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

RESULTS

Patients

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

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

EFS and OS

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

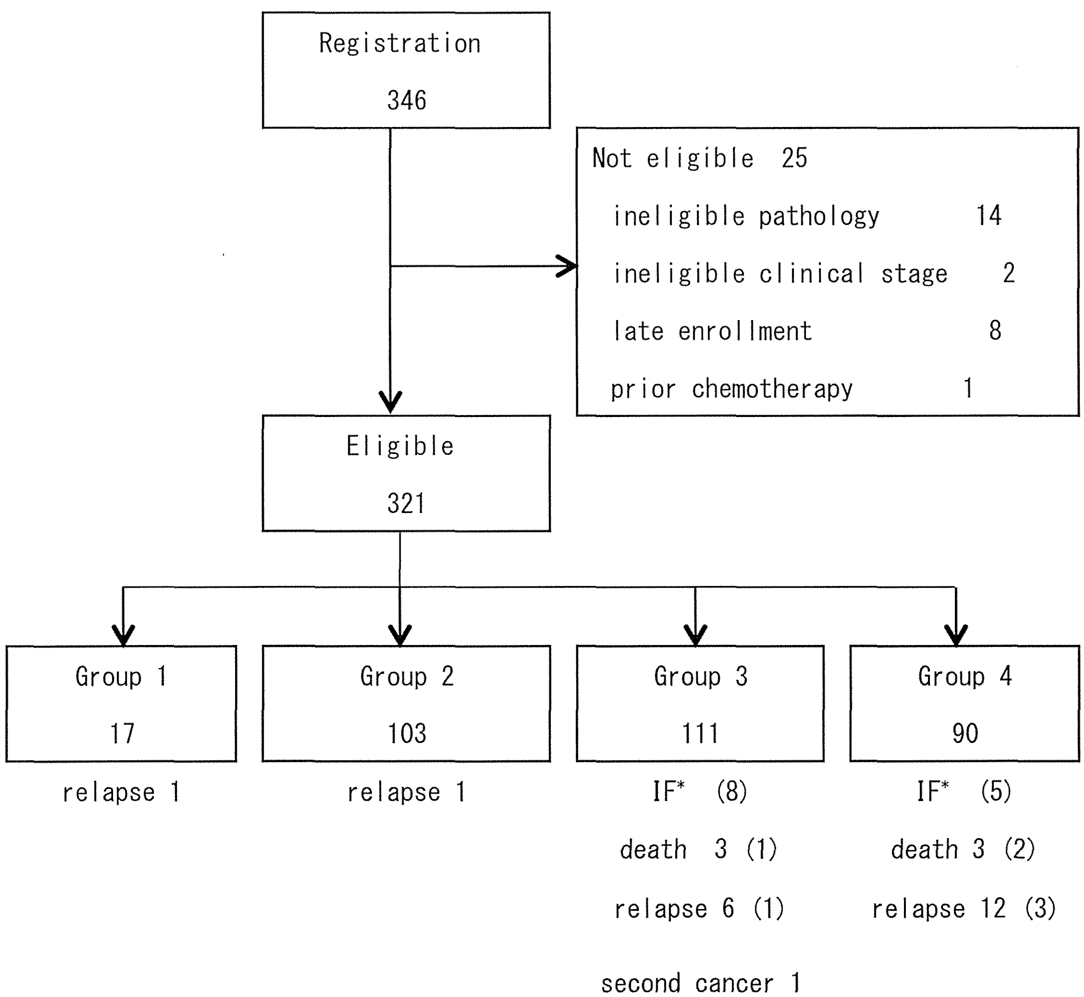

Fig. 2. Patient flow chart and events according to the treatment group. There were 40 events which consisted of each one in Group 1 and 2, 18 in Group 3, and 20 in Group 4. Number in parentheses indicates events occurred during protocol chemotherapy. Number in parenthesis indicates events occurred during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

TABLE II. Patients Characteristics

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

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

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

Treatment Failure Events

Forty patients experienced an event and 25 have died (Fig. 2). The cause of death was tumor progression in 14, infection in 7, stem cell transplantation-related death in 3, and pulmonary bleeding in 1. The 40 events consisted of 13 induction failures, 6 deaths, 20 relapses, and one second cancer. Of the13 patients (6 in Group 3 and 7 in Group 4) who failed the initial treatment, 4 patients in Group 3 received salvage therapy and achieved CRu. At the time of the last analysis, 8 patients (4 in Group 3 and 4 in Group 4) were alive without tumor. Death in remission occurred in 3/321 (1%) patients: two died of infection and one died of pulmonary bleeding. The longest duration before relapse from the start of therapy was 38.9 months in DLBCL and 13.6 months in Burkitt histology. Relapse sites were 10 in local, 6 in BM, 2 in BM+CNS, one in local + CNS, and one in CNS. All CNS relapse occurred in patients with BL, but not with DLBCL. Thus, isolated CNS failure was only one among 38 patients with CNS involvement. Of the 20 relapsed patients, 11 died and 9 survived without tumor. A second cancer occurred among the patients who failed the initial treatment: a 12-year-old male with BL developed a secondary malignancy with acute myeloid leukemia (FAB M5) 17 months after the initial diagnosis.

Toxicity

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

DISCUSSION

During the last two decades, the survival outcome of children with B-NHL has been markedly improved through consecutive

