tional allo-HSCT due to the lack of an HLA-identical sibling or a suitable unrelated donor, age >50 years old and/or organ dysfunction (generally attributable to previous intense chemo- and/or radiotherapy).

All of the patients provided written informed consent in accordance with the requirements of the Institutional Review Board.

HLA Typing and Donor Matching. An unrelated donor was searched through the Japan Marrow Donation Program (13) for patients without an HLA-identical sibling donor. When no appropriate donor was identified, the Japan Cord Blood Bank Network (14) was searched. CB units, which were ≥4 of 6 HLA-antigen matched and contained at least 2×10^7 nucleated cells/kg of recipient body weight before freezing were used. CB units were not depleted of T lymphocytes.

Preparative Regimen. The preparative regimen was composed of fludarabine 25 mg/m^2 on days −7 to −3, melphalan 80 mg/m^2 on day −2, and 4 Gy TBI in 2 fractions on day −1.

Supportive Cares. All of the patients were managed in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis carinii* prophylaxis. Fluoroquinolone and fluconazole were administered for prophylaxis of bacterial and fungal infections, respectively. Prophylaxis of herpes virus infection with acyclovir was also given (15). Neutropenic fever was managed according to the guidelines (16, 17). Cytomegalovirus (CMV) pp65 antigenemia was monitored once a week. If positive results were identified, preemptive therapy with foscarnet was initiated. Hemoglobin and platelet counts were maintained at >7 g/dl and $>10 \times 10^9$/liter, respectively, with in-line filtered and irradiated blood transfusions.

Management of GVHD. GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. Acute and chronic GVHD were graded according to the established criteria (18, 19).

GVHD prophylaxis was a continuous infusion of cyclosporin 3 mg/kg from day −1 until the patients tolerated oral administration. It was tapered off from day 100 until day 150. If grade II-IV acute GVHD developed, 1 mg/kg/day of prednisolone was added to cyclosporin and tapered from the beginning of clinical response.

Chimerism Analysis. Chimerism was assessed using fluorescent *in situ* hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, PCR for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10% (20).

Whole blood and CD3-positive cell chimerism was assessed at the time of granulocyte engraftment. When engraftment was delayed, chimerism was assessed on day 30. For those who died before engraftment, chimerism was assessed at least once during life.

Engraftment. Engraftment was defined as WBC counts $> 1.0 \times 10^9$/liter or absolute neutrophil counts $> 0.5 \times 10^9$/liter for 2 consecutive days. Granulocyte colony stimulating factor (Filgrastim) 300 μg/m^2/day was administered i.v. from day 1 until neutrophil engraftment.

Graft failure was defined as peripheral cytopenia and marrow hypoplasia occurring later than day 60, without detection of donor markers by cytogenetic and/or molecular techniques.

Table 1 Patient characteristics (n = 30)

Age (y), median (range)	58.5 (20–70)
Weight (kg), median (range)	52 (38–75)
Male/female	16/14
Diagnosis	
Malignancy	
Acute myeloid leukemia	14
Myelodisplastic syndrome	1
Acute lymphoblastic leukemia	3
Adult T-cell leukemia	5
Plasma cell leukemia	1
Chronic myeloid leukemia	1
Malignant lymphoma	1
Benign	
Severe aplastic anemia	4
Disease status at transplantation (malignancy)	
Remission	1
Refractory to previous chemotherapy	25

RRT and Transplantation-Related Mortality (TRM). RRT was defined as any nonhematological organ dysfunction from day 0 to day 28 and was graded according to the Bearman's criteria (2). TRM was defined as death without the primary disease progression.

Endpoints and Statistical Analysis. Primary end points were composed of the rates of durable engraftment and TRM within day 100. Secondary end points were the rates of RRT, acute and chronic GVHD, infections, event-free survival (EFS), and overall survival (OS).

Acute GVHD was analyzed for engrafted patients. Chronic GVHD was analyzed for patients who survived ≥100 days.

EFS was defined as the duration of survival after transplantation without disease progression, relapse, graft failure, or death. The probabilities of OS and EFS were shown by the Kaplan-Meier method as of January 31, 2004. Surviving patients were censored on the last day of follow-up. Cox regression analysis was used to determine the effect of various variables on OS.

RESULTS

Patient Characteristics. Median age was 58.5 years (range, 20–70 years), and median weight was 52 kg (range, 38–75 kg; Table 1). All of the patients were CMV-seropositive.

The malignancies of 25 patients were refractory to cytotoxic chemotherapies except acute myeloblastic leukemia (n = 1) in first CR. The remaining 4 patients had transfusion-dependent severe aplastic anemia.

CB Characteristics. Twenty-four and 6 patients received 4 of 6 and 5 of 6 HLA-antigen-matched CB, respectively. Twenty-one patient CB pairs were sex-mismatched. Median infused total nucleated cell dose and CD34-positive cell dose before freezing were 3.1×10^7/kg (range, $2.0–4.3 \times 10^7$/kg) and 0.74×10^5/kg (range, $0.17–2.5 \times 10^5$/kg), respectively.

Engraftment. Twenty-six patients [87%; 95% confidence interval (95% CI), 75–99%] achieved primary neutrophil engraftment, among whom median day of engraftment was 17.5 days (range, 10–54 days; Fig. 1). Their engraftment was durable

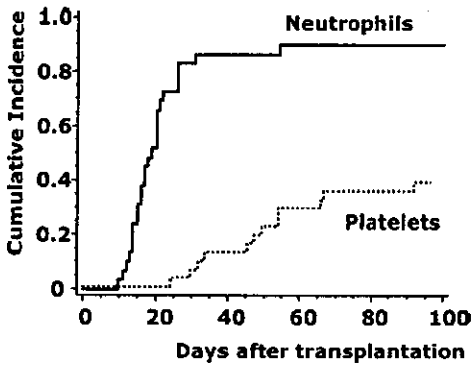

Fig. 1 Engraftment of neutrophils and platelets. Twenty-six (87%; 95% confidence interval, 75–99%) and 16 patients (40%; 95% confidence interval, 25–57%) achieved primary neutrophil and platelet engraftment, respectively.

Table 2 Neutrophil engraftment, chimerism, and overall survival

Neutrophil engraftment Variable	n	% (95% CI)[a]	*P*
Total cell dose			
≧3 × 10^7/kg	16	94% (82–100%)	
<3 × 10^7/kg	14	79% (57–100%)	0.25
HLA disparities			
HLA 5/6 match	6	67% (29–100%)	
HLA 4/6 match	24	92% (81–100%)	0.24
100% Donor chimerism			
Total cell dose			
≧3 × 10^7/kg	16	100%	
<3 × 10^7/kg	14	86% (67–100%)	0.63
HLA disparity			
HLA 5/6 match	6	83% (54–100%)	
HLA 4/6 match	24	96% (88–100%)	0.31
Overall survival			
Total cell dose			
≧3 × 10^7/kg	16	54% (24–83%)	
<3 × 10^7/kg	14	52% (6.6–87%)	0.70
HLA disparities			
HLA 5/6 match	6	63% (20–100%)	
HLA 4/6 match	24	51% (20–81%)	0.60

[a] CI, confidence interval.

without requiring readministration of Filgrastim. Two patients died of TRM within 28 days of transplant. Primary graft failure occurred in the remaining 2 patients, who underwent second RI-UCBT with the same preparative regimen and GVHD prophylaxis and achieved neutrophil engraftment and complete donor chimerism. No patients experienced a decrease in neutrophil <0.5 × 10^9/liter during the follow-up.

Platelet counts >20 × 10^9/liter were achieved by 16 patients (40%; 95% CI, 25–57%) on a median day of 39 days (range, 25–95 days). No other patient achieved platelet recovery until the last day of follow-up.

No significant association was found between neutrophil engraftment and either infused cell dose or HLA disparity (Table 2).

Chimerism Analysis. Chimerism data were obtained from all of the 30 patients. Cumulative incidence of complete donor chimerism at day 60 was 93% (95% CI, 84–100%), and median time to complete donor chimerism was 22 days (range, 13–56 days; Fig. 2). The 2 patients who died of TRM within 28 days had complete donor chimerism before neutrophil engraftment. All of the surviving patients were monitored for chimerism every 3 months, followed the cyclosporine tapering schedule from day 100 to day 150, and maintained complete donor chimerism during the follow-up even after the discontinuation of immunosuppressants.

No significant association was identified between complete donor chimerism and either infused cell dose or HLA disparity (Table 2).

RRT and TRM. Four patients (13%) developed grade III RRT. No patient had grade IV RRT. The most commonly involved organs were the gut and kidney (Table 3).

TRM within 100 days of RI-UCBT was 27%. Primary causes of death were interstitial pneumonitis (n = 2), acute GVHD (n = 2), gastrointestinal bleeding (n = 1), acute heart failure (n = 1), limbic encephalopathy (n = 1), and sepsis (n = 1).

GVHD. Grade II-IV and III-IV acute GVHD occurred in 27% (95% CI, 11–43%) and 23% (95% CI, 7.4–39%) of the patients, respectively. Median onset of grade II-IV acute GVHD was day 36 (range, day 17–66; Fig. 3).

Of the 13 patients who survived >100 days, 3 (23%) developed chronic GVHD.

