IV.　研究成果の刊行物・印刷

Cancer Medicine

Open Access

ORIGINAL RESEARCH

Imatinib use immediately before stem cell transplantation in children with Philadelphia chromosome-positive acute lymphoblastic leukemia: Results from Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) Study Ph$^+$ALL04

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Keywords
Ph+ALL, children, imatinib, HSCT, MRD

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Funding Information
This study was supported by a grant from the Ministry of Health, Labour and Welfare and a grant from the Children's Cancer Association of Japan.

Received: 16 September 2014; Revised: 28 October 2014; Accepted: 31 October 2014

doi: 10.1002/cam4.383

This study was presented at the 53rd annual meeting of the American Society of Hematology (ASH), San Diego in December, 2011.

Abstract

Incorporation of imatinib into chemotherapeutic regimens has improved the prognosis of children with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ALL). We investigated a role of imatinib immediately before hematopoietic stem cell transplantation (HSCT). Children with Ph$^+$ALL were enrolled on JPLSG Ph$^+$ALL 04 Study within 1 week of initiation of treatment for ALL. Treatment regimen consisted of Induction phase, Consolidation phase, Reinduction phase, 2 weeks of imatinib monotherapy phase, and HSCT phase (Etoposide+CY+TBI conditioning). Minimal residual disease (MRD), the amount of BCR–ABL transcripts, was measured with the real-time PCR method. The study was registered in UMIN-CTR: UMIN ID C000000290. Forty-two patients were registered and 36 patients (86%) achieved complete remission (CR). Eight of 17 patients (47%) who had detectable MRD at the beginning of imatinib monotherapy phase showed disappearance or decrease in MRD after imatinib treatment. Consequently, 26 patients received HSCT in the first CR and all the patients had engraftment and no patients died because of complications of HSCT. The 4-year event-free survival rates and overall survival rates among all the 42 patients were 54.1 $\pm$ 7.8% and 78.1 $\pm$ 6.5%, respectively. Four of six patients who did achieve CR and three of six who relapsed before HSCT were salvaged with imatinib-containing chemotherapy and subsequently treated with HSCT. The survival rate was excellent in this study although all patients received HSCT. A longer use of imatinib concurrently with chemotherapy should eliminate HSCT in a subset of patients with a rapid clearance of the disease.

Introduction

Progress in childhood leukemia treatment has raised the 5-year survival rate to as high as 80–90%, however, outcomes in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) patients still remain poor [1, 2]. Arico et al. reported results of an international retrospective study comprising 610 Ph+ALL children treated with intensive chemotherapy without tyrosine-kinase inhibitors and observed 7-year event-free survival (EFS) and overall survival (OS) to be 32% was 45%, respectively [3]. They also showed that allogeneic hematopoietic stem cell transplantation (HSCT) was beneficial. In another study which was the first large prospective cohort study of pediatric patients treated with chemotherapy and tyrosine-kinase inhibitor (TKI), the Children's Oncology Group (COG) assessed increased exposure to imatinib combined with chemotherapy in five cohorts [4]. Forty-four children, who received continuous imatinib from consolidation to the end of treatment, had 3-year EFS of 80%. In this group, which excluded patients with induction failure, the outcome of children treated with HSCT was not better than those treated with chemotherapy plus imatinib. The excellent outcome of this cohort of patients was recently updated: 5-year EFS of 28 patients treated with chemotherapy alone was 70% [5]. Results of an additional study were recently reported by the European intergroup study (EsPhALL) [6]. They adopted a risk-stratified approach for treatment of patients on the basis of early response to therapy and found that the combination of imatinib and Berlin-Frankfurt-Munster (BFM) backbone intensive treatment was safe and possibly beneficial to patients, although 77% received HSCT.

While results of these previous reports showed overall improved outcomes associated with imatinib plus intensive chemotherapy in children and adolescents with Ph-positive ALL, a poor prognosis is still observed for some Ph+ALL patients. The variations in the response to therapy suggest that Ph+ALL is heterogeneous with regard to sensitivity to chemotherapy, TKI and HSCT [7]. The amount of minimal residual disease (MRD) at HSCT was shown to be associated with the outcome of children with ALL after HSCT [8]. In this context, serial analyses of MRD may aid in the selection of patients who could be treated with intensive chemotherapy protocols including a tyrosine-kinase inhibitor.

Here, we report results of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) Ph+ALL04 study, which was conducted in the same era as the COG and EsPhALL studies. Our main objectives were to investigate the potential therapeutic role of using imatinib immediately before HSCT and to evaluate the utility of quantitative MRD assessments on EFS and OS.

Patients and Methods

Patients

Children diagnosed with untreated Ph+ALL between the age of 1 and 18 years were consecutively enrolled from November 2004 to May 2008 onto the JPLSG Ph+ALL04 study. Written informed consent was obtained from the parents or guardians and from the patients as appropriate for their age and conceptual ability.

Diagnosis of ALL was based on morphological, biochemical, and flow cytometric features of leukemic cells, including lymphoblast morphology on May- or Wright-Giemsa-stained bone marrow smears, negative staining for myeloperoxidase, and reactivity with monoclonal antibodies to B- or T-lineage-associated lymphoid differentiation antigens.

All patients with ALL were screened for diagnosis of Ph+ALL using RT-PCR. The presence of Ph-chromosome was further confirmed by standard karyotyping and/or FISH analysis for BCR–ABL fusion gene. Forty-four children with Ph+ALL were enrolled into the JPLSG Ph+ALL04 study within 1 week of initiation of treatment for ALL. However, two patients were not evaluable because Ph-chromosome was not detected either with standard karyotyping or FISH analysis; therefore, 42 patients were eligible for analysis. The median patient follow-up period was 5.2 years (range: 0.6–7.5 years).

Treatment protocol

The protocol was approved by the institutional review boards of all participating institutions and by the Clinical Research Assessment Committee of the Japanese Society of Pediatric Hematology, which merged with the Japan Society of Pediatric Oncology and became the Japanese Society of Pediatric Hematology/Oncology in January 2012. Details of the treatment regimen of this single arm study are outlined in Figure 1 and Table 1. Chemotherapy regimen was based on the high-risk arm of TCCSG (Tokyo Children's Cancer Study Group) L99-15 [9]. Briefly, after five-drug induction therapy, consolidation therapy with high-dose cytarabine with asparaginase and BFM Ib-type was administered, followed by reinduction therapy with four-drug. After completion of reinduction therapy, imatinib monotherapy phase (2 weeks of imatinib at a dose of 340 mg/m^2) was started, and all patients received allogeneic HSCT after imatinib phase. The conditioning regimen

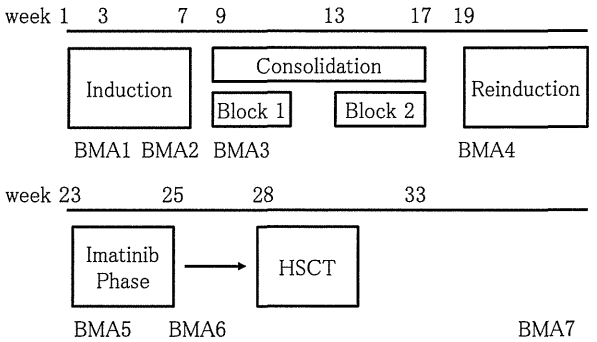

Figure 1. Ph+ALL04 protocol and timing of MRD detection. BMA1, day15; BMA2, day29; BMA3, before consolidation; BMA4, before reinduction; BMA5, before imatinib mesylate; BMA6, after imatinib mesylate; BMA7, 3 months after HSCT; BMA, bone marrow aspiration; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease.

of HSCT was uniform across all patients and consisted of etoposide, cyclophosphamide, and total body irradiation [10, 11]. Prophylactic cranial irradiation was not employed. Imatinib was not used after HSCT. Remission was defined as the presence of fewer than 5% blasts with the recovery of hematopoiesis. Before and after each phase, MRD defined as the amount of BCR–ABL transcripts, was measured with the real-time PCR method (cut-off 50 copies/μg RNA). Time points for MRD detection are shown in Figure 1.

