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Allogeneic hematopoietic stem cell transplantation with the modified myeloablative conditioning regimen for children with chronic active Epstein–Barr virus infection

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ABSTRACT

Importance: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered the only effective treatment for chronic active Epstein–Barr virus infection (CAEBV). The clinical efficacy and safety of allo-HSCT with different conditioning regimens in children with CAEBV remain unclear.

Objective: To evaluate the effectiveness and safety of allo-HSCT with the modified myeloablative conditioning (MAC) regimen for children with CAEBV and also the factors affecting the outcomes.

Methods: We retrospectively analyzed children with CAEBV who underwent allo-HSCT with the modified MAC regimen at Beijing Children's Hospital, Capital Medical University from October 2016 to June 2021. Data related to the clinical manifestations, engraftment, and outcome were extracted from the medical records.

Results: The cohort comprised 41 patients (24 males, 17 females) with a median transplantation age of 92.6 (60.4, 120.7) months and a median follow-up time of 28.2 (15.3, 40.2) months. Four patients (9.8%) died, among which three died from primary disease relapse, and one died from grade IV acute graft-versus-host diseases (aGVHD) after stopping treatment. The 3-year overall survival (OS) and 3-year event-free survival (EFS) rates were 88.8% ± 5.4% and 85.0% ± 5.7%, respectively. The 3-year OS and EFS did not significantly differ between the patients with hemophagocytic lymphohistiocytosis (HLH) and the patient without HLH (87.7% ± 6.8% *vs.* 91.7% ± 8.0%, $P = 0.790$; 85.0% ± 6.9% *vs.* 84.6% ± 10.0%, $P = 0.921$), or among the patients with complete remission, partial remission, and activity disease before HSCT (all $P > 0.05$). Multivariate analysis showed that grade III–IV aGVHD was a risk factor for mortality (Hazards ratio: 11.65, 95% confidence interval: 1.00, 136.06; $P = 0.050$).

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Interpretation: Allo-HSCT with the modified MAC regimen is safe and effective for pediatric CAEBV. This treatment benefits patients with HLH or active disease. Patients with Grade III–IV aGVHD may be associated with worse outcomes.

KEYWORDS
Myeloablative conditioning, Chronic active Epstein–Barr virus infection, Hematopoietic stem cell transplantation, Children

INTRODUCTION

Epstein–Barr virus (EBV) is a double-stranded DNA virus that belongs to the γ herpesvirus subfamily and is also known as human herpesvirus-4. EBV was first identified in 1964 in lymphoblastoid cells cultured from Burkitt's lymphoma.[1,2] Primary infection with EBV occurs primarily in childhood and is mostly asymptomatic. Some patients with EBV infection develop infectious mononucleosis and increased numbers of atypical lymphocytes in peripheral blood. Most such cases resolve spontaneously with supportive care only.[3] However, other patients may develop chronic and recurrent infectious mononucleosis-like symptoms after EBV infection and progress to chronic active EBV infection (CAEBV). T cells or NK cells infected by EBV can clonally proliferate and infiltrate into multiple organs. The clinical outcome varies from inert and self-limited diseases to aggressive and fatal diseases. CAEBV has both malignant and immunodeficient aspects, and its prognosis is usually poor.[4] If untreated, CAEBV may lead to life-threatening complications, including hemophagocytic lymphohistiocytosis (HLH), multiple organ failure, or lymphoma, and most patients die at 10–15 years of age.[5] At present, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered the only way to eliminate EBV-infected T cells or NK cells. CAEBV can be cured by transplanting normal donor immune cells into patients to rebuild the patient's immune system.[4–7]

At present, most large-scale clinical studies on CAEBV are conducted in Japan, while there are few reports on the treatment of CAEBV by allo-HSCT in China, especially in children. Furthermore, there is no unified conditioning regimen of allo-HSCT for CAEBV. The clinical efficacy and safety of allo-HSCT with different conditioning regimens in children with CAEBV are unclear, and the factors affecting the outcome are undetermined. Since 2016, our hospital has adopted the modified myeloablative conditioning (MAC) regimen in allo-HSCT for the treatment of children with CAEBV. This study aimed to evaluate the safety and clinical effectiveness of the MAC regimen and analyze the factors affecting the outcome of children with CAEBV.

METHODS

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Beijing Children's Hospital, Capital Medical University ([2021]-A-123-R). Written informed consent was obtained from the parents or guardians of all patients.