Infection. Twelve patients developed infections: bacteremia (n = 8), invasive aspergillosis (n = 3), and pulmonary tuberculosis (n = 1). Nine of them had been treated with

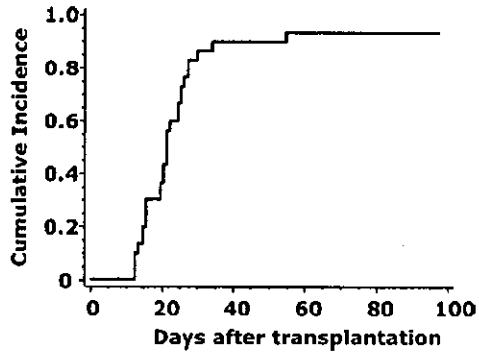

Fig. 2 Achievement of complete donor chimerism. Cumulative incidence of complete donor chimerism at day 60 after reduced-intensity unrelated cord-blood transplantation (*RI-UCBT*) was 93% (95% confidence interval, 84–100%), and median time to complete donor chimerism was day 22 (range, day 13–56).

Table 3 Regimen-related toxicity within 28 days (Bearman's score)

Score	Diarrhea	Kidney	CNS[a]	Liver	Lung
Grade 0	18	18	26	22	27
Grade 1	8	5	0	3	2
Grade 2	4	6	1	4	0
Grade 3	0	1	3	1	1
Grade 4	0	0	0	0	0

[a] CNS, central nervous system.

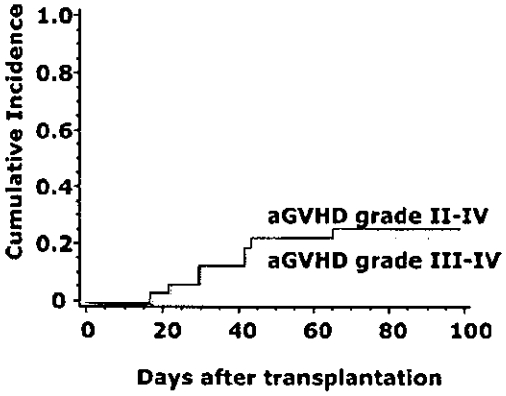

Fig. 3 Development of acute graft-*versus*-host disease (GVHD). Grade II-IV and III-IV acute GVHD developed in 27% (95% confidence interval, 11–43%) and 23% (95% confidence interval, 7.4–39%) of the patients, respectively. Median onsets of grade II-IV and III-IV acute GVHD were day 36 (range, day 17–66) and day 30 (range, day17–44), respectively.

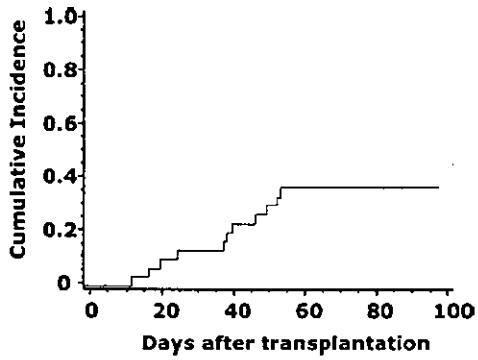

Fig. 4 Development of cytomegalovirus reactivation. Reactivation of cytomegalovirus was documented in 11 patients (37%) on a median of day 40 (range, day 13–55).

corticosteroids at the onset of infections. Reactivation of CMV was documented in 11 patients (37%) on a median of day 40 (range, day 13–55; Fig. 4). Eight of them had been treated with corticosteroids at the onset of CMV antigenemia. None of them developed CMV-related diseases. One patient developed hemorrhagic cystitis with adenovirus and BK virus infection.

Pre-Engraftment Noninfectious Fever. Seven patients with documented infection before engraftment were excluded from the analysis of pre-engraftment reaction (Table 4). Eighteen patients developed noninfectious fever before neutrophil engraftment (Fig. 5). Noninfectious high-grade fever often coexisted with eruption, diarrhea, and weight gain, starting on a median of day 9. Pathological examination of eruption from 8 patients revealed nonspecific inflammatory reactions and was not compatible with GVHD.

Survival. As of January 2004, a total of 11 patients remained alive. Median follow-up of the survivors and all of the enrolled patients were 238 days (range, 169–485) and 125 days (range, 26–485), respectively. Primary diseases recurred in 3 patients. Estimated 1-year OS and EFS were 32.7% (95% CI, 14.3–51.1%; Fig. 6) and 22.2% (95% CI, 5.9–38.5%; Fig. 7), respectively. Neither cell dose nor HLA disparity was associated with OS (Table 2).

DISCUSSION

Because CB contains a small amount of hematopoietic stem cells and stem cell boost or donor lymphocyte infusion is not available after UCBT, graft failure has been a major concern in adult UCBT. The present study demonstrated the feasibility of RI-UCBT for adult patients, in addition to pediatric patients (21). In this study, 26 of the 30 patients (87%) achieved durable engraftment, and 28 patients achieved complete donor chimerism by day 60, including 2 patients who died before engraftment. Interestingly, 4 patients with severe aplastic anemia, which has been associated with a high incidence of graft rejection (22), achieved complete chimerism after our reduced-intensity regimen. These findings suggest that the combination of fludarabine, melphalan, and low-dose TBI might be more immunosuppressive than conventional myeloablative regimens, creating niche for CB to engraft. Alternatively, CB may exert a strong graft-*versus*-host effect, making room for stable engraftment of stem cells.

Delayed hematopoietic recovery and infection during neutropenia are the significant concerns in adult UCBT. Laughlin *et*

Table 4 Characteristics of pre-engraftment reaction (n = 23)

Temperature	
38.0–38.9°C	2
39.0–39.9°C	10
≥40.0°C	7
Day of peak body temperature	9 (5–12)
Serum levels of CRP[a] (mg/dl)	13.8 (0.5–18.9)
Day of peak serum levels of CRP	10 (8–16)
Diarrhea	11
Eruption	10
Jaundice	5
Use of corticosteroid	13
Good response to corticosteroid	7

[a] CRP, C-reactive protein.

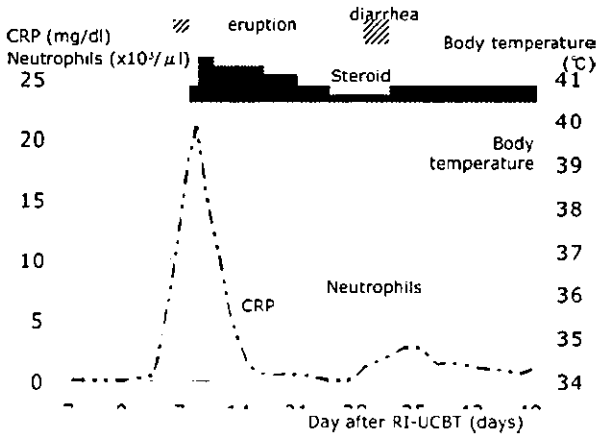

Fig. 5 Clinical course of a patient who developed pre-engraftment fever. Immune-reactions display two peaks, at around day 9 and day 18.

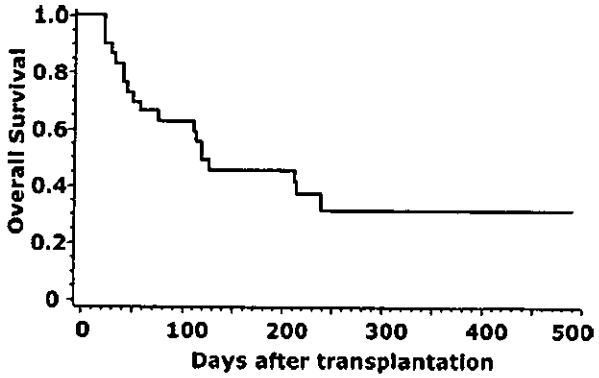

Fig. 6 Probability of overall survival after reduced-intensity unrelated cord-blood transplantation. Estimated 1-year overall survival was 32.7% (95% confidence interval, 14.3–51.1%).

al. (23) reported neutrophil recovery in 90% of patients by a median of 27 days after UCBT, which was significantly delayed compared with allo-HSCT. The delay has been attributed to the limited cell dose in the reports on myeloablative UCBT. The median nucleated cell dose in our study (3.1×10^7/kg) was greater than those in some reports from Western countries (2.1×10^7/kg; Ref. 9). The low median body weight (52 kg) in the Japanese population may favor neutrophil engraftment, whereas our results showed no association between the cell dose and engraftment in the small sample size. In the present study, median time to engraftment was 17.5 days (range, 10–54 days), which was much faster than that reported in previous studies on myeloablative UCBT (7–9). Our results were comparable with the report on adult RI-UCBT by Barker *et al.* (21). Their results showed neutrophil engraftment on a median of 26 days after busulfan/fludarabine/TBI 2 Gy and 9.5 days after cyclophosphamide/fludarabine/TBI 2 Gy. Whereas the reason for the difference remains unclear, these findings suggest that fludarabine-based reduced-intensity regimens enable rapid and stable engraftment.

TRM within 100 days was 27% in this study, which is lower than those reported on myeloablative UCBT (Refs. 7, 9, 24; 32–51% in pediatric patients and 56–63% in adults). Given the relatively old age (median, 58.5 years) and advanced stages of the primary diseases, our reduced-intensity preparative regimen probably decreased TRM. Our TRM within 100 days is comparable with that of 28% in adult RI-UCBT by Barker *et al* (21).

