**Hematopoietic Stem Cell Transplantation Based on Minimal Residual Disease with a Unified Conditioning Regimen Comprising Total Body Irradiation, Etoposide and Cyclophosphamide: Results from the JPLSG ALL-R08-II Trial, the First Nationwide Prospective Study for Children with Intermediate-Risk Relapsed Acute Lymphoblastic Leukemia in Japan**

**Running short title**: HSCT w/TBI, ETP and CY for Children with IR Relapsed ALL

Hideaki Ueki1, Chitose Ogawa2, Hiroaki Goto3, Masanori Nishi4, Junko Yamanaka5, Shinji Mochizuki5, Takuro Nishikawa6, Tadashi Kumamoto2, Ritsuo Nishiuchi7, Atsushi Kikuta8, Shohei Yamamoto9, Shunji Igarashi1, Atsushi Sato10, Toshinori Hori11, Akiko Moriya Saito12, Tomoyuki Watanabe13, Takao Deguchi14, Atsushi Manabe15, Keizo Horibe12, Hidemi Toyoda16

1Department of Pediatric Haematology/Oncology, Japanese Red Cross Narita Hospital, Narita, Japan

2Department of Pediatric Oncology, National Cancer Centre Hospital, Tokyo, Japan

3Division of Hematology/Oncology, Kanagawa Children's Medical Centre, Yokohama, Japan

4Department of Pediatrics, Faculty of Medicine, Saga University, Saga, Japan

5Department of Pediatrics, National Centre for Global Health and Medicine, Tokyo, Japan

6Department of Pediatrics, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

7Department of Pediatrics, Kochi Health Sciences Centre, Kochi, Japan

8Department of Pediatric Oncology, Fukushima Medical University Hospital, Fukushima, Japan

9Department of Pediatrics, Tokai University School of Medicine, Isehara, Japan

10Department of Hematology/Oncology, Miyagi Children’s Hospital, Sendai, Japan

11Department of Pediatrics, Aichi Medical University School of Medicine, Nagakute, Japan

12Clinical Research Centre, National Hospital Organization Nagoya Medical Centre, Nagoya, Japan

13Department of Nutritional Science, Faculty of Psychological and Physical Science, Aichi Gakuin University, Nisshin, Japan

14Division of Cancer Immunodiagnostics, Children’s Cancer Centre, National Centre for Child Health and Development, Tokyo, Japan

15Department of Pediatrics, Hokkaido University, Sapporo, Japan

16Department of Pediatrics, Mie University, Tsu, Japan

# Address correspondence to: Hidemi Toyoda, M.D., Ph.D.

Department of Pediatrics, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan

Tel: +81-59-232-1111, Fax: +81-59-231-5127, E-mail: [htoyoda@med.mie-u.ac.jp](mailto:htoyoda@med.mie-u.ac.jp)

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**Abbreviations**

|  |  |
| --- | --- |
| ALL | acute lymphoblastic leukemia |
| allo | allogeneic |
| HSCT | hematopoietic stem cell transplantation |
| MRD | minimal residual disease |
| JPLSG | the Japanese Pediatric Leukemia/Lymphoma Study Group |
| CTCAE v3.0 | Common Terminology Criteria for Adverse Events version 5.0 |
| TBI | total body irradiation |
| ETP | etoposide |
| CY | cyclophosphamide |
| EFS | event-free survival |
| OS | overall survival |
| HLA | human leukocyte antigen |
| GVHD | Graft-versus-host disease |
| MTX | methotrexate |
| CsA | cyclosporine |
| RQ-PCR | real-time quantitative polymerase chain reaction |
| BMT | bone marrow transplantation |
| CBT | cord blood transplantation |