The study was registered in UMIN-CTR (Medical Information, University hospital Medical Information Network—Clinical Trials Registry, URL: http://www.umin.ac.jp/ctr/index-j.htm): UMIN ID C000000290.

Statistical analysis

The primary endpoint of this study was to examine EFS and OS in the overall patient series and determine the efficacy of imatinib mesylate in children with Ph+ALL assessed by a molecular quantification technique. The sample size was determined by the Simon's two-stage minimax design [12]. The lower limit of interest in the response probability was 20% and the desirable target level of response probability was 40%. The required sample size of eligible patients for the analysis was 33 for the alpha error at 0.05 and beta error at 0.20. The secondary objective was to evaluate the proportion of patients who received HSCT in the first complete remission (CR).

The duration of EFS was defined as the time from the initiation of therapy to either treatment failure (relapse, death, or diagnosis of secondary cancer) or the last day the patient was confirmed to be under remission. Patients who did not achieve CR after the first induction phase were considered to have failed at day 1. The probability of EFS and OS was estimated by the Kaplan–Meier method. All data analyses were performed using STATA® statistical software (version 11.0; StataCorp LP, College Station, TX). Follow-up data were actualized as of 31 May 2012.

Role of the funding source

Novartis provided the study drug (imatinib mesylate). The sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for decision to submit for publication.

Results

Patient characteristics and overall outcome of patients

Of the 42 patients registered in the Ph+ALL04 study from 2004 to 2008 and included in this analysis, nine were girls and 33 were boys and the median age at diagnosis was 7 years (range 2–15 years) (Table 2). Minor BCR–ABL fusion gene was detected in 33 children, whereas the major BCR–ABL fusion gene was detected in nine children. All patients had B-cell precursor ALL. Prednisolone

Table 1. Treatment scheme of Ph+ALL04.

Induction	Prednisolone 60 mg/m^2 for 5 weeks, Vincristine 1.5 mg/m^2 for five times, Daunorubicin 25 mg/m^2 for four times, Cyclophosphamide 1200 mg/m^2 for twice, L-asparaginase 6000 U/m^2 for nine times, TIT for three times
Consolidation Block 1	High-dose cytarabine (2 g/m^2 for eight times) with L-asparaginase 10,000 U/m^2 once, TIT once, methylprednisolone 125 mg/m^2 for eight times
Consolidation Block 2	Cyclophosphamide 1200 mg/m^2 once, cytarabine 75 mg/m^2 for 15 times, 6MP 60 mg/m^2 for 21 days, TIT for three times
Reinduction	Dexamethasone 6 mg/m^2 for 14 days, Vincristine 1.5 mg/m^2 for four times, Doxorubicin 25 mg/m^2 for four times, L-asparaginase 10,000 U/m^2 for four times, TIT once
Imatinib monotherapy phase	Imatinib 340 mg/m^2 for 14 days, TIT once
HSCT	TBI 12 Gy, Etoposide 60 mg/kg (BW <30 kg) or 1800 mg/m^2 (BW ≥30 kg), Cyclophosphamide 60 mg/kg for twice

TIT, triple intrathecal therapy (MTX + Ara-C + hydrocortisone). Cranial irradiation was not given. HSCT, hematopoietic stem cell transplantation.

Table 2. Characteristics of children with Ph+ALL (n = 42).

Median age at diagnosis (range)	7 years (2–15 years)
Girls/boys	9/33
White blood cell at diagnosis (range)	39 × 10^9/L (1 – 681 × 10^9/L)
CNS involvement at diagnosis yes[1]/no	3/39
Minor BCR–ABL/major BCR–ABL	33/9
Prednisolone response PGR[2]/PPR[3]	33/9
4-year EFS	54.1 ± 7.8%
4-year OS	78.1 ± 6.5%

[1]CNS involvement was observed in three patients: all the three patients had blasts in the CSF.
[2]PGR, prednisolone good responder (less than 1000/μL blasts in the peripheral blood after 7 days of prednisolone treatment).
[3]PPR, prednisolone poor responder (equal or more than 1000/μL blasts in the peripheral blood after 7 days of prednisolone treatment).

response was assessed on day eight of steroid treatment. Thirty-three patients (79%) had less than 1000/μL blasts in the peripheral blood and nine patients (21%) had equal or more than 1000/μL blasts. Of the 42 patients, 36 (86%) achieved CR and 11 of these 36 patients also achieved MRD-negative after induction phase. A median follow-up period was 5.4 years. The 4-year OS (Fig. 2A) and EFS (Fig. 2B) rates among all patients were 78.1 ± 6.5% and 54.1 ± 7.8%, respectively.

The efficacy of imatinib monotherapy

A flow diagram of the enrolled patients is shown as Figure 3. Of 36 patients who achieved CR at the end of induction, the effects of the imatinib monotherapy phase was evaluable in 30 patients, as six patients excluded due to relapse (n = 1), transferring to non-JPLSG hospital (n = 2), and withdrawal (n = 3). There were 13 patients who had no MRD at the beginning of this phase, all of whom remained MRD-negative after imatinib monotherapy with the exception of one patient who had 100 copies/μg RNA of BCR–ABL transcripts after 2 weeks of imatinib monotherapy. There were five patients who showed clearance of BCR–ABL transcripts after imatinib: the copy number of transcripts/μg RNA of these patients was 450, 280, 250, 130, and 77, respectively. In the remaining 12 patients, three showed decrease of more than 1 – log transcripts: from 6600 to 140, from 39,000 to 1700, and from 1500 to 76, whereas four patients relapsed after this phase. Imatinib was well tolerated in all the patients.

HSCT in the first CR

After 2-weeks of imatinib monotherapy, 26 patients underwent HSCT in the first CR, including 17 patients who were MRD-negative at the time of HSCT (Fig. 2). The grafts were bone marrow from related donors for 10 patients, unrelated bone marrow for 10 patients, related cord blood for 1 patient, and unrelated cord blood for five patients. All the patients achieved engraftment, and no patients died because of complications of HSCT.

Five patients relapsed, all of whom were MRD negative before HSCT, Only two of the patients are alive after the second HSCT whereas the other three patients died because of treatment-related mortality. Twenty-one patients continued to be in 1st CR and MRD-negative for a median of 5.2 years after diagnosis. Of note, all the five patients who were treated with unrelated cord blood transplantation continued to be in 1st CR.

