Low toxicity of a conditioning with 8-Gy total body irradiation, fludarabine and cyclophosphamide as preparative regimen for allogeneic hematopoietic stem cell transplantation in pediatric hematological malignancies

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Abstract

We here report the efficacy and toxicity of a conditioning regimen with fractionated 8-Gy total body irradiation (TBI), fludarabine, and cyclophosphamide in allogeneic hematopoietic stem cell transplantation (HSCT) for pediatric hematological malignancies. Among 22 children who received related or unrelated HSCT, nine were transplanted with refractory disease and/or from HLA two- or more loci-mismatched family donors. None of the patients developed graft failure. The Seattle grading system revealed that 18 patients had no regimen-related toxicity (RRT), and the remaining patients had grade I gastrointestinal toxicity alone. The estimated overall survival and leukemia-free survival at 2 years were 57.1% and 48.0 %, respectively, in 10 patients with acute lymphoblastic leukemia; 91.7% and 71.3%, respectively, in 12 patients with myeloid leukemia. The incidence of treatment-related mortality (TRM) was 4.8% at 2 years. The rates of RRT above grade II and TRM in an 8-Gy TBI-containing regimen were significantly lower than the data of historical control patients who underwent 12-Gy TBI and cyclophosphamide with or without etoposide. The intermediate-dose TBI-based conditioning regimen may confer successful engraftment combined with minimized RRT, although its efficacy should be further evaluated.

Introduction

Allogeneic HSCT is one of the most effective treatments to cure pediatric acute leukemia in CR2 or with non-CR. TBI-containing regimens have been demonstrated to confer a better outcome, as compared with non-TBI regimens, in HLA-identical sibling BMT for children with ALL (1, 2). On the other hand, the use of BU plus CY was proposed as a new preparative conditioning alternative to TBI-based regimen for pediatric AML patients (3). In adult patients with AML, TBI plus CY appeared to be superior to BU plus CY in terms of OS and LFS (4). Additionally, transplant mortality and relapse were substantially less frequent in patients undergoing TBI in the study.

In the historical experience, increasing the dose of TBI from 12 to 15.75 Gy significantly reduced the probability of post-transplant relapse in patients with AML, but did not improve survival because of increased mortality from causes other than relapse (5). During the last 10 years, multiple studies using reduced-intensity conditioning followed by allogeneic HSCT have been reported mainly in adult recipients. Low-dose TBI-containing regimens may overcome the risk of fatal regimen-related complications (6). The tendency for graft failure, grade II – IV acute GVHD, and chronic GVHD, however, increases with HLA disparity, according to the results of a retrospective analysis of 341 Japanese patients with hematological malignancies who underwent HSCT from related donors after FLU-based reduced-intensity conditioning with 2-Gy to 4-Gy TBI (7). In that report, 2-year OS in recipients of two- to three-loci-mismatched transplants was significantly worse than that of patients who received one-locus-mismatched transplants and HLA-matched transplants. Non-myeloablative approach with FLU, MEL and antithymocyte globulin has been applied to children with non-malignant disease, whereas there are a few reports of FLU-based regimen for children with malignant disease.

To elucidate what degree of intensity conditioning was adequate for a reduced disease recurrence and, at the same time, reduced graft failure and RRT in pediatric patients with hematological malignancies, the present trial decreased the dose of TBI to 8 Gy by supplementation with FLU, which has been shown to augment the cytotoxic effects of irradiation by increasing the amount of residual DNA and chromosome damage (8). We describe here the engraftment rate, hematopoietic chimerism, frequency of TRM, and outcome of this conditioning regimen in allogeneic HSCT for pediatric hematological malignancies including HSCT in non-CR and/or from donors with mismatched HLA antigens.

Methods

Patient selection

A total of 22 children with leukemia, 1 – 13 years of age, were transplanted between December 2002 and May 2007 (Table 1A). Since 17 patients had a diagnosis of either hematological relapse in acute leukemia or of JMML, we considered HSCT to be indicated. HSCT was also applied to 5 patients with acute leukemia in CR1 who had a poor prognosis, i.e., Case 3, precursor NK-cell ALL (9); Case 7, a high white blood cell count (>100,000/μl) on presentation and poor response to initial treatment with prednisolone (10); Cases 20 to 22, M6 or M7 (11, 12). When the conditioning treatment was initiated, 2, 6, and 2 patients with ALL were in CR1, CR2, and non-CR (> 30% marrow blasts), respectively. In CR2 and non-CR, the interval between the initial diagnosis and first relapse was <36 months in 5 patients, and ≥36 months in 3 patients. Three, 5 and 3 patients with AML were transplanted in CR1, CR2, and non-CR (10% to 63% marrow blasts), respectively. The study protocol was approved by the institutional review board of The Shinshu University School of Medicine, and written informed consent was obtained from the parents of all patients.