Patients

Children with CAEBV who received allo-HSCT with the modified MAC regimen at Beijing Children's Hospital, Capital Medical university from October 2016 to June 2021 were included. The assessed data were the source of hematopoietic stem cells, conditioning regimen, adverse effects, and outcome. The follow-up time was defined as the number of days between transplantation and final follow-up.

Diagnostic criteria of CAEBV and HLH

CAEBV diagnosis was made based on the diagnostic criteria proposed by Okano et al.[8] and Ohshima et al.[9] HLH diagnosis was based on the HLH-04 criteria proposed by the International Histiocyte Society. Also, the patients with primary HLH were excluded.[10] EBV load was measured using real-time quantitative PCR, and the lower limit of detection was 500 copies/ml in peripheral blood mononuclear cells (PBMCs) or plasma.

Pre-transplant chemotherapy

Thirteen patients diagnosed with EBV-HLH received the HLH-94 regimen to suppress activated T cells, NK cells, and macrophages before they were diagnosed with CAEBV.[11] All patients were treated with 1−4 courses of the L-DEP regimen to reduce/eliminate EBV-infected T/NK cells and suppress the disease activity after they were diagnosed with CAEBV: liposomal doxorubicin $25\,mg{\cdot}m^{-2}$ on day 1; etoposide $100\,mg{\cdot}m^{-2}$ on the first day of each week. PEG-asparaginase $2000\ U{\cdot}m^{-2}$ on day 5; methylprednisolone $15\,mg{\cdot}kg^{-1}{\cdot}d^{-1}$ on days

1−3, 2 mg·kg^{-1}·d^{-1} on days 4−7 and 1 mg·kg^{-1}·d^{-1} on days 8−14 followed by gradual tapering the following week.[12,13] Seven patients, whose initial pathologies were highly suspected of tumorigenesis or whose disease remained active or who had a high burden of residual lesions after 1−3 courses of L-DEP therapy, received ESCAP regimen: etoposide 150 mg·m^{-2} on day 1; cytosine arabinoside 1.5 mg·m^{-2} twice/d ×8 times starting from the night of day 1; L-asparaginase 6000 U·m^{-2} once every other day ×5 times starting from day 5; methylprednisolone 2.5 mg·kg^{-1} twice/d ×8 times starting from the night of day 1, 2 mg·kg^{-1}·d^{-1} on days 6−12, 1 mg·kg^{-1}·d^{-1} on days 13−15, 0.5 mg·kg^{-1}·d^{-1} on days 16−18, 0.25 mg·kg^{-1}·d^{-1} on days 19−21.[5,13] Before the initiation of conditioning, two patients with active disease received L-DEC regimen: low-dose etoposide 30 mg·m^{-2}·d^{-1} and cytosine arabinoside 20 mg·m^{-2}·d^{-1} were continuously administered for 24 h for 0.5−2 weeks.[5,13]

Transplantation

Evaluation of CAEBV disease state[12]: the disease state prior to allo-HSCT was assessed according to clinical characteristics and EBV load before being divided into active disease (AD), partial remission (PR), and complete remission (CR). AD: persistent inflammatory manifestations, such as fever, lymphadenectasis, hepatosplenomegaly, pancytopenia, progressive skin damage, and persistent positive plasma EBV-DNA. PR: partial remission of the above manifestations. CR: a significant decrease in EBV-DNA in the absence of the above manifestations.

Peripheral blood stem cells (PBSC) were used as a graft source in a matched unrelated donor (MUD) and matched sibling donor (MSD) transplantation, while bone marrow and PBSC were used as a combined graft source in the mismatched related donor (MMRD)-HSCT transplantation.[14] The bone marrow volume was 10−20 ml·kg^{-1} and the maximum volume was 800 ml. The target total CD34$^+$ cell count was (6−10) ×10^6/kg. The day of bone marrow infusion was defined as day 01, and PBSC infusion was defined as day 02.