Outcome of patients who did not have CR or who relapsed before HSCT

Of six patients who suffered induction failure, five patients were switched to an imatinib-containing chemotherapy treatment. Four of the five patients achieved CR,

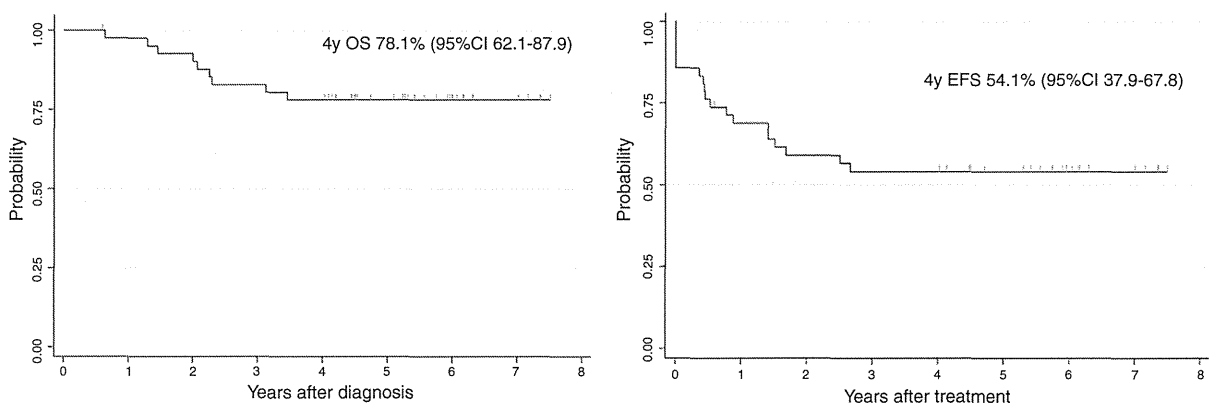

Figure 2. Overall survival of children with Ph+ALL (n = 42) (A) and event-free survival of children with Ph+ALL (n = 42) (B).

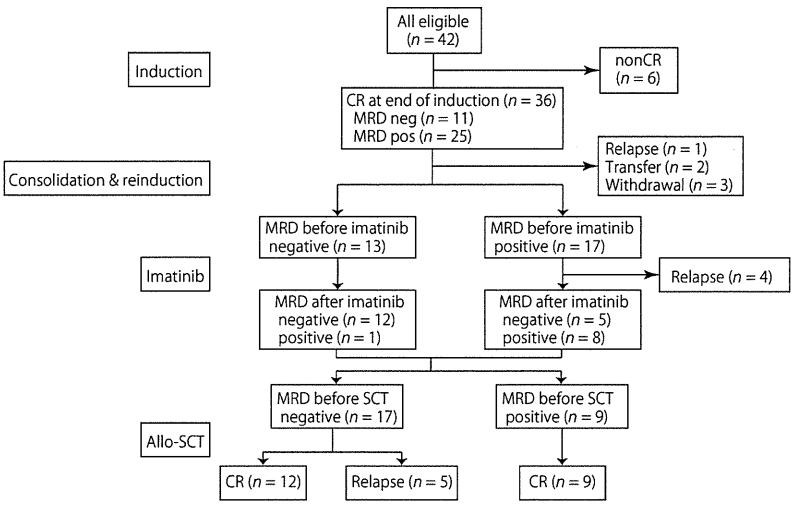

Figure 3. Flow diagram and MRD status of patients. MRD, minimal residual disease.

and all of these four patients received cord blood transplantation and remain in continued CR at 36, 42, 44, and 61 months after HSCT. Five patients relapsed before HSCT: one after consolidation and four after imatinib therapy. Three of the five patients achieved CR after imatinib-containing chemotherapy, and two of them are alive at 49 and 54 months after HSCT.

Discussion

Addition of new drugs to conventional chemotherapy regimens is a challenging issue in treatment of leukemia. During the planning phase of the study, imatinib was not being used in children with Ph+ALL. The amount of MRD at HSCT was reported in relation to outcomes of children with ALL after HSCT [8]. Therefore, we tested the hypothesis that the use of imatinib immediately before HSCT might be beneficial for children with Ph+ALL since it may reduce the amount of MRD at HSCT. We also measured BCR–ABL transcripts as a biomarker for imatinib response, to clarify its effect even when the disease was in CR. In this study, the chemotherapy regimen we employed was based on the previous high-risk treatment protocol of the TCCSG L-99-15 Study [9]. The treatment strategy was effective in inducing MRD-negative status in 13 patients at the time of the imatinib phase and 26 of 42 patients (62%) achieved first CR at the time of HSCT (around 25–28 weeks after diagnosis). In consequence, it was not possible to perform a robust assessment of the efficacy of imatinib because the number of patients with detectable MRD (n = 17) at the beginning of the imatinib phase was much smaller than expected. Nevertheless, the imatinib therapy appeared to have antileukemic effects indicated by the observation that 47% of patients with detectable MRD at the beginning of this phase transitioned to MRD negative status after the short course imatinib treatment.

Twenty-six patients received HSCT in the first CR. Among them, MRD was negative at HSCT in 17 patient and all the five patients who relapsed after HSCT were MRD-negative at HSCT. In contrast, relapse was not observed in nine patients with a detectable level of MRD at HSCT. This suggests that the detection of MRD at HSCT was not related to the occurrence of relapse after HSCT in children with Ph+ALL. In adults with Ph+ALL, Lee et al. also described that the level of MRD at HSCT had little association with relapse after HSCT [13]. However, in the Japan Adult Leukemia Study Group (JALSG) Ph+ALL202 protocol, the relapse rate was significantly lower among patients who were MRD negative at HSCT [14]. More sensitive techniques, such as a deep-sequencing approach, may help to elucidate the significance of MRD at HSCT in patients with Ph+ALL [15].

The amount of MRD at the early phase of treatment for children with ALL distinguishes patients with good prognoses from those with poor prognoses [16, 17]. In our study, the amounts of BCR–ABL transcripts were prospectively monitored. In our cohort, among 26 patients who received HSCT at the first CR, 11 had MRD-negativity at the end of induction therapy, and two of the 11 patients relapsed after HSCT, while three of 15 patients with MRD-positivity at the end of induction suffered relapse after HSCT. Although the number of patients is small, the high/low status of MRD at the end of induction therapy did not seem to be correlated with relapse after HSCT. However, these data should be

interpreted with caution because the method to detect MRD in our study and in JALSG was PCR detection of BCR–ABL transcripts, not an immunoglobulin/T-cell receptor (Ig/TCR) DNA-based technique or flow cytometry. In fact, Jeha, et al. recently reported that MRD detected with flow cytometry at the end of induction was dramatically reduced when TKI was incorporated into induction regimens [18].

In contrast, five of six patients who relapsed before HSCT had a high level of MRD of more than 10,000 copies/μg RNA of BCR–ABL at least 1 month before hematological relapse. Zaliova, et al. also reported BCR–ABL transcript-based MRD enabled better and earlier prediction of relapse compared to DNA-based MRD [19]. Taken together, the value of BCR–ABL-transcript-based-MRD has not yet been fully defined. Prospective studies in Ph+ALL patients comparing several methods of MRD assessment including BCR–ABL transcript, Ig/TCR-DNA, and flow cytometry is warranted. Although response to treatment based on MRD is considered essential for risk group stratification in current protocols for childhood ALL, the innate characteristics of leukemic cells, including additional karyotypic abnormalities[5] and deletion of IKZF-1 [20], might also be informative for the prediction of outcomes in patients with Ph+ALL.

Although OS was excellent in this study, an 86% induction rate appears unsatisfactory, in addition to six out of 36 patients in CR after induction phase experiencing a relapse before HSCT. The use of imatinib in the earlier phase of treatment, even in the induction phase, may be beneficial in children with Ph+ALL. Indeed, imatinib has been used in the induction phase of adult trials and has demonstrated an increase in CR rate [21]. Furthermore, imatinib was successfully used in children from day 15 of induction in a recent SHOP study from Spain, but results were based on a small number of patients (n = 16) [22]. In our study, all the nine patients treated with imatinib-containing chemotherapy as a salvage therapy achieved CR. Hyper-CVAD with imatinib was employed in seven of these nine patients. Hyper-CVAD with imatinib, which is widely used for adults with Ph+ALL [23], may be an alternative option for children with Ph+ALL as a salvage therapy. Detailed clinical course of these patients will be reported separately.