Donor selection

HLA antigens for A, B, C, and DRB1 were determined by high-resolution DNA typing (13).

The primary criterion for donor selection was HLA compatibility, defined as a 6/6 allele match (at the A, B, and DRB1 loci) or a 5/6 allele match (single-antigen mismatch at the A, B, or DRB1 locus), with preference for a 6/6 match. If matched related donors were not available, unrelated donors were sought. When the patient lacked an available related or unrelated donor who had at least 5 of 6 HLA antigens that matched the recipient at the allele level, an HLA-haploidentical related donor was selected. If a transplant was urgently needed, unrelated umbilical cord blood was chosen according to the number of nucleated cells per the recipient's body weight and HLA compatibility. Only 2 (Cases 1 and 15) of 22 patients were transplanted from donors who had mismatching at HLA-C locus.

Conditioning regimen and transplantation

Preparative conditioning consisted of TBI (2 Gy per day at a rate of 8-9 cGy/min, day –7 to day –4), FLU (30 mg/m^2 per day, day –8 to day –4), and CY (60 mg/kg per day, day –3, –2).

In BMT, the median numbers of total nucleated cells, $CD34^+$ cells, and $CD3^+$ cells infused were 4.7 x 10^8 (range, 3.0 – 15.6 x 10^8), 4.3 x 10^6 (2.1 – 15.0 x 10^6), and 3.6 x 10^7 (1.5 – 12.4 x 10^7) cells per kilogram of the recipient's weight, respectively. T cell depletion was not performed. The pre-thawed nucleated cell and $CD34^+$ cell doses of unrelated cord blood were 3.2 – 4.0 x 10^7, and 0.21 – 0.3 x 10^6 cells per kilogram, respectively.

GVHD prophylaxis and treatment

When the stem cell source was an HLA-identical sibling, short-term methotrexate (10 mg/m^2 intravenously on day 1 and 7 mg/m^2 on days 3 and 6) and cyclosporine A (starting at 3 mg/kg/day on day –1 for target concentration to 150 – 200 ng/ml) were used for prophylaxis of acute GVHD. When bone marrow cells were obtained from a mismatched sibling, a parent, or an unrelated donor, we used short-term methotrexate (15 mg/m^2 intravenously on day 1 and 10 mg/m^2 on days 3, 6, and 11) and FK506 (starting at 0.03 mg/kg/day on day –1 for target concentration to 10 – 15 ng/ml).

Additionally, methylprednisolone was given at 0.5 to 1 mg/kg/day in most of the transplants from HLA-mismatched donors. After engraftment, the dose of methylprednisolone was tapered and discontinued up to day 30 in the absence of acute GVHD. In cord blood transplantation, FK506 and methylprednisolone were given intravenously as prophylactics. Diagnoses of GVHD were based on clinical manifestations and the histology of biopsy samples of affected tissues. Acute GVHD was graded according to standard criteria (14). Chronic GVHD was graded according to the criteria described previously (15). Grade II – IV acute GVHD was treated with 1 – 2 mg/kg/day of methylprednisolone with or without 20 mg/kg/day of mycophenolate mofetil.

Supportive care

All patients stayed in a laminar airflow-equipped room. G-CSF (5 μg/kg/day) was administered intravenously to ALL patients from day 5, and its use was discontinued after the neutrophil count exceeded 1,500/μl. Oral antibiotics were administered to sterilize the bowel. Acyclovir (10 mg/kg, daily) and micafangin (2 mg/kg, daily) were given intravenously during the peritransplant period. Intravenous gamma globulin was administered at 200 mg/kg every two weeks up to day 60, and thereafter monthly until 1 year. Trimethoprim/sulfamethoxazole was used after engraftment for prophylaxis of *Pneumocystis carinii* infections. Cytomegalovirus pp65 antigenemia was routinely monitored once a week. When antigenemia was detected, preemptive therapy with ganciclovir was initiated. Routine surveillance of EB virus, adenovirus, and BK virus reactivation based on real-time PCR was performed before HSCT, weekly from day 7 to day 100, and every 2 weeks from day 101 to day 180.