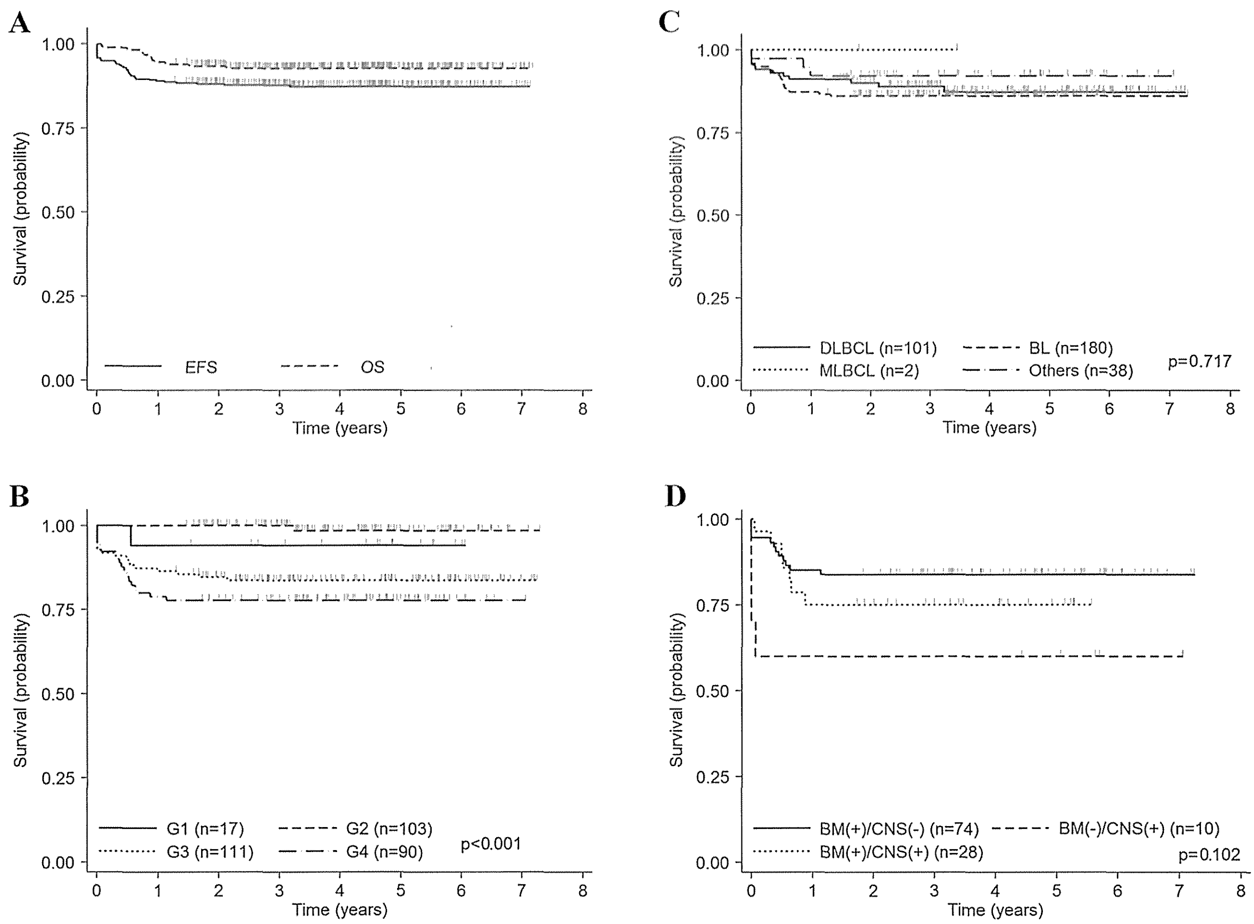

Fig. 3. Kaplan–Meier curves for OS and EFS of all patients (**A**). Kaplan–Meier curves for EFS according to treatment group (**B**), histology (**C**), and BM/CNS involvement (**D**).

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

This suggests that the 5 g/m^2 HDMTX over 24 hour-infusion is equally as effective to the CNS-positive disease as the aforementioned 8 g/m^2 HDMTX over 4 hours infusion, and reinforces the possibility that CNS irradiation could be omitted without jeopardizing the outcome of patients with CNS disease by using systemic and it MTX therapy [3,5,9].

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

Several factors associated with poor outcome in the high-risk group in childhood B-NHL have been reported. Cairo et al. has shown a significantly inferior outcome (4-year EFS 61% ± 6%) of the subgroup of children with combined BM and CNS involvement at diagnosis as compared with children with BM or CNS only [5]. However, our results in Group 4 showed that the outcome (4-year EFS 75% ± 8%) of this subgroup with BM+/CNS+ was not significantly inferior than that of the subgroup with BM+ (83% ± 4%) or CNS+ (60% ± 1%). Failure to initial therapy is also known to be a strong, unfavorable prognostic factor. Past studies in LMB 89/96 have shown that non-responders to pre-phase therapy (COP regimen) suffer a significantly inferior outcome as compared with responders or incomplete responders [3,5]. In our study, an appropriate evaluation of tumor regression just after pre-phase therapy was difficult for many patients, such that we compared the outcome according to response at the final evaluation time after two or three courses of therapy. These results showed that 4-year EFS of patients who did not achieve CR/CRu was only $15\% \pm 10\%$, which was as dismal as the outcome of poor-responders to COP regimen in the FAB/LMB 96 study [5]. To rescue the poor-responders in our study, we employed salvage therapy with high-dose Ara-C and VP16 to patients who did not achieve remission after 2 or 3 courses of therapy in Group 2 or 3, as in the BFM90 or FAB96 study [2,4]. As a result, 4 of 6 patients in Group 3 received salvage therapy and survived without tumor. This response rate was similar to that of FAB96 study, in which 10 out of 16 patients who received the second phase treatment intensification after the consolidation phase were alive. Thus, our results reconfirmed the efficacy of the salvage therapy.

Management of acute toxicity by short-pulse intensive chemotherapy is essential to successfully carry out the treatment protocol for childhood B-NHL. In our study, grade IV neutropenia occurred in almost all patients, but the rate of grade IV infection was quite low. Consequently, therapy-related death was less than 1% in all patients, and 2.1% in Group 4 patients. These results show the safety and feasibility of our treatment protocol. Anthracycline cardiotoxicity and secondary malignancy by alkylating agents are serious late events in pediatric cancer treatment [17,18]. To reduce the risk of cardiotoxicity, we employed THP-adriamycin (pirarubicin) instead of ADR. Pirarubicin is a derivative of ADR with reportedly less cardiotoxicity in adults [19–24]. Recently, we have reported that no significant cardiac dysfunction was detected in long-term survivors of children with acute lymphoblastic leukemia who received THP treatment [25–27]. In the present study, there were no patients with cardiac insufficiency or cardiac myopathy during the 7-year observation period. These results suggest that late-onset cardiotoxicity induced by pirarubicin is uncommon in childhood lymphoid malignancies, at least up to the cumulative dose of $240\,mg/m^2$. In our study, there was one male with a second cancer with acute myeloid leukemia, although the correlation between his second cancer and the protocol treatment is uncertain because he was resistant to the pre-phase followed by arbitrary treatment.