Both the COG and EsPhALL studies, which were contemporary to our study, showed that the use of imatinib concurrently with standard chemotherapy for ALL was safe and tolerable. Conceivably, HSCT may be omitted in a subset of patients who achieve deep remission status if earlier and longer use of imatinib is applied. In our study, all nine patients who were in first CR with a detectable level of MRD at HSCT continue to be in the first CR with negative MRD after HSCT. Based on our data, HSCT itself was safe and effective for children with Ph+ALL. Among 26 patients who were transplanted, no patients experienced treatment-related mortality in spite of the use of unrelated grafts in more than half of patients. It might be due to a uniform use of preconditioning regimen, a good selection of donors and an appropriate timing of HSCT. Eckert et al. also described the importance of standardization of HSCT procedure in the ALL REZ BFM 2002 trial [17]. Since the late effects of HSCT are substantial, the indication of HSCT should be limited. However, HSCT is still an important modality for patients who are at high-risk for relapse, and conditioning regimen consisting of TBI, VP and CY may become a standard regimen for HSCT.

In conclusion, we interpret our results to suggest that the brief use of imatinib monotherapy on leukemic cells prior to HSCT may have a potential therapeutic effect which was demonstrated by 47% of MRD-positive patients transitioning to MRD negative status by the end of this phase. In addition, this was the first prospective trial to conduct HSCT in all children with Ph+ALL in first CR with a uniform conditioning treatment. Use of this protocol achieved an OS of approximately 80%. This result could serve as a basis for future trials aiming to reduce the rate of children who need be treated without HSCT. Finally prospective studies of Ph+ALL are warranted for the comparison of various MRD assessment methods, including BCR–ABL transcript, Ig/TCR-DNA and flow cytometry.

Acknowledgments

We thank Tomoyuki Watanabe for statistical assistance, Emiko Nishioka for preparing and refining the data of patients and Kevin Urayama for editing the manuscript. We also thank the members of the Ph+ALL Committee of the JPLSG: Soichi Adachi, Kazuko Hamamoto, Makoto Yoshida, Keiko Yagi, Akira Ohara, Koichiro Ikuta, Masahiro Tsuchida, Hiroyuki Takahashi, Atsushi Kikuta, Arata Watanabe, Motoaki Chin, Tsutomu Watanabe, Michiko Kajiwara, and Tetsuya Takimoto. Finally, we thank all pediatricians and nurses who participated in the treatment and follow-up of patients in this study. This study was supported by a grant from the Ministry of Health, Labour and Welfare and a grant from the Children's Cancer Association of Japan.

Conflict of Interest

None declared.

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Supporting Information

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

Figure S1. Kinetics of MRD during treatment. Each dot indicates each patient. Time points are shown in Figure 1. MRD, minimal residual disease.

Pediatr Blood Cancer 2015;62:279–284

Statistical Analysis of Relation Between Plasma Methotrexate Concentration and Toxicity in High-Dose Methotrexate Therapy of Childhood NonHodgkin Lymphoma

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Background. Plasma monitoring of Methotrexate (MTX) levels is a standard approach to predict MTX-related toxicities in a high-dose (HD) MTX monotherapy for childhood acute lymphoblastic leukemia. However, it is uncertain whether plasma MTX levels can predict MTX-related toxicity in the HDMTX plus additional chemotherapy for childhood B-cell nonHodgkin lymphoma (B-NHL). ***Procedures.*** To statistically analyze the relationship between MTX pharmacokinetic parameters and MTX-related toxicities, we collected data from patients with delayed MTX elimination ($\geq$1 μM at 48 hr and/or $\geq$0.5 μM at 72 hr) in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) BNHL 03 study. Blood MTX levels were measured at 24, 48, and 72 hr after 3 or 5 g/m^2 HD-MTX administration for 24 hr. ***Results.*** Three hundred and four patients received 2–4 courses of the HDMTX plus additional chemotherapy, and delayed MTX elimination was observed in 165 courses of 127 patients. In those, nephrotoxicity was significantly correlated with plasma MTX levels for each patient ($P=0.03$), and also for each course ($P=0.009$), but no other toxicities were correlated. Another analysis according to HDMTX courses showed no significant correlation between the first high plasma MTX levels and subsequent MTX levels in later course. It also showed that incidence of liver and gastrointestinal toxicities was most frequent in the first HDMTX course, and then sharply decreased in later courses ($P<0.001$). ***Conclusions.*** Our results suggest that plasma MTX level is not a reliable predictor for adverse events except for nephrotoxicity in multiple HDMTX therapy courses in childhood B-NHL. Pediatr Blood Cancer 2015;62:279–284. © 2014 Wiley Periodicals, Inc.

Key words: childhood; HDMTX; nonHodgkin lymphoma; toxicity

INTRODUCTION

In the past two decades, treatment outcome of childhood B-cell nonHodgkin Lymphoma (B-NHL) has been greatly improved by using a short intensive multiagent regimen including high-dose methotrexate (HDMTX), intermediate-dose cyclophosphamide (CPA) and anthracycline [1–4]. Since this treatment rationale is based on rapid elimination of tumor cells with short cell cycle time by subsequent administration of multiple anticancer agents, imprudent prolongation of treatment intervals or dose reduction according to drug toxicity may increase the risk of treatment failure [5–8]. Therefore, the balance between efficacy and adverse events is one of the major clinical challenge to achieve a high cure rate of the disease. Among the multiple drugs, MTX-related toxicity may possibly be predicted based on plasma MTX levels in childhood acute lymphoblastic leukemia (ALL), because HDMTX is used as monotherapy in intensification and maintenance phases [9,10]. However, it might be difficult to predict what kinds of toxicities are associated with plasma MTX levels in a HDMTX plus additional chemotherapy for childhood BNHL, because CPA and anthracycline which are concomitantly used with HDMTX, also induce various toxicities similar to MTX toxicities. In addition, it is unknown whether high plasma MTX level is associated to a particular patient, in other words, the first high MTX level is likely to repeat in later HDMTX courses in a particular patient.

In this study, to answer those clinical issues, we statistically analyzed the relationship between MTX pharmacokinetic parameters and MTX-related toxicities in patients with B-NHL treated by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) B-NHL03 protocol study [4].

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

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Grant sponsor: Ministry of Health, Labor and Welfare of Japan; Grant numbers: H14-Koka(Gan)-031; H15-Koka(Gan)-024; H16-GanRinsho-004; H17-GanRinsho-004; H20-GanRinsho-Ippan-017; H23-GanRinsho-Ippan-014

Conflict of interest: Nothing to declare.

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Received 22 May 2014; Accepted 15 September 2014

DOI 10.1002/pbc.25305
Published online 30 October 2014 in Wiley Online Library (wileyonlinelibrary.com).

PATIENTS AND METHODS

Patients and Protocol Treatment

The protocol was conducted in 112 hospitals of the Japanese Pediatric Leukemia/Lymphoma Study group (JPLSG) after approval by each institution's review board, and written informed consent was provided by patients or legal guardians before treatment. A total of 346 untreated B-NHL patients under 18 years of age, were registered to participate in the JPLSG B-NHL03 study (University hospital Medical Information Network Japan, UMIN ID: C000000317) between November 2004 and January 2011. Patients were stratified into four therapy groups (G1, G2, G3, G4) based on Murphy's stage, tumor resectability and BM/CNS involvement. Chemotherapy regimens are shown in supplemental Table SI. HDMTX was administered to patients in regimen A (2A for G2, 3A for G3, and 4A1 and 4A2 for G4). G2 received four courses (2A → 2B, ×2), G3 and G4 received six courses (3A → 3A → 3B, ×2; 4A1 → 4A2 → 4B, ×2) of chemotherapy regimens. Regimen A consisted of HDMTX, dexamethasone, vincristine, intermediate-dose cyclophosphamide (CPA), and pirarubicin (THP-adriamycin, THP). Patients in G2 and G3 received 3 g/m^2 HDMTX and those in G4 received 5 g/m^2 HDMTX. HDMTX was administered for 24 hr and intravenous hydration at a rate of 100 ml/m^2/hr with 4.3% glucose, $NaHCO_3$ 33 mEq/L, L-Lactate 20 mEq/L, NaCl 35 mEq/L, and KCL 20 mEq/L was initiated 12 hr before the MTX infusion and was maintained for 48 hr after the infusion. During this period, acetazolamide (125 mg <5 years old or 250 mg ≥5 years old) was administered every 12 hr. Urine pH was checked with each void and a bolus of $NaHCO_3$ (8.4 mEq in 20 ml) was administered if the pH was <7.0. After 12 hr of MTX infusion, leucovorin (LV) 15 mg/m^2 was given orally every 6 hr for a total of seven doses. When patients showed high plasma MTX levels (≥0.2 μM) at 72 hr, LV rescue was continued until MTX concentration level decreased to less than 0.2 μM.

