Biol Blood Marrow Transplant 20 (2014) 214–221

Umbilical Cord Blood as an Alternative Source of Reduced-Intensity Hematopoietic Stem Cell Transplantation for Chronic Epstein-Barr Virus–Associated T or Natural Killer Cell Lymphoproliferative Diseases

Akihisa Sawada*, Masami Inoue, Maho Koyama-Sato, Osamu Kondo, Kayo Yamada, Mariko Shimizu, Kanako Isaka, Tomiko Kimoto, Hiroaki Kikuchi, Sadao Tokimasa, Masahiro Yasui, Keisei Kawa

Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

Article history:
Received 15 August 2013
Accepted 30 October 2013

Key Words:
Chronic active EBV infection
Hypersensitivity to mosquito bites
Severe-type hydroa vacciniforme
Reduced-intensity conditioning
Cord blood transplantation

A B S T R A C T

Chronic Epstein-Barr virus–associated T/natural killer cell lymphoproliferative diseases represented by chronic active Epstein-Barr virus infection are lethal but are curable with several courses of chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT). Recently, we reported that reduced-intensity conditioning (RIC) provided better outcomes than myeloablative conditioning because RIC was less toxic. However, it was unclear whether cord blood transplantation (CBT) works in the context of RIC. We retrospectively analyzed 17 patients who underwent RIC followed by bone marrow transplantation (RIC-BMT) and 15 patients who underwent RIC followed by CBT (RIC-CBT). The representative regimen was fludarabine and melphalan based. The overall survival rates with RIC-BMT and RIC-CBT were 92.9% ± 6.9% and 93.3% ± 6.4%, respectively ($P = .87$). One patient died of lung graft-versus-host disease after RIC-BMT, and 1 patient died of multiple viral infections after RIC-CBT. Although cytotoxic chemotherapy was also immunosuppressive and might contribute to better donor cell engraftment after RIC-HSCT, the rate of engraftment failure after RIC-CBT was still higher than that after RIC-BMT (not significant); however, patients who had experienced graft failure were successfully rescued with a second HSCT. Unrelated cord blood can be an alternative source for RIC-HSCT if a patient has no family donor.

INTRODUCTION

Epstein-Barr virus (EBV), also known as human herpesvirus 4, is a B cell lymphotropic and oncogenic virus and occasionally causes a variety of B cell lymphoproliferative diseases (LPDs) [1]. However, latent EBV infection can occur in T cells and/or natural killer cells (T/NK cells), resulting in T/NK cell LPDs [2,3]. Chronic EBV-associated T/NK cell LPDs (EBV$^+$ T/NK cell LPDs) are 1 of 3 distinct types of EBV$^+$ T/NK cell LPDs (acute type, chronic type, and malignant type) [1,4]. Chronic EBV$^+$ T/NK cell LPDs are represented by chronic active EBV infection (CAEBV) that is sometimes accompanied by hypersensitivity to mosquito bites or severe-type hydroa vacciniforme, but hypersensitivity to mosquito bites and severe-type hydroa vacciniforme also occur independently of CAEBV [5].

Chronic EBV$^+$ T/NK cell LPD is not a simple chronic disease, but its flare could result in a rapid, irreversible, and fatal clinical course. A study found that more than 60% of patients died over several years, mainly due to organ failure (such as liver and heart), hemophagocytic syndrome (HPS), and lymphoma [6]. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only cure for the patients with chronic EBV$^+$ T/NK cell LPD [7], although rare exceptions were reported in which complete molecular remission was achieved with multidrug chemotherapy [8].

Reduced-intensity conditioning (RIC) and umbilical cord blood transplantation (CBT) were developed in the 2000s. RIC is less toxic than myeloablative conditioning in regard to reducing transplant-related mortality, allowing growth and development in children, and to retaining fertility in young adults [9]. In our previous report on chronic EBV$^+$ T/NK cell LPD, RIC followed by HSCT (RIC-HSCT) brought about better outcomes (survival rate > 90%) than myeloablative conditioning-HSCT, and we found that CBT, as well as bone marrow transplantation (BMT), might lead to a cure in these patients [10]. However, the number of cases of RIC-CBT was too small to discuss its efficacy in that report. Unrelated CBT is potentially promising because, as opposed to unrelated bone marrow, unrelated cord blood (CB) is immediately available. However, CB lymphocytes are naive and do not have specific immune memories to EBV, whereas most adult lymphocytes have been exposed to and are immune to EBV; it is possible that this difference can influence the post-transplant clinical courses and immune reconstruction to EBV. In addition, donor lymphocyte infusion is not available after CBT.

The aim of this retrospective analysis using our latest institutional registration and follow-up data was to clarify whether RIC-CBT could be an alternative treatment for the patients with chronic EBV$^+$ T/NK cell LPD if there is no family donor. This study was approved by the Research Ethics Committee of Osaka Medical Center and Research Institute for Maternal and Child Health.

Financial disclosure: See Acknowledgments on page 221.

* Correspondence and reprint requests: Akihisa Sawada, MD, PhD, Associate Director, Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo, Izumi City, Osaka 594-1101, Japan.