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

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

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ORIGINAL ARTICLE

Early use of allogeneic hematopoietic stem cell transplantation for infants with *MLL* gene-rearrangement-positive acute lymphoblastic leukemia

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Sixty-two infants with *MLL* gene-rearrangement-positive acute lymphoblastic leukemia (MLL-r ALL) were treated with the MLL03 protocol of the Japanese Pediatric Leukemia/Lymphoma Study Group: short-course intensive chemotherapy followed by early allogeneic hematopoietic stem cell transplantation (HSCT) within 4 months of the initial induction. The 4-year event-free survival and overall survival rates were 43.2% (95% confidence interval (CI) = 30.7–55.1%) and 67.2% (53.8–77.4%), respectively. A univariate analysis showed younger age (<90 days at diagnosis), central nervous system disease and poor response to initial prednisolone therapy significantly associated with poor prognosis ($P<0.05$). In a multivariate analysis, younger age at diagnosis tended to be associated with poor outcome (hazard ratio = 1.969; 95% CI = 0.903–4.291; $P = 0.088$). Although the strategy of early use of HSCT effectively prevented early relapse and was feasible for infants with MLL-r ALL, the fact that substantial number of patients still relapsed even though transplanted in their first remission indicates the limited efficacy of allogeneic HSCT for infants with MLL-r ALL. Considering the risk of severe late effects, indications for HSCT should be restricted to specific subgroups with poor risk factors. An alternative approach incorporating molecular-targeted drugs should be established.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) in infants younger than 1-year old accounts for 2.5–5% of childhood ALL and carries clinical and biological features distinct from those of ALL in older children.[1] Patients have a high frequency (up to 80%) of 11q23 translocations/*MLL* gene rearrangements (MLL-r) and a highly distinctive gene expression profile, and majority of infants with MLL-r ALL are characterized by a high white blood cell count and marked hepatosplenomegaly at presentation, and by a pro-B-cell phenotype of their leukemic cells, which lack CD10 expression.[2] The prognoses of these patients are very poor, as illustrated by recent published long-term event-free survival (EFS) and overall survival (OS) rates of 42–54% and 45–61%, respectively.[3–10] In a large international study, Interfant-99, the presence of MLL-r, a very high white blood cell count, age <6 months and a poor response to prednisolone prophase were associated with inferior outcomes. Notably, the 4-year EFS of MLL-r patients was only 36.9%, which is much poorer than the 74.1% EFS of *MLL*-germline patients.[3]

Between 1995 and 2001, we conducted two consecutive Japanese nationwide studies of infant ALL, designated MLL96 and MLL98, in which we stratified patients according to their *MLL* gene configurations; all MLL-r ALL infants were assigned intensive chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) at their first remission.[4] Because of the high rate of early relapse before the time for HSCT was reached, which is frequently observed in infants with ALL, the overall outcomes of these MLL-r patients were far from satisfactory. However, an encouraging result in our study for 3-year posttransplantation EFS (64.4%) in patients receiving HSCT at their first remission prompted us to speculate whether an intervention with more-effective chemotherapy and HSCT in an earlier phase could prevent early relapse and produce a better outcome.[11] Therefore, we planned the MLL03 study and analyzed the outcomes of infants with MLL-r ALL.

MATERIALS AND METHODS

Patients

Between February 2004 and January 2009, 92 consecutive infants younger than 1 year with suspected newly diagnosed ALL from 126 centers and hospitals in Japan were assessed for their eligibility for MLL03. This study included more than 90% of the same patients as the national study of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). Patients with germline *MLL* gene, mature B-cell ALL, Down syndrome or congenital ALL cases with gestational age of less than 37 weeks were excluded according to the eligibility criteria of the study (Supplementary Table 1). The diagnosis of ALL was established based on bone-marrow morphology (or peripheral

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blood morphology, if bone-marrow aspiration resulted in a dry tap), cytochemical staining and immunophenotyping, which were confirmed with a central review system. The leukemic cell karyotypes were determined with a cytogenetic analysis using a G-banding technique. The *MLL* gene configuration of each patient was determined with a Southern blotting analysis, as described previously,[12] and the chimeric genes *MLL–AF4*, *MLL–AF9*, *MLL–AF6* and *MLL–ENL* were also examined with real-time PCR. With these strategies, 30 patients were excluded because they did not meet eligible criteria of the study as follows (Supplementary Table 1): *MLL*-germline ALL ($n=20$), mature B-cell ALL ($n=1$), congenital ALL cases with immature gestational age ($n=3$), death before diagnostic confirmation of ALL ($n=1$), unable to start protocol therapy within 7 days after the registration ($n=1$), refusal to participate by the guardian ($n=2$), the patient was registered before the study approval by the institutional review board ($n=1$) and the patient was transferred to a non-JPLSG member hospital ($n=1$; Figure 1). Ultimately, 62 patients with MLL-r ALL were eligible and were enrolled in the protocol study. Written informed consent was obtained from the guardians of the patients according to the Declaration of Helsinki, and institutional review board approval was obtained for all aspects of the study.

Treatment

The details of the therapeutic regimen used in the MLL03 study are described in Table 1. The patients were non-randomly assigned to commence 7-day prednisolone monotherapy. The prednisolone response and the leukemic status of the central nervous system (CNS) were evaluated on day 8, and a prednisolone good responder was defined as an infant with a peripheral blood blast count of less than 1000/μl and a poor responder as an infant with ⩾1000/μl. CNS involvement was defined as > 5/μl mononuclear cells with a leukemic morphology. As most other study groups evaluate CNS status on day 1 before any treatment is given, CNS status in the current study might be influenced by the prednisolone prophase. In addition, day 8 evaluation of prednisolone prophase in this study is unique compared with other studies that usually assess peripheral blood blasts after 1 week of prednisolone concurrent with single intrathecal injection of chemotherapy.