All of the patients tolerated our preparative regimen without grade IV RRT (Bearman's criteria; Ref. 2). Four patients developed grade III RRT with common involvements of the gut, kidney, and liver (Table 3). We used melphalan, which has dose-limiting toxicities of the gut and liver (25). These remained mild without hepatic veno-occlusive disease. Because renal toxicities of fludarabine, busulfan, and TBI 4 Gy are reportedly minimal, the high incidence of renal toxicity might be attributable to concomitant administration of nephrotoxic agents such as cyclosporin and antibiotics. Elderly patients might be susceptible to RRT. We plan to investigate optimal dosages of cyclosporin in RIST for elderly patients. Because TBI, even at a low dose, sometimes causes significant late toxicities in the lung (22), long-term follow-up is required.

Little information on GVHD after RI-UCBT is available. In the present study, the incidences of grade II-IV and III-IV acute GVHD and chronic GVHD were 27%, 23%, and 23%, respectively, whereas some reported those to be 33–44%, 11–22%, and 0–25%, respectively, in myeloablative UCBT (7, 8, 26). There are no significant differences in the incidences of GVHD between myeloablative UCBT and RI-UCBT. This is similar to the GVHD incidences in myeloablative allo-HSCT and RIST (27). Median onset of acute GVHD was 36 days (range, 17–66 days) in the present study, which was comparable with that of myeloablative UCBT (7, 8, 26). In contrast, the achievement of complete donor chimerism and the onset of acute GVHD are delayed in RIST compared with myeloablative allo-HSCT (27, 28). CB might have a potential of intense graft-*versus*-host effect, allowing niche for early engraftment. The characteristics of GVHD after RI-UCBT remain to be investigated, including different organ involvements and response to immunosuppressive treatment.

Interestingly, 20 patients developed inflammatory reactions before engraftment (Table 4). These reactions included noninfectious high-grade fever, eruption, diarrhea, and jaundice, starting on a median of day 9. Because the reactions preceded engraftment (median, day 17.5), we speculated that some form of immune reaction that is not categorized as acute GVHD occurs after RI-UCBT without achieving engraftment. The pre-engraftment fever has been reported on rare occasions in previous reports of UCBT and might be similar to those observed after haploidentical transplantations. Antithymocyte globulin and corticosteroids, which have strong immunosuppressive properties, were commonly used in previous studies on UCBT (9), whereas neither was used in the present study. Immunosuppressive treatment with corticosteroids was effective for the pre-engraftment fever. These findings support that immune-mediated reactions after UCBT might manifest easily with the present regimen. The doubling time of cultured CB $CD34^+$ cells is 7–10 days, which is several hundred-fold faster than that of cultured adult marrow cells (29). Mononuclear cells from CB display a unique cytokine profile such as comparable levels of

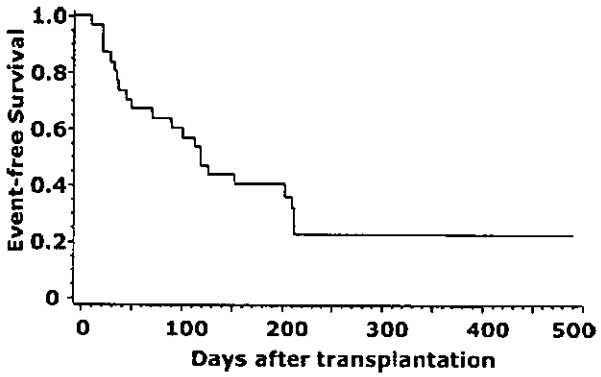

Fig. 7 Probability of event-free survival after reduced-intensity unrelated cord-blood transplantation. Estimated 1-year event-free survival was 22.2% (95% confidence interval, 5.9–38.5%).

interleukin (IL) 2, IL-6, and tumor necrosis factor α, reduced levels of IFN-γ and IL-10, and complete absence of IL-4 and IL-5 (30, 31). Pre-engraftment fever is possibly attributable to a cytokine storm induced by massive proliferation of cells with a unique cytokine profile. Another possibility is homeostasis-driven proliferation of naive T cells in highly immunosuppressed individuals, as demonstrated in murine models (32, 33). This reaction is reportedly associated with cytotoxic cytokines (32, 33). Fever as a transient response to contamination with maternal blood or cells during CB collection cannot be excluded (34). Reactivation of human herpesvirus 6 might be associated with this complication (35). If pre-engraftment fever exerts some antitumor effects, it is reasonable that patients with advanced and chemorefractory hematological diseases achieved long-term remission after RI-UCBT in the present study.

Infection is a common and significant problem in myeloablative UCBT (8, 9, 24), but little is known in RI-UCBT. The present study demonstrated that infection is also problematic in RI-UCBT. Twelve patients developed infection in this study, 9 of whom had been on corticosteroid therapy. Eight of 11 patients with CMV antigenemia had received corticosteroids. Delayed immunological reconstitution with or without GVHD, pre-engraftment fever, and corticosteroids may be risk factors for infection. Appropriate management of GVHD and pre-engraftment fever warrants additional investigation.

One-year OS was 35% in the present study, showing that some patients with advanced hematological malignancies can achieve durable remission after RI-UCBT. Contrary to our prediction, primary diseases recurred only in 3 patients. The candidates for RI-UCBT have extremely poor prognosis with conventional salvage chemotherapy. These findings suggest that RI-UCBT exerts strong antitumor activity and is promising for patients with refractory hematological malignancies without an HLA-identical sibling or an unrelated donor. In contrast, it is premature to apply RI-UCBT to low-risk diseases.

In conclusion, our study demonstrated the feasibility of RI-UCBT for adult patients with advanced hematological diseases, although the limitations included the small sample size and short follow-up. If CB is feasible for adults as an alternative stem cell source, RI-UCBT may become the choice of treatment for patients with advanced hematological diseases that are incurable with conventional treatments. RI-UCBT is particularly appealing for patients who require urgent treatments. Although RI-UCBT is currently associated with a high TRM, this study provided a rationale for continuing our clinical trials. Additional investigations need to focus on minimizing adverse effects including RRT, GVHD, and pre-engraftment immune reactions, whereas preserving graft-*versus*-leukemia effects.

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Disseminated tuberculosis following reduced-intensity cord blood transplantation for adult patients with hematological diseases

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Summary:

Allogeneic hematopoietic stem cell transplantation (allo-SCT) recipients are prone to infections. The incidences of mycobacterial infections after allo-SCT in several case series vary from less than 0.1–5.5%. However, no study has been published on tuberculosis following unrelated cord blood transplantation (UCBT). We retrospectively reviewed medical records of 113 adult patients with a median age of 54 years who underwent reduced-intensity UCBT (RI-UCBT) at Toranomon Hospital from March 2002 to May 2004. *Mycobacterium tuberculosis* infections were diagnosed in three patients (2.7%), of these two patients developed primary infection and one patient developed reactivation of latent tuberculosis. The interval between RI-UCBT and the diagnosis of tuberculosis was 34, 41 and 61 days. All the patients had disseminated disease at diagnosis. Histological examination showed the lack of granuloma in caseous necrosis. Combination antituberculous treatments showed limited efficacy, and two patients died immediately after diagnosis. *M. tuberculosis* caused life-threatening illness, rapidly progressing in RI-UCBT recipients. The lack of granuloma in caseous necrosis suggests the impaired T-cell function in early post transplant phase of RI-UCBT. We should consider *M. tuberculosis* in the differential diagnoses of fever of unknown source after RI-UCBT.
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Unrelated cord blood transplantation (UCBT) is an attractive alternative for patients with hematologic malignancies who do not have a matched related or unrelated donor. Myeloablative UCBT for adult patients achieves engraftment in 90% of the cases and is associated with 15% of transplant-related mortality, mostly attributable to infection.[1] The feasibility of UCBT using reduced-intensity regimens (RI-UCBT) for adult patients with hematologic diseases has been reported.[2–4] Studies on immune recovery following UCBT suggested that RI-UCBT recipients may have reduced incidences of graft-versus-host disease (GVHD) and infectious complications.[5] However, little information is available on infections following RI-UCBT as well as myeloablative UCBT.[6–8]

Mycobacterium tuberculosis (*M. tuberculosis*) is a common pathogen in the world. It is endemic to East Asia including Japan. Japan conducts a nationwide program of *Bacillus Calmette-Guerin* (BCG) vaccination in infants and revaccination in school children at the age of 6, 7, 13 and 14 years since 1951 and 1954, respectively. Annual incidence of tuberculosis decreased from 698 per 100 000 in 1951 to 31 per 100 000 in 2000. Tuberculosis mostly affects people at the age of 60 years or older; 82% of the patients are 40 years or older.[9,10] Reactivation of latent tuberculosis is common in immunodeficient patients such as AIDS patients and organ transplant recipients.[11,12] Recent studies on tuberculosis following allogeneic hematopoietic stem cell transplantation (allo-SCT) have demonstrated that this is a significant problem in endemic countries with an incidence of 0.1–5.5%.[13–21]

This is the first report, to our knowledge, on tuberculosis following RI-UCBT. Detailed description of this complication would be informative in the management of RI-UCBT.