E-mail address: asawada@mch.pref.osaka.jp (A. Sawada).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.10.026

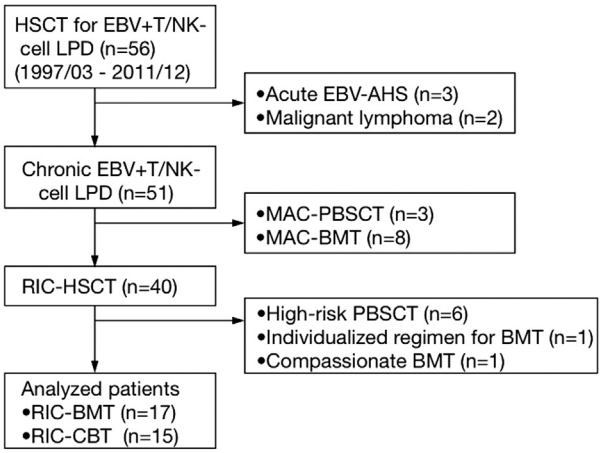

Figure 1. Patient selection. Fifty-six patients with EBV$^+$ T/NK cell LPD were treated with allogeneic HSCT at our institute. Selection criteria for the current study were chronic EBV$^+$ T/NK cell LPD, RIC, and BMT or CBT. Two patients who underwent RIC-BMT were also excluded because of individualized less-intensity conditioning for kidney dysfunction (n = 1) and compassionate transplantation for uncontrollable fulminant hemophagocytic lymphohistiocytosis (n = 1). EBV-AHS indicates EBV-associated hemophagocytic syndrome; MAC, myaloablative conditioning; PBSCT, peripheral blood stem cell transplantation.

METHODS

Patients

Patients with CAEBV met the criteria proposed in 2005 [11]. Fifty-six patients with EBV$^+$ T/NK cell LPD were treated with allogeneic HSCT between March 1997 and December 2011 at our institute (Figure 1). We excluded 5 patients from the analysis because their diagnoses were hemophagocytic lymphohistiocytosis as acute EBV infection (n = 3) or malignant lymphoma (n = 2). Of the 51 patients with chronic EBV$^+$ T/NK cell LPD, 44 had a diagnosis of CAEBV, 5 had a diagnosis of hypersensitivity to mosquito bites, and 2 had a diagnosis of severe-type hydroa vacciniforme without clinical manifestation of CAEBV.

Because all CBTs were performed after RIC and because the overall survival (OS) rate after myeloablative conditioning (median ± standard error, 54.5% ± 15.0%) was significantly inferior to that after RIC in regard to toxicity [10], patients who underwent myeloablative conditioning-HSCT (n = 11) were excluded from further analysis. There were 40 cases of RIC-HSCT. Six patients who underwent peripheral blood stem cell transplantation were excluded because peripheral blood stem cell transplantation tended to be used in patients with severe complications or other high-risk conditions: poorly controlled pneumonia with *Pseudomonas aeruginosa* (n = 1), severe heart dysfunction (n = 2), persistent HPS with febrile neutropenia (n = 1), and HLA 3/6 allele-mismatched HSCT (n = 2). The hematopoietic cell transplantation-specific comorbidity index (HCT-CI) of these 6 patients was a median of 1 (range, 0 to 4) [12].

Two additional patients were excluded: 1 was treated with individualized less-intensity conditioning for kidney dysfunction (including fludarabine [Flu] 100 mg/m^2 and melphalan [LPAM] 50 mg/m^2) and experienced donor-cell rejection after BMT (HCT-CI = 0), and 1 had uncontrolled fulminant CAEBV-associated HPS with adenovirus hemorrhagic cystitis at the initiation of RIC and died of progressive primary disease at 4 days after compassionate BMT (HCT-CI = 6). As a result, the analysis includes 17 patients who underwent RIC-BMT (related, n = 11; unrelated n = 6) and 15 patients who underwent unrelated RIC-CBT. The HCT-CI of these 17 and 15 patients were all 0.

Detection of EBV

Identification of EBV-infected cells and measurement of EBV load have been described elsewhere [10]. Briefly, for identification, the EBV genome was amplified by PCR in DNA extracted from each lymphocyte subset in peripheral blood, or EBV-encoded small ribonucleic acid (EBER) and surface

