Children With Acute Lymphoblastic Leukemia—A Retrospective Study by the Children's Cancer and Leukemia Study Group in Japan

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We analyzed the minimal residual disease (MRD) in 50 children with acute lymphoblastic leukemia (ALL) by amplifying the clonally rearranged T-cell receptor (TCR) γ/δ chain and/or immunoglobulin (Ig) κ chain gene using the allele-specific-PCR method. All children were treated according to the protocols of the Children's Cancer and Leukemia Study Group of Japan (CCLSG). The patients were stratified into four risk-groups according to the leukocyte count and age at diagnosis. We prospectively sampled the patients' bone marrow at 1 month (point 1) and 3 months (point 2) after the initiation of chemotherapy and quantitated the MRD retrospectively. The results of MRD were closely related with the clinical outcome. The relapse rate of the patients MRD-positive at points 1 and 2 was 46% (6/13) and 86% (6/7), respectively, whereas those MRD-negative results at point 1 and 2 were 13% (3/13) and 3% (3/30), respectively. We found significant differences in the event-free survival between MRD-positive children and MRD-negative children like the reports, which have been made by BFM and EORTC groups. We conclude that MRD in an early phase of chemotherapy can be a good predictor of the prognosis of childhood ALL regardless of the protocol of chemotherapy or race.

Keywords: Acute lymphoblastic leukemia (ALL); Childhood leukemia; Minimal residual disease (MRD); T-cell receptor gene; Immunoglobulin gene

INTRODUCTION

To date, more than 90% of children with acute lymphoblastic leukemia (ALL) achieve complete remission and more than 70% have a long-term, relapse-free survival [1–4]. Modulation of the therapy based on the tumor burden may help to further raise the cure rate, and reduce the risk of side effects, such as secondary malignancies and sterility, due to the overdosed chemotherapy [5–8]. Intensified therapy might contribute to eradicate the residual leukemic cells. Alternatively, reduction of therapy based on the negative results might improve the risk for therapy-related toxicity. The number of leukemic cells in minimal residual disease (MRD) is not large enough to observe by conventional microscopy.

In this decade, accurate and very sensitive quantitation of MRD became possible by amplifying tumor-specific chimeric transcripts and clonally rearranged genes of T-cell receptor (TCR) γ/δ and immunoglobulin (Ig) heavy/light chains in ALL patients [9–16]. It is a

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powerful tool to estimate the tumor burden which remains even after intensive chemotherapy [17,18].

Among the number of tumor markers, the junctional sequences of rearranged genes for Ig and TCR have been exploited as valuable clonal markers for MRD in childhood ALL, because childhood ALL lacks common leukemia-specific chimeric transcripts such as BCR/ABL which is found in more than 30% of ALL in adulthood [19,20]. Amplification of rearranged TCR/Ig gene using the allele-specific oligonucleotide (ASO) which is complementary to the junctional sequences allow us to detect one leukemic cell in 10^3–10^6 normal cells. Recently, several groups have reported that MRD in the early phase of chemotherapy is an independent prognostic factor in childhood ALL [21–23]. They addressed the possibilities that ALL in children can be stratified for their risks according to the amount of MRD at completion of induction therapy. In this study, we verified the relation between MRD and prognosis of ALL in Japanese children. We also considered the possibility of applying this method to a new strategy of chemotherapy which includes the modification of post-remission protocol according to the MRD level.

MATERIALS AND METHODS

Patients and Chemotherapy

Fifty-eight children with ALL were enrolled in the retrospective analysis for MRD in this study. They were selected based on the following criteria, enrolled in the 911-protocol of Children's Cancer and Leukemia Study Group in Japan (CCLSG) [3], and bone marrow cells sampled at 1 or 3 months after the initiation of the chemotherapy available. The diagnosis of ALL was based on morphologic, cytochemical, and immunological characteristics of leukemic cells. Immunophenotyping using flowcytometry was performed in all patients. We classified the ALL into five groups, undifferentiated ALL (CD19+, CD10 −), common ALL (CD19+, CD10+), Pre-B ALL (CD19+, CD10+, cIgμ+), B-ALL (sIg+, sIg κ or λ+), and T-ALL (CD2+, CD5+).