Patients and methods

Data collection

Medical records of 113 recipients who underwent RI-UCBT between March 2002 and May 2004 at Toranomon Hospital were reviewed for the diagnosis of tuberculosis. Their characteristics were shown in Table 1. All the patients

Table 1 Patients' characteristics and transplantation procedures

Variables	*Number*
Patients characteristics	
Age (median, range)	54 (17–79)
Sex (male/female)	65/48
Primary diseases	
AML/MDS	46
Malignant lymphoma	41
Acute lymphoblastic leukemia	15
Severe aplastic anemia	5
Chronic myeloid leukemia	1
Plasma cell neoplasms	4
Others	1
Risk of underlying diseases[a] (high/low)	108/5
Transplantation procedures	
GVHD prophylaxis (cyclosporine/tacrolimus)	89/24
Number of infused CD34+cells ($\times 10^7$/kg) (median, range)	2.9 (1.7–5.2)
HLA disparity (0/1/2)	2/15/96

[a]We divided the risk of transplantation into two groups. The low-risk group was as follows: acute myeloid or lymphoid leukemia in first and second remission, chronic myelogeneous leukemia in chronic phase and myelodysplastic syndrome refractory anemia. The other patients were defined as having high-risk diseases.
TBI = total body irradiation; AML = acute myeloid leukemia; MDS = myelodysplastic syndromes.

had received BCG vaccines according to the national vaccination program.[10] No patients had undergone anti-tuberculosis treatments prior to RI-UCBT.

The following data of the patients with tuberculosis were collected: demographics, past medical history including tuberculosis, the primary hematologic diseases, the presence of acute or chronic GVHD, chest radiographs, diagnostic methods and the primary site of *M. tuberculosis* infection, and the outcome of tuberculosis treatment.

Transplantation procedures and supportive care

The pretransplant evaluation of the respiratory system included chest radiographs, high-resolution computed tomographies (CT) and pulmonary function tests. Sputum smears and cultures for acid-fast bacilli were not routinely obtained.

A cord blood (CB) unit was searched through the Japan Cord Blood Bank Network. CB units, which were available for UCBT were 4/6 or more serologically HLA-antigen matched and contained at least 2×10^7 nucleated cells/kg of recipient body weight before freezing.

Transplantation procedures were shown in Table 1. The RI conditioning regimen consisted of fludarabine 25 mg/m^2 on days −6 to −2, melphalan 40 mg/m^2 on days −3 and −2, and total body irradiation (TBI) 4 Gy on day −1. Granulocyte colony-stimulating factor was administered from day 1 until neutrophil engraftment.

GVHD prophylaxis was either tacrolimus 0.03 mg/kg or cyclosporine 3 mg/kg in a continuous infusion starting on day −1. The diagnosis of GVHD was made based on clinical judgment and skin or gut biopsy results to support the clinical diagnosis. Acute GVHD was graded according to the consensus criteria.[22] When patients developed grade II–IV acute GVHD, we initiated 1–2 mg/kg/day of methylprednisolone in addition to tacrolimus or cyclosporine.

Patients were managed in reverse isolation in laminar airflow-equipped rooms. All patients received prophylaxis with trimethoprim-sulfamethoxazole against *Pneumocystis carinii* infection. They received tosufloxacine 450 mg/day, fluconazole 200 mg/day and acyclovir 600 mg/day for the prophylaxis of bacterial, fungal and herpesvirus infection, respectively. Broad-spectrum antibiotics were initiated when neutropenic fever developed. All patients were monitored for cytomegalovirus pp65 antigenemia once a week. When the antigenemia turned positive, pre-emptive therapy with foscarnet was initiated as described previously.[23]

Definition of M. tuberculosis *infection*

A diagnosis of tuberculosis was based on the identification of *M. tuberculosis* on acid-fast bacilli stain of sputum, endotracheal aspirates, bone marrow aspirates or bronchoalveolar lavage (BAL), or the presence of caseous granulomas on hematoxylin–eosin staining of tissue specimens.[24] All of the BAL specimens were subject to polymerase chain reactions (PCR) using *M. tuberculosis*-specific primers[25] and COBAS AMPLICOR™ system (Roche Diagnostics KK, Tokyo, Japan).

Disease control for tuberculosis

All the staff members were screened for tuberculosis by chest X-ray annually. Anyone with positive chest X-ray and suggestive symptoms are not on service.

Any patients with suspected tuberculosis are placed in respiratory isolation until three consecutive sputum samples are negative for acid-fast bacilli. Patients with the diagnosis of tuberculosis receive the standard antituberculosis treatment with rifampicin, isoniazid and ethambutol, with or without pyrazinamide. Since rifampicin may decrease cyclosporine concentration, cyclosporine dose was adjusted by monitoring its blood levels.

Results

Three of the 113 RI-UCBT recipients (2.7%) developed tuberculosis. They had no different risk factors for tuberculosis compared with the other 110 patients. Their detailed clinical characteristics are shown in Table 2.

Patient 1

A 67-year-old man with chronic myeloid leukemia in blastic crisis underwent RI-UCBT in July 2003. He had no family history of tuberculosis. Screening chest CT before transplantation was normal. He developed a pre-engraftment immune reaction on day 9,[3] which was successfully treated by methylprednisolone 0.25 mg/kg. He achieved neutrophil engraftment on day 19. He developed grade II acute GVHD on day 24, which responded to methylprednisolone. He developed a high-grade fever on day 61, and chest CT

Table 2 Clinical features of three patients who developed disseminated tuberculosis following RI-UCBT

	Patient 1	*Patient 2*	*Patient 3*
Primary disease	CML	AML	AML
Disease status	Blastic crisis	Primary induction failure	Second remission
Age (years)/sex	67/male	64 female	20/female
Past and family history of tuberculosis	None/none	None/none	None/none
Screening chest CT scan	Normal	Small calcified nodules in right lower lobe	Normal
Pre-engraftment immune reaction	Present	Present	Absent
Maximal grade of acute GVHD	Grade 1	Absent	Absent
Corticosteroid use	Yes	Yes	No
Onset of tuberculosis	Day 63	Day 34	Day 46
Initial symptoms	Fever, fatigue	Fever, cough, fatigue	Fever, chest pain
Chest CT finding at diagnosis	Multiple cavitations	Infiltration in the right lower lobe, wall thickening of bronchus	Mediastinal lymphadenopathy
Type of tuberculosis	Miliary tuberculosis	Miliary tuberculosis	Miliary tuberculosis
Treatment	INH/RFP/EB/PZA	INH/RFP/EB	INH/RFP/EB
Outcomes	Died of miliary tuberculosis on day 116	Died of miliary tuberculosis on day 57	Improved, alive on day 180

CML = chronic myeloid leukemia; AML = acute myeloid leukemia; INH = isoniazid; RFP = rifampicin; EB = ethambutol; PZA = pyrazinamide.

on day 62 revealed small nodules in bilateral lungs, which were 2 mm in diameter. The lesions were considered to be bacterial or fungal infection and we initiated empiric treatments. The fever persisted despite broad-spectrum antibiotics and antifungal agents. Follow-up CT on day 75 showed multiple cavities in bilateral lungs, which were 12 mm in diameter (Figure 1a). Ziehl–Neelsen stain and PCR of BAL specimens on day 77 were positive for *M. tuberculosis*. Subsequently, *M. tuberculosis* was cultured from BAL fluid and bone marrow aspirates. On day 61, white blood cell count was 7.3×10^9/l (neutrophil 91.0%) with 95 CD4 + T-cells/μl; serum IgG was 306 mg/dl. Cyclosporine was tapered and a combination therapy with isoniazid, rifampicin, ethambutol and pyrazinamide was initiated. GVHD aggravated after tapering of cyclosporine, which was successfully treated with methylprednisolone 1 mg/kg. On day 89, he developed respiratory failure due to interstitial pneumonitis, requiring mechanical ventilation. He died of multiple-organ failure on day 116. In the post-mortem examination, almost all organs showed necrosis without granulation (Figure 2a). Ziehl–Neelsen stain identified acid-fast bacilli in the necrosis.

Patient 2

A 64-year-old woman with refractory acute myeloid leukemia underwent RI-UCBT in August 2003. She had no history of tuberculosis. Screening chest CT showed a small calcification nodule in the right lower lobe, which was 10 mm in diameter. She developed a pre-engraftment immune reaction on day 9,[3] which was successfully controlled by methylprednisolone 0.25 mg/kg. She achieved neutrophil engraftment on day 23. The clinical course had been uneventful until day 34, when a high-grade fever developed. Chest CT on day 34 showed infiltration in the right lower lobe, thickening of bronchial wall and pericardial effusion (Figure 1b). The patient refused bronchoscopy at this point. The fever persisted despite broad-spectrum antibiotics and antifungal drugs. BAL specimens obtained on day 43 were positive for *M. tuberculosis* on PCR. The calcified nodule in the right lobe indicated reactivation of latent tuberculosis. Phenotype of the peripheral lymphocytes on day 43 was shown in Table 2. Combination therapy with isoniazid, rifampicin and ethambutol was immediately initiated. However, it was discontinued due to hepatic toxicity. She died of adult respiratory distress syndrome on day 57. In the post-mortem examination, almost all organs had necrosis without granulation, and Ziehl–Neelsen stain revealed acid-fast bacilli in the necrotic lesions (Figure 2b). She also had disseminated cytomegalovirus infection.