Measurements of Plasma MTX Concentration

Plasma MTX concentrations were determined by each institute, and the measurements were performed by a monoclonal antibody-based immunoassay (fluorescence polarization immunoassay, FPIA) in 91 institutes, or by an enzyme multiplied immunoassay technique (EMIT) in 21 institutes. Delayed MTX elimination was defined as plasma MTX concentration ≥1 μM at 48 hr and/or ≥0.5 μM at 72 hr after MTX administration. Since only one third of the data of MTX concentrations at 24 hr after MTX administration (the end of 24-hr infusion) was available and there were also no sufficient sampling points between 24 and 48 hr to calculate the pharmacokinetic parameters of MTX, we could not analyze the appropriate pharmacokinetic parameters including systemic clearance (CLSYS) based on the two-compartmental model. We therefore calculated the basic two parameters of MTX (elimination rate constant (ke) and terminal half-life ($t_{1/2}$)). The terminal slope of MTX concentration (C) versus time (t), which represents ke, was calculated as $ke = [\ln(C1) \cdot \ln(C2)]/(t2 \cdot t1)$, where C1 and C2 were concentrations at t1 (48 hr) and t2 (72 hr), respectively. The $t_{1/2}$ was calculated by dividing 0.693 by ke.

Statistics

Plasma MTX levels and toxicity data were prospectively collected for each treatment phase and toxicity severity was graded according to National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.0. Continuous variables were summarized as the mean ± standard deviation (SD) or median (minimum, maximum) and categorical variables were presented as numbers and percentages. Correlation between the two variables was estimated by Spearman's correlation coefficient. The plasma MTX concentrations in patients with an adverse event (AE) were compared to those in patients without the AE by using Wilcoxon's rank sum test. In this analysis, one observation for each patient was taken into account. The observation with the AE and the highest concentration at 48 hr was preferentially used if a patient received more than one course and had more than one observation. Furthermore, log-transformed MTX concentrations were compared between patients with and without AE using generalized estimating equations (GEE) method [11] including AE (yes vs. no) and course as factors, in order to take into account repeated measures of the same patient. The presence (≥grade 3) of toxicity (hepatic toxicity, stomatitis, and infection) were analyzed using the GEE with repeated-measures logistic regression model including nephrotoxicity (yes vs. no) and course as factors. We assumed an exchangeable covariance matrix for the repeated-measures in the GEE analyses. All tests were two-sided, and p values less than 0.05 were considered to indicate statistical significance. Statistical analyses were carried out using SAS 9.3 (SAS Institute, Inc., Cary, NC).

RESULTS

Pharmacokinetic Parameters

One hundred twenty seven patients out of a total of 304 patients who received HDMTX therapy showed delayed MTX elimination. MTX concentrations in patients with delayed MTX elimination are summarized in Table I. Percentages of patients with delayed MTX elimination by treatment groups were 26.2% in G2, 40.5% in G3, and 62.2% in G4, respectively. The male to female ratio in patients with delayed MTX elimination was more than double than patients without delayed MTX elimination (107/20 = 5.35 vs. 123/54 = 2.27,

TABLE I. Summary of High Plasma MTX Concentrations at 48 and 72 hr After MTX Dosing*

			MTX concentration at 48 hr		MTX concentration at 72 hr	
Group	No. of patients	No. of courses	Mean ± SD	Median (Min, Max)	Mean ± SD	Median (Min, Max)
2	26	27	2.63 ± 2.25	1.86 (0.99, 11.00)	0.66 ± 0.52	0.61 (0.08, 1.90)
3	45	53	3.41 ± 5.19	1.77 (0.80, 29.70)	1.02 ± 1.55	0.44 (0.17, 8.23)
4	56	85	3.80 ± 6.29	1.82 (0.93, 48.00)	1.06 ± 1.79	0.54 (0.05, 11.00)

*≥1 μM at 48 hr and/or ≥0.5 μM at 72 hr after MTX administration.

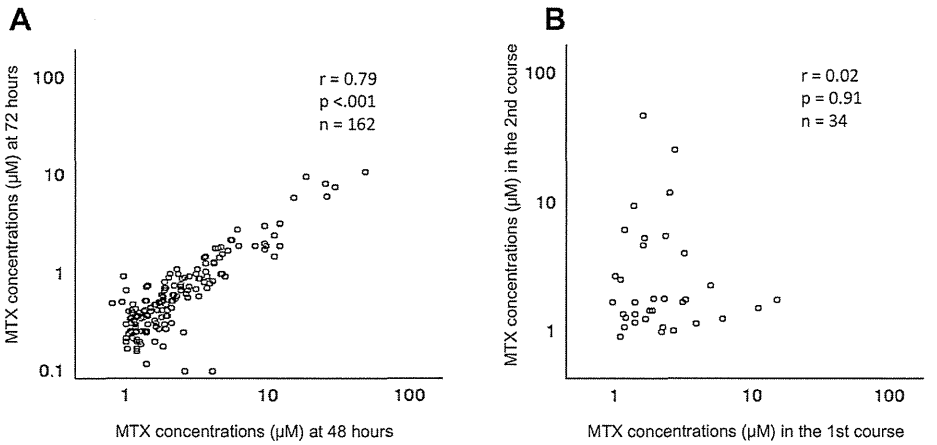

Fig. 1. Correlation between blood MTX concentrations at 48 and 72 hr in patients with delayed MTX clearance (**A**). Correlation between blood MTX concentrations in first and second courses at 48 hr (**B**). r denotes Spearman's rank correlation coefficient.

$P = 0.004$ by Fisher's exact test), and the ratios according to treatment group were 5.5 in G2, 10.2 in G3, and 3.6 in G4. Thus, males with G3 showed the highest risk of delayed MTX elimination. However, there was no significant difference in age between the two groups (mean of years 8.8 vs. 8.8). MTX concentrations were widely variable between patients at either dosage. MTX concentrations at 48 hr ranged from 0.99 to 29.7 μM in the 3 g/m^2 HDMTX group, and 0.93 to 48 μM in the 5 g/m^2 HDMTX group. There was a significantly positive correlation between MTX concentrations at 48 and 72 hr in each patient (Fig. 1A). On the other hand, there was no significant

TABLE II. Relationship Between Plasma MTX Pharmacokinetics (MTX Concentration and MTX Half-Life) and Adverse Events