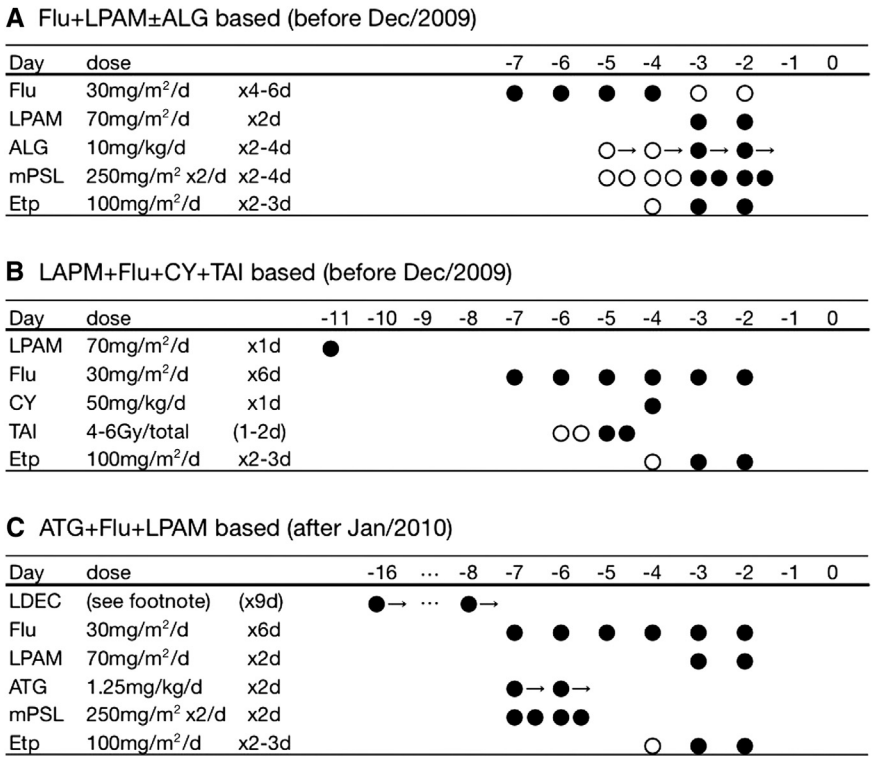

Figure 2. Conditioning regimen. Closed circles indicate fixed administration, and open circles indicate optional administration. (A) Standard conditioning regimen, which tended to be used for BMT. The problems were a high rate of EBV$^+$ post-transplant LPD at a total antilymphocyte globulin dose of 40 mg/kg in patients with variable diseases other than EBV$^+$ T/NK cell LPD and then no availability of antilymphocyte globulin afterward. (B) Conditioning regimen for high risk of rejection, which tended to be used for CBT. The problem was a usage of irradiation, which might induce a higher rate of subsequent neoplasms compared with drug administration. (C) Current regimen universally administered for BMT and CBT since January 2010. ALG indicates anti-lymphocyte globulin LDEC, low-dose Etp 30 mg/m^2/d and CA 20 mg/m^2/d were administered for 24 hours continuously for a median of 9 days (range, 4 to 14 days) just before RIC with Flu + LPAM; TAI, thoracoabdominal irradiation; mPSL, methylprednisolone; CY, cyclophosphamide.

markers were stained on thin-sliced tissue from involved organs such as liver and skin. EBV load was measured using real-time quantitative PCR in a commercial laboratory (BML Inc., Tokyo, Japan), and the lower limit of detection was 200 copies/mL in whole blood [13].

Treatment before HSCT

Patients with active disease such as fever and hepatitis were initially treated with immunochemotherapy including prednisolone (PSL) 1 to 2 mg/kg/d, cyclosporine A 3 mg/kg/d, and etoposide (Etp) 150 mg/m^2/wk from 1 to several weeks until symptoms were controlled. Patients were then treated with at least 2 courses of combination chemotherapy such as a modified CHOP regimen, high-dose cytosine arabinoside (CA), L-asparaginase, or their reduced doses in combination [10]. Modified CHOP consisted of cyclophosphamide 750 mg/m^2/d (day 1), pirarubicin 25 mg/m^2/d (days 1 and 2), vincristine 2 mg/m^2/d (maximum dose, 2 mg) (day 1), and PSL 50 mg/m^2 (day 1). High-dose CA consisted of CA 1.5 g/m^2 twice a day (days 1 to 6) and PSL 30 mg/m^2/d (days 1 to 6). VPL consisted of Etp (VP-16) 150 mg/m^2/d (day 1), PSL 30 mg/m^2/d (days 1 to 7), and L-asparaginase 6000 U/m^2/d (days 1 to 7). Capizzi consisted of CA 3 g/m^2 4 times (every 12 hours) and L-asparaginase 10,000 U/m^2 once (4 hours post-CA) and PSL 30 mg/m^2/d (days 1 and 2). ESCAP consisted of Etp 150 mg/m^2/d (day 1), CA 1.5 g/m^2 8 times (every 12 hours), L-asparaginase 6000 U/m^2/d for 5 days (4 hours post-CA and following days), methylprednisolone 125 mg/m^2/d (days 1 to 4), and PSL 30 mg/m^2/d (days 5 to 8).

As the initial combination chemotherapy, CHOP therapy was administered to 82.4% of patients in the RIC-BMT group and 93.3% of patients in the RIC-CBT group. As the second chemotherapy, ESCAP, Capizzi, and VPL were administered to 47.1%, 29.4%, and 11.8% of patients in the RIC-BMT group, respectively, and ESCAP, Capizzi, and VPL were administered to 40.0%, 26.7%, and 13.3% of patients in the RIC-CBT group, respectively. Patients in the RIC-BMT group underwent a median of 3 courses of pretransplant chemotherapy (range, 2 to 6), and patients in the RIC-CBT group underwent a median of 3 courses as well (range, 2 to 5).

HSCT Procedure

The conditioning regimen for patients treated with extensive courses of chemotherapy was Flu 120 to 180 mg/m^2 and LPAM 140 mg/m^2 with or without antilymphocyte globulin 20 to 40 mg/kg (Figure 2). The conditioning regimen for other patients was Flu 180 mg/m^2, LPAM 70 mg/m^2, cyclophosphamide 50 mg/kg, and thoracoabdominal irradiation 4 to 6 Gy with or without antilymphocyte globulin 20 to 40 mg/kg. Since January 2010, a revised universal conditioning regimen of Flu 180 mg/m^2, LPAM 140 mg/m^2, and antithymocyte globulin 2.5 mg/kg was administered for both BMT and CBT.

Since 2009, most patients were treated before conditioning with intravenous low-dose Etp 30 mg/m^2/d and CA 20 mg/m^2/d for 24 hours continuously for a median of 9 days (range, 4 to 14 days) as a pre-RIC treatment to reduce the burden of EBV$^+$ T/NK cells and the risk of rejection. Most patients received Etp 100 mg/m^2/d for 2 to 3 days to delete activated histiocytes (HPS induced by conditioning-related tumor lysis) and to reduce the incidence of graft-versus-host disease (GVHD). GVHD prophylaxis included cyclosporine A for HLA-matched related BMT; tacrolimus and a short course of methotrexate for HLA-mismatched CBT; HLA-mismatched related BMT and HLA-matched unrelated BMT; and tacrolimus, short course of methotrexate, and methylprednisolone for HLA-mismatched unrelated BMT.