Patient 3

A 20-year-old woman with acute myeloid leukemia in second remission underwent RI-UCBT in November 2003. Screening chest CT was normal. She achieved neutrophil engraftment on day 28. The clinical course had been uneventful until she developed a high-grade fever on day 46. She had not developed either a pre-engraftment fever or acute GVHD.[3] Chest CT showed mediastinal lymphadenopathy, which was 15 mm in diameter (Figure 1c). The fever did not respond to empiric broad-spectrum antibiotics. Bone marrow aspirates on day 65 were positive for *M. tuberculosis* on PCR and culture. She subsequently developed cutaneous tuberculosis (Figure 3) with disseminated intravascular coagulation. She was diagnosed with primary infection of *M. tuberculosis*. On day 46, white blood cell count and serum levels of IgG were 5.6×10^9/l (neutrophil 84.5%) and 1180 mg/dl, respectively. Phenotype of the peripheral lymphocytes on day 46 was shown in Table 2. After initiation of isoniazid, rifampicin and ethambutol, these symptoms improved gradually. She is alive without reactivation of tuberculosis on day 180.

Figure 1 Chest CT scans. (a) Patient 1: follow-up CT on day 75 showed multiple cavities in bilateral lungs, which were 12 mm in diameter. (b) Patient 2: chest CT on day 34 showed infiltration in the right lower lobe, thickening of bronchial wall and pericardial effusion. (c) Patient 3: enlarged mediastinal lymph nodes.

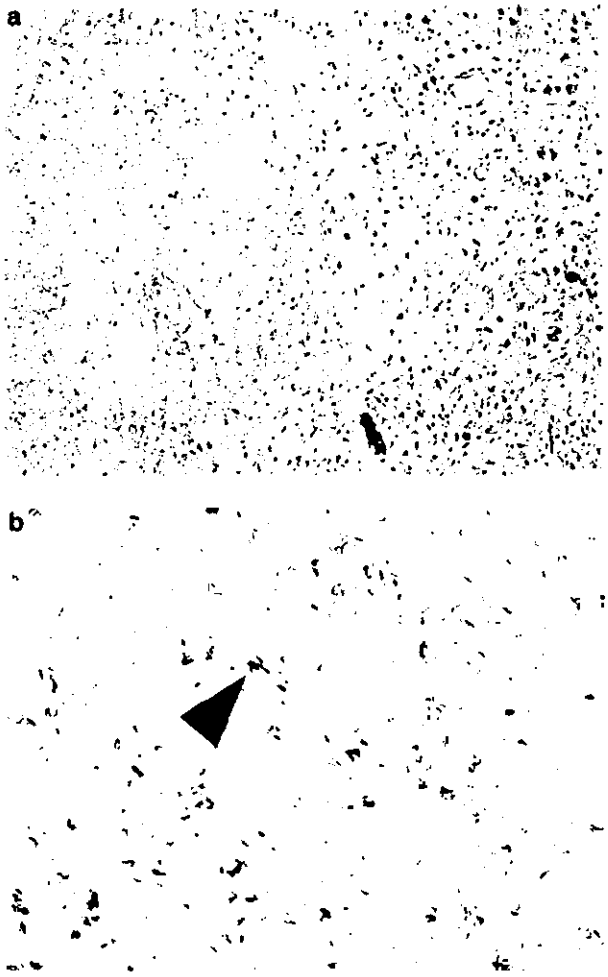

Figure 2 Pathologic features of tuberculosis infection. (a) Caseous necrosis without granulation in the lung. Paraffin section with hematoxylin–eosin stain; original magnification × 100. (b) Acid-fast bacilli (arrowhead) in caseous necrosis in the liver. Paraffin section with Ziehl–Neelsen stain; original magnification × 400.

Discussion

Japan has been an endemic country of tuberculosis. Among the newly diagnosed patients in 2001, 57.1% were at the age of 60 years and above and 17.5% were in their 20's and 30's. Many patients above 50 have a history of tuberculosis. Even without obvious history of tuberculosis, latent infection after unknown exposure is common in the generation. Therefore, Patients 1 and 2 were considered to have reactivation of latent tuberculosis. Transmission from them to Patient 3 was unlikely because Patient 3 was admitted to the hospital 20 and 25 days after patients 1 and 2 had died, respectively. No patients had tuberculosis during her hospitalization. Owing to her young age, her tuberculosis was considered a primary infection rather than reactivation of latent infection.

The incidence of tuberculosis following RI-UCBT is 2.5% in our hospital, and the median age was 54 years in this study. A high incidence of tuberculosis might be

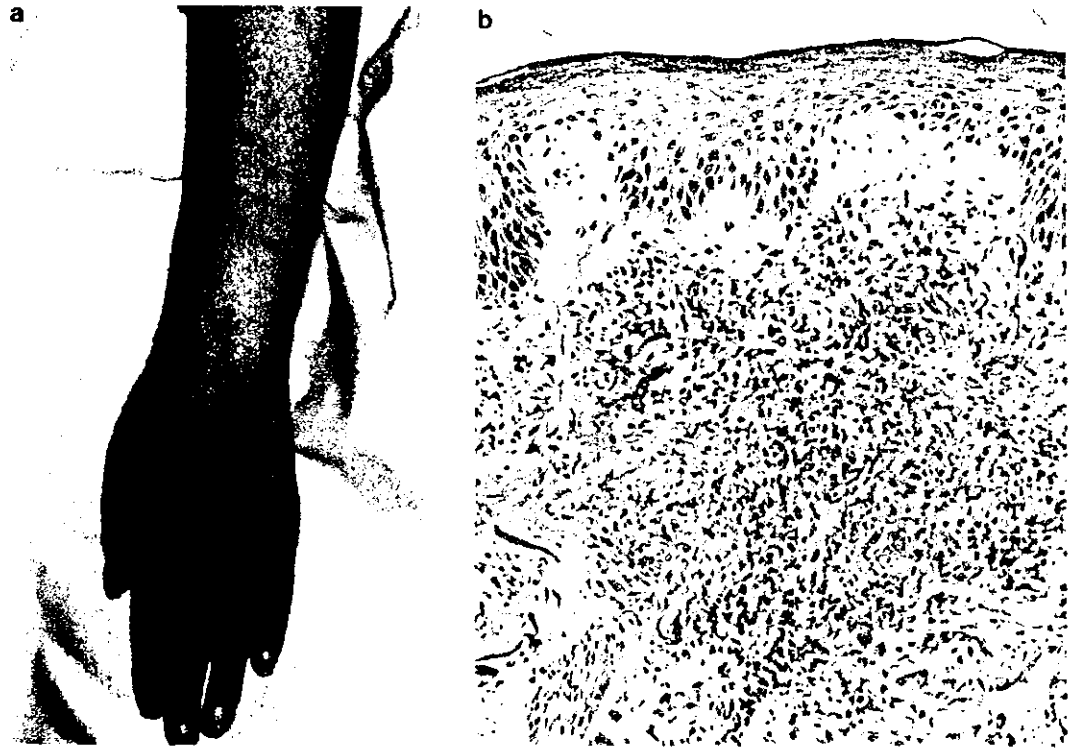

Figure 3 Macroscopic and pathologic features of cutaneous tuberculosis. (a) Disseminated tuberculosis (arrowhead) in the forearm. (b) Caseous necrosis and granuloma in the dermis. Paraffin section with hematoxylin–eosin stain; original magnification × 100.

expected in RI-UCBT patients due to patients' age and prolonged immunosuppression associated with the use of CB as a stem cell source; however, the incidence of tuberculosis following RI-UCBT was comparable to that following allo-HSCT in previous reports from endemic countries.[15,18,21] Tuberculosis is less frequent in RI-UCBT recipients than in the immunodeficient patients of different etiologies such as solid organ transplantation and AIDS. Immunosuppression after RI-UCBT is transient,[11] while AIDS patients and solid organ transplant recipients have a life-long immunosuppression. Alternatively, the frequent and prolonged use of several antibiotics may suppress reactivation of *M. tuberculosis*. Fluoroquinolones are active against *M. tuberculosis*, and are occasionally used as antituberculous prophylaxis.[16]

Clinical features of tuberculosis following RI-UCBT might be different from those following conventional allo-HSCT.[13,21] Tuberculosis following conventional allo-HSCT usually occurs several months after transplantation; the median time to presentation with tuberculosis was 324 days post transplant.[18] It usually follows indolent clinical courses. The lung is the most common site of the disease, and the common manifestations are fever and cough. If treated adequately, most patients are cured without relapse. The clinical courses of our patients were different from those in the previous reports.[13,21] Tuberculosis developed within 100 days of RI-UCBT, either reactivation or primary infection, and disseminated rapidly to various organs. It should be noted that all the three patients developed miliary tuberculosis, and two of the three patients were refractory to antituberculosis therapy and finally died. Such a rapid extrapulmonary progression of tuberculosis has been reported in solid organ recipients or AIDS patients.[26,27]

Table 3 Phenotypic analysis of peripheral lymphocytes at the onset of tuberculosis

	Patient 2	*Patient 3*
White blood cells (× 10⁹/l)	2.0	1.5
Lymphocyte (× 10⁹/l)	0.28	0.12
CD4+ T cell (× 10⁹/l)	0.09	0.04
CD8+ T cell (× 10⁹/l)	0.07	0.034
CD4/8 ratio	1.24	1.14
CD4+, 45RA+ (× 10⁹/l)	0.044	0.011
CD4+, CD45RO+ (× 10⁹/l)	0.01	0.022
CD4+, CD69+ (× 10⁹/l)	0.0	0.0011
CD4+, CD25+ (× 10⁹/l)	0.06	0.021
CD20+ (× 10⁹/l)	0.004	0.001
CD3−, CD56+ (× 10⁹/l)	0.08	0.019

Although limited data are available on immune reconstitution of RI-UCBT recipients, it is clear their cellular immunity is extremely impaired (Table 3). While recovery of the immune system depends on peripheral expansion of mature T- and B-lymphocytes transferred with the graft,[28] CB does not contain antigen-experienced cells, leading to a slow immune reconstitution and an increased risk of infectious complications. Functional immune recovery begins 3 months after conventional UCBT in adult patients, and T-cell reconstitution begins by 18 months.[29]

Chronic GVHD, immunosuppressive therapy, TBI and T-cell depletion have been shown to be risk factors for tuberculosis following conventional allo-HSCT.[16,17,30] We used a TBI-containing preparative regimen, and two patients developed either GVHD or pre-engraftment immune reaction, requiring corticosteroid. Prolonged immunosuppression results in failure to acquire adoptive immunity against tuberculosis, and TBI hampers normal function of alveolar macrophages. Both might have contributed to an increased susceptibility to early-onset tuberculosis.