The induction phase consisted of dexamethasone, vincristine, doxorubicin, cyclophosphamide and triple intrathecal chemotherapy with methotrexate, cytarabine (Ara-C) and hydrocortisone, followed by an intermediate dose of Ara-C and etoposide (VP-16). Based on the *in vitro* drug sensitivity data presented by Pieters *et al.*[13] showing that the lymphoblasts of infant ALL show high sensitivity to Ara-C, each of the two consolidation courses were intensified with high-dose Ara-C to prevent early relapse. However, L-asparaginase was not included throughout the therapy because of its low sensitivity in infants. All the patients received two initial courses (*induction* and *consolidation-1*) and their remission status was evaluated after each course. Complete remission (CR) was defined by testing bone marrow with less than 5% leukemic cells, regeneration of hematopoiesis and no evidence of leukemia cells elsewhere after either the *induction* or *consolidation-1* course.

Because the main objective of the MLL03 study was to evaluate the efficacy and safety of allogeneic HSCT in the early phase of the disease (within 4 months of the initial induction), all the patients with continuous CR were prescribed the following HSCT after *consolidation-2*. The donors were restricted to two types: human leukocyte antigen ⩾4/6 serologically matched unrelated cord blood or human leukocyte antigen ⩾5/6 matched related donor. The conditioning was a nonirradiation myeloablative regimen with busulfan (BU), VP-16 and cyclophosphamide. An oral formulation of BU was used until October 2006, when the intravenous formulation became available in Japan. Regardless of the drug formulation, the dose of BU was determined according to individual pharmacokinetic tests, with a targeted average steady-state concentration of 600–900 ng/ml.[14] The prophylaxis for graft-versus-host disease was either cyclosporine or tacrolimus combined with short-term methotrexate.

Statistical analyses

All the analyses were performed by the intention-to-treat approach; all the 62 eligible patients were fully analyzed even for the cases that dropped out

Table 1. Treatment for infant ALL with a rearranged *MLL* gene in MLL03 study

Phase and drug	*Delivery, duration*	*Dosage*	*Dose schedule*
PSL prophase			
PSL	IV	60 mg/m^2	Days 1–7
Induction			
DEX	IV	10 mg/m^2	Days 8–21
VCR	IV	0.05 mg/kg	Days 8, 15
CPA	IV,2 h	1 200 mg/m^2	Day 9
DXR	IV, 1 h	25 mg/m^2	Days 10, 12
TIT		age-adjusted[a]	Days 8, 22[b]
VP-16	IV, 2 h	100 mg/m^2	Days 22–25
Ara-C	IV, 4 h	500 mg/m^2	Days 22–25
Consolidation-1			
MIT	IV, 1 h	10 mg/m^2	Day 1
VP-16	IV, 2 h	100 mg/m^2	Days 1–5
Ara-C	IV, 4 h	3000 mg/m^2	Days 1–5
TIT		Age-adjusted[a]	Days 1, 8
Consolidation-2			
VCR	IV	0.05 mg/kg	Day 1
MTX	IV, 12 h	3 000 mg/m^2	Day 1
Leucovorin	IV	15 mg/m^2	36 hr after start of MTX, 7 times
Ara-C	IV, 3 h	3000 mg/m^2 × 2	Days 4, 5
TIT		Age-adjusted[b]	Days 1, 8
Conditioning regimen for hematopoietic stem cell transplantation			
BU	PO/IV	Adjusted based on PK results	Days −8, −7, −6, −5
VP-16	IV, 12 h	60 mg/kg	Day −4
CPA	IV, 2 h	60 mg/kg	Days −3, −2

Abbreviations: ALL, acute lymphoblastic leukemia; Ara-C, cytarabine; BU, busulfan; CPA, cyclophosphamide; DEX, dexamethasone; DXR, doxorubicin; IV, intravenously; MIT, mitoxantrone; MTX, methotrexate; PO, orally; PK, pharmacokinetics; PSL, prednisolone; TIT, triple intrathecal therapy; VCR, vincristine; VP-16, etoposide. The dose of each drug except VCR, PSL, and DEX were reduced by one-third in patients younger than 60 days and by one fourth in those 61–120 days of age. [a]Doses were adjusted according to the patient's age at administration as follows: 90 days old or younger, MTX 3 mg, hydrocortisone (HDC) 10 mg, Ara-C 6 mg; younger than 1 year old, MTX 6 mg, HDC 10 mg, Ara-C 15 mg; 1 year and older, MTX 8 mg, HDC 15 mg, Ara-C 20 mg. [b]Additional TITs on days 15 and 29 for patients with CNS disease.