According to the white blood cell count and age at the time of diagnosis, they were classified into four groups, namely high-high-risk group (HHRG), high-risk group (HRG), intermediate-risk group (IRG), and low-risk group (LRG). The stratification of patients and regimens are schematically indicated in Table I and Fig. 1.

The induction protocol for LRG, IRG and HRG was VLPA consisting of vincristine (VCR), prednisolone (PSL), pirarubicin hydrochloride (THP) and L-asparaginase (L-Asp). For HHRG patients, VP-16 and cytocine arabinocide (Ara-C) was added after a VLPA. After the induction was achieved in IRG, HRG, and HHRG, CCM consisting of cyclophosphamide (CPM), Ara-C, and mercaptopurine (6-MP) was administered followed by re-induction with VLPA, and another CCM. After the induction was achieved in LRG, a high-dose methotrexate (MTX) was administered three times followed by VPMA-M, consisting of VCR, PSL, THP, 6-MP and MTX every 5 weeks.

TABLE I Stratification of children with ALL by CCLSG

WBC (/L)	Age (years old)			
	1–4	4–6	6–10	>10
<5000	LRG	LRG	IRG	HRG
5001–10,000	LRG	IRG	HRG	HRG
10,001–50,000	IRG	IRG	HRG	HRG
50,001–100,000	HRG	HRG	HRG	HRG
100,000 <	HHRG	HHRG	HHRG	HHRG

Bone marrow cells for the MRD quantitation were sampled at one and/or three months after the initiation of chemotherapy. In all cases, point 1 falls in the recovery phase after the induction therapy, VLPA. Point 2 is just before the beginning of the second CCM in IRG, HRG, and HHRG. In LRG, point 2 bone marrow was sampled at the recovery phase of the third high-dose MTX. The MRD analysis was made in case the sampled bone marrow was in morphological CR by microscopic observation.

Screening for the Clonal Rearrangements of TCR and Ig Genes by Heteroduplex Analysis

High-molecular weight DNA was extracted according to a standard method from heparinized bone marrow cell samples and bone marrow smears in some cases [15,24]. The samples for screening of tumor markers were collected at diagnosis, when more than 80% of blasts were contained.

All samples were investigated for the major recombination patterns of TCRδ, γ, and Ig κ chain genes. For the TCR δ chain gene, six major recombinations, Vδ2–Dδ3,

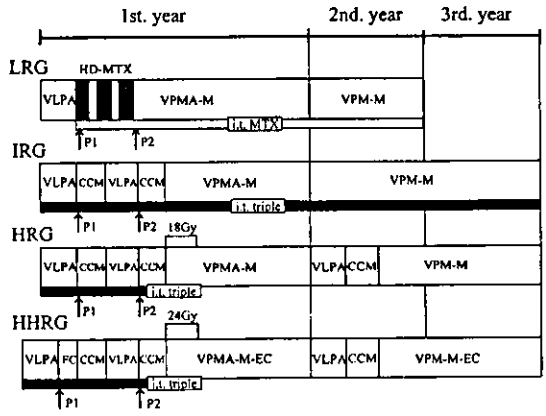

FIGURE 1 Scheme of protocols of CCLSG ALL 911. The scale in the figure is not the precise time. VLPA; VCR, L-asparaginase, PSL, and THP, HD-MTX; high-dose MTX, VPMA-M; VCR, PSL, 6-MP, THP, and MTX, VPM-M; VCR, PSL, 6-MP, and MTX, CCM; CPM, Ara-C, and 6-MP, EC; VP-16 and Ara-C, VPMA-M-EC; VPMA-M plus EC. Boxes with oblique lines show cranial irradiation. Arrows indicate the points for bone marrow sampling, P1; point 1, and P2; point 2. i.t., Intrathecal injection; triple, MTX, Ara-C and PSL.