Toxicity grading

RRT was assessed according to the Seattle criteria in the heart, lungs, liver, kidneys, mucosa, gut, bladder, and nervous system (16). Toxicities were graded from 0 (none) to 4 (fatal). The criteria exclusively assessed RRT until day 28 (and on day 100 for lungs). We also used NCI-CTC version 2.0 (17). NCI-CTC evaluated toxicities due to all post-transplant events, including GVHD and infection, as well as RRT. The grade of organ toxicity was evaluated on days –9, 0, 7, 14, 21, and 28 (and on day 100 for lungs). According to these criteria, grade 0 indicates no organ toxicity, and grades 1 to 4 indicate increasing levels of toxicity.

Assessment of engraftment

Myeloid recovery was defined as the first of three consecutive days with an absolute neutrophil count ≥500/μl, and platelet recovery as the day the platelet count reached 50,000/μl without platelet transfusion. Hematopoietic chimerism was evaluated in bone marrow or peripheral blood cells by fluorescent in situ hybridization with the simultaneous application of probes specific to chromosomes X and Y for sex-mismatched transplants or analysis of DNA microsatellite polymorphisms by PCR (18). Chimerism was assessed within 1 month of HSCT and subsequently at least every third month during the first 2 years.

Definition of engraftment syndrome

Engraftment syndrome was defined using diagnostic criteria described previously (19 – 21), which included the development of 2 or more of the following symptoms 4 days before and after the start of neutrophil recovery (absolute neutrophil count >100/μl): (1) fever (temperature >38.5°C) without an identifiable infectious cause; (2) weight gain ≥2.5% over the pretransplantation baseline weight; (3) erythematous rash not

attributable to a medication; and (4) hypoxia, pulmonary infiltrates, or both, not attributable to infection, thromboembolism, pulmonary hemorrhage, fluid overload, or cardiac disease. Most of the patients did not require any therapy.

Statistical analysis

Actuarial estimates of TRM, OS, LFS, and RR were made with the Kaplan-Meier technique. Patients were censored for OS if they were alive at last follow-up. Patients were censored for LFS if they were alive and in remission at last follow-up. Follow-up was through November 1, 2007. Analysis was performed with SPSS version 11.0.

To determine the significance of difference between two independent groups, we used the unpaired *t*-test or Mann-Whitney-*U* test when the data were not normally distributed. The chi-square test were used to determine the frequencies of type (ALL vs myeloid malignancies) and stage (CR1 plus CR2 vs CR3 plus non-CR) of leukemia, the frequency of stem cell source, and the extent of HLA disparity in A, B and DRB1 loci (3 allele-mismatched plus 2 allele-mismatched vs 1 allele-mismatched plus no allele-mismatched). The level of significance was defined as a *P*-value of less than 0.05.

Results

Patients and disease characteristics

A total of 22 patients received the preparative regimen consisting of fractionated 8-Gy TBI, FLU (150 mg/m^2), and CY (120 mg/kg). Patient characteristics are listed in Table 1A. At the time of transplantation, the median age of the patients was 6.5 years (range, 1 – 13 years). The diagnoses were ALL (n=10), AML (11), and JMML (n=1). Twenty patients with acute leukemia received BMT from 3 matched siblings, 2 matched parents, 9 mismatched related, 3 matched unrelated, and 3 mismatched unrelated donors. One patient (Case 22) was transplanted with one-locus-mismatched umbilical cord blood. Accordingly, eight of 21 patients with acute leukemia received BMT in non-CR and/or from related donors with mismatches of 2 or 3 HLA antigens. A patient with JMML (Case 11) underwent a transplant of one-locus-mismatched umbilical cord blood because of hematological deterioration during treatment with 6-mercaptopurine and cytarabine. The median follow-up for the 18 living patients was 26 months (range, 6 – 56 months).

Engraftment and chimerism

All of the 22 patients achieved sustained neutrophil engraftment after a median of 15 days (range, 9 – 16 days) in those receiving G-CSF, and after a median of 22 days (15 – 42 days) in those receiving no G-CSF. Twenty-one of 22 patients (95.5%) reached a platelet count of greater than 50,000/μl without transfusion support after a median of 23 days (14 – 92 days). Case 16 could not be evaluated for platelet engraftment because of early death. Donor cell chimerism of higher than 99% was achieved within 1 month of transplantation in all patients. Neither mixed chimerism nor secondary graft failure was observed.

Infection

No patients developed clinically or microbiologically documented bacteremia or fungal infection during the clinical course. Four patients had cytomegalovirus-antigenemia 43 to 180 days after BMT, but no patients developed cytomegalovirus disease, including pneumonia. Two patients had adenovirus-associated hemorrhagic cystitis and BK virus-associated hemorrhagic

cystitis 24 days and 28 days after BMT, respectively.