# Abstract

# Background: In children with intermediate-risk relapsed acute lymphoblastic leukemia (ALL), allogeneic hematopoietic stem cell transplantation (allo-HSCT) has markedly improved the outcome of patients with poor minimal residual disease (MRD) response. However, there is no consensus on the optimal conditioning regimen for allo-HSCT. Procedure: We prospectively analyzed the efficacy and safety of allo-HSCT with a unified conditioning regimen for children with intermediate-risk relapsed ALL, based on MRD in the bone marrow after induction, in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) ALL-R08-II nationwide cohort. The conditioning regimen for allo-HSCT comprised total body irradiation (TBI), etoposide (ETP) and cyclophosphamide (CY) (UMIN000002025). Results: Twenty patients with post-induction MRD ≥ 10−3 and two with MRD that could not be evaluated underwent allo-HSCT. Engraftment was confirmed in all patients. No transplantation-related mortality was observed. The 3-year event-free survival and overall survival after transplantation were 86.4% ± 7.3% and 95.5% ± 4.4%, respectively. Conclusion: Allo-HSCT based on post-induction MRD with TBI + ETP + CY conditioning was highly effective and feasible for Japanese children with intermediate-risk relapsed ALL.

# Introduction

# Treatment outcomes of children with acute lymphoblastic leukemia (ALL) have improved in recent years but are still unsatisfactory. In a retrospective survey of children in Japan who developed ALL from 1989–1999 and relapsed up to 2003 (n = 356), the 3-year event-free survival (3y-EFS) and 3-year overall survival (3y-OS) in the intermediate-risk (S2) group of the multicenter ALL-REZ BFM trial stratification of relapsed ALL [1] were 40.6% ± 3.8% and 54.2% ± 3.8% (n = 169), respectively (unpublished data).

In children with intermediate-risk relapsed ALL, the efficacy of allogeneic hematopoietic stem cell transplantation (allo-HSCT), assessed as minimal residual disease (MRD), has been recognized in recent years. The probability of EFS in patients with post-induction MRD ≥ 10−3 who were allocated to allo-HSCT as reported by the Berlin-Frankfurt-Münster group was 64% ± 5% in the ALL-REZ BFM 2002 study but was 18% ± 7% in the predecessor ALL-REZ BFM P95/96 trial (*P* = 0.001) [2]. However, there is no consensus on the optimal conditioning regimen for allo-HSCT for MRD-positive children in this risk group.

Conditioning regimens comprising total body irradiation (TBI) + etoposide (ETP) + cyclophosphamide (CY) have been studied for treating childhood ALL, with promising outcomes reported [3-5]. Historically, the outcome of allo-HSCT using TBI + CY had not been satisfactory, with an EFS of 35% to 55% for children with late relapse of ALL [6-10]. Allo-HSCT with TBI + ETP conditioning for children with ALL in second remission reportedly showed EFS of 60% to 76% [7,11-13]. The mechanisms of anti-leukemia activity for ETP and CY are different. ETP is cytotoxic by interfering with the repair of double-stranded DNA breaks by topoisomerase II [14]. The alkylating agent, CY, damages leukemic cells through DNA crosslinking [15]. In addition, ETP reportedly has synergistic activity with CY in vitro [16-18]. A previous study reported the outcomes of 28 children with ALL in second remission. The children received TBI (12 Gy), ETP and CY as a conditioning regimen for allo-HSCT, and the 2-year disease-free survival was 80.2% [4].

In this study, we conducted a prospective, non-randomized nationwide multicenter trial to investigate the efficacy and safety of allo-HSCT conditioned with the TBI + ETP + CY regimen for children with intermediate-risk first-relapsed ALL who had an unsatisfactory MRD response.

# Methods

# *Study design*

# The Japanese Pediatric Leukemia/Lymphoma Study Group’s (JPLSG) ALL-R08 study was the first nationwide prospective multicenter trial for children with first relapse of ALL in Japan. The ALL-R08 study contained two parts: ALL-R08-I observational study and ALL-R08-II clinical trial. The ALL-R08-II trial was for children with intermediate-risk (S2) relapsed ALL and was registered in the University Hospital Medical Information Network Clinical Trials Registry in Japan as UMIN000002025. The intermediate-risk (S2) relapsed ALL included children with an early (≥18 months after primary diagnosis and <6 months after completion of primary therapy) or late (≥6 months after completion of primary therapy) combined bone marrow relapse, late isolated bone marrow relapse, very early (<18 months after primary diagnosis and <6 months after completion of primary therapy) and early isolated extramedullary relapse. Patients with MRD < 10−3 in the bone marrow after induction therapy group were planned to be treated without allo-HSCT, whereas patients with MRD ≥ 10−3 were transplanted at the end of therapy.