This study demonstrates that tuberculosis is a significant complication of RI-UCBT. There is a considerable delay from the onset of symptoms to the diagnosis of tuberculosis. Unless we have enough information on the complication, it is difficult to make an early diagnosis and to initiate an antituberculous treatment. A delay in establishing a diagnosis of tuberculosis in RI-UCBT recipients could have a pivotal impact on their survival. While needs for antituberculous prophylaxis are controversial in solid organ and marrow transplantation,[31] identification of high-risk patients and prophylaxis in the subgroup may be beneficial in RI-UCBT. We suggest screening before RI-UCBT with PPD skin test in addition to chest CT, especially in the endemic areas. Further studies are warranted to investigate clinical features of tuberculosis following RI-UCBT, and to identify its optimal management.

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Pediatric Transplantation

Comparison of the outcomes of allogeneic bone marrow transplantation from partially mismatched related donors, matched sibling donors, and matched unrelated donors in Japanese pediatric patients: A single center result

Nagatoshi Y, Kawano Y, Okamura J. Comparison of the outcomes of allogeneic bone marrow transplantation from partially mismatched related donors, matched sibling donors, and matched unrelated donors in Japanese pediatric patients: A single center result.
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Abstract: Human leukocyte antigen-disparity is an essential factor in selecting a suitable donor for allogeneic bone marrow transplantation (BMT), and selection criteria may differ between countries or races and between adults and children. We investigated the usefulness of partially mismatched related donors (PMRD) for Japanese children in comparison with matched sibling donors (MSD) and matched unrelated donors (MUD). Eighteen patients were transplanted from PMRD, who consisted of 12 parents, five siblings, and one cousin. Five of these 18 patient–donor pairs were serologically two-loci mismatched and 13 were one-locus mismatched. The probability of engraftment from PMRD was not different from that using BMT from MSD (n = 59) or MUD (n = 28). Severe acute graft-versus-host disease (GVHD) (≥grade III) developed more frequently in PMRD (25.5 ± 11.0%) than in MSD (0.0%), but was seen just as often as in MUD (21.9 ± 7.9%). The probabilities of chronic GVHD in PMRD (56.7 ± 14.3%) and MUD (41.7 ± 11.4%) were significantly higher than that in MSD (18.7 ± 5.7%, p = 0.01). However, there was no difference in the probability of event-free survival among the three groups. We conclude that PMRD (up to two-loci mismatch) could become suitable donors in BMT to the same extent as MUD for pediatric patients in Japan.

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Key words: bone marrow transplantation – partially mismatched related donor – pediatric patients – graft-versus-host disease – human leukocyte antigen-disparity

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Abbreviations: ACV, acyclovir; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; BMT, bone marrow transplantation; BUS, busulfan; CBT, cord blood transplantation; CMV, cytomegalovirus; CR, complete remission; CSA, cyclosporine A; CY, cyclophosphamide; EFS, event-free survival; FK, tacrolimus; G-CSF, granulocyte colony-stimulating factor; GCV, ganciclovir; GVHD, graft-versus-host disease; HDCA, cytarabine; HF, hyper-fractionated; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; L-PAM, L-phenylalanine mustard; MDS, myelodysplastic syndrome; m-PSL, methyl-prednisolone; MSD, matched sibling donor; MTX, methotrexate; MUD, matched unrelated donor; PMRD, partially mismatched related donor; TBI, total body irradiation; TCD, T-cell depletion; TRM, treatment-related mortality.

Most patients who require allogeneic HSCT do not have HLA-MSDs. In 1985, in the early development of HSCT, the Seattle group tried allogeneic BMT from a PMRD without TCD (1). They found that acute GVHD developed more frequently and was more severe in PMRD than in BMT from a MSD. This has been the only report to compare BMT from PMRD without TCD to that from MSD as a single-center experience. Since then, BMT from PMRD has shifted toward the use of TCD to avoid fatal complications, and while this has reduced the incidence of severe GVHD, it has increased the rates of both rejection and disease relapse (2–4).

More recently, patients who lack an MSD have been able to receive BMT from a MUD through the establishment of marrow donor programs. However, the incidence of acute GVHD after BMT from MUD, especially the severe form, has been reported to be much higher than that from MSD, which has resulted in a higher incidence of TRM (5, 6). Furthermore, considerable time and effort is needed to coordinate patient–donor pairs, compared with related donors, and there is a substantial economic burden from the time of registration to BMT.

On the other hand, purified CD34-positive cells as an alternative to T-cell-depleted grafts have been used for HSCT from PMRD, based on progress in cell manipulation technologies (7, 8). In this setting, donors are available immediately, and the results have been satisfactory to some extent. However, the procedures used for the mobilization and separation of CD34-positive cells are still expensive for routine use, and the application of this procedure using pediatric donors is still ethically controversial.

We analyzed a single institute's experience with BMT from PMRD without TCD in a pediatric population to confirm the importance of related donors by considering the probabilities of acute GVHD, chronic GVHD, TRM, and EFS, compared to those with BMT from MSD or MUD.

Materials and methods

Patients and donors

Between December 1987 and August 2000, 18 patients underwent BMT from PMRD at the National Kyushu Cancer Center, Fukuoka, Japan. Their clinical courses and outcomes were compared with those of 59 and 28 patients who underwent BMT from MSD and MUD, respectively, in the same period. HLAs were determined by serological typing before 1994, when we became able to type HLAs at the DNA level. Molecular testing was not carried out in MSD because haplotypes were clearly identified in family studies to result from inheritance. Acute leukemias and MDS were diagnosed according to the criteria of the French–American–British cooperative group (9, 10). The characteristics and HLA disparities of the patients and donors in the PMRD group are shown in Table 1. The distributions of age and gender in the PMRD group were identical to those in the control groups (MSD and MUD), as shown in Table 2. Both the PMRD and MUD groups contained more high-risk patients than the MSD group. All of the patients were thought to be candidates for BMT at the time they received PMRD or MUD and lacked HLA-identical siblings. All cases were approved by the Institutional Review Board, and written informed consent was obtained from the patients and donors or their guardians.

Conditioning regimen

Lymphoid malignancies were conditioned with a HF-TBI-based regimen as previously reported (11). The HF-TBI consisted of 1.2 Gy/fraction × 3 fractions/day for a total of 13.2 Gy (i.e. 11 fractions) when the patient was over 5 yr of age, and 1.2 Gy/fraction × 2 fractions/day for 5 days, for a total of 12 Gy, when the child was under 5 yr of age. Lungs were shielded to a calculated dose of 12 Gy. Cytotoxic and immunosuppressive agents in addition to TBI consisted of four doses of HDCA (3 g/m^2 × 2/day) and two consecutive days of CY (60 mg/kg/day, total dose 120 mg/kg) for lymphoid malignant diseases. A combination of TBI (13.2 Gy), L-PAM (90 mg/m^2/day for 2 days, total dose 180 mg/m^2), and BUS (4 mg/kg/day for 2 days, total dose 8 mg/kg) was used for some patients with higher-risk features, such as primary induction failure, ALL not in first or second CR, AML not in first CR, relapse and refractory disease in both types of leukemia. Patients with other leukemias received BUS (4 mg/kg or 140 mg/m^2 for 4 days, total dose 16 mg/kg or 560 mg/m^2) and L-PAM (70 mg/m^2/day for 3 days, total dose 210 mg/m^2).

Prophylaxis and diagnosis of GVHD

Prophylaxis for GVHD consisted of a combination of short-term MTX (15, 10, 10, and 10 mg/m^2 on days +1, +3, +6, and +11) and either CSA (3 mg/kg/day, divided into two doses) (12) or FK (0.03 mg/kg/day, continuous infusion) (13). All cases in the MSD group received a single agent: CSA (14) or MTX (15). CSA and FK were given intravenously from day −1 with careful monitoring of serum trough levels, where the target levels during the first 30 days were 200–400 and 10–20 ng/mL, respectively. Acute and chronic GVHD were diagnosed according to the criteria of Glucksberg et al. (16) and Schulman et al. (17). No grafts were manipulated further.

Treatment of acute and chronic GVHD

Oral prednisone (2 mg/kg/day) was used as the first choice for treating acute and chronic GVHD. It was continued for 2–4 wk depending on the clinical response and then tapered by 0.5 mg/kg/2–4 wk. If the patient could not take oral medications, a soluble form of prednisone or mPSL was given intravenously. When the initial therapy was not sufficient, high-dose mPSL therapy at 30 mg/kg/day (max 1 g) over 3 days was applied as the second-line therapy.