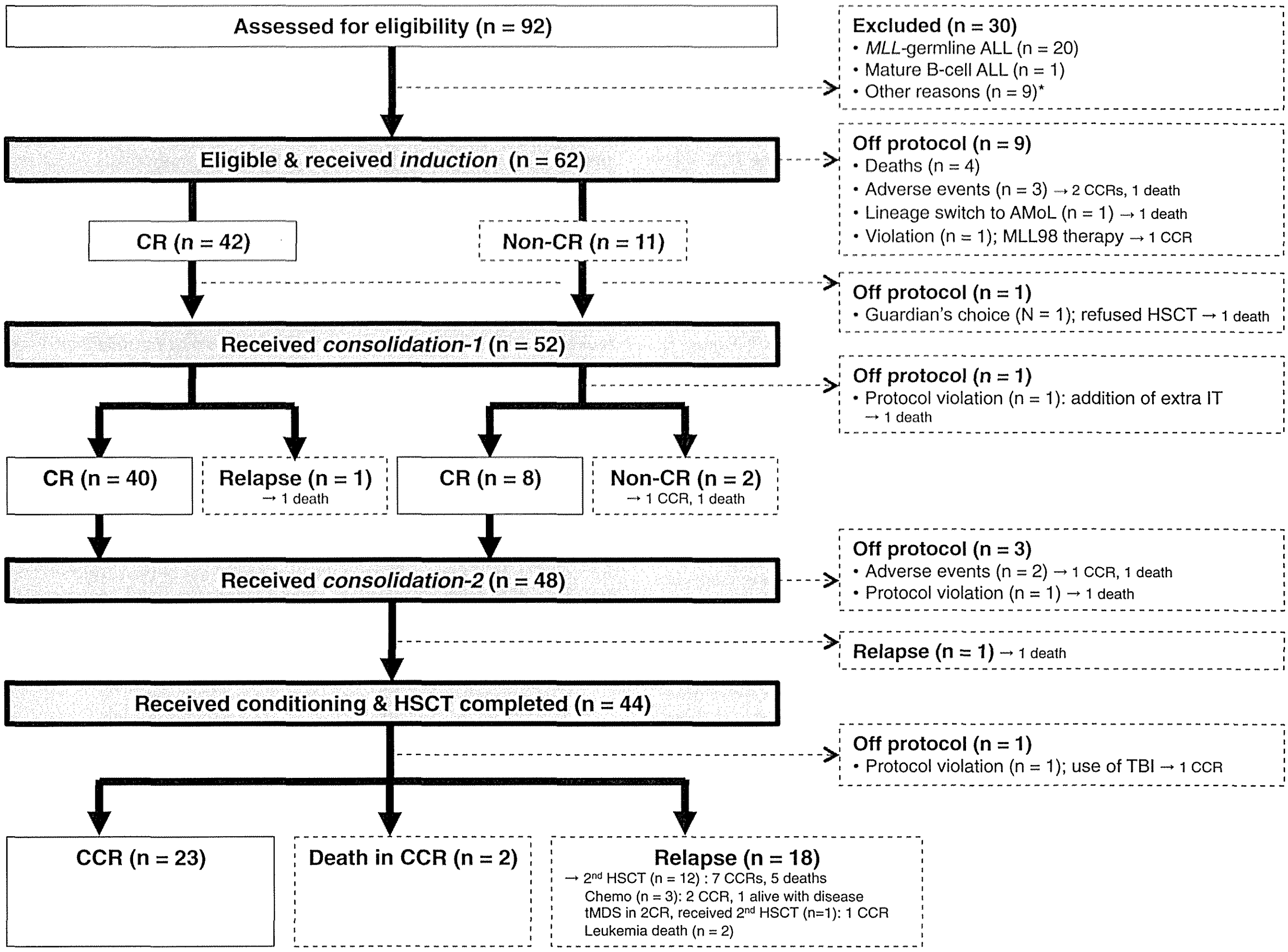

Figure 1. Patient flow chart in the MLL03 study. ALL, acute lymphoblastic leukemia; AMoL, acute monocytic leukemia; CR, complete remission; CCR, continuous CR; HSCT, hematopoietic stem cell transplantation; IT, intrathecal therapy; TBI, total-body irradiation; tMDS, therapy-related myelodysplastic syndrome; 2CR, second CR. *Reasons for exclusion of the nine patients are as follows: congenital ALL cases with immature gestational age ($n=3$), death before diagnostic confirmation of ALL ($n=1$), unable to start protocol therapy within 7 days after the registration ($n=1$), refusal to participate by the guardian ($n=2$), the patient was registered before the study approval by the institutional review board ($n=1$) and the patient was transferred to a non-JPLSG member hospital ($n=1$).

of the study before completing the protocol-specified therapy for various reasons (Table 1). EFS was defined as the length of time from the diagnosis of ALL to the last follow-up or first event (failure to achieve remission, relapse, secondary malignancy or death from any cause). OS was defined as the length of time from the diagnosis of ALL to death from any cause. The probabilities of EFS and OS were estimated with the Kaplan–Meier method and standard errors (s.e.) with the Greenwood formula, and were then compared with the log-rank test; 95% confidence intervals (CIs) were computed. A Cox proportional hazards regression model was used to identify the risk factors associated with the EFS rate. Variables including age at initial diagnosis (<90 days vs ⩾90 days), white blood cell count at initial diagnosis (⩾100 000/μl vs <100 000/μl), CNS disease (positive vs negative), cytogenetics (t(4;11)(q21;q23) vs others) and response to initial prednisolone monotherapy (poor vs good responders) were considered for inclusion in the model. The significant variables associated with the EFS rate were then identified. No statistical adjustment was made for the performance of multiple tests, but two-sided P values greater than 0.05 were interpreted with caution. All data analyses were performed with the STATA statistical software (version 11.0; StataCorp LP, College Station, TX, USA).

RESULTS

Patient characteristics

The characteristics of the 62 enrolled infants with MLL-r ALL are shown in Table 2. Notably, the proportion of younger infants aged <180 days (6 months) at diagnosis was very high (68% (41/62)) in the present report compared with those in previous reports, in which they usually constituted as much as 50%.[3–5]

Treatment outcomes

Remission induction results. The prednisolone response was evaluated in 59 out of 62 patients (95%): 43 (69%) infants were good responders and 16 (26%) were poor responders. Forty-two patients (67.7%) achieved CR after the initial induction therapy, four patients died (because of sepsis ($n=2$), acute respiratory distress syndrome after respiratory syncytial virus infection ($n=1$) and liver failure ($n=1$)) and five patients dropped out of the protocol with one of the following reasons: severe adverse events ($n=3$: heart failure or renal failure from tumor lysis syndrome and respiratory syncytial virus bronchiolitis. The patient with heart failure eventually died of leukemia progression, and the other two are alive in continuous CR.), lineage switch to acute monocytic leukemia ($n=1$: died of leukemia) and protocol violation ($n=1$: alive with continuous CR after HSCT following MLL98 chemotherapy; Figure1). Notably, five of these eight patients (excluding the protocol violation case) were less than 90 days of age (six were <180 days of age) at diagnosis. In addition, one patient, although achieved CR after *induction*, dropped out of the protocol because

Table 2. Characteristics of 62 MLL-r ALL infants enrolled on study MLL03

	No. of patients (%)
Sex	
Male	27 (44)
Female	35 (56)
Age, days	
<90	22 (36)
90 to <180	20 (32)
180 to <366	20 (32)
WBC count, 10^9/L	
<100	34 (55)
100 to <300	11 (18)
⩾300	17 (27)
Immunophenotype	
Pro-B	42 (68)
Pre-B	4 (6)
Common B	9 (14)
AMLL	6 (10)
AUL	1 (2)
11q23 abnormality	
t(4;11)(q21;q23) or *MLL-AF4*	31 (50)
t(9;11)(p22;q23) or *MLL-AF9*	4 (6)
t(11;19)(q23;p13) or *MLL-ENL*	3 (5)
Other 11q23 abnormalities	5 (8)
Other abnormalities	7 (11)
Normal karyotype	9 (15)
Not evaluable	3 (5)
CNS disease	
Positive	11 (18)
Negative	48 (77)
Not evaluable	3 (5)