Treatment-related organ toxicity

The regimen was very well tolerated. The Seattle grading system revealed that 4 patients had grade I gastrointestinal toxicity alone (approximately 500 to 1,000 ml/m^2/day of diarrhea), as shown in Table 2. The remaining 18 patients had no RRT in eight organs. On the other hand, according to NCI-CTC, proposed to evaluate toxicity due to all post-transplant events, Grade II or higher toxicity was observed in the liver (36.4%), mucosa (9.1%), and gastrointestinal tract (22.7%), whereas no toxicity was observed in the heart, lungs, kidneys, and nervous system. Hepatic toxicity was found almost 14 days after HSCT, and was attributed in part to engraftment syndrome and/or acute GVHD. Although hepatic toxicity was graded as I or II in most patients, Grade III to IV toxicity related to acute GVHD occurred in 3 patients. Hepatic veno-occlusive disease was not observed. Gastrointestinal toxicity caused by RRT appeared on day 0 and improved on day 7 in most cases. On the other hand, gastrointestinal toxicity associated partly with engraftment syndrome and/or acute GVHD appeared 7 days after HSCT, and was graded as I or II in all but Case 4. Grade II to III stomatitis seen in 2 patients was attributed to acute GVHD. Two patients had grade III toxicity of the bladder due to virus-associated hemorrhagic cystitis. All complications found within 1 month after HSCT were reversible.

Graft-versus-host disease

Seven of 22 patients did not experience acute GVHD. The number of patients who had grade I, II, and III acute GVHD was 4, 4, and 7, respectively (Table 1A). No patients experienced grade IV acute GVHD. In 21 patients who could be evaluated for chronic GVHD, limited and extensive chronic GVHD occurred in 4 and 5 patients, respectively. Case 9 died of hepatic failure induced by chronic GVHD 6 months after BMT.

Response and survival

Although 6 patients (two with ALL, three with AML, and one with JMML) had active disease at the start of preparative conditioning, the days 30 and 60 bone marrow examination (including fluorescence in situ hybridization analysis using probes specific for X and Y chromosomes, and chimerism assessment using a polymerase chain reaction-based analysis of polymorphic DNA sequences) revealed complete remission in twenty-one of 22 patients.

The estimated OS, LFS, and RR in all patients with hematological malignancies at 2 years were 73.8% (95%CI, 53.6 – 94.0%), 59.7% (37.7 – 81.7%), and 37.2% (14.9 – 59.4%), respectively. In 10 patients with ALL, 2-year OS, LFS, and RR were 57.1% (95%CI, 25.0 – 89.2%), 48.0% (95%CI, 15.9 – 80.1%), and 46.0% (12.1 – 79.9%), respectively. Five patients are alive and currently in complete remission after BMT. On the other hand, in 12 patients with myeloid leukemia, OS, LFS, and RR at 2 years were 91.7% (95%CI, 76.0 – 100%), 71.3% (95%CI, 43.6 – 99.0%), and 28.7% (1.0 – 56.4%), respectively. Nine patients, including 2 patients with active disease at the time of transplant, are alive and currently in complete remission after HSCT. Seven patients (four ALL, three AML) relapsed at a median of 8 months (range, 1.5 – 15 months) after BMT. Among them, 4 patients succumbed to their disease at a median of 11.5 months (2 – 19 months) after BMT. Because of the one GVHD-related death mentioned above, the rate of TRM at 2 years was 4.8%.

Comparison of engraftment, toxicity, and response/survival between 8-Gy

TBI-containing regimen and 12-Gy TBI-containing regimen.