Conditioning regimen (Figure 1): all patients received modified MAC. (1) Busulfan (Bu) (3.2−4.8) mg·kg^{-1}·d^{-1}, intravenous, d−8 to d−6; (2) Fludarabine (Flu) 30 mg·m^{-2}·d^{-1}, intravenous, d−13 to d−9; (3) Etoposide (VP-16) 300 mg·m^{-2}·d^{-1}, intravenous, d−13 to d−11; (4) Cyclophosphamide (Cy) 30 mg·kg^{-1}·d^{-1}, intravenous, d−5 to d−2; (5) Rabbit anti-human thymocyte immunoglobulin (ATG) was used for patients receiving MUD/MMRD transplantation (2.5 mg·kg^{-1}·d^{-1}, d−5 to d−2) and PR/AD patients receiving MSD transplantation (1.25 mg·kg^{-1}·d^{-1}, d−5 to d−2). Patients received porcine anti-human lymphocyte globulin (ALG) or CD25 monoclonal antibody instead of ATG if they could not tolerate ATG. One patient received post-transplant high-dose cyclophosphamide (PT-Cy) instead of ATG because he had high fever and hypotension when he used ATG. While, one of the active patients received 20 mg·kg^{-1} ATG and another patient received 200 mg·kg^{-1} ALG to suppression of activated T cells. One patient received 12 Gy total marrow and lymphoid irradiation, 150 mg·m^{-2} Flu, and 120 mg·kg^{-1} Cy in her secondary allo-HSCT after transplant rejection. To distinguish between donor and recipient cells, engraftment analyses were conducted according to genetic alteration in the 19 autosomal short tandem repeat loci and Amelogenin loci on the sex chromosome. Full chimerism was defined by the detection of ≥ 95% and mixed chimerism 5%–95% of donor hematopoietic stem cells in the recipient bone marrow or peripheral blood.[15] The date of neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count exceeding 500/μl. The date of platelet recovery was determined as the first of seven consecutive days when the platelet count exceeded 20 × 10^9/L without platelet infusion.

Treatment for complications: All the MMRD-HSCT and MUD-HSCT recipients received prophylactic cyclosporine A (CsA), mycophenolate mofetil (MMF), methotrexate (MTX) and ATG for prophylaxis for acute graft-versus-host disease (aGVHD), while all the MSD-HSCT recipients received prophylactic CsA and MTX for GVHD. The GVHD standard referred to the Mount Sinai Acute GVHD International Consortium grading system.[16] Chronic GVHD (cGVHD) grading standard referred to the National Institutes of Health (NIH) grading system.[17] The first-line treatment for aGVHD was methylprednisolone. The second-line treatment was CD25 monoclonal antibody and ruxolitinib. Ursodeoxycholic acid, low molecular weight heparin calcium, and alprostadil were used to prevent veno-occlusive disease (VOD) at the beginning of conditioning. Defibrotide was used when VOD[18] or transplant-associated thrombotic microangiopathy (TA-TMA)[19] was diagnosed.

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation or the median (interquartile range). The SPSS software package (IBM, Armonk, NY, USA), version 24.0, was employed for all statistical analyses. The chi-square test or Fisher's exact test was performed to compare categorical variables and the Mann-Whitney *U* test or *t*-test was used to compare quantitative variables. The survival rate was estimated using the Kaplan-Meier method and was assessed by the log-rank test. The multivariate Cox proportion regression model was used to determine the independent prognostic factors influencing overall survival (OS) and event-free survival (EFS). The OS was defined as the duration from HSCT to any death. Patients remaining