# *Patients*

# Children with intermediate-risk first-relapsed ALL were registered in the ALL-R08-II trial between June 1, 2009 and October 31, 2013. The inclusion criteria for this trial were as follows: intermediate-risk (S2) first relapse of non-T-cell ALL; age < 18 years at the onset of ALL; age < 20 years at diagnosis of relapse; no evident abnormality of liver, kidney and heart; Eastern Cooperative Oncology Group performance status score of 0–2 or a score of 3 caused by ALL (baseline performance status was scored 0–2, but if the exacerbation of performance status was attributed to leukemia, a score of 3 was allowed).

# The exclusion criteria were as follows: mature B-cell ALL; Ph+ ALL; infant leukemia with rearrangement of mixed lineage leukemia gene; Down syndrome; bleeding ≥ CTCAE v3.0 grade 3 of the central nervous system; uncontrollable infections; pregnancy or possibility of pregnancy; past history of congenital or acquired immunodeficiency; contraindication for any agent planned to use in the trial; any other condition that physicians consider inadequate and relapses after HSCT. Written informed consent was obtained from the patients aged ≥16 years and/or from the legal guardians according to the Helsinki Declaration and the ethical guidelines for clinical research in Japan.

# *Treatment protocol*

# The treatment protocol was based on the ALL-REZ BFM P95/96 trial. An outline of the therapy is shown in Fig. 1. Following a cytoreductive pre-phase with dexamethasone, remission induction therapy was administered. The induction therapy consisted of two blocks (F1 and F2). MRD in the bone marrow was evaluated after block F2, and patients with positive MRD (≥10−3) were assigned to the allo-HSCT arm. Patients with unevaluable MRD were assigned to either the chemotherapy arm or allo-HSCT arm at the physicians’ discretion. Patients assigned to allo-HSCT arm underwent five courses of block chemotherapy (R2/R1/R2/R1/R2, Table S2) followed by allo-HSCT. Patients who were not in hematological remission (≥5% leukemic cells in bone marrow) after the first R1 course were excluded from the study.

# *Hematopoietic stem cell transplantation*

# A standard donor for allo-HSCT in the trial was defined as follows: related donor (bone marrow or peripheral blood stem cell) who was at least five out of six human leukocyte antigen (HLA) serologically identical with typing of HLA A, B and DR; non-related donor obtained from the Japan Marrow Donor Program; six out of six HLA serologically identical or a non-related cord blood donor who was at least four out of six HLA serologically identical with typing of HLA A, B and DR, with a nuclear cell count ≥ 2.5 × 107/kg.

# The conditioning regimen for allo-HSCT was as follows: TBI 12 Gy + ETP 60 mg/kg × 1 day + CY 60 mg/kg × 2 days for patients <30 kg; TBI 12 Gy + ETP 1800 mg/m2 × 1 day + CY 60 mg/kg × 2 days for patients ≥30 kg. In patients who obtained no standard donor, the donor selection and conditioning regimen were at the discretion of the institution where the patient was hospitalized.

# *Graft-versus-host disease prophylaxis*

# Graft-versus-host disease (GVHD) prophylaxis was performed as follows. For patients aged <10 years who received transplantation from HLA identical siblings, either methotrexate (MTX) only or cyclosporine (CsA) only were administered. MTX was administered at a dose of 15 mg /m2 intravenously on day +1 and 10 mg/m2 on days +3, 6, 11, 18, 25 and once a week subsequently to day +60. CsA was started at 3 mg/kg/day delivered in two intravenous infusions over 2 hours with a target trough level of 150–250 ng/mL or in a continuous infusion with a target trough level of 200–300 ng/mL. For the patients aged ≥10 years who received transplantation from HLA identical siblings, MTX and CsA were administered (MTX was administered intravenously at 15 mg/m2 on day +1 and at 10 mg/m2 on days +3, 6 and 11. The protocol for CsA administration was the same as above). For the patients from other types of standard donors, MTX (15 mg/m2 on day +1 and 10 mg/m2 on days +3, 6 and 11) and tacrolimus (started at 0.02 mg/kg/day in continuous intravenous infusion, adjusted for a target trough level of 5–15 ng/mL) were administered. GVHD prophylaxis for patients who received transplantation from a non-standard donor was not regulated.