From September 1993 to June 2002, we used a combination of 12-Gy TBI (six equally divided fractions at a rate of 8-9 cGy/min, twice daily, with a minimal 8 hours interval between fractions) and CY (120 mg/kg) with or without etoposide (50 mg/kg) as a preparative regimen of HSCT for 19 patients with hematological malignancies shown in Table 1B. Accordingly, we compared the engraftment rate, frequencies of RRT and TRM, and outcome between HSCT with 8-Gy TBI-containing regimen and HSCT with 12-Gy TBI-containing regimen. The results are presented in Table 3. While the historical control included peripheral blood stem cell transplantation at higher incidence, both groups showed no significant difference in the frequency of type/stage of leukemia and the extent of HLA disparity. In 19 patients who underwent 12-Gy TBI-containing conditioning, graft failure occurred in two patients. According to the Seattle grading system, nine (47.4%) had RRT above grade II, and three died of either renal toxicity or hepatic failure within 1 month of HSCT. The frequencies of grade II – IV toxicity were 16%, 21%, 16%, 26%, 16%, 11%, 0% and 16% in the heart, lungs, liver, kidneys, mucosa, gastrointestinal tract, bladder, and nervous system, respectively. The rate of TRM at 2 years was 39.2%. The estimated OS, LFS, and RR at 2 years were 42.1% (95%CI, 19.9 – 64.3%), 36.8% (15.1 – 58.5%), and 37.8% (11.5 – 64.1%), respectively. In 9 patients with ALL and 10 patients with myeloid malignancies, 2-year OS were 33.3% (95%CI, 2.5 – 64.1%) and 50.0% (95%CI, 19.0 – 80.1%), respectively. Consequently, the 8-Gy TBI-containing regimen appeared to be superior in the rates of RRT above grade II and TRM, and OS, as compared with the 12-Gy TBI-containing regimen.

Discussion

Graft failure is a drawback of HSCT after FLU-based reduced-intensity conditioning with 2-Gy to 4-Gy TBI. According to a Japanese study of HSCT from related donors (7), the incidence of graft failure was 3.7% in recipients with an HLA-matched donor, 5.7% in those with a one-locus-mismatched donor, and 10.3% in those with a two- to three-loci-mismatched donor. In a report of Kletzel et al. (22), fully donor chimerism was achieved in nine of 11 ALL children who received FLU, BU and antithymocyte globulin, and four patients were alive and in remission. In the present study, none of the patients developed graft failure, even though 9 of 22 patients received either two- to three-loci-mismatched related BMT or one-locus-mismatched unrelated BMT. Taken together with sustained neutrophil engraftment in 27 of 29 patients who received HSCT (including 3 patients from mismatched related donors, and 19 patients from mismatched unrelated cord blood units) after the 9-Gy TBI + FLU + MEL regimen (23), satisfactory engraftment may be an advantage of the intermediate-dose TBI-based conditioning regimen. In addition, HLA incompatibility between donor and recipient does not appear to be an important risk factor of graft failure for our 8-Gy TBI-containing regimen.

The current preparative regimen consisting of 8-Gy TBI, FLU, and CY was very well tolerated by all patients, and no patients died from RRT. According to the Seattle grading system, which was designed to measure toxicity due only to the transplant regimen (16), no organs except the gastrointestinal tract was affected. Four patients had gastrointestinal toxicity, but its grade was I. According to the NCI-CTC for evaluating toxicity due to all post-transplant events, such as GVHD, infections, and

other complications (17), grade II or higher toxicity in the liver, mucosa, and gastrointestinal tract among eight organs was documented in approximately 10 – 35% of patients in the 8-Gy TBI-containing regimen. Time-course study revealed that gastrointestinal toxicity caused by RRT appeared on day 0 and improved on day 7 in most cases. On the other hand, gastrointestinal toxicity and hepatic toxicity associated partly with engraftment syndrome and/or acute GVHD appeared 7 days and 14 days after HSCT, respectively. All the complications were reversible, except in Case 9 in which the patient died of hepatic failure induced by chronic GVHD. When TRM for patients with acute leukemia in CR1+CR2 was compared, the rate was 6.3% in 8-Gy TBI-containing regimen (n=16), and 37.5% in 12-Gy TBI-containing regimen (n=8). The other investigators reported that the frequency of TRM in the myeloablative conditioning was 12.9% to 29.6% in children with ALL in CR1 and/or CR2, and 15.3% to 25% in children with AML in CR1 and/or CR2 (2, 24-28). On the other hand, the TRM rate was 39.3% to 60% in children with ALL in CR3 and non-CR, and 35.5% in children with AML in non-CR. Although approximately 40% of patients received HSCT in non-CR and/or from HLA 2 or more-loci-mismatched related donors in the present study, the incidence of TRM of all patients was 4.8% at 2 years in our regimen. Thus, mild RRT and low TRM were outstanding characteristics of our 8-Gy TBI-containing regimen.