Dδ2–Dδ3, Dδ2–Jδ1, Vδ2–Jδ1, Vδ1–Jδ1, and Vδ3–Jδ1, were screened. For the TCR γ chain gene, Vγ I–$Jγ_{1.1/2.1}$, Vγ I–$Jγ_{1.3/2.3}$, Vγ II–$Jγ_{1.3/2.3}$, Vγ III–$Jγ_{1.3/2.3}$, Vγ IV–$Jγ_{1.1/2.1}$, and Vγ IV–$Jγ_{1.3/2.3}$, and for the Ig κ chain gene, Vκ I–κ deleting element (κDE), Vκ II–κDE, Vκ III–κDE, Vκ IV–κDE, and RS–κDE were screened.

For the screening of these recombinations, we used the primers described previously by us and others [15,16,24–26]. For TCR γ/δ and Ig κ chain gene, we adopted the multiplex PCR method [26,27].

We determined the monoclonality of rearranged TCR γ/δ and Ig κ genes by heteroduplex PCR strategy [28]. PCR was essentially performed as described previously [15]. In each 100 μl of reaction mixture, 500 ng of high-molecular weight DNA, 200 mM dNTP, 20 pmol of forward and reverse oligonucleotide primers, 10 mM Tris–HCl pH 8, 30 mM $MgCl_2$, 50 mM KCl, and 1 U of Taq DNA polymerase (Takara Shuzo, Shiga, Japan), were contained. The PCR program consisted of an initial 5 min denaturing at 94°C followed by 35 cycles of amplification, 30 s at 94°C, 1 min at 56°C and 1 min at 72°C using a thermal cycler (OMN-E, Hybaid, Ashford, UK). After the last cycle we added a 10 min extension at 72°C. The reaction was performed with appropriate positive and negative controls.

For heteroduplex formation analysis, the PCR products were denatured at 94°C for 5 min and subsequently cooled to room temperature in 60 min to induce heteroduplex formation. The products were immediately loaded on 6% non-denaturing polyacrylamide gel in 1 × Tris–boric acid–EDTA buffer and stained with ethidium bromide after completion of electrophoresis.

PCR products which turned out to have clonal recombination by heteroduplex analysis were purified on a 2% agarose gel using a QIAX II kit (Qiagen, Hilden, Germany). Sequence analysis of the junction region of recombined genes was carried out by the dideoxynucleotide method and analyzed on autosequencer.

We designed ASO-primers so that their 3′ end span the junctional region of the patient-specific recombination.

Detection of MRD by ASO-PCR

The methods of MRD detection, a two-step (nested) PCR using the ASO-primer in the second round of amplification, were basically the same as previously reported [15,29]. We used only the ASO-primers which were confirmed to reach a sensitivity of 10^{-4} or more for the MRD detection. The bone marrow DNA sampled at point 1 and/or 2 were amplified together with the serially (with 10-fold dilution step, 10^{0}–10^{-5}) diluted initial leukemic DNA in the buffy coat DNA from seven healthy volunteers. The quantitation of MRD was done by comparing the intensities of band-signals on an agarose gel stained with ethidium bromide.

Statistical Analysis of Prognosis

Actuarial probabilities of event-free and disease-free survivals were calculated by the method of Chaplain and Meier where an event was defined as relapse or death.

RESULTS

Characteristics of Patients With Clonal TCR/Ig Gene Rearrangements Eligible for the MRD Detection

Using polyacrylamide gel electrophoresis (PAGE), we determined clonal recombination in 50 patients (86%). The remaining eight patients (14%) had no clonal rearrangement. Forty-two (84%) had more than one clonal rearrangement whose junctional sequences could be determined. Subsequent sequence analysis was successful in all cases examined.

Among the 50 patients with clonal rearrangements, we found B-cell precursor ALL in 45 children (34 common ALL, and 11 pre-B ALL), one undifferentiated ALL (u-ALL), and four T-lineage ALL. Age at diagnosis ranged from 1.0 to 14.2 years (median, 8.4 years). Concerning the risk-groups, the numbers of patients enrolled in HHRG, HRG, IRG and LRG were 7, 17, 15 and 11, respectively.

Clonal rearrangements of TCR δ, TCR γ, and Ig κ chain gene were observed in 34 (68%), 27 (54%) and 21 (42%) patients, respectively (Table II).