	Adverse event (number of patients base)			Adverse event (number of courses base)		
	No, n = 118	Yes, n = 9	P^a	No, n = 156	Yes, n = 9	P^b
Nephrotoxicity ≥grade 2[c]						
48 hr	1.93 (0.80, 25.50)	6.17 (0.99, 48.00)	0.0307	1.81 (0.80, 25.50)	6.17 (0.99, 48.00)	0.009
72 hr	0.53 (0.08, 9.89)	1.82 (0.35, 11.00)	0.0023	0.50 (0.05, 9.89)	1.82 (0.35, 11.00)	<0.001
$t_{1/2}$ (hr)	12.3 (4.5, 35.4)	14.1 (9.9, 42.6)	0.373	12.7 (4.5, 1571.7)	14.1 (9.9, 42.6)	0.318
	Adverse event (number of patients base)			Adverse event (number of courses base)		
	No, n = 84	Yes, n = 42	P^a	No, n = 120	Yes, n = 44	P^b
Hepatic toxicity ≥grade 3/4[c]						
MTX 48 hr	1.98 (0.80, 48.00)	1.91 (0.95, 15.00)	0.65	1.82 (0.80, 48.00)	1.82 (0.95, 15.00)	0.95
MTX 72 hr	0.58 (0.08, 11.00)	0.59 (0.16, 6.00)	0.96	0.51 (0.05, 11.00)	0.53 (0.16, 6.00)	0.58
$t_{1/2}$ (hr)	12.6 (4.5, 35.4)	13.0 (8.0, 1,571.7)	1.00	12.8 (4.5, 35.4)	12.9 (8.0, 1,571.7)	0.26
	Adverse event (number of patients base)			Adverse event (number of courses base)		
	No, n = 68	Yes, n = 58	P^a	No, n = 98	Yes, n = 66	P^b
Oral mucositis ≥grade 3/4[c]						
MTX 48 hr	1.86 (0.80, 48.00)	2.28 (0.95, 25.50)	0.38	1.69 (0.80, 48.00)	2.23 (0.95, 25.50)	0.25
MTX72 hr	0.49 (0.08, 11.00)	0.60 (0.10, 9.89)	0.35	0.45 (0.05, 11.00)	0.59 (0.10, 9.89)	0.23
$t_{1/2}$ (hr)	12.9 (5.7, 35.4)	12.6 (4.5, 1,571.7)	0.83	12.8 (5.4, 35.4)	12.7 (4.5, 1,571.7)	0.23
	Adverse event (number of patients base)			Adverse event (number of courses base)		
	No, n = 23	Yes, n = 103	P^a	No, n = 37	Yes, n = 127	P^b
Infection ≥grade 3/4[c]						
MTX 48 hr	2.03 (0.80, 48.00)	1.90 (0.93, 29.70)	0.49	1.90 (0.80, 48.00)	1.81 (0.93, 29.70)	0.56
MTX 72 hr	0.70 (0.22, 11.00)	0.56 (0.08, 9.89)	0.31	0.50 (0.22, 11.00)	0.52 (0.05, 9.89)	0.25
$t_{1/2}$ (hr)	13.0 (10.0, 35.4)	12.4 (4.5, 27.7)	0.48	12.9 (8.5, 35.4)	12.6 (4.5, 1,571.7)	0.74

Data are presented as median (min, max). n: number of patients or courses. [a]Wilcoxon's rank sum test. [b]Generalized estimating equations method for repeated log-transformed MTX concentrations. [c]NCI-CTC version 2.0.

correlation between MTX concentrations at 48 hr in the first and next HDMTX courses in each patient (Fig. 1B).

Toxicities

In order to clarify what kinds of MTX toxicities are closely associated with MTX pharmacokinetic parameters, we statistically analyzed the correlation between the parameters (plasma MTX levels and half-life ($t_{1/2}$)) and MTX-related toxicities (stomatitis, nephrotoxicity, hepatic toxicity, and infection). In this study, we excluded hematological toxicity and CNS toxicity from the analysis, because neutropenia ≥grade 3 was observed in almost all (>98%) patients regardless of MTX levels, and CNS toxicity ≥grade 3 occurred in only one case. In general, adverse events (AEs) ≥grade 3 were collected for analysis, but serum creatinine and proteinuria ≥grade 2 were used for nephrotoxicity because the number of nephrotoxic AEs ≥grade 3 was very few (n = 4) and proteinuria has been shown to be a HDMTX-related nephrotoxicity [12]. The number of patients with nephrotoxicity ≥grade 2 was nine: five in grade 2, one in grade 3 and one in grade 4 with high serum creatinine levels, and two in grade 2 with proteinuria. As shown in Table II, only nephrotoxicity was significantly correlated with higher MTX levels for each patient, and also for each course, but other toxicities had no correlations to MTX levels. MTX half-life showed no significant relation to any of the MTX-related toxicities. We also analyzed statistical difference in the frequency of other toxicities, such as hepatic toxicity, stomatitis and infection between patients with nephrotoxicity and patients without (Table III). These results showed that patients with nephrotoxicity tended to have higher frequencies of hepatic toxicity, although the difference did not reach significant levels.

Lastly, we studied the difference in incidences of severe toxicities according to HDMTX courses in all patients of group 3 and group 4 (Fig. 2). Incidences of hematological toxicities did not vary widely during the four courses. However, incidences of non-hematological toxicities such as liver and gastrointestinal toxicities showed a large variation during the courses: the incidence was the greatest in the first course, and then sharply decreased in later courses in both groups ($P < 0.001$). In addition, the incidences seemed to be unrelated with plasma MTX levels.

Modification of Protocol Treatments

In our study, treatment modifications according to delayed MTX elimination were reported in 15 patients (2 in group 2, 4 in group 3, and 9 in group 4). Eleven of which had suffered from MTX-induced nephrotoxicity with high creatinine levels (6 in grade 1, 3 in grade 3, 1 in grade 3 and 1 in grade 4). The modifications were as follows: dose reduction or prolongation of treatment intervals of CPA and THP in 8, withdrawal of CPA and THP in 2, reduction of HDMTX dose (from 5 to 3 g/m^2) in the next HDMTX course in 3 (2 between 1st and 2nd course, one between 2nd and 3rd course), and exchanging course 4A with course 4B without HDMTX in 2. Of the 15 patients, 14 patients except one, who had CNS involvement, survived without diseases.

TABLE III. Incidence of MTX-Related Toxicities According to Nephrotoxicity

		Nephrotoxicity ≥grade 2		
Group		No	Yes	P^a
Hepatic toxicity				
Total	Grade 3	36 (23.2)	3 (33.3)	
	Grade 4	2 (1.3)	2 (22.2)	
	≥grade 3/4	38 (24.5)	5 (55.6)	0.051
2	Grade 3	5 (20.0)	1 (50.0)	
	Grade 4	0 (0.0)	0 (0.0)	
	≥grade 3/4	5 (20.0)	1 (50.0)	0.34
3	Grade 3	10 (20.4)	0 (0.0)	
	Grade 4	1 (2.0)	1 (33.3)	
	≥grade 3/4	11 (22.4)	1 (33.3)	0.79
4	Grade 3	21 (25.9)	2 (50.0)	
	Grade 4	1 (1.2)	1 (25.0)	
	≥grade 3/4	22 (27.2)	3 (75.0)	0.10
Oral mucositis				
Total	Grade 3	57 (36.8)	3 (33.3)	
	Grade 4	5 (3.2)	1 (11.1)	
	≥grade 3/4	62 (40.0)	4 (44.4)	0.84
2	Grade 3	5 (20.0)	1 (50.0)	
	Grade 4	1 (4.0)	0 (0.0)	
	≥grade 3/4	6 (24.0)	1 (50.0)	0.42
3	Grade 3	20 (40.8)	1 (33.3)	
	Grade 4	0 (0.0)	1 (33.3)	
	≥grade 3/4	20 (40.8)	2 (66.7)	0.46
4	Grade 3	32 (39.5)	1 (25.0)	
	Grade 4	4 (4.9)	0 (0.0)	
	≥grade 3/4	36 (44.4)	1 (25.0)	0.39
Infection				
Total	Grade 3	119 (76.8)	7 (77.8)	
	Grade 4	1 (0.6)	0 (0.0)	
	≥grade 3/4	120 (77.4)	7 (77.8)	0.87
2	Grade 3	20 (80.0)	1 (50.0)	
	Grade 4	0 (0.0)	0 (0.0)	
	≥grade 3/4	20 (80.0)	1 (50.0)	0.28
3	Grade 3	38 (77.6)	3 (100.0)	
	Grade 4	0 (0.0)	0 (0.0)	
	≥grade 3/4	38 (77.6)	3 (100.0)	NC
4	Grade 3	61 (75.3)	3 (75.0)	
	Grade 4	1 (1.2)	0 (0.0)	
	≥grade 3/4	62 (76.5)	3 (75.0)	0.75

[a]Generalized estimating equations method for repeated adverse event (≥grade 3/4). NC: not calculated. Data are n (%).