Table 1
Patient Characteristics

	Graft Source	
	BMT (n = 17)	CBT (n = 15)
Age at onset		
0-9 yr	6 (35%)	13 (87%)
10-19 yr	4 (24%)	0
20-29 yr	2 (12%)	1 (7%)
≥30 yr	5 (29%)	1 (7%)
Sex		
Male	7 (41%)	5 (33%)
Female	10 (59%)	10 (67%)
Diagnosis		
CAEBV	13 (76%)	6 (40%)
CAEBV+HMB	1 (6%)	5 (33%)
HMB	3 (18%)	2 (13%)
SHV	0	2 (13%)
EBV-infected subset		
T	6 (35%)	7 (47%)
NK	9 (53%)	8 (53%)
T+NK	2 (12%)	0
Donor relation		
Related MS	7 (41%)	0
Non-MS	4 (24%)	0
Unrelated	6 (35%)	15 (100%)

HMB indicates hypersensitivity to mosquito bite; SHV, severe-type hydroa vacciniforme; MS, HLA-matched sibling donor.

Patients were prophylactically treated with ganciclovir at 5 mg/kg intravenously every 12 hours before HSCT (from days −8 to −1) when they were serologically positive for cytomegalovirus (CMV), and all patients received acyclovir at 600 mg/m^2/d orally after HSCT [13]. CMV antigenemia was monitored with antibodies C10/C11 against CMV pp65 every week (high-risk patients) or every other week after engraftment. Patients were preemptively treated with ganciclovir when the pp65-positive cell number was ≥10/150,000 WBCs. Patients with CMV or human herpesvirus 6 disease were immediately treated with ganciclovir or foscarnet.

Endpoints and Definition

The primary endpoint was OS, defined as the duration from HSCT to any death. Second HSCT was not censored in OS. Patients remaining alive at the last follow-up were censored. Event-free survival (EFS) was defined as the duration from HSCT to any event: recurrence of disease, transplant-related mortality, and second HSCT (due to engraftment failure or loss of donor chimerism). EBV-associated LPD of donor-cell origin was regarded as infectious disease but not as recurrence of disease or subsequent primary malignancy.

The date of neutrophil recovery was considered the first of 3 consecutive days the absolute neutrophil count exceeded 500/μL. Engraftment failure

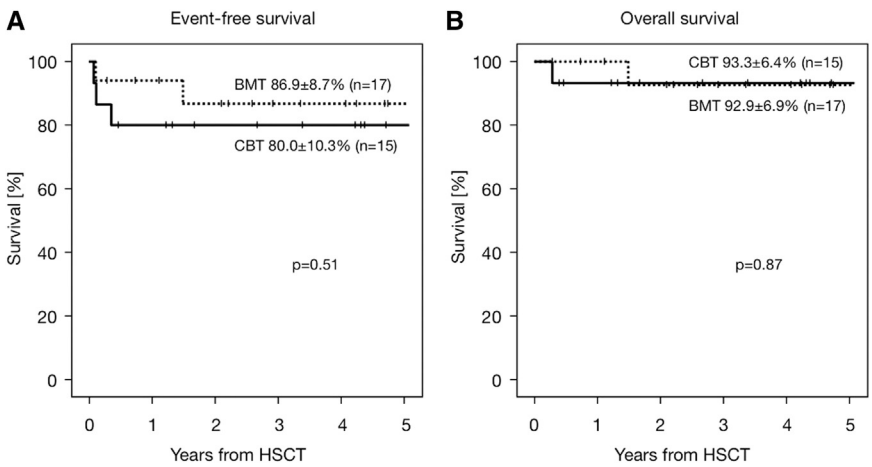

Figure 3. Survival rates after HSCT. (A) EFS and (B) OS after HSCT. Solid lines represent CBT, and dotted lines represent BMT.

was defined as an absolute neutrophil count < 500/μL or donor chimerism < 5% in WBCs from peripheral blood at day 28 after HSCT. Continuous complete donor chimerism was defined as a neutrophil count > 500/μL and donor-type WBCs > 95% in peripheral blood at day 28 and later, and mixed chimerism was defined as a neutrophil count > 500/μL and donor-type WBCs 5% to 95% in peripheral blood at day 28 or later.

Statistics

Statistical analyses were performed using the SPSS version 14 (SPSS Inc., Chicago, IL). Survival rate was estimated with the Kaplan-Meier method and assessed with the log-rank test. Chi-square test was used for univariate analysis.

RESULTS

The characteristics of the 32 patients included in the study are shown in Table 1. Fifteen of 32 patients were included in our previous report [10]. There was a significant difference in age between patients in the RIC-BMT and RIC-CBT groups. In these 32 patients, the EFS rate was 83.4% ± 6.8% and the OS rate was 92.8% ± 4.9%.

The EFS rate of patients treated with RIC-BMT and RIC-CBT was 86.9% ± 8.7% and 80.0% ± 10.3%, respectively, which was not statistically significant ($P = .51$, Figure 3A). The OS rate of patients treated with RIC-BMT and RIC-CBT was 92.9% ± 6.9% and 93.3% ± 6.4%, respectively, again not statistically significant ($P = .87$, Figure 3B). However, interestingly, there were some differences in the clinical courses after HSCT between patients treated with RIC-BMT and patients treated with RIC-CBT, as shown in the following text.