Abbreviations: AMLL, acute mixed-lineage leukemia; AUL, acute undifferentiated leukemia; CNS, central nervous system; MLL-r ALL, *MLL* gene-rearrangement-positive acute lymphoblastic leukemia; WBC, white blood cell.

the guardian refused the HSCT strategy and withdrew the consent. As a result, total 52 patients received *consolidation-1* and 40/41 patients continued to be CR, 8 extra cases entered CR, 1 relapsed (died of leukemia), 2 failed to achieve CR (one is alive in continuous CR and the other died of leukemia progression) and 1 patient dropped out of the study because of protocol violation ($n = 1$: died of leukemia after the second relapse).

Thus, the overall CR rate (CR after either *induction* or *consolidation-1*) was 80.6% (50/62).

Transplantation outcome. Total 48 patients received *consolidation-2*, and another 3 patients dropped off the study because of severe adverse events ($n = 2$: one is alive in continuous CR and the other died of leukemia progression) and protocol violation ($n = 1$: died of leukemia after the second relapse), and 1 patient relapsed (died of leukemia after the second relapse; Figure1). Thus, 44 patients received HSCT in their first remission (1CR), however, one case dropped out of the study because of protocol violation using total-body irradiation as a conditioning regimen. Among the 43 patients who received HSCT per protocol, 31 patients underwent unrelated cord blood transplantation and 12 patients underwent related bone-marrow transplantation. Although the median infused cell dose was higher in the related bone-marrow transplantation group and the median days to platelet engraftment was longer in the unrelated cord blood transplantation group, there were no differences between the two groups in the incidence of acute or chronic graft-versus-host disease, relapse,

Table 3. Comparison of results of HSCT by different donor sources in MLL03 study

	UCBT, n = *31*	*RBMT*, n = *11*[a]	P *value*
Infused cell dose, x10^7/kg			
Median (range)	10.7 (5.00–21.5)	48.0 (8.70–119)	0.01
Neutrophil engraftment			
n	31 (100%)	11 (100%)	
Median days (range)	16 (14–30)	16 (11–31)	0.65
Platelet engraftment			
n	30 (97%)	11 (100%)	
Median days (range)	40.5 (16–69)	25 (11–52)	0.04
Acute GVHD			
I–II	19	6	
III–IV	2	0	0.67
Chronic GVHD	6	1	0.75
Relapse			
Total	13	5	0.87
BM relapse	10[b]	2	
Isolated EM relapse	1	1	
Combined BM/EM relapse	2	2	
Non-relapse death	1	0	0.58
CCR	17 (54%)	6 (54%)	0.73

Abbreviations: BM, bone marrow; CCR, continuous complete remission; EM, extramedullary; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; RBMT, related bone marrow transplantation; UCBT, unrelated cord blood transplantation. [a]Data not available for one RBMT case, because of sudden death on day +125. [b]One case developed therapy-related myelodysplastic syndrome 4 years after the second CR.

non-relapsed death or the percentage of continuous CR (Table 3). Eighteen patients relapsed after HSCT: twelve in the bone marrow, two in an extramedullary site and four in both. Among these patients, 13 underwent a second HSCT: 7 are alive in continuous CR for median follow-up of 4.4 years (range = 2.5–5.4 years) post second HSCT, 1 is alive but has developed secondary myelodysplastic syndrome and 5 died (3 of disease-related and 2 of HSCT-related causes). Of the remaining five patients, two with isolated subcutaneous relapse are alive in CR after chemotherapy with or without local irradiation, one is alive but relapsed after salvage chemotherapy and two died of disease progression.

Analysis of overall outcome. The median follow-up period of all the 62 patients was 4.0 years (range = 0–7.4 years). The 18-month EFS rate, a primary end point of this study, was 53.2% (95% CI = 40.1–64.6%). The 4-year EFS and OS rates were 43.2% (95% CI = 30.7–55.1%) and 67.2% (95% CI = 53.8–77.4%), respectively (Figure 2). The 4-year EFS rates according to the different risk factors are presented in Table 4; younger age at diagnosis (<90 days), CNS disease and poor response to initial prednisolone monotherapy were significantly associated with a poor prognosis in the univariate analysis. In the multivariate analysis, only younger age at diagnosis tended to be associated with a poorer EFS rate (hazard ratio = 1.969 (95% CI = 0.903–4.291); $P = 0.088$), but the association was not statistically significant, probably because the number of infants analyzed was small. Other associations, such as with CNS disease (hazard ratio = 1.243 (95% CI = 0.421–3.655); $P = 0.694$) and poor prednisolone response (hazard ratio = 1.078 (95% CI = 0.507–2.291); $P = 0.875$), were also statistically insignificant.

Outcome by minimal residual disease (MRD). The significance of MRD was evaluated by measuring selected *MLL*-fusion transcripts in several patients using real-time quantitative PCR in an add-on study. Unfortunately, MRD could not be consistently monitored and the results were not used to guide therapy. Only 11 samples