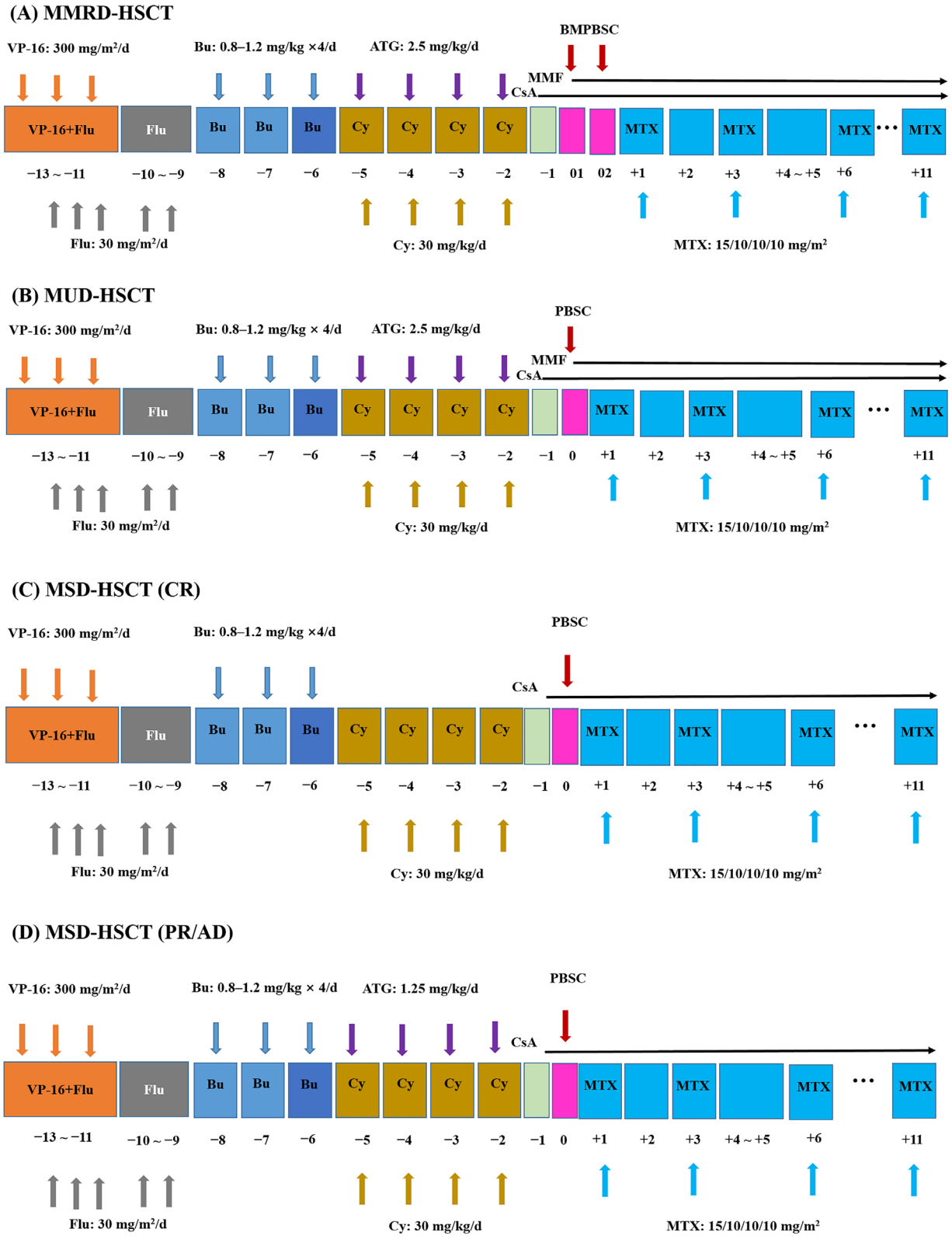

FIGURE 1 Modified myeloablative conditioning and treatment for complications for HSCT. (A) MMRD-HSCT, (B) MUD-HSCT, (C) MSD-HSCT for CR patients, (D) MSD-HSCT for PR/AD patients. HSCT, hematopoietic stem cell transplantation; MMRD, mismatched unrelated donor; MUD, matched unrelated donor; MSD, matched sibling donor; CR, complete remission; PR, partial remission; AD, activity disease; Flu, fludarabine; Bu, busulfan; Cy, cyclophosphamide; VP-16, etoposide; ATG, anti-human thymocyte immunoglobulin; BM, bone marrow; PBSC, peripheral blood stem cell; CsA, cyclosporine A; MMF, mycophenolate mofetil; MTX, methotrexate.

alive at the last follow-up were censored. EFS was defined as the duration from HSCT to any event: recurrence of primary disease, transplant-related mortality, and second HSCT (due to engraftment failure or donor chimerism loss).

RESULTS

Patient information

A total of 41 patients were enrolled, including 24 males (58.5%) and 17 females (41.5%). Twenty-eight patients (68.3%) were diagnosed with EBV-related T lymphocyte proliferative diseases, while 13 (31.7%) were diagnosed with EBV-related T/NK lymphoproliferative diseases. The pathological grade was grade I in 20 patients, grade II in five patients, grade III in 14 patients, and unknown in two patients. The median ages at onset, diagnosis, and transplantation were 73.0 (44.5, 94.5) months, 82.0 (54.0, 109.0) months, and 92.6 (60.4, 120.7) months, respectively. The median time from onset to transplantation was 8.5 (5.0, 16.3) months. Twenty-eight patients (68.3%) had concomitant HLH at the onset. The median number of courses of the pre-transplant chemotherapy regimen was three. Before transplantation, 19 patients (46.3%) achieved clinical CR, 12 (29.3%) achieved clinical PR, and 10 (24.4%) were still in the active state and underwent emergency transplantation. Five patients (12.2%) received MSD transplantation, four (9.8%) MUD transplantation, and 32 (78.0%) MMRD-HSCT. The MMRD was a sibling in four cases and a parent in 28 cases. The median HLA match in the MMRD cases was 5/10 (5/10, 6/10) (Table 1).