Engraft Failure

The HSCT procedure and results are shown in Table 2. Four patients experienced rejection, mixed chimerism, or autologous hematological recovery: 1 in the RIC-BMT group and 3 in the RIC-CBT group. Of these 4 patients, elevation of EBV load was documented in 2 (unique patient number [UPN] 463 and 605). A girl (UPN 463) experienced neutrophil recovery of complete autologous cells (donor 0%) day 35 after RIC-CBT using regimen B shown in Figure 2. However, she was continuously dependent on red blood cell and platelet infusion and granulocyte colony-stimulating factor afterward, and EBV load was elevated 2×10^4 copies/mL (whole blood) without symptoms. She successfully underwent a second RIC-CBT [10]. Three other patients (1 treated with RIC-BMT and 2 treated with RIC-CBT) experienced rejection without neutrophil recovery after the current regimen C for conditioning (Table 3). A girl (UPN 573) had a homozygote of HLA allelotype that resulted in no HLA mismatched to graft-versus-host direction and 2 alleles mismatched only to rejective direction. She successfully underwent RIC-peripheral blood stem cell transplantation from the same donor (her father) of the first RIC-BMT. A girl (UPN 569) had bidirectional 2 allele mismatches, and a boy (UPN 605) had bidirectional 3 allele mismatches to the CBT donor; here, no anti-HLA antibody or antibody against donor cells, which might be related to lower neutrophil and platelet recovery, was detected in their plasma [14]. CB contained a sufficient number of WBCs and hematopoietic stem/progenitor cells. These patients underwent a second RIC-CBT and had complete donor chimerism.

Graft-versus-Host Disease

No differences were found in the occurrence of acute and chronic GVHD between patients treated with RIC-BMT and those treated with RIC-CBT. A high ratio of severe acute and extensive chronic GVHD was observed in the RIC-BMT group, but the significance was statistically demonstrated only with extensive chronic GVHD (Table 2). No differences were seen in the occurrence and the severity of acute and chronic GVHD between HLA-matched related donors and unrelated or HLA-mismatched related donors; indeed, 1 patient who underwent matched-sibling BMT died of GVHD (UPN 563, mentioned later).

Table 2
Events and Causes of Death

	BMT		CBT (n = 15)	BMT vs. CBT *P*
	MS or R (6/6) (n = 10)	U or R (5/6) (n = 7)		
Regimen				
Flu+LPAM±ALG based	5	2	3	
LAPM+Flu+CY+TAI based	3	1	6	—
ATG+Flu+LPAM based	2	4	6	
Engraftment failure				
Autorecovery/ persistent neutropenia	0/1	0	1*/2	.23
→Second HSCT	→1	—	→3	
→Complete donor chimerism	→1	—	→3	
Acute GVHD				
Grades II-IV/ assessable number	6/9 (67%)	3/7 (43%)	6/12 (50%)	.74
Grades III-IV/ assessable number	2/9 (22%)	2/7 (29%)	1/12 (8%)	.25
Chronic GVHD				
Lim+Ext/assessable number	6/9 (67%)	3/7 (43%)	5/12 (42%)	.74
Ext/assessable number	6/9 (67%)	2/7 (29%)	1/12 (8%)	.02
Viral reactivation/ infection				
CMV antigenemia	2	5	10†	.17
CMV disease	1	0	2†	.47
EBV+PTLD	1	1	1	.62
HHV6 diseases	1	0	2	.47
BKV hemorrhagic cystitis	1	0	5†,‡	.05
Death				
GVHD	1 (BO)	0	0	—
Viral disease	0	0	1 (CMV+BKV)†	—

R(6/6) indicates a related donor who was not an HLA-matched sibling but had serologically matched HLA-A, -B, and -DR for graft-versus-host (GVH) direction; R(5/6), related donor who had serologically one-mismatched HLA for GVH direction; assessable patient number in acute and chronic GVHD, patients who achieved complete donor chimerism after first HSCT; MS, matched sibling; ALG, antilymphocyte globulin; ATG, antithymocyte globulin; TAI, thoracoabdominal irradiation; PTLD, post-transplant lymphoproliferative disease; BO, bronchiolitis obliterans as a lung GVHD.
HHV6 diseases were encephalitis (after BMT), skin rash (CBT) and pneumonitis (CBT).
* One patient showed recipient chimerism 100% but had incomplete hematopoiesis.
† One patient with BKV cystitis developed lethal systemic BKV infection and CMV pneumonitis.
‡ PCR for BKV detection was not performed in 1 of 5 patients with hemorrhagic cystitis.

Viral Infection and Reactivation

There was no difference in the occurrence of CMV, EBV, and human herpesvirus-6 diseases between patients treated with RIC-BMT and those treated with RIC-CBT. A high ratio of significance appeared only with the occurrence of BK virus