Petropoulos et al. (23) demonstrated that HSCT after a preparative regimen of 9-Gy TBI, FLU, and MEL yielded 31% of 3-year OS in 22 children with ALL and 83% of the OS in 6 children with AML. In that report, the frequencies of grade II – IV toxicity evaluated with the Seattle criteria were 0%, 3%, 7%, 7%, 14%, 14%, 0%, and 3% in the heart, lungs, liver, kidneys, mucosa, gastrointestinal tract, bladder, and nervous system, respectively. The TRM rate was 24%. They stopped the concurrent study for adult patients, because the regimen was not tolerated. When 8-Gy TBI plus FLU was applied to HSCT for 71 adult patients with AML, the incidence of grade II – IV toxicity assessed with the NCI-CTC was 14% to 75% in the heart, lungs, liver, kidneys, mucosa, gastrointestinal tract, and nervous system (29). The frequency of TRM was 8% in CR, and 37% in non-CR. When compared with their results, the absence of any serious RRT in our study may be due to the use of CY, not MEL, in the preparative regimen and to the durability of pediatric patients. Despite the high incidence of grade II to III acute GVHD (50%) and extensive chronic GVHD (23.8%), the TRM rate was very low in the present study. Reduced RRT may preserve the capacity of patients to face the later phase of post-transplant organ damage.

Recent studies of pediatric HSCT revealed that the probability of LFS for ALL was approximately 60 – 70% after 3 – 5 years in CR1, 40 – 60% after 3 – 8 years in CR2, and 10% after 3 years in non-CR (2, 25 – 28, 30 – 34). In AML, the 2 – 5-year probability of LFS was approximately 40 – 70% in CR1, 45 – 60% in CR2, and 10 – 20% in non-CR. In the present study, the 2-year LFS rate was 48.0% in children with ALL, and 71.3% in children with myeloid leukemia. The outcome of our preparative regimen appears to be favorable for the treatment of myeloid leukemia. However, it is not appropriate to judge about the efficacy of this preparative study, because the small number of patients with a heterogenous diagnosis and hematopoietic stem cell source were followed for a short period. Additionally, long-term follow-up is necessary in regard to the possible occurrence of growth retardation, endocrine disturbances, sterility, and second tumors.

Abbreviations

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; BMT, bone marrow transplantation; BU, busulfan; CR, complete remission; CR1, first complete remission; CR2, second complete remission; CR3 third complete remission; CY, cyclophosphamide; FK506, tacrolimus; FLU, fludarabine; G-CSF, Granulocyte colony-stimulating factor; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; JMML, juvenile myelomonocytic leukemia; LFS, leukemia-free survival; MEL, melphalan; NCI-CTC, National Cancer Institute Common Toxicity Criteria; non-CR, refractory disease; OS, overall survival; PCR, polymerase chain reaction; RR, relapse rate; RRT, regimen-related toxicity; TBI, total body irradiation; TRM, treatment-related mortality, non-relapsed death.

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Table 1. Characteristics of patients

(A) 8-Gy TBI-containing regimen

Patient No.	Age/ Sex	Disease	Disease Status	Interval up to HSCT[a)]	Donor/ Source	HLA-mismatch[b)]		GVHD		Outcome (Interval after HSCT)	Lansky Score (%)
						GVH	HVG	acute	chronic		
1	11/M	ALL	non-CR	57mo	U / BM	1(A)	1(A)	III	Ex	Relapse (15mo), Death (19mo)	NA
2	6/F	ALL	non-CR	22mo	S / BM	None	None	I	No	Relapse (8mo), Death (10mo)	NA
3	10/F	NK-ALL	CR1	9mo	S / BM	2(B,DR)	2(B,DR)	II	Ex	CR (+56mo)	100
4	5/F	ALL	CR2	23mo	M/ BM	1(DR)	1(DR)	II	Lo	CR (+40mo)	100
5	7/M	ALL	CR2	64mo	S / BM	1(DR)	1(DR)	0	No	CR (+30mo)	100
6	9/F	ALL	CR2	22mo	U / BM	None	None	0	No	Relapse (6mo), Death (13mo)	NA
7	9/F	ALL	CR1	5mo	M/ BM	None	None	0	No	Relapse (11mo), 2nd HSCT (24mo), Alive (+28mo)	100
8	13/F	ALL	CR2	92mo	U / BM	1(DR)	1(DR)	0	Lo	CR (+26mo)	100
9	6/M	ALL+DS	CR2	23mo	U / BM	1(DR)	1(DR)	III	Ex	Death (6mo) associated with GVHD	NA
10	9/M	ALL	CR2	46mo	U / BM	None	None	III	Ex	CR (+12mo)	100
11	1/F	JMML	non-CR	3mo	U / CB	None	1(A)	0	No	CR (+45mo)	100
12	9/F	AML(M2)	non-CR	12mo	M/ BM	2(B,DR)	3(A,B,DR)	II	Ex	CR (+32mo)	80
13	2/F	AML(M4)	CR2	18mo	M/ BM	1(DR)	1(DR)	III	Lo	CR (+31mo)	90
14	9/M	AML(M2)	CR2	18mo	S / BM	None	None	0	No	CR (+27mo)	100
15	5/M	AML(M7)	non-CR	6mo	M/ BM	2(B,DR)	3(A,B,DR)	III	No	Relapse (7mo), Alive (+23mo)	90
16	1/F	AML(M7)	non-CR	5mo	M/ BM	2(A,DR)	2(A,DR)	III	NA	Relapse (1.5mo), Death (2mo)	NA
17	3/M	AML(M2)	CR2	10mo	M/ BM	3(A,B,DR)	3(A,B,DR)	I	Lo	CR (+20mo)	100
18	12/M	AML(M2)	CR2	20mo	M/ BM	1(B)	3(A,B,DR)	III	No	CR (+17mo)	100
19	10/F	AML(M2)	CR2	7mo	F / BM	None	None	0	No	Relapse (14mo), Alive (+15mo)	100
20	1/F	AML(M7)	CR1	5mo	U / BM	None	None	II	No	CR (+14mo)	100
21	4/F	AML(M6)	CR1	4mo	S / BM	None	None	I	No	CR (+6mo)	100
22	3/F	AML(M7)	CR1	9mo	U / CB	1(DR)	1(DR)	I	No	CR (+6mo)	100