Engraftment and chimerism

The median infused mononuclear cell count was 8.1 (6.5, 10.6) $\times 10^8$/kg, and the median infused $CD34^+$ cell count was 9.8 (7.9, 10.3) $\times 10^6$/kg. All 41 patients were engrafted successfully, including seven patients with mixed chimerism who eventually converted to the full donor type after treatment. In one patient, the donor chimerism rate was 99.1% on day 20, and transplant rejection occurred on day 30. This patient underwent successful secondary allo-HSCT, and the subsequent donor chimerism rate remained more than 95% until the end of the follow-up. The lowest donor chimerism rate of the other six patients was 61.1%–95.0% and was converted to full donor type after reducing the dose of immunosuppressive agents and/or reinfusion with mesenchymal stem cells.

EBV-DNA loads

The median EBV-DNA loads at onset was 1.05 (0.50, 1.88) $\times 10^7$ copies/ml in PBMCs and 9.86 (2.35, 116.00) $\times 10^3$ copies/ml in plasma. After chemotherapy, the EBV-DNA load decreased but was still present in PBMCs, while there was no EBV-DNA detected in plasma in 23 patients (56.1%) before allo-HSCT. The median EBV-DNA loads

TABLE 1 Demographic and clinical characteristics of the patients with chronic active Epstein–Barr virus infection

Variables	Patients ($n = 41$)
Sex (male/female)	24/17
Age at onset (months)	73.0 (44.5, 94.5)
Age at diagnosis (months)	82.0 (54.0, 109.0)
Age at transplantation (months)	92.6 (60.4, 120.7)
Time from onset to HSCT (months)	8.5 (5.0, 16.3)
Follow-up time (months)†	28.2 (15.3, 40.2)
EBV-infected cells	
T	28
T+NK	13
Pathological grade	
I	20
II	5
III	14
Unknown	2
Patients with HLH	28
Pre-transplant chemotherapy courses	3 (2, 3)
Disease status	
CR	19
PR	12
AD	10
Donor type	
MSD	5
MUD	4
MMRD	32
HLA match	5/10 (5/10, 8/10)
Infused cells	
MNC ($\times 10^8$/kg)	8.1 (6.5, 10.6)
$CD34^+$ cell ($\times 10^6$/kg)	9.8 (7.9, 10.3)
Time of neutrophil engrafted (days)	12.0 (11.0, 14.0)
Time of platelet engrafted (days)	15.0 (12.0, 22.5)
Donor chimerism	
Full	34
Mixed-full	7
Outcome	
Dead	4
Alive	37

Data were shown as *n* or median (Q1, Q3). †The follow-up time is the time from the transplantation to the last visit or death. Abbreviations: AD, activity disease; CR, complete remission; EBV, Epstein-Barr virus; HLA, human leukocyte antigen; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; MNC, mononuclear cell; MSD, matched sibling donor; MUD, matched unrelated donor; MMRD, mismatched related donor; PR, partial remission.

TABLE 2 Transplant-related complications

Complications	Patients (n = 41)
aGVHD	
I	7 (17.1)
II	6 (14.6)
III	3 (7.3)
IV	7 (17.1)
cGVHD	
Mild	11 (26.8)
Moderate	3 (7.3)
Severe	0 (0.0)
CMV infection	17 (17.1)
CMV pneumonia	1 (2.4)
Septicemia	6 (14.6)
TA-TMA	9 (22.0)
VOD	4 (9.8)
CLS	7 (17.1)

Data were shown as n (%). Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CLS, capillary leakage syndrome; CMV, cytomegalovirus; TA-TMA, transplant-associated thrombotic microangiopathy; VOD, veno-occlusive disease.

before allo-HSCT was 2.51 (0.25, 7.63) $\times 10^6$ copies/ml in PBMCs and < 500 copies/ml in plasma. After allo-HSCT, there was no EBV-DNA detected in PBMCs in 26 patients (63.4%), and the median EBV-DNA loads in PBMCs at final follow-up was < 500 copies/ml. After allo-HSCT, there was no EBV-DNA detected in the plasma of all patients (Table S1). Repeat EBV-DNA plasma testing was positive in three patients who were confirmed to have a recurrence of the primary disease. No patients had a post-transplant lymphoproliferative disorder.