Table 3
Current Regimen and Engraftment

UPN	Age (yr)	Sex	Body Weight (kg)	Source	No. Mismatched HLA				Conditioning				No. Courses Chemotherapy	EBV Load at RIC (Copies/mL)	Conditioning-Associated HPS	GVHD Prophylaxis	Infused Cells		Anti-HLA Ab	Results	
					Rejection Direction		GVH Direction														
					ABDR	Cw	ABDR	Cw	LDEC (Days)	ATG (mg/kg)	Flu+LPAM (mg/m^2)	Etp (mg/m^2)					ANC ($\times 10^7$/kg)	CD34+[$\times 10^5$/kg]		Neutrophil Recovery	Engraftment (Chimerism)
563	36	F	51	MS-BM	0	0	0	0	4	2.5	F180+L140	200	2	8×10^5	–	CsA	33.9	92.7	ND	+	Donor
607	11	M	33	U-BM	0	0	1	0	10	2.5	F180+L140	200	3	2×10^4	–	CsA/sMTX/mP	36.7	33.6	ND	+	Donor
588	37	F	47	U-BM	0	0	0	0	7	2.5	F180+L140	200	4	1×10^4	–	Tac/sMTX	32.5	39.1	ND	+	Donor
556	25	F	54	U-BM	0	1	0	1	9	-	F180+L140	300	3	<200	–	Tac/sMTX	32.3	24.1	ND	+	Donor
595	15	M	53	U-BM	1	1	1	0	6	2.5	F180+L210*	200	3	7×10^4	–	Tac/sMTX/mP	27.0	27.6	ND	+	Donor
573	11	F	33	R-BM	2	1	0	0	12	-	F180+L140	300	2	1×10^4	–	Tac/sMTX	50.6	67.3	ND	–	Failure
584	23	F	46	U-CB	1	1	1	1	11	2.5	F180+L140	300	5	3×10^4	–	Tac/sMTX	3.41	0.96	Neg	+	Donor
582	11	F	42	U-CB	2	0	2	1	9	2.5	F180+L140	300	4	2×10^4	–	Tac/sMTX	4.19	1.63	Neg	+	Donor
569	10	F	42	U-CB	2	0	2	0	14	2.5	F180+L140	300	2	2×10^4	+	Tac/sMTX	3.36	0.84	Neg	–	Failure
571	9	M	44	U-CB	2	0	2	0	14	2.5	F180+L140	300	2	5×10^4	+	Tac/sMTX	2.37	0.80	ND	+	Donor
605	9	M	28	U-CB	2	1	2	1	7	2.5	F180+L140	200	2	4×10^4	+	Tac/sMTX	3.93	1.46	Neg	–	Failure
603	38	M	61	U-CB	2	2	2	1	8	2.5	F180+L140	300	2	3×10^4	+	Tac/sMTX	1.64	1.23	Neg	+	Donor

UPN indicates unique patient number; F, fludarabine; L, LPAM; LDEC, low-dose Etp and cytosine arabinoside as a pre-RIC treatment; ATG, antithymocyte globulin; GVH, graft-versus-host; ANC, all nucleated cells; CD34+, CD34-positive cells; Ab, antibody; MS, matched sibling; R, HLA-mismatched related; U, unrelated; BM, bone marrow; CsA, cyclosporine A; sMTX, short course of methotrexate; mP, methylprednisolone; Tac, tacrolimus; neg, negative; ND, not done.

The numbers of mismatched-HLA alleles (genotype) in HLA-A + B + DRB1 (ABDR) and in HLA-Cw are separately shown. The order of patients is from low risk to high risk of rejection; that is, ascending in rejection direction, then descending in GVHD direction. Age and body weight are at the initiation of conditioning.

* One day of additional LPAM (70 mg/m^2/d $\times$ 3 days in total) because more than 1 year had passed since the latest chemotherapy.