# *Local therapy for extramedullary lesion*

# In patients with unilateral testicular involvement on physical examination, orchiectomy of the involved side was performed. If the biopsy result of the contralateral testis was negative, the testis was irradiated at 3 Gy prior to TBI. If the biopsy result of the contralateral testis was positive, the testis was irradiated at 6 Gy prior to TBI. In patients with bilateral testicular involvement on physical examination, the bilateral testes were removed or irradiated with 12 Gy prior to TBI.

# Patients with central nervous system involvement at diagnosis of relapse were boosted with 6 Gy of cranial irradiation prior to TBI.

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# *MRD assessment*

# MRD of bone marrow samples was quantified using real-time quantitative polymerase chain reaction (RQ-PCR) at the end of induction (after course F2). The data of RQ-PCR were analyzed on the basis of the EuroMRD Consortium guidelines [19]. An MRD level ≥ 10−3 was considered positive in this study.

# *Statistical methods*

EFS and OS were estimated using the Kaplan–Meier method and calculated from the date of allo-HSCT until the event date. Data were censored on October 31, 2016. Patients who were lost to follow-up were censored at the last-contact date. The 95% confidence interval was calculated using Greenwood’s formula. Second relapses, deaths from any causes and secondary malignancies were considered as events for EFS calculation. OS was calculated from the date of allo-HSCT to death from any cause. The calculations were performed in StataCorp 2015 software (Stata Statistical Software: Release 14. College Station, TX, USA: StataCorp LP).

# Results

# *Patient characteristics*

# From June 2009 to October 2013, 81 patients from 34 centers in Japan were enrolled in the ALL-R08-II trial. The trial profile is shown in Fig. 2. Four patients who did not meet the inclusion criteria after enrolment were excluded. Of 73 patients who underwent bone marrow aspiration 3 at the end of induction, MRD was positive in 31 patients and not evaluable in eight patients. Finally, 20 of 31 patients with positive MRD, and two of eight patients with non-evaluable MRD (assigned to the allo-HSCT arm at the physicians’ discretion) underwent allo-HSCT. The characteristics of the patients who underwent transplantation are shown in Table 1.

# *Engraftment*

# Table 2 shows the engraftment profile. Engraftment (absolute neutrophil counts ≥ 5.0 × 108/L) was confirmed in all patients who underwent transplantation. For patients who underwent unrelated bone marrow transplantation (BMT, n = 14) and unrelated cord blood transplantation (CBT, n = 8), the median time to reach an absolute neutrophil count of 5.0 × 108/L was 18 (range, 12–28) days and 19 (range, 13–65) days, respectively. The median time to platelets ≥ 2.0 × 1010/L was 27 (range, 12–43) days for patients who underwent BMT, and 39.5 (range, 26–109) days for patients who underwent CBT, respectively. The median time to reticulocytes ≥ 1% was 20.5 (range, 15–84) days and 24.5 (range, 16–77) days.

# *Safety outcomes*

# Table 3 shows the incidence of GVHD. Acute GVHD was observed in 11 (79%) of 14 patients who underwent BMT and 6 (75%) of 8 patients who underwent CBT. Grade III–IV acute GVHD was observed in 3 (21%) of 14 patients who underwent BMT and 1 (13%) of 8 patients who underwent CBT. The incidences of chronic GVHD observed in the BMT and CBT groups were five (36%) and two (25%), respectively.

# Table S1 shows the incidence of adverse effects (grade 3–4, CTCAE version 3.0) from the start of conditioning until day 28. Grade 4 allergy and grade 3 mucositis were observed in one patient each. No other grade 4 non-hematological toxicity was observed in this period. In later follow-up, thrombotic microangiopathy was observed in one patient on day 67. Neither sinusoidal obstruction syndrome nor post-HSCT lung disease was reported. No transplantation-related mortality was observed.

# *Survival*

# Three of the 22 patients who underwent allo-HSCT relapsed and died eventually. The cause of death was relapse of ALL in three of three patients. With a median follow-up of 4.0 years (range, 1.6 to 6.5 years), the 3y-EFS and 3y-OS after transplantation were 86.4% ± 7.3% and 95.5% ± 4.4%, respectively (Fig. 3).