Complications

In our study, 13 patients developed grade I–II aGVHD (skin stage 1–3 and/or gastrointestinal stage 1) and 10 patients developed grade III–IV aGVHD (skin stage 2–4 and/or gastrointestinal stage 2–4, with or without liver stage 2–3). Fourteen patients (34.1%) developed cGVHD. The cGVHD was classified as mild in 11 patients presenting with local manifestations of the skin, oral region, and eyes, with an NIH score of 1–2. Three patients had moderate lung cGVHD, with an NIH score of 1. One patient died of grade IV aGVHD after he abandoned treatment for financial reasons. The GVHD of the other patients was controlled by anti-GVHD and supportive therapy (Table 2).

Nine patients (22.0%) were diagnosed with TA-TMA. Eight patients were improved after treatment, while one patient was left with the sequela of moderate renal insufficiency. Four patients (9.8%) had combined VOD, all of whom survived after treatment. Seven patients (17.1%) developed early manifestations of capillary leakage syndrome during the treatment with ATG, which improved after discontinuation of ATG and replacement therapy with ALG, CD25 monoclonal antibody, or post-cyclophosphamide (Table 2).

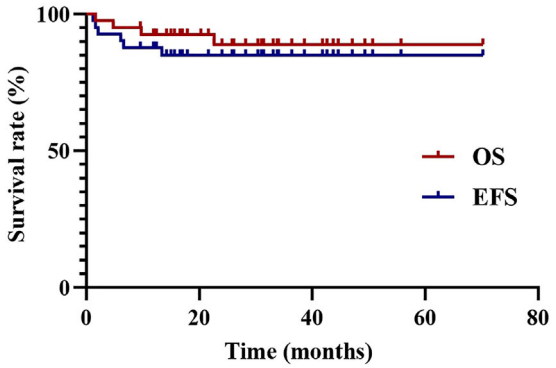

FIGURE 2 The expected 3-year OS of pediatric patients with CAEBV after allo-HSCT was 88.8% ± 5.4%, and the expected 3-year EFS was 85.0% ± 5.7%. Allo-HSCT, allogeneic hematopoietic stem cell transplantation; OS, overall survival; EFS, event-free survival; CAEBV, chronic active Epstein–Barr virus.

Six patients (14.6%) developed septicemia during the empty bone marrow stage and recovered with anti-infection treatment. Cytomegalovirus (CMV) infection occurred in 17 patients (41.5%), one of whom developed CMV pneumonia. After antiviral treatment and reduction of immunosuppressants, the whole-blood CMV-DNA test result became negative, and the CMV pneumonia was cured.

Follow-up and survival

As of March 15, 2022, the median follow-up time was 28.2 (15.3, 40.2) months, and the longest follow-up time was 70.2 months. Relapse occurred in five patients (12.2%), of which three patients died, one patient survived after chemotherapy with the L-DEP regimen and donor EBV-specific cytotoxic T lymphocyte infusion, and one patient survived after a second allo-HSCT. Four patients (9.8%) died, among which three patients died of primary disease relapse, and one died of grade IV aGVHD after stopping treatment. Totally, 37 patients (90.2%) survived and 35 (85.4%) survived without events, giving a 3-year OS of 88.8% ± 5.4% and 3-year EFS of 85.0% ± 5.7% (Figure 2).

Based on the presence of concomitant HLH at the onset of treatment for CAEBV, patients were divided into the HLH group (n = 28) and the non-HLH group (n = 13). There were no significant differences between the HLH and non-HLH groups in 3-year OS (87.7% ± 6.8% *vs.* 91.7% ± 8.0%, P = 0.790), 3-year EFS (85.0% ± 6.9% *vs.* 84.6% ± 10.0%, P = 0.921), and recurrence rates (11.8% ± 6.4% *vs.* 15.4% ± 10.0%, P = 0.692) (Figure 3). Based on the pre-transplant status, patients were divided into CR, PR,