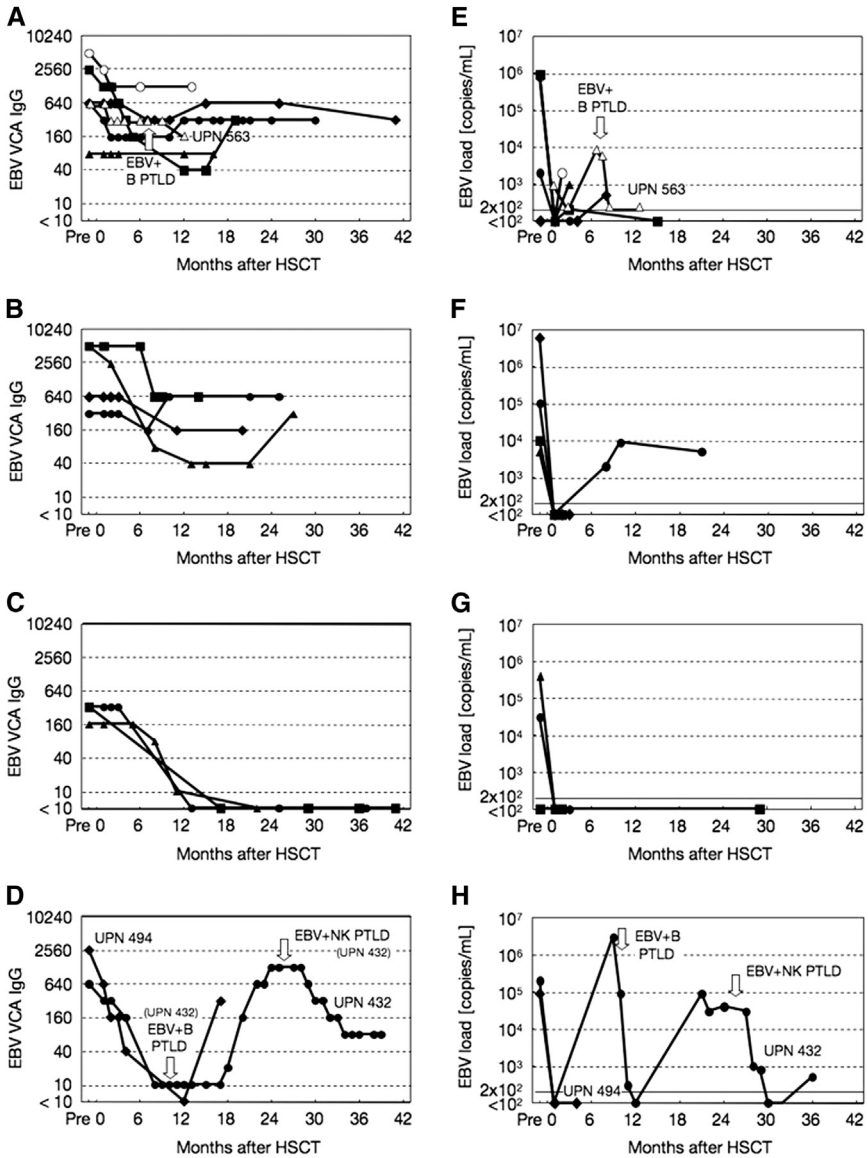

Figure 4. Anti-EBV antibody titers after HSCT. (A-D) The changes in anti–EBV-VCA IgG titers, followed for at least 1 year after HSCT, are shown. (A) BMT from a donor whose EBV-VCA IgG was known (n = 6, all were positive for EBV-VCA IgG). Open triangles represent UPN 563, who had EBV$^+$ B cell post-transplant LPD (PTLD). (B-D) CBT (n = 9). (B) EBV-VCA IgG titer has never achieved an undetectable level (<1:10) in 4 of 9 recipients after CBT. Engrafted B cells might be infected with recipient-derived EBV, because CB is regarded as free from EBV. The lowest titers were observed during 6 to 18 months after BMT and CBT (A and B). (C) EBV-VCA IgG titer remained negative 12 months after CBT in 3 recipients. Recipient EBV might be eradicated by CBT. (D) UPN 432 had severe PTLD after secondary primary EBV infection: EBV$^+$ B cell PTLD followed by EBV$^+$ NK cell PTLD. Those lymphocytes arise from engrafted donor cells. UPN 494 showed no symptoms. (E-H) Changes in EBV load are shown. (E) Changes in EBV load of patients in A, (F) those of patients in B, (G) those of patients in C, and (H) those of patients in D. PTLD indicates post-transplant LPD.

(BKV) hemorrhagic cystitis, which was statistically higher after RIC-CBT. One patient experienced lethal viral disease after RIC-CBT (UPN 569, mentioned later).

Anti–EBV virus capsid antigen (VCA) immunoglobulin G (IgG) titer was followed and measured with a fluorescent antibody test in a commercial laboratory. The changes in the titer followed for at least 1 year after HSCT are shown in Figure 4A-D, and the changes in EBV load are shown in Figure 4E-H. Three patients experienced EBV-associated post-transplant LPD (UPN 563 and 607 after BMT and UPN 432 after CBT). UPN 563 (Figure 4A,E) and UPN 607 (not shown) experienced simple EBV$^+$ B-cell LPD that was successfully treated with rituximab 7 and 4 months after BMT, respectively. However, UPN 432 had severe post-transplant LPD (Figure 4D,H); she experienced symptoms of primary EBV infection such as sore throat, jaundice, fever, and lymphadenopathy with EBV$^+$ B-cell LPD in the central nervous system and left eyeground 10 months after CBT [15] and EBV$^+$ NK cell LPD an additional 16 months later. Those cells were derived from donor-originated lymphocytes and were fortunately susceptible to R-CHOP and high-dose CA with or without L-asparaginase, respectively. However, we failed to prove the EBV was derived from a third party other than the recipient or the donor [16].

Relapse and Causes of Death

No patient who achieved complete donor chimerism after the first or second HSCT experienced a relapse of primary

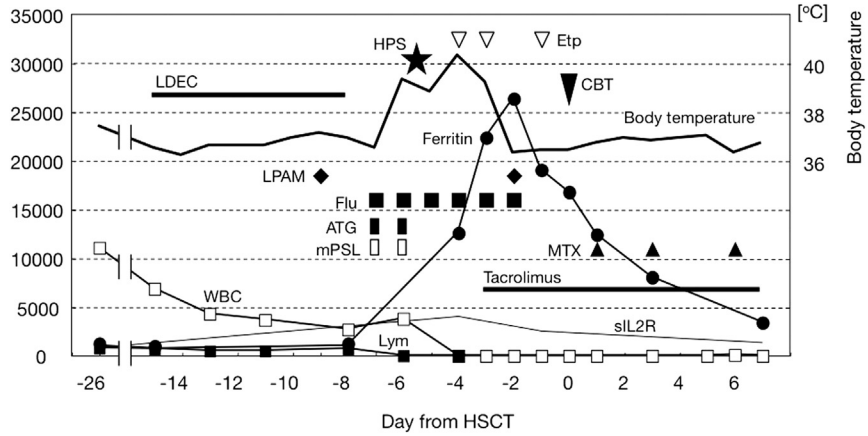

Figure 5. Conditioning-associated HPS. Typical conditioning-associated HPS in UPN 603 is shown. HPS (closed star) was controlled by Etp. WBC indicates white blood cell (/μL); Lym, lymphocyte (/μL); Ferritin, serum ferritin level (ng/mL); sIL2R; soluble interleukin-2 receptor (U/mL); mPSL, methylprednisolone; ATG, antithymocyte globulin; MTX, methotrexate.

disease. One of the 3 patients who underwent RIC-CBT twice, 1 patient (UPN 569) died of multiple viral diseases (hemorrhagic BKV cystitis, CMV pneumonitis, and BKV pneumonitis) 61 days after the second RIC-CBT. One woman (UPN 563) died of bronchiolitis obliterans as lung GVHD 541 days after RIC-BMT.