# Four patients were excluded from the study and did not undergo HSCT at the physicians’ discretion (Fig. 2). Of these four patients, two had second relapse later and two are alive without second relapse. Two patients with non-evaluable MRD did not undergo HSCT at the physicians’ discretion, according to the protocol. Of these two patients, one had a second relapse and one remained alive without relapse.

# Discussion

# In this study, we showed that allo-HSCT based on MRD after induction therapy with a unified TBI + ETP + CY-conditioning regimen had excellent EFS and OS in children with intermediate-risk first-relapsed ALL in the JPLSG ALL-R08-II trial. The feasibility of the conditioning regimen was also shown.

To the best of our knowledge, there are no reports of a unified allo-HSCT-conditioning regimen for treatment of children with relapsed ALL with positive post-induction MRD. This trial showed that allo-HSCT conditioned with a TBI + CY + ETP regimen remarkably improved the outcome in children with intermediate-risk first-relapsed ALL for whom post-induction MRD was positive (n = 20) or non-evaluable (n = 2). The 3y-EFS and 3y-OS were 86.4% and 95.5%, respectively, much better than the historical results in Japan of 40.6% and 54.2% even though they were not stratified by the MRD response.

We hypothesize that the following factors may have contributed to the improved outcomes. First, the alkylator CY and the topoisomerase II inhibitor ETP have been reported to have different mechanisms of their anti-leukemia effects and to act synergistically [17,18], which may have improved the efficacy of the TBI + CY + ETP regimen. In addition, the major acute non-hematological adverse effects of CY and ETP might not have synergy.

This study had several limitations. First, this was not a randomized controlled trial because of the small cohort. Second, selection bias must have existed concerning four patients who were excluded at the physician’s discretion out of 31 patients with positive MRD after induction therapy (Fig. 2). Finally, because of the different backgrounds, such as supportive therapy for infections, it is difficult to simply compare the outcomes of this study and those of previous studies.

In conclusion, allo-HSCT based on post-induction MRD with a unified conditioning regimen of TBI + ETP + CY was highly effective and feasible for children with intermediate-risk relapsed ALL in the JPLSG ALL-R08-II cohort. Because of the small number of children with relapsed ALL in each country, an international study using the allo-HSCT regimen is needed to optimize the regimen for children with intermediate-risk relapsed ALL.

**Conflict of Interest**

The authors have no conflict of interest.

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**Data Availability Statement**

# The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Figure Legends**

**Figure 1. Outline of JPLSG ALL-R08-II clinical trial.** Following the cytoreductive pre-phase, remission induction therapy (F1 and F2) was performed. MRD was evaluated in BMA 3, and patients with MRD ≥ 10−3 were assigned to the allo-HSCT arm. Patients with non-evaluable MRD were assigned to either the chemotherapy arm or the allo-HSCT arm at the physicians’ discretion. Patients assigned to the allo-HSCT arm underwent five courses of block chemotherapy (R2/R1/R2/R1/R2) followed by allo-HSCT. Each block of chemotherapy is shown in Table S2 in detail. ALL, acute lymphoblastic leukemia; BMA, bone marrow aspiration; CNS, central nervous system; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; PP, pre-phase

**Figure 2. Trial profile.** Of 81 patients registered for the JPLSG ALL-R08-II trial, 22 underwent HSCT in total, which included 20 patients with MRD ≥ 10−3 and 2 patients with MRD not evaluable at the physician’s discretion. ALL, acute lymphoblastic leukemia; BMA, bone marrow aspiration; CR, complete remission; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease

**Figure 3. Kaplan–Meier curves of EFS (A) and OS (B) of patients who underwent transplantation in JPLSG ALL-R08-II.** EFS and OS were calculated from HSCT. The 3y-EFS and 3y-OS were 86.4% (95% CI: 63.4–95.4) and 95.5% (95% CI: 71.9–99.4), respectively. The median follow-up was 49 (range, 20–80) months. CI, confidence interval; EFS, event-free survival; OS, overall survival