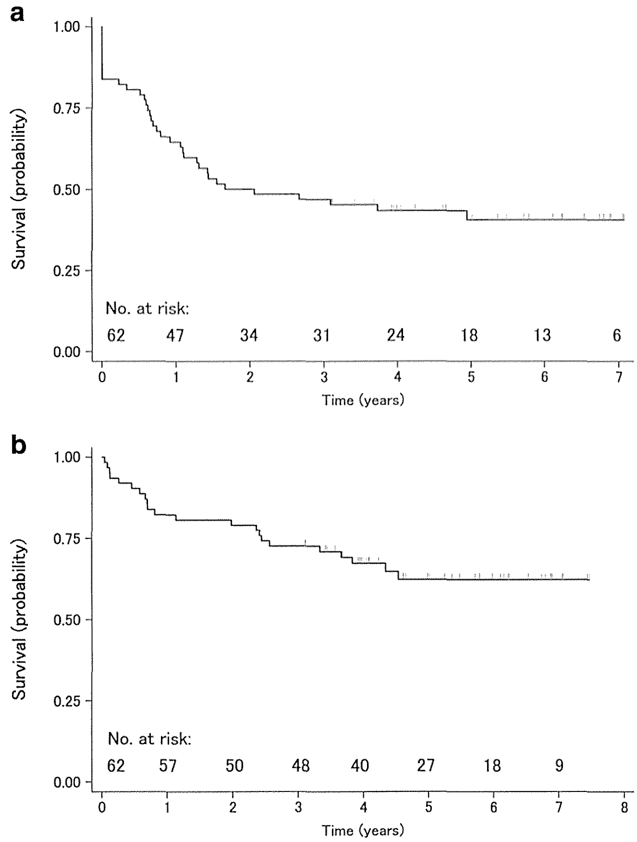

Figure 2. Outcomes of infants with ALL and *MLL*-gene rearrangements enrolled in the MLL03 study. (**a**) Event-free survival (EFS). (**b**) Overall survival.

Table 4. Comparison of 4-year EFS according to the risk factors in 62 MLL-r ALL in MLL03 study

	No. of patients	*4-Year EFS rate, %*	*95% CI*	*P value*
Age, days				
<90	22	22.7	8.2–41.4	0.016
⩾90	40	54.4	37.7–68.4	
<180	42	35.3	21.3–49.7	0.102
⩾180	20	60.0	35.7–77.6	
WBC count, $\times 10^9/l$				
<100	34	46.4	29.0–62.1	0.328
⩾100	28	39.2	21.6–56.5	
CNS disease				
Positive	11	27.2	6.5–53.8	0.046
Negative	48	49.7	34.9–62.8	
Karyotype				
t(4;11)(q21;q23)	31	41.9	24.6–58.3	0.613
Others	31	47.4	28.3–64.2	
Prednisolone response				
PGR	43	53.1	37.2–66.7	0.013
PPR	16	25.0	7.7–47.1	

Abbreviations: CI, confidence interval; CNS, central nervous system; EFS, event-free survival; MLL-r ALL, *MLL* gene-rearrangement-positive acute lymphoblastic leukemia; PGR, prednisolone good responder; PPR, prednisolone poor responder; WBC, white blood cell.

were available at the end-of-induction time point and 10 samples at the transplantation time point because it was difficult to collect paired samples. At the end-of-induction time point, two patients were MRD positive and one eventually relapsed. The 4-year EFS rate for the nine patients negative for MRD after induction was 40.0% (95% CI = 12.2–67.0%; $P = 0.052$). At the pretransplantation time point, two patients were MRD positive and ultimately relapsed. Of the eight patients with negative MRD at HSCT, five relapsed and three cases are in continuous CR; the 4-year EFS rate was 37.5% (95% CI = 8.7–67.4%; $P = 0.081$).

Safety analysis

The grade 3 and 4 toxicities in each treatment phase, evaluated according to the second version of the National Cancer Institute Common Toxicity Criteria, are described in Table 5. Hematological toxicities and nonhematological toxicities, such as diarrhea, elevated liver transaminases and infections, were quite common throughout all treatment phases. Serious complications, such as pulmonary or neurologically related complications or hemorrhage, were predominantly observed during the *induction* phase. Notably, tumor lysis syndrome was observed in 40% of patients presumably because rasburicase, a recombinant urate oxidase, was not available in Japan during the study period.

DISCUSSION

Infant MLL-r ALL is one of the most difficult to cure of all the subtypes of childhood acute leukemia, and the EFS rates are estimated to be less than 40%, even in recently reported studies of patients treated with intensive chemotherapy with or without HSCT.[3–5] The major factor responsible for the high failure rate is the high relapse rate in the early postremission phase of treatment. In fact, more than half treatment failures occurred before HSCT in our previous MLL96 and MLL98 studies, which made it difficult to assess the true impact of allogeneic HSCT on infants with MLL-r ALL.[4] Therefore, in the present study, we intensified the pretransplantation chemotherapy with high-dose Ara-C and assigned all eligible patients to receive allogeneic HSCT in the early postremission phase, within 4 months of the initial induction therapy.

This strategy was feasible because nearly 90% (44/50) of those who achieved remission were able to undergo allogeneic HSCT in 1CR. However, the low overall CR rate, attributable to high induction toxicity, and the substantial number of patients who still relapsed after HSCT resulted in a 4-year EFS rate of 43.2%, which is no better than the previous reports including the study Interfant-99; only 12% (37/297) of the MLL-r cases in the Interfant-99 study received allo HSCT in 1CR.[3] One factor that affected the outcome of this study was an unexpectedly high proportion of younger infants, less than 180 days of age, at diagnosis. It is well known that a younger age at diagnosis is associated with a higher risk of induction toxicity and relapse, and is definitely a poor prognostic factor in MLL-r infants with ALL.[3–5,15] The 4-year EFS rate of the 42 patients <180 days old was 35.3%, whereas that of the 20 patients ⩾180 days old was 60.0%. Another potentially associated factor was the lower treatment potential of the pretransplantation chemotherapy given in this study. We completely eliminated the use of L-asparaginase because its activity against infant MLL-r ALL is low, and this strategy could have adversely affected the outcome, despite the treatment intensification with high-dose Ara-C. One should realize that *in vitro* resistance to a certain drug does not mean absolute resistance to that drug, but is a relative to that of other types of ALL. Furthermore, our strategy of minimizing the chemotherapy courses given before HSCT could have meant that they were insufficient to reduce the leukemic burden, which might have resulted in post-HSCT relapse in some cases. The correlation between MRD and treatment outcome was evaluated in a small proportion of patients in this study, and those with

Table 5. Grade 3 and 4 toxic events by different treatment phases in 62 MLL-r ALL infants in MLL03 study