DISCUSSION

As a source of HSCT that follows the RIC regimen, there was no inferiority with use of CB in the present analysis. At the time of HSCT, the median age of patients in the RIC-BMT group (15 years; range, 5 to 38 years) was older than that of patients in the RIC-CBT group (9 years; range, 3 to 38 years). This was simply attributed to the availability of CB (smaller body weight in younger patients); however, this might potentially confer a survival advantage to the CBT group, because adult patients tend to have more complications and organ dysfunctions than children.

CB may be a favorable source in allogeneic HSCT because of its easy and immediate accessibility. CBT might also have superiority because of a lower occurrence rate and lower severity of GVHD. The adverse results might be mainly attributed to engraftment failure and disease relapse after CBT. CB lymphocytes are naive and do not have specific immune memories to EBV, whereas most adult lymphocytes have been exposed to and are therefore immune to EBV. However, no relapse was observed in the patients so far who achieved complete donor chimerism. The occurrence of engraftment failure was slightly higher in the RIC-CBT group than in the RIC-BMT group, but this was not significant. Given that the EBV load was regarded as a minimal residual disease marker for the efficacy of chemotherapy, most patients failed to achieve complete molecular remission after chemotherapy (data not shown); however, chemotherapy might have more influence on reducing the engraftment failure after RIC-CBT. For better engraftment, 3 or more courses of chemotherapy may be needed (see Table 3); otherwise, 1 day of additional treatment with LPAM may be required (LPAM 210 mg/m^2 in total, as in UPN 595).

The donors for BMT were all serologically positive for EBV-VCA IgG and regarded as having EBV latent infection. The EBV-VCA IgG titer in serum from recipients (n = 6) was reduced in the first 6 months and was maintained to be positive with a slight increase thereafter (Figure 4A). The recipients were regarded as harboring persistent (and permanent) EBV latent infection, but we could not determine the present EBV as of recipient origin or donor origin. Four of 9 patients treated with CBT had a similar pattern of EBV-VCA IgG (Figure 4B); however, because donor CB is regarded as free from EBV, engrafted B cells might be infected with endogenous EBV derived from recipient cells (or reinfected with a third-party EBV from blood transfusion). On the other hand, the EBV-VCA IgG level decreased and remains negative (<1:10) in 3 of 9 recipients after CBT (Figure 4C). Because the EBV load in blood from the 3 recipients also remains negative (Figure 4G), it is possible that the endogenous EBV in these patients was eradicated at the time of RIC-CBT. However, these patients might be infected with third-party–derived EBV (secondary primary infection) by chance; if such an infection occurred before the patient's immunity was fully reconstructed (early after CBT), EBV$^+$ post-transplant LPD would be life threatening, as seen in UPN 432 (Figure 4D,H). This phenomenon (eradication of endogenous EBV and reinfection with exogenous EBV later) was also observed in 2 patients after BMT from an EBV-seronegative donor [17].

Viral infection/reactivation other than EBV may also be a critical issue, and donor lymphocyte infusion is not available after CBT, although, in our series, the higher occurrence rate of viral infection was proven only in BKV hemorrhagic cystitis. However, 1 patient died of multiple viral diseases (CMV and BKV) in the RIC-CBT group. Despite such a high rate of viral infection after RIC-CBT, complete donor chimerism prevents relapse of primary disease (chronic EBV$^+$ T/NK cell LPD). Allogeneic immunity instead of antiviral immunity may be the chief mechanism for controlling the primary disease.

Finally, conditioning-associated HPS was seen in 1 patient with EBV$^+$ T cell LPD and in 3 patients with EBV$^+$ NK cell LPD. As shown in Figure 5, for example, UPN 603 experienced typical conditioning-associated HPS and needed earlier and longer administration of Etp (days −4, −3, and −1) than scheduled (days −3 and −2). There was no relation between EBV load just before RIC and occurrence of conditioning-associated HPS (Table 3). Attention is required for HPS not only after HSCT [18] but also after treatment with antithymocyte globulin or an initiation of conditioning in patients with chronic EBV$^+$ T/NK cell LPD.

In conclusion, CB can be an alternative source for RIC-HSCT in patients with chronic EBV^+ T/NK cell LPD when there is no HLA-matched sibling donor. No differences in EFS and OS rates were found between patients treated with RIC-BMT and those treated with RIC-CBT. The occurrence of engraftment failure was slightly high in the RIC-CBT group, but this was not significant. The ratio of severe acute GVHD might be smaller, and the rate of extensive chronic GVHD was statistically lower after RIC-CBT. A higher incidence of BKV hemorrhagic cystitis was documented after RIC-CBT. There were no deaths from GVHD, but viral infection/reactivation would be life threatening after RIC-CBT. Attention to conditioning-associated HPS is necessary in patients with chronic EBV^+ T/NK cell LPD.

ACKNOWLEDGMENTS

The authors thank all patients and staff included in this study for their participation.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: A.A. analyzed and interpreted data and wrote the manuscript. M.I. interpreted data and reviewed the manuscript. M.K.-S., O.K., K.Y., M.S., K.I., T.K., H.K., and S.T. enrolled patients and reported clinical data. M.Y. interpreted data. K.K. interpreted data and reviewed the manuscript.

Financial disclosure: The authors have nothing to disclose.

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