Detection of MRD and Clinical Outcome

Seventy-three bone marrow samples were analyzed for MRD, i.e. 36 samples at point 1 and 37 samples at point 2. We defined those whose quantities of MRD more than or equal to 10^{-4} as MRD-positive and those less than 10^{-4} as MRD-negative. Because we warranted all the patients the sensitivity of detection to 10^{-4}. Table III summarizes the results of MRD at points 1 and 2 and the clinical outcome.

TABLE II Patient characteristics

Total number	50
Age median (year)	8.4 (range 1.0–15.0)
Sex	
Female	19
Male	31
Phenotype	
Percursor B ALL	45
Common ALL	34
Pre-B ALL	11
T-ALL	4
u-ALL	1
Risk groups	
HHRG	7
HRG	17
IRG	15
LRG	11
Clonal rearrangements	
TCR δ chain gene	34 (68%)
TCR γ chain gene	27 (54%)
Igκ chain gene	21 (42%)

TABLE III MRD results and clinical outcome

Time point	MRD results	Clinical outcome		Total
		Relapsed	CR	
Point 1	Positive	6	7	13
	Negative	3	20	23
Point 2	Positive	6	1	7
	Negative	1	29	30

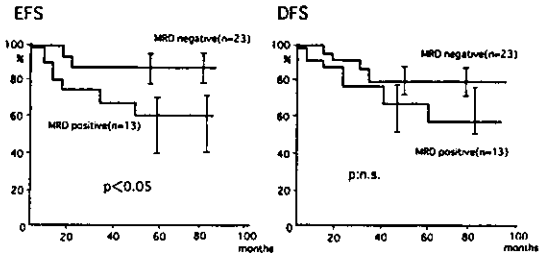

FIGURE 2 (A) Event-free survival (EFS) of the patients according to MRD at point 1. (B) Disease-free survival (DFS) of the patients according to MRD at point 1.

At point 1, 13 of the 36 patients (36%) were MRD-positive. Six of the 13 patients (46%) MRD-positive at this point relapsed, whereas only three of the 23 patients (13%) MRD-negative had a relapse. There was a significant difference in event-free survival between patients MRD-positive and those MRD-negative at point 1 ($p < 0.05$) (Fig. 2A).

More clear-cut results were observed at point 2. Six of the seven patients (86%) MRD-positive had a relapse, as compared with only one of the 30 MRD-negative patients (3%). There were significant differences in both event-free survival and disease-free survival between patients MRD-positive and those MRD-negative at point 2 ($p < 0.0005$ and $p < 0.0001$, respectively) (Fig. 3A and B).

In Table IV, we summarize the characteristics of nine patients who had a relapse. All patients were MRD-positive either at point 1 or 2. They were five HHRG, two HRG and two LRG patients. Seven patients had a relapse in the bone marrow while the rest (HH12 and L17) in the

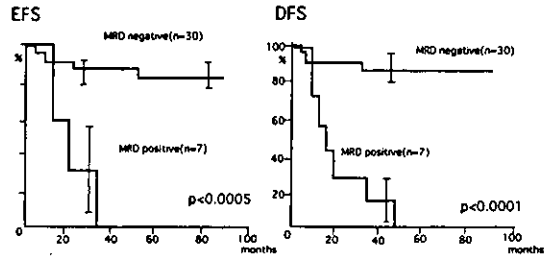

FIGURE 3 (A) Event-free survival of the patients according to MRD at point 2. (B) Disease-free survival of the patients according to MRD at point 2.

central nervous system. Time to relapse varied from 4 to 40 months after diagnosis. Eight children relapsed during the course of chemotherapy and one (case L13) had a relapse after completion of chemotherapy. Case HH17 showed an increase of leukemic cells at point 2 and relapsed only 1 month later. Remarkably, four of the five HHRG patients were MRD-positive at both points 1 and 2, and had a relapse in spite of treatment with the most intensive post-remission therapy.