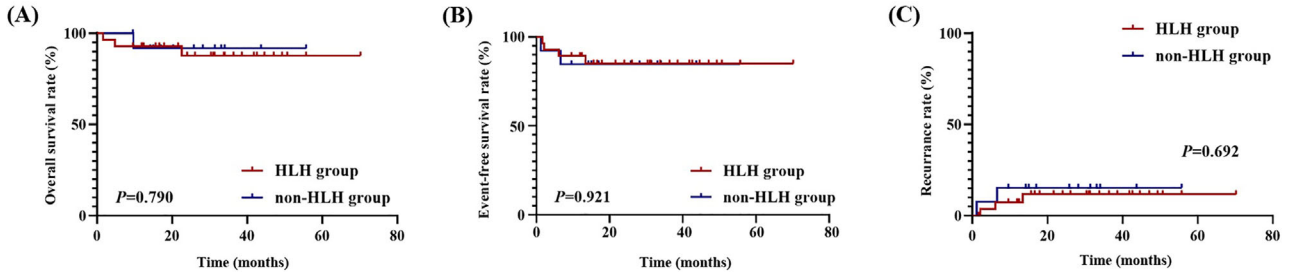

FIGURE 3 There were no significant differences between the hemophagocytic lymphohistiocytosis (HLH) and non-HLH groups in 3-year OS (87.7% ± 6.8% *vs.* 91.7% ± 8.0%, $P = 0.790$), 3-year EFS (85.0% ± 6.9% *vs.* 84.6% ± 10.0%, $P = 0.921$), and recurrence rates (11.8% ± 6.4% *vs.* 15.4% ± 10.0%, $P = 0.692$). OS, overall survival; EFS, event-free survival.

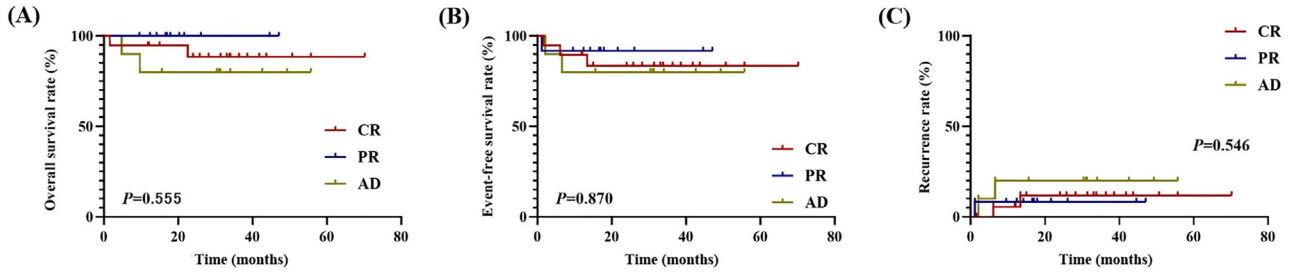

FIGURE 4 There were no significant differences between the CR, PR, and AD groups in 3-year OS (88.8% ± 7.8% *vs.* 100% ± 0.0% *vs.* 80.0% ± 12.6%, $P = 0.555$), 3-year EFS (83.5% ± 8.7% *vs.*91.7% ± 8.0% *vs.* 80.0% ± 12.6%, $P = 0.870$), and recurrence rates (11.9% ± 7.9% *vs.* 3% ± 8.0% *vs.* 20.0% ± 12.6%, $P = 0.546$). CR, complete remission; PR, partial remission; AD, activity disease; OS, overall survival; EFS, event-free survival.