Toxicities *Patients assessed*	*Induction* n = *62 (%)*	*Cons-1* n = *52 (%)*	*Cons-2* n = *48 (%)*	*Conditioning* n = *44 (%)*[a]
Blood/bone marrow				
Hemoglobin	58 (94)	44 (85	41 (85)	40 (91)
Leukocytes (total WBC)	59 (95)	52 (100)	48 (100)	44 (100)
Neutrophils/granulocytes	62 (100)	52 (100)	48 (100)	44 (100)
Platelets	59 (95)	50 (96)	46 (96)	40 (91)
Gastrointestinal				
Stomatitis/pharyngitis	7 (11)	2 (4)	0 (0)	18 (41)
Vomiting	0 (0)	2 (4)	0 (0)	4 (9)
Diarrhea	13 (21)	15 (29)	6 (13)	12 (27)
Constipation	1 (2)	0 (0)	0 (0)	1 (2)
Pancreatitis	0 (0)	0 (0)	0 (0)	0 (0)
Hepatic				
Total bilirubin	7 (11)	1 (2)	2 (4)	8 (18)
AST/ALT	27 (44)	7 (13)	26 (54)	4 (9)
Metabolic/laboratory				
Amylase	0 (0)	0 (0)	0 (0)	—
Hyperglycemia	2 (3)	0 (0)	0 (0)	—
Renal/genitourinary				
Creatinine	1 (2)	0 (0)	0 (0)	0 (0)
Proteinuria	1 (2)	0 (0)	0 (0)	
Cardiovascular				
Thrombosis/embolism	1 (2)	0 (0)	0 (0)	0 (0)
Other cardiovascular	1 (2)	0 (0)	0 (0)	
Pulmonary				
Dyspnea	10 (16)	0 (0)	0 (0)	3 (7)
Hypoxia	15 (24)	1 (2)	0 (0)	5 (11)
Infection/febrile neutropenia				
Infection	45 (73)	36 (69)	17 (35)	32 (73)
Allergy/immunology				
Allergic reaction/ hypersensitivity	0 (0)	0 (0)	0 (0)	0 (0)
Syndromes				
Tumor lysis syndrome	25 (40)	0 (0)	0 (0)	—
Thrombotic microangiopathy	—	—	—	0 (0)
Veno-occlusive disease	—	—	—	6 (14)
Dermatology/skin				
Rash/desquamation	1 (2)	0 (0)	0 (0)	5 (11)
Neurology	4 (6)	0 (0)	0 (0)	1 (2)
Hemorrhage	6 (10)	0 (0)	1 (2)	1 (2)

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; *Cons-1, consolidation-1*; *Cons-2, consolidation-2*; MLL-r ALL, *MLL* gene-rearrangement-positive acute lymphoblastic leukemia; WBC, white blood cell. [a]One patient who discontinued the study because of protocol violation (use of total body irradiation) is included.

residual MRD at both the postinduction and pretransplantation time points tended to show a worse outcome. Moreover, the fact that nearly half the patients ultimately relapsed even though transplanted in 1CR indicates the limited efficacy of allogeneic HSCT itself for infants with MLL-r ALL.

Although the EFS rate of our study cohort was not satisfactory, their 4-year OS rate of 67.2% was relatively good. This can be explained by the low HSCT-related mortality rate and the relatively high salvage rate after relapse compared with those in our previous MLL96 and MLL98 studies.[16] Among the 43 patients who received allogeneic HSCT per protocol, only 2 (4.6%) died of non-HSCT toxicities (1 of transfusion-related lung injury and 1 with unknown sudden death), although this death rate was high (15.0%, 8/53) in our previous MLL96 and MLL98 studies.[4] Several reasons can be proposed to explain this observation. One is the introduction of an appropriate dose of BU in the conditioning regimen, based on individual pharmacokinetic studies. It is well recognized that the pharmacokinetics of BU vary widely among infants, which may lead to severe post-HSCT organ damage, including lung injury, hepatic veno-occlusive disease, etc.[14,17] Second, the minimum course of pre-HSCT chemotherapy might have reduced the potential organ damage that could have occurred if the patient were instead heavily treated with multiple courses of chemotherapy. Therefore, our strategy of including short-course chemotherapy and the early use of HSCT with individually tailored doses of BU could have reduced both the early-relapse rate and the transplantation-related deaths. The fact that 7 out of 13 cases of post-HSCT relapse were salvaged with a second HSCT may also reflect the low toxic potential of the early HSCT strategy, as mentioned above. Of course, late effects are yet to be evaluated in this study and must be observed especially carefully in these cases.

Because of the limited effectiveness of HSCT and the potential risk of late effects, alternative strategies with novel targeted therapies should be explored for infants with MLL-r ALL.[4,18,19] Recent research has demonstrated that the aberrant epigenetic status, induced by a reciprocal *MLL* translocation via the H3K79 methyltransferase DOT1L, has a central role in MLL-r leukemogenesis.[20–22] The clinical development of epigenetic modifiers, such as DNA methyltransferase inhibitors and/or histone deacetylase inhibitors, is currently in progress. A small-molecule inhibitor of DOT1L is also in clinical development. Meanwhile, HSCT should be restricted to patients at higher risk of relapse, who are likely to benefit from this treatment modality.[23] This stratification is currently being evaluated in our ongoing JPLSG MLL-10 study.

In conclusion, short-course chemotherapy and the early use of HSCT in our study was feasible for infants with MLL-r ALL. However, given the limited effects of HSCT and the potential risk of late effects, the indication for HSCT should be restricted to specific subgroups with poor risk factors, and an alternative approach incorporating molecular-targeted drugs should be established in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

K Koh, DT, TM, MH, Y Takahashi, AO, K Kato, KS and EI (principal investigator) participated actively in the study conception and design; K Koh, DT and EI reviewed the data analysis and interpretation and were the main authors of the manuscript; AMS and TW conducted the statistical analysis; TS was responsible for the busulfan pharmacokinetic study; TD and MT were responsible for the immunophenotyping diagnostics; YH was responsible for coordinating the molecular biology analyses; K Koh, K Kato, JT and Y Takeshita recruited patients; MT, KH and SM contributed to the financial and administrative support of the study; and all authors contributed to the conduct of the trial and were involved in the review of the results and the final approval of the manuscript.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)