DISCUSSION

Recent pharmacokinetic and pharmacogenetic studies of HDMTX treatment in childhood lymphoid malignancies have shown significant relations between polymorphisms in genes coding for enzymes involved in folate metabolisms and MTX-related toxicities. However, individual prediction of MTX toxicity and dose adjustment of HDMTX based on pretreatment genotyping do not reach a practical use [13–15] and routine monitoring of plasma MTX concentrations still has an important role to predict MTX toxicities in clinical practice.

In the present study, we analyzed the relation between MTX pharmacokinetics and MTX-related toxicities in the HDMTX plus additional chemotherapy for childhood B-NHL. We found that plasma MTX levels were significantly correlated with nephrotoxicity (creatinine and/or proteinuria ≥grade 2), but not other toxicities. MTX half-life was not associated with any toxicity. These results suggest that MTX-induced nephrotoxicity could be

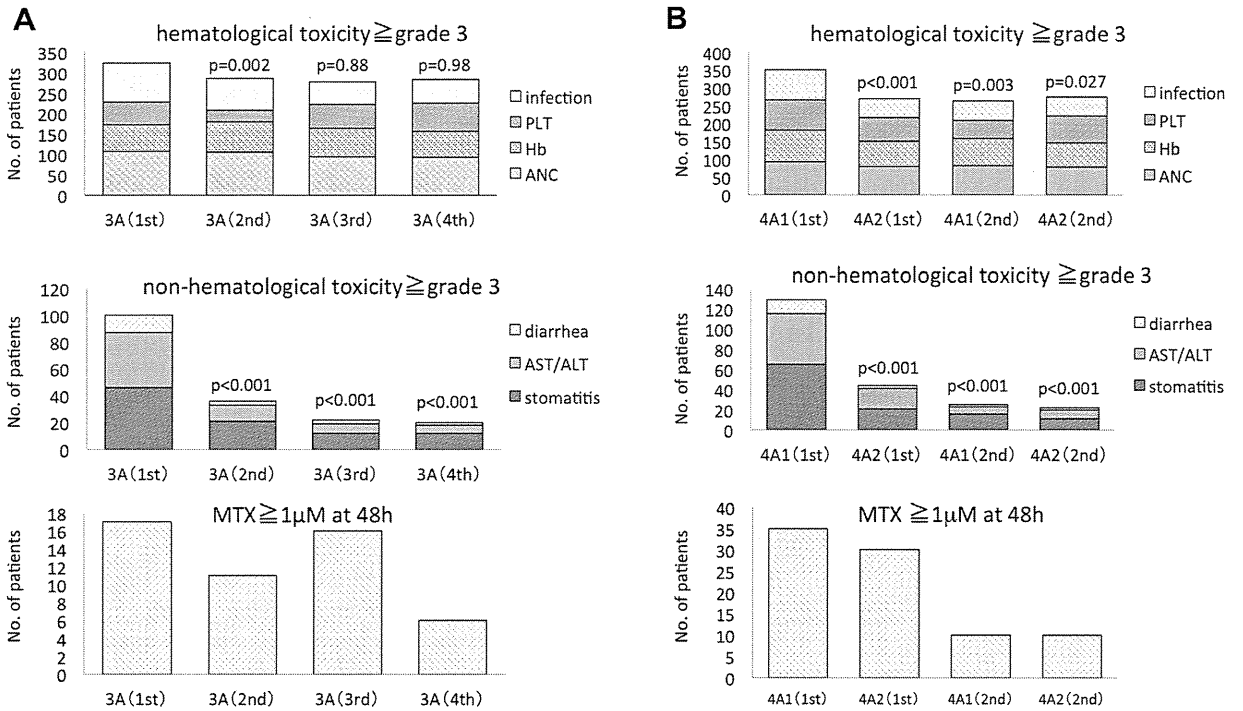

Fig. 2. Incidence of MTX-related toxicities and delayed MTX elimination according to HDMTX course. Left panel (**A**) for group 3; Right panel (**B**) for group 4. Number in vertical axis shows number of patients with hematological toxicities (**upper panel**), non-hematological toxicities (**middle panel**), and delayed MTX elimination (≥1 μM at 48 hr) (**lower panel**). Number of patients who received HDMTX therapy of each course was 108 in 1st 3A1, 106 in 2nd 3A1, 95 in 3rd 3A1, and 94 in 4th 3A1 in group 3, and 94 in 1st 4A1, 83 in 1st 4A2, 79 in 2nd 4A1, and 78 in 2nd 4A2 in group 4, respectively. *P* values reported from Dunnett's test based on the generalized estimating equation method comparing the toxicity count by course (reference group is 3A (1st) or 4 A1 (1st)).

caused by the long-time exposure to high plasma MTX levels during 48–72 hr, but is not related with MTX half-life determined in the elimination phase in our study.

Very few studies have been reported on nephrotoxicity of HDMTX in lymphoma patients [12,16]. May et al. [16] retrospectively studied the incidence of nephrotoxicity in adults with lymphoma, and reported a 21% (37/179 courses) incidence of nephrotoxicity with creatinine ≥grade 2 in patients associated with delayed MTX elimination. This was five times higher than 4% (7/165 courses) incidence of nephrotoxicity in our study. This discrepancy may be due to the difference in age of patients between the two studies. They also suggested that renal toxicity was not related to delayed MTX elimination, because the ratio (20%) of nephrotoxicity of patients who do not have was the almost same as patients with delayed MTX elimination. However, this is not consistent with our findings, because the incidence of nephrotoxicity ≥grade 2 in patients without delayed MTX elimination was 0% in our study (data not shown). Lack of correlation between delayed MTX elimination and other toxicities was rather unexpected. This finding suggests that MTX-related toxicities such as stomatitis, hepatic toxicity and infection are affected by CPA and THP as well as MTX in the HDMTX courses in childhood B-NHL treatment.

In our study, delayed MTX elimination was significantly associated with male sex. This finding is inconsistent with some HDMTX studies in childhood ALL, in which female sex has been reported to be associated with high MTX concentrations or low MTX clearance [17,18], whereas other studies have shown that gender is not significantly associated with MTX concentrations or pharmacokinetic polymorphism in childhood ALL [19,20]. Thus, the role of gender in MTX pharmacokinetics still remains to be elucidated in childhood ALL. In childhood NHL, our results may provide actionable observation that male sex has two times higher risk than female to suffer delayed elimination of MTX in HDMTX therapy, although male sex was not an unfavorable prognostic factor in outcome [4].