DISCUSSION

The prognosis of childhood ALL has been improved by protocols based on the risk stratification at diagnosis. Patients with poor risk factors, for example, age older than 10 years or white blood cell count of more than 50,000/μl at diagnosis in the CCLSG protocol, are classified into the HRG and treated with an intensive chemotherapy throughout the protocol. Conversely, patients in the LRG are treated with a less intensive and shorter protocol than those at high risk. Although such stratification has indeed improved the prognosis as a whole, a considerable number of the patients classified into the LRG still have a relapse (e.g. 22% in CCLSG 911 protocol at 8 years).

In recent years, it has been demonstrated that an early response to chemotherapy is an independent prognostic factor in childhood ALL by other groups [21–23]. The patients responding rapidly to chemotherapy have an

TABLE IV Characteristics and MRD results of the patients with relapse

Case no.	Risk group	Phenotype	MRD results		Site of relapse	Time to relapse (months)
			Point 1	Point 2		
HH8	HHR	T-ALL	Positive	Positive	BM	13
HH12	HHR	T-ALL	Positive	Positive	CNS	12
HH17	HHR	c-ALL	Negative	Positive	BM	4
HH19	HHR	u-ALL	Positive	Positive	BM	6
HH22	HHR	T-ALL	Positive	Positive	BM	6
H44	HR	Pre-B ALL	Positive	Not done	BM	9
H57	HR	c-ALL	Not done	Positive	BM	6
L13	LR	Pre-B ALL	Positive	Negative	BM	40
L17	LR	c-ALL	Positive	Positive	CNS	14

HHR: High-high risk; HR: High risk; IR: Intermediate risk; LR: Low risk; c-ALL: common ALL; u-ALL: undifferentiated ALL; BM: bone marrow, CNS: central nervous system.

excellent prognosis and those with a slow response have a poor prognosis. The present results are consistent with these reports. The patients MRD-positive (more than or equal to 10^{-4}) showed a higher relapse rate than those MRD-negative, 13 vs. 46% at point 1 and 3 vs. 86% at point 2. There were differences in event-free survival between MRD-positive children and MRD-negative children.

In the present study, MRD was detected in 13 of the 36 (36%) patients at the end of induction therapy (point 1). This rate was much lower than that reported by zur Stadt *et al.* [30] (64/76, 85%) but comparable with that reported by van Dongen *et al.* [21] (60%) and Cave *et al.* [22] (42%). The induction protocol in the latter two studies consisted of a four-drug regimen (PSL, VCR, daunorubicin (DNR), and L-Asp) including a 1-week PSL prephase, while that in the former study lacked L-Asp. The protocol in our study also consisted of four drugs, PSL, VCR, THP, and L-Asp. Possibly, the differences in MRD levels between these groups may result from the omission of L-Asp in the induction therapy.

Most of the patients MRD-positive at point 2 relapsed within 12 months in our study. Although the study population was small, these results suggest that modification of the post-remission therapy might help eradicate residual leukemic cells in MRD-positive patients. It might be possible to enhance the intensity of the post-remission therapy for the MRD-positive patients in the lower risk group. Nachman *et al.* reported that intensification of early post remission therapy improves the clinical outcome in patients with a slow response to remission induction therapy [31]. It is noteworthy that five HHRG patients who had a relapse had already been receiving the most intensive therapy. These patients do not seem to be cured just by intensifying the post-remission therapy. They might have needed allogenic stem cell transplantation, which induces a graft vs. leukemia effect. The recent findings on MRD in the early phase of chemotherapy in childhood ALL including the present findings that the response to therapy based on MRD can be used to stratify the patients after achieving CR.

Van Dongen *et al.* suggested that it would be possible to construct a new strategy of chemotherapy according to MRD-based risk stratification defined by MRD levels at 5 and 12 weeks after the initiation of therapy [21]. In order to include a PCR-based MRD detection in the prospective clinical study, rapid determination of the clonal recombination of TCR/Ig genes of leukemic cells is needed. Using the heteroduplex PAGE analysis, we could identify the clonal rearrangements in a short time with a high accuracy.

We conclude that an early response to chemotherapy is a predictor of good prognosis of childhood ALL regardless of the protocol of chemotherapy or race. By amplification of the clonally recombined TCR/Ig gene, we can monitor MRD from early phase of chemotherapy and design the therapeutic protocol for each patient based on the residual leukemic cells.

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