Table 1. Characteristics of PMRD and the results of GVHD

UPN	Age/ gender	Disease: Diagnosis	Disease: Status	Disease: Duration (months)	Relationship	HLA disparity	GVH direction	HVG direction	Acute GVHD: Stage: Skin	Stage: Gut	Stage: Liver	Grade	Response to treatment	Chronic GVHD	Response to treatment
023	17/F	AML	CR2	63	Sibling	1 locus (A)	1	1	NE	NE	NE	NE	NE	NE	
025	11/M	CML	CP	10	Mother	1 locus (A)	0	1	3	0	0	II	Good	None	
040	13/M	CML	CP	36	Mother	1 locus (A)	0	1	2	0	0	I		NE	
042	16/M	CML	CP	17	Mother	1 locus (DR)	0	1	3	0	0	II	Poor	NE	
066	12/F	ALL	RP2	27	Sibling	1 locus (B)	1	1	0	0	0	0		None	
071	4/M	AML	CR2	26	Sibling	1 locus (B)	1	1	NE	NE	NE	NE	NE	NE	
077	3/F	ALL	CR3	20	Sibling	1 locus (A)	1	1	2	0	0	I		None	
082	16/F	ALL	CR2	39	Mother	1 locus (A)	1	0	3	0	0	II	Good	None	
113	2/M	JMML		0	Mother	1 locus (B)	1	1	3	0	0	II	Good	None	
127	1/M	AML	CR2	9	Cousin	1 locus (A)	1	0	3	0	0	II	Good	Limited	Poor
128	4/M	AML	CR1	6	Father	1 locus (A)	1	1	0	0	0	0		None	
152	0.8/F	ALL	CR1	5	Father	2 loci (A, DR)	0	2	3	1	0	II	Poor	Extensive	Good
170	4/M	ALL	RP2	17	Mother	2 loci (B, DR)	2	2	3	1	0	II	Poor	Extensive	Poor
174	10/F	ALL	CR2	46	Mother	1 locus (DR)	1	1	2	0	0	I	Good	Extensive	Poor
177	9/M	ALL	CR3	35	Mother	2 loci (A, DR)	2	2	3	4	0	IV	Poor	Extensive	Poor
178	4/F	NHL	CR4	35	Father	2 loci (A, B)	1	1	3	3	0	III	Good	Extensive	Good
179	12/M	AML	IF	5	Sibling	1 locus (A)	0	1	0	0	0	0		NE	
192	2/M	ALL	CR1	5	Father	2 loci (B, DR)	1	2	3	0	0	II	Poor	None	

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CML, chronic myelogeneous leukemia; JMML, juvenile myelomonocytic leukemia; NE, not evaluable; NHL, non-Hodgkin's lymphoma; CR, complete remission; CP, chronic phase; RP, relapse; IF, induction failure; HLA, human leukocyte antigen; GVH, graft-versus-host; HVG, host-versus-graft.

Table 2. Patient characteristics

	PMRD	MSD	MUD	p-value
Age				0.9*
Median (yr)	6	8	9	
Range	0.7–17	1–16	1–21	
Gender				0.75*
Male	11	33	18	
Female	7	26	10	
Diagnosis				0.02†
ALL	8	25	15	
AML	5	20	1	
CML	3	1	3	
MDS	1	11	9	
NHL	1	2	0	
Disease status (leukemia)				0.12†
Standard risk‡	7	33	11	
High risk	9	13	8	

*Kruskal–Wallis test.
†Chi-square test.
‡ALL ≤ CR2; AML in CR1; CML in CP1.
PMRD, partially mismatched related donor; MSD, matched sibling donor; MUD, matched unrelated donor; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CML, chronic myelogeneous leukemia; MDS, myelodysplastic leukemia; NHL, non-Hodgkin's lymphoma.

Supportive care

All of the patients were nursed in a high-particulate filtered airflow single room and managed with mask and gown isolation. A combination of vancomycin, polymixin B, and amphotericin B was given orally to the patients for gut decontamination. All of the patients received intravenous hyperalimentation and blood products when necessary. All blood products were irradiated and filtered to deplete contaminating WBC. ACV was administered at 1000 mg/day orally or 5 mg/kg intravenously every 12 h from day −7 to day +30. Intravenous immunoglobulin 250 mg/kg was given bi-weekly from day 0 to day +100. G-CSF (400 μg/m^2 of filgrastim, Sankyo Co., Tokyo, or 5 μg/kg of lenograstim, Chugai Pharmaceutical Co., Tokyo) was given intravenously over 1 h from day +7 for patients who were diagnosed with lymphoid malignancy and who underwent BMT. GCV (10 mg/kg/day divided into two doses) was administered to patients who showed > 10 positive cells per 50 000 WBC by a test of CMV antigenemia since 1995 (18) even if they had no clinical symptoms.

Statistical analysis

Probabilities of engraftment, acute and chronic GVHD, TRM, EFS, and overall survival were estimated by the Kaplan–Meier method (19). Differences in these end points were analyzed statistically using the log-rank test and Bonferroni method. Differences in patient age, transplanted cell doses, and time from diagnosis to transplant among the three groups were analyzed by the Kruskal–Wallis test. The distributions of patient gender, diagnoses and disease status were analyzed by the chi-square test.

Results

Doses of grafts

The number of bone marrow nucleated cells transplanted in PMRD [median (range): 4.1 (1.4–6.6) × 10^8/kg of recipient's body weight] was not significantly different from those in MSD [4.3 (0.9–8.4) × 10^8/kg] and MUD [3.2 (0.4–7.8) × 10^8/kg].

Engraftment

One patient (UPN071) died of complications before engraftment and another patient (UPN023) did not engraft. Therefore, 16 of 17 evaluable patients were engrafted and the probability of engraftment in PMRD was 94.4 ± 5.4% (±s.e.), which was not significantly different from those in MSD (98.1 ± 1.9%) and MUD (100.0 ± 0.0%). The median number of days for the neutrophil count to reach $>0.5 \times 10^9$/L was 21 (range: 12–35), 16 (9–40), and 19 (14–39) in PMRD, MSD, and MUD, respectively. Secondary graft failure was not observed. The probability of platelet engraftment in PMRD was also similar to those in MSD and MUD. The median number of days for the platelet count to reach $>20 \times 10^9$/L was 26 (range: 16–37), 21 (5–81), and 28 (11–93) in PMRD, MSD, and MUD, respectively.

Graft-versus-host disease

The details of acute and chronic GVHD in the PMRD group are shown in Table 1. Notably, severe acute GVHD (grade III or IV) developed in only three of 16 evaluable patients in PMRD. There was no sign of chronic GVHD in six patients. The results of comparisons with the other groups were as follows:

Acute GVHD

Acute GVHD of any grade occurred in 13 of 16 evaluable patients in PMRD. The probability of acute GVHD (76.3 ± 10.3%) was not significantly higher than that in MSD (58.3 ± 6.5%) or MUD (80.8 ± 7.7%) (Fig. 1a). However, the probability of acute GVHD greater than or equal to grade II in PMRD (60.6 ± 13.2%) was significantly higher than that in MSD (22.6 ± 6.5%) ($p = 0.04$) (Fig. 1b). This value in MUD (71.7 ± 10.2%) was not significantly different from that in PMRD. A similar trend was observed when the probabilities of severe acute GVHD (≥grade III) were compared among the three groups (PMRD, 25.5 ± 11.0%; MSD, 0.0%; MUD, 21.9 ± 7.9%) (Fig. 1c).

Chronic GVHD

The probability of chronic GVHD (limited or extensive) in PMRD was significantly higher than that in MSD (56.7 ± 14.3% vs. 18.7 ± 5.7%; $p = 0.01$), but not significantly different from that in MUD (41.7 ± 11.4%) (Fig. 1d). The incidence of extensive chronic GVHD was not significantly different between PMRD (27%) and MUD (23%).

Complications

In general, complications after BMT were related to the type of conditioning regimen and the occurrence of infections. There were no early complications specific to the type of graft in the present study.

CMV infection occurred in seven of 17 evaluable cases in PMRD, of which one case (UPN 042)

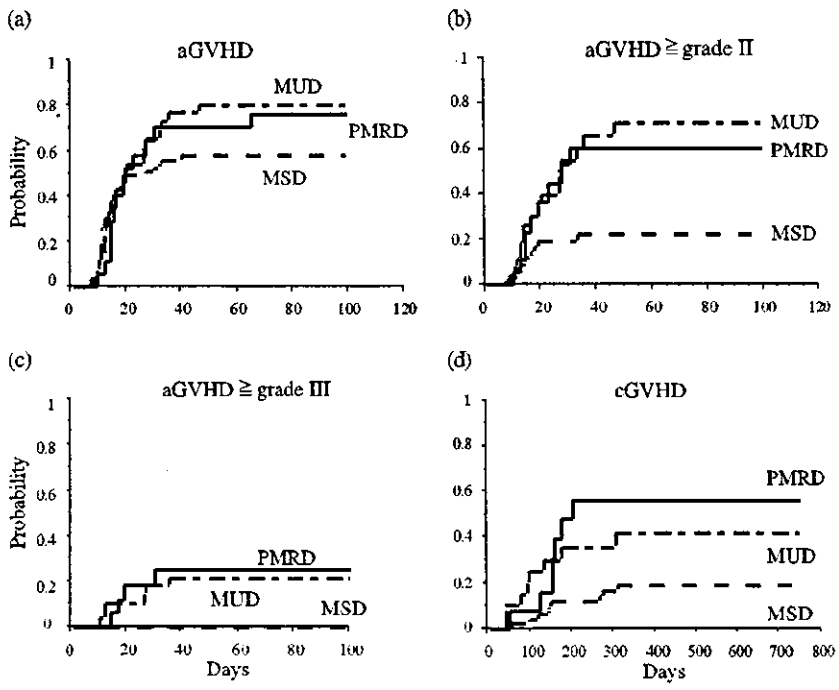

Figure 1. Probabilities of GVHD. The probabilities of acute GVHD of any grade in PMRD were not significantly different from those in either MSD or MUD (a: $p = 0.38$). The probability of acute GVHD (≥grade II) in PMRD was significantly higher than that in MSD ($p = 0.002$), but was similar to that in MUD (b). The probability of severe acute GVHD (grade III or IV) in PMRD was similar to that in MUD, while none of the patients in MSD developed severe acute GVHD (c). The probability of chronic GVHD in PMRD was similar to that in MUD, but significantly higher than that in MSD (d: $p = 0.01$). The log-rank test was used for statistical evaluation.