There was no significant relation between the first high plasma MTX levels and subsequent MTX levels in the later HDMTX course. This finding showed that there was a wide intra-individual variability of blood MTX levels as previously described by others [21]. Since MTX is primarily eliminated by kidney, creatinine clearance may reflect blood MTX levels. However, there have been controversial studies for relation between creatinine concentrations and plasma MTX levels. One study of children who received 3 or 5 g/m^2 of HDMTX has shown a positive correlation between serum creatinine concentrations and blood MTX levels [22] whereas, another study for children who received 1 or 2 g/m^2 failed to show the positive association [21]. Although creatinine clearance is not steady, it is unlikely that creatinine clearance may change during administration of HDMTX, since all patients were strictly monitored and maintained a high urine output and urinary alkalization during HDMTX administration in our study. From the point of view of clinical practice, we

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

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

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

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

ACKNOWLEDGEMENTS

We thank the patients who enrolled in the B-NHL03 study and their families, and Dr. T. Hasegawa for measurements of MTX pharamacokinetic parameters. This study was supported by Grants for Clinical Cancer Research from the Ministry of Health, Labor and Welfare of Japan; H14-Koka(Gan)-031, H15-Koka(Gan)-024, H16-GanRinsho-004, H17-GanRinsho-004, H20-GanRinsho-Ippan-017, H23-GanRinsho-Ippan-014.

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Leukemia (2015) **29**, 290–296

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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.

Leukemia (2015) **29,** 290–296; doi:10.1038/leu.2014.172

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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Received 19 March 2014; revised 14 May 2014; accepted 28 May 2014; accepted article preview online 3 June 2014; advance online publication, 24 June 2014

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.

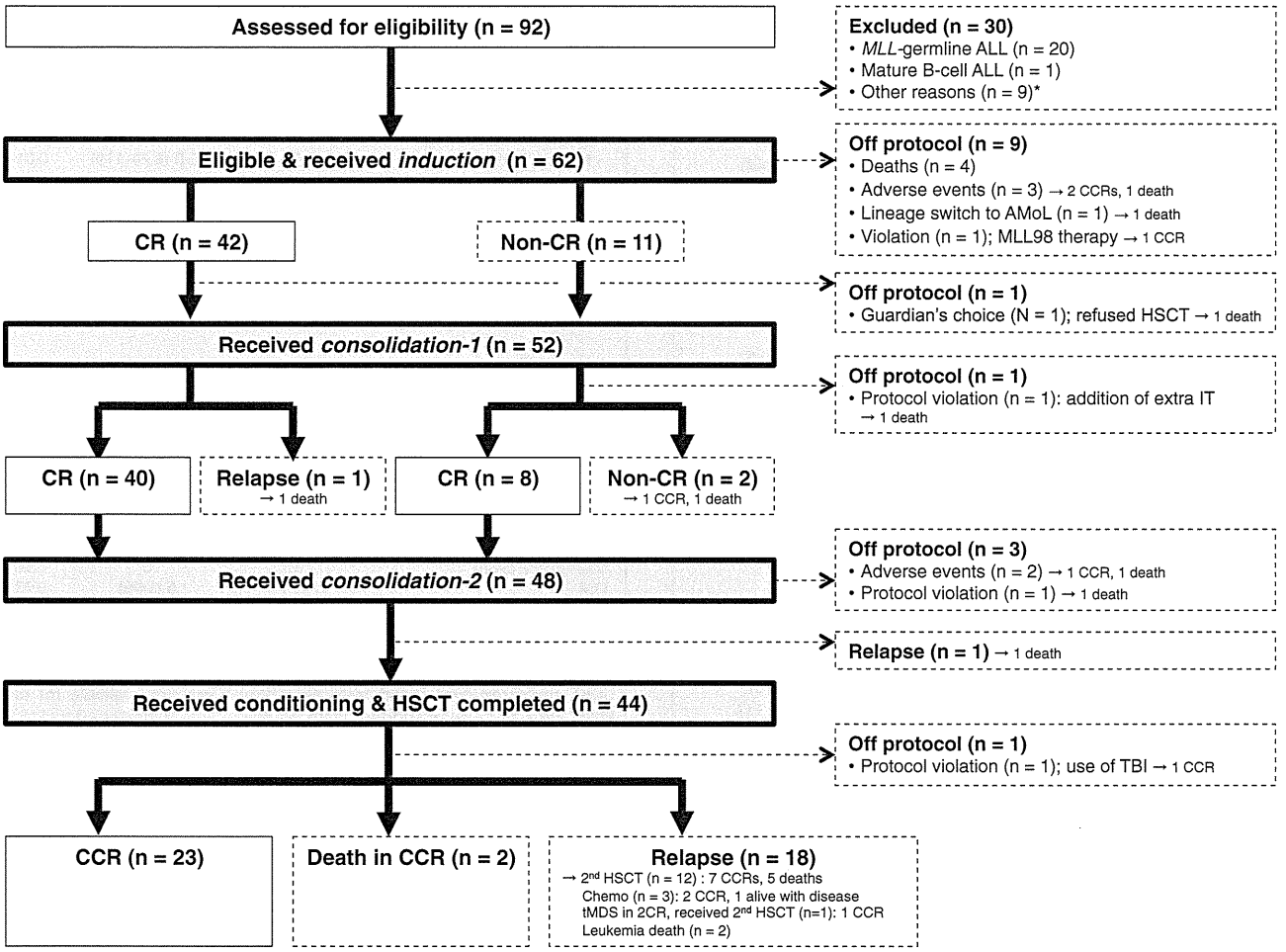

Figure 1. Patient flow chart in the MLL03 study. ALL, acute lymphoblastic leukemia; AMoL, acute monocytic leukemia; CR, complete remission; CCR, continuous CR; HSCT, hematopoietic stem cell transplantation; IT, intrathecal therapy; TBI, total-body irradiation; tMDS, therapy-related myelodysplastic syndrome; 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 $\geqslant 90$ days), white blood cell count at initial diagnosis ($\geqslant 100\,000/\mu l$ vs $<100\,000/\mu l$), 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

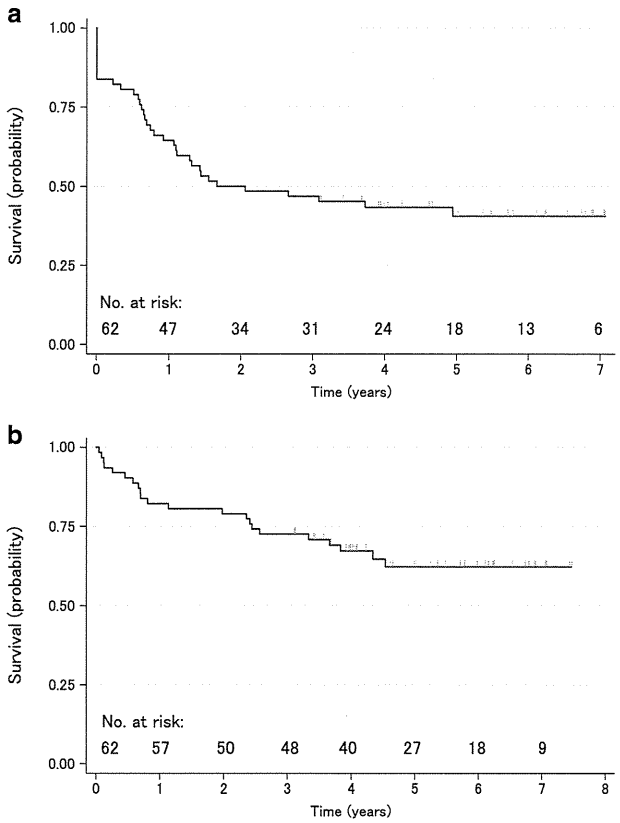

Figure 2. Outcomes of infants with ALL and *MLL*-gene rearrangements enrolled in the MLL03 study. (**a**) Event-free survival (EFS). (**b**) Overall survival.

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 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.