(B) 12-Gy TBI-containing regimen

Patient No.	Age/ Sex	Disease	Disease Status	Interval up to HSCT[a)]	Donor/ Source	HLA-mismatch[b)]		GVHD		Outcome (Interval after HSCT)	Lansky Score (%)
						GVH	HVG	acute	chronic		
1	19/F	ALL	CR2	40mo	S / BM	None	None	II	Lo	CR (+176mo)	100
2	19/F	ALL	CR3	72mo	S / PB	None	None	NA	NA	Death (0.5mo) associated with RRT	NA
3	4/M	ALL	CR2	22mo	U / BM	None	None	II	No	CR (+132mo)	100
4	0/M	MLL$^+$ALL	non-CR	8mo	F / PB	None	None	II	No	Relapse (6mo), Death (9mo)	NA
5	3/M	ALL	non-CR	21mo	S / PB	None	None	I	NA	Death (3.5mo) associated with TMA	NA
6	3/M	Ph1$^+$ALL	CR1	7mo	U / CB	1(A)	1(A)	NA	NA	Graft failure, 2nd HSCT (7mo), 3rd HSCT(23mo), Death (25mo) associated with infection	NA
7	7/F	ALL	non-CR	30mo	S / PB	None	None	II	No	CR (+120mo)	100
8	1/M	MLL$^+$ALL	CR1	7mo	U / CB	2(B,DR)	2(B,DR)	NA	NA	Death (1.2mo) associated with RRT	NA
9	14/M	ALL	non-CR	2mo	S / PB	None	None	II	Ex	Relapse (6mo), Death (9mo)	NA
10	8/M	AML(M2)	CR1	7mo	S / BM	None	None	II	No	Relapse (9mo), Alive (+232mo)	100
11	2/M	JMML	non-CR	7mo	U / BM	None	None	0	No	Relapse (5mo), Death (12mo)	NA
12	15/M	Ph1$^+$AML(M0)	CR2	11mo	U / BM	None	None	NA	NA	Death (0.8mo) associated with RRT	NA
13	7/M	MDS(RAEB)	non-CR	12mo	U / BM	None	None	0	No	Relapse (3mo), Death (6mo)	NA
14	9/F	AML(M2)	CR1	11mo	M/ BM	None	None	0	No	CR (+147mo)	100
15	19/F	MDS(RAEB)	non-CR	62mo	U / BM	None	None	III	Lo	Death (4mo) associated with infection	NA
16	7/M	AML(M2)	CR2	18mo	S / PB	None	None	II	Lo	CR (+111mo)	100
17	3/M	JMML	non-CR	45mo	U / BM	None	None	NA	NA	Graft failure, Death (0.8mo) associated with RRT	NA
18	4/F	AML(M2)	non-CR	15mo	S / PB	None	None	0	No	CR (+97mo)	100
19	0/F	JMML	non-CR	12mo	U / CB	2(B,B)	2(B,B)	III	Lo	CR (+93mo)	100