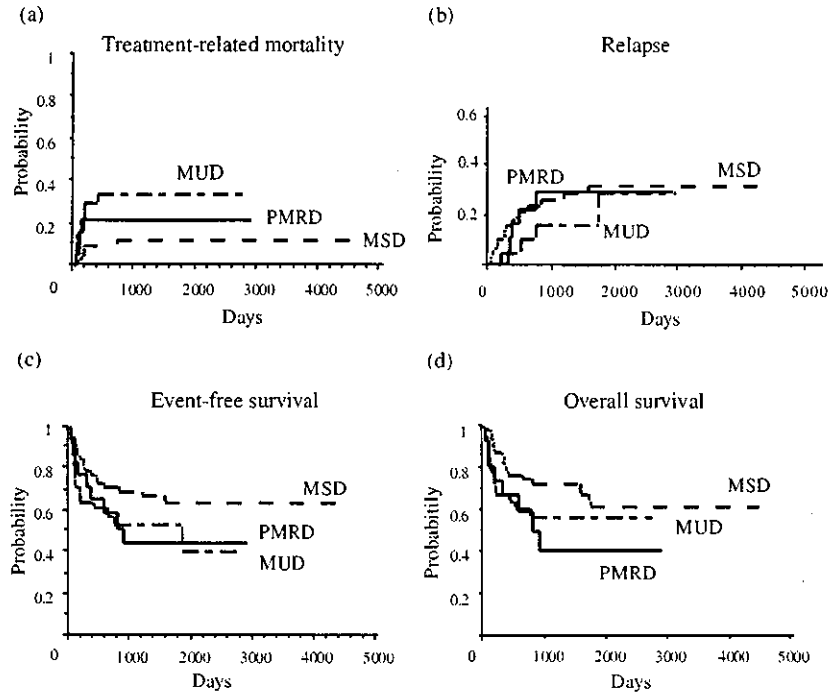

Figure 2. Probabilities of treatment-related mortality (TRM), relapse, event-free survival (EFS), and overall survival (OS). The probabilities of TRM among PMRD, MSD, and MUD were significantly different (a: $p = 0.04$). In contrast, there were no significant differences among the groups with regard to relapse rate (b: $p = 0.7$), EFS (c: $p = 0.33$), or OS (d: $p = 0.23$). Differences were examined by the log-rank test.

developed CMV pneumonitis and was diagnosed by open lung biopsy in 1991. The other patients were monitored by antigenemia and treated successfully with preemptive therapy by GCV. Therefore, none of the other patients developed CMV disease. In these seven patients, acute GVHD of grade I or II was found in one and four patients, respectively. CMV infection was not found in two patients who showed severe acute GVHD. On the other hand, six of 59 cases in MSD and 11 of 30 in MUD developed CMV infection. The incidence of CMV infection in PMRD (39%) was significantly higher than that in MSD (8%) ($p = 0.008$) and similar to that in MUD (36%).

Hemorrhagic cystitis was observed in 21 cases (three in PRMD, five in MUD, and 13 in MSD). The etiology of cystitis was CY in one case (UPN 025) in PMRD, but was not confirmed in the other two cases (UPN 042 and 082). CY-induced hemorrhagic cystitis was found in two cases each in MUD and MSD, while adenovirus-induced cystitis was found in one case in MUD. The etiologies of the remaining cases could not be identified.

Treatment-related mortality

The probability of TRM in PMRD was 22.6 ± 10.1%, which was not significantly higher than that in MSD (11.9 ± 4.6%) ($p = 0.16$), and lower than that in MUD (33.5 ± 9.1%) ($p = 0.52$), as shown in Fig. 2a. The cause of death in PMRD included hepatic failure of unknown etiology, CMV pneumonitis, gastrointestinal hemorrhage, and bronchiolitis obliterans.

Relapse and survival rate

The probabilities of relapse in PMRD, MSD, and MUD were 40.0% (95%CI, 12.2–67.8%), 32.5% (95% CI, 19.0–46.0%), and 40.1% (95% CI, 3.7–76.5%), respectively (Fig. 2b). The differences were not statistically significant. The probability of EFS in PMRD was 45.9% (95% CI, 21.4–70.4%; median follow-up, 26 months), which was not significantly different from those in MSD (59.5%; 95% CI, 46.0–73.0%, 57 months) and MUD (39.8%; 95% CI, 15.3–64.3%, 58 months) (Fig. 2c). Similar results were found regarding the probabilities of overall survival in the three groups (45.9%, 95% CI, 21.2–70.6%; 65.1%, 52.2–78.0%; 57.1%, 38.7–75.5%, in PMRD, MSD, and MUD, respectively; $p = 0.23$) (Fig. 2d).

Discussion

As about two-thirds of patients who require allogeneic HSCT do not have HLA-matched siblings, PMRD has been used for more than 20 yr for patients who lack an MSD. Recently, alternative donor sources have expanded, such as MUD and unrelated cord blood. However, there are still problems regarding the selection of suitable donors, as an alternative stem cell source

has several disadvantages for patients. For example, it usually takes 5–6 months from registration to BMT from MUD in the Japan Marrow Donor Program, and patients or their guardians must pay $6000 as a coordination fee and for laboratory tests in addition to ordinary medical costs. CBT from a banking system does not require a great deal of time for coordination or strict HLA-matching between the patient and donor. Recently, extremely favorable results of CBT have been reported in an adult population (20). On the other hand, other previous large-scale studies reported that the results of CBT were not superior to those of transplantation from other than cord blood (21, 22).

In this report, we retrospectively analyzed the usefulness of unmanipulated marrow from PMRD in a Japanese pediatric population. We demonstrated that unmanipulated marrow grafts from PMRD could reconstitute and sustain hematopoiesis as well as those from MUD and MSD. The prophylaxis for GVHD in patients transplanted from PMRD was as intense as that in MUD, and the probabilities of both acute and chronic GVHD in PMRD were not significantly different from those in MUD. In addition, the severity of chronic GVHD in PMRD was not different from that in MUD. On the other hand, both acute and chronic GVHD were more severe than those in MSD. In the present study, we typed HLA serologically in seven of 18 patient–donor pairs before 1994. Therefore, we cannot directly compare our results regarding the probability and severity of GVHD with data analyzed by the type of HLA disparity (23, 24). In another report, it was pointed out that HLA-C locus mismatch played a significant role in acute GVHD among patients in a combined pediatric and adult population who were transplanted from unrelated donors (25). Unfortunately, we could not evaluate which locus is more important in the development of acute and chronic GVHD, due to limited data regarding HLA-C and the change in the typing method during the study period. As our study was combined with transplantation from parents to their children and between mismatched siblings, we could not reach any definite conclusions, but the genetic homogeneity in the Japanese population may affect these results (26).

In the present series, the probability of relapse was not significantly different among the three groups. The percentage of patients with high-risk features was higher in the PMRD group (50%) than in the MSD group (28%), although this difference was not statistically significant. A potential graft-versus-leukemia effect might be expected to occur more often in PMRD than in MSD.

Viral infection including CMV reactivation is a major concern in post-transplant complications. It has been reported to occur frequently in patients with acute GVHD greater than or equal to grade II (27) and CMV-IP was considered fatal before 1988. However, in addition to the establishment of an appropriate treatment (28), the introduction of a method for the early detection of CMV infection (29) has made CMV reactivation both successfully treatable and preventable (30). Although acute GVHD was fairly frequent in our series of BMT from PMRD, CMV infection occurred in seven of 17 evaluable patients, which was a lower incidence than we expected. Only one patient died of CMV-IP, when GCV was not available in Japan. In addition, there were no other complications induced by severe immune incompetence, including EB virus-related lymphoproliferative disorders, in our setting.

In the present study in a pediatric population, the probabilities of EFS and overall survival in PMRD were not significantly different from those in the other two groups, even though these patients developed severe acute GVHD and chronic GVHD more frequently than those in MSD. In a Japanese adult population, the results of transplant from PMRD were reported to be similar to ours (31).

Conclusions

We conclude that unmanipulated marrow from PMRD might be an acceptable graft as an alternative to that from MUD in some situations, due to its immediate availability and because it requires less work and less cost. A prospective study is needed to determine whether or not these results are due to genetic homogeneity within the Japanese population.

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