[a)] Interval between the initial diagnosis and HSCT; [b)] HLA antigens were determined by high-resolution DNA typing.
Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; BM, bone marrow; CB, cord blood; CR, complete remission; CR1, first complete remission; CR2, second complete remission; CR3, third complete remission; DS, Down syndrome; Ex, extensive type; F, father; GVH, graft-versus-host; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; HVG, host-versus-graft; JMML, juvenile myelomonocytic leukemia; Lo, localized type; M, mother; MDS, myelodysplastic syndrome; MLL$^+$ALL; acute lymphoblastic leukemia with MLL gene rearrangements, NA, not applicable; NK-ALL, precursor NK-cell acute lymphoblastic leukemia; non-CR, refractory disease at the time of HSCT; PB, peripheral blood; Ph1$^+$, Philadelphia chromosome-positive; RAEB, refractory anemia with excess of blasts; RRT, regimen-related toxicity; S, sibling; TBI, total body irradiation; TMA, thrombotic microangiopathy; U, unrelated.

Table 2. Toxicity grading according to the Seattle criteria and NCI-CTC version 2.0

Organ toxicity	Seattle criteria					NCI-CTC version 2.0				
	0	1	2	3	4	0	1	2	3	4
Cardiac toxicity	22	0	0	0	0	22	0	0	0	0
Pulmonary toxicity	22	0	0	0	0	22	0	0	0	0
Hepatic toxicity	22	0	0	0	0	8	6	5	2	1
Renal toxicity	22	0	0	0	0	22	0	0	0	0
Stomatitis	22	0	0	0	0	20	0	1	1	0
Gastrointenstinal toxicity	18	4	0	0	0	4	13	4	1	0
Bladder toxicity	22	0	0	0	0	20	0	0	2	0
Nervous system toxicity	22	0	0	0	0	22	0	0	0	0

Table 3. Comparison of the engraftment, toxicity, and response/survival between 8-Gy TBI-containing regimen and 12-Gy TBI-containing regimen

	8-Gy+FLU+CY	12-Gy+CY+/-etoposide	P-value
Number of patients	22	19	
Median follow-up (range, months)	18 (2 – 56)	12 (0.5 – 232)	0.969
Age, median (range, years)	6.5 (1 – 13)	7 (0 – 19)	0.896
Male/Female	8/14	12/7	0.087
Diagnosis			
ALL	10	9	0.903
CR1+CR2 vs CR3+non-CR at HSCT	2+6 vs 0+2	2+2 vs 1+4	0.170
AML	11	5	0.121
CR1+CR2 vs non-CR at HSCT	3+5 vs 3	2+2 vs 1	>0.999
MDS, JMML	1	5	0.080
Stem cell source			
BM vs PB vs CB	20 vs 0 vs 2	9 vs 7 vs 3	0.002
HLA-mismatch			
3/6+2/6 vs 1/6+0/6	4+2 vs 8+8	0+2 vs 1+16	0.249
Graft failure	0/22	2/19	0.209
RRT above grade II	0/22	9/19	<0.001
2-year TRM	4.8%	39.2%	0.015
Acute GVHD			
grade 0 – I	11/22	5/14	0.400
grade II – IV	11/22	9/14	
Chronic GVHD			
None/ Lo	16/21	12/13	0.370
Ex	5/21	1/13	
2-year OS (95% CI)	73.8% (53.6 – 94.0%)	42.1% (19.9 – 64.3%)	0.029
2-year LFS (95% CI)	59.7% (37.7 – 81.7%)	36.8% (15.1 – 58.5%)	0.078
2-year RR (95% CI)	37.2% (14.9 – 59.4%)	37.8% (11.5 – 64.1%)	0.843

The frequencies of type (ALL vs myeloid malignancies) and stage (CR1 plus CR2 vs CR3 plus non-CR) of leukemia, the frequency of stem cell source, and the extent of HLA disparity in A, B and DRB1 loci (3 allele-mismatched plus 2 allele-mismatched vs 1 allele-mismatched plus no allele-mismatched), engraftment rate, the frequencies of RRT, TRM and GVHD, and outcome were compared between 8-Gy TBI-containing regimen and 12-Gy TBI-containing regimen.

Abbreviation: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; BM, bone marrow; CB, cord blood; CR1, first complete remission; CR2, second complete remission; CR3, third complete remission; CY, cyclophosphamide; Ex, extensive type; FLU, fludarabine; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; JMML, juvenile myelomonocytic leukemia; LFS, leukemia-free survival; Lo, localized type; MDS, myelodysplastic syndrome; non-CR, refractory disease; OS, overall survival; PB, peripheral blood; RR, relapse rate; RRT, regimen-related toxicities according to the Seattle criteria; TBI, total body irradiation; TRM, treatment-related mortality.