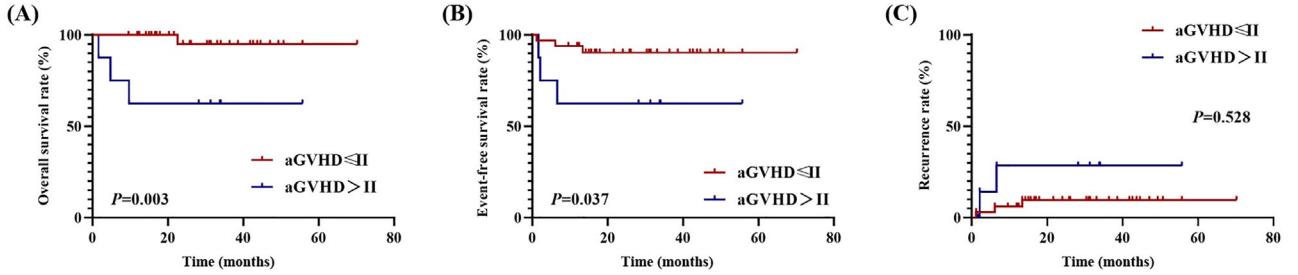

FIGURE 5 Patients with grade III–IV aGVHD had lower 3-year OS and EFS rates than those without aGVHD or with grade I–II aGVHD (62.5% ± 17.1% *vs.* 95.0% ± 4.9%, $P = 0.003$; 62.5% ± 17.1% *vs.* 90.3% ± 5.3%, $P = 0.037$). The recurrence rate did not significantly between the groups (28.6% ± 7.1% *vs.* 9.73% ± 5.3%, $P = 0.528$). aGVHD, acute graft-versus-host disease; OS, overall survival; EFS, event-free survival.

and AD groups; there were no significant differences among the three groups in 3-year OS (88.8% ± 7.8% *vs.* 100% ± 0.0% *vs.* 80.0% ± 12.6%, $P = 0.555$), 3-year EFS (83.5% ± 8.7% *vs.* 91.7% ± 8.0% *vs.* 80.0% ± 12.6%, $P = 0.870$), and recurrence rates (11.9% ± 7.9% *vs.* 8.3% ± 8.0% *vs.* 20.0% ± 12.6%, $P = 0.546$) (Figure 4). Patients with grade III–IV aGVHD had lower 3-year OS and EFS rates than those without aGVHD or with grade I–II aGVHD (62.5% ± 17.1% *vs.* 95.0% ± 4.9%, $P = 0.003$; 62.5% ± 17.1% *vs.* 90.3% ± 5.3%, $P = 0.037$) (Figure 5). The recurrence rate did not significantly between the group with grade III–IV aGVHD and the group without aGVHD or with grade I–II aGVHD. Donor type and TA-TMA/VOD combination had no effect on 3-year OS, 3-year EFS, or recurrence rates.

Analysis of prognostic factors

The sex, age at onset, age at diagnosis, age at allo-HSCT, time from onset to diagnosis, time from diagnosis to treatment, time from treatment to allo-HSCT, type of infected cells, presence of HLH at the onset, state before allo-HSCT (CR/PR/AD), EBV-DNA loads before allo-HSCT in PBMCs and in plasma, pathological grade, the cycle of chemotherapy, use of ATG in the conditioning regimen, type of HSCT (MSD/MUD/MMRD), amount of mononuclear cell and $CD34^+$ cell infusion, time of granulocyte recovery, time of platelet recovery, whether the patient has chimerism, aGVHD, cGVHD, CMV infection, septicemia, TA-TMA, or VOD were analyzed by univariate analysis for effects on OS and EFS. The covariates with $P < 0.1$

TABLE 3 Univariate and multivariate analysis of factors affecting overall survival (OS) and event-free survival (EFS) in patients with chronic active Epstein–Barr virus infection

	OS				EFS			
Factors	**Univariate HR (95%CI)**	***P***	**Multivariate HR (95%CI)**	***P***	**Univariate HR (95%CI)**	***P***	**Multivariate HR (95%CI)**	***P***
Gender								
Male	0.66 (0.09, 4.72)	0.678	–	–	0.75 (0.15, 3.71)	0.722	–	–
Female	Reference				Reference			
Age at onset (months)	1.03 (1.00, 1.06)	0.036	1.01 (0.96, 1.06)	0.850	1.03 (1.01, 1.05)	0.016	1.01 (0.97, 1.05)	0.569
Age at diagnosis (months)	1.03 (0.99, 1.05)	0.079	1.22 (0.64, 2.28)	0.568	1.03 (1.00, 1.05)	0.022	1.33 (0.57, 3.09)	0.512
Age at HSCT (months)	1.02 (0.99, 1.05)	0.093	0.85 (0.45, 1.60)	0.608	1.03 (1.00, 1.05)	0.027	0.77 (0.34, 1.75)	0.547
Complication at onset								
With HLH	0.75 (0.08, 7.21)	0.802	–	–	0.94 (0.17, 5.12)	0.941	–	–
Without HLH	Reference				Reference			
State before HSCT (CR/PR/AD)	–	0.798	–	–	–	0.826	–	–
Conditioning regimen								
With ATG	0.25 (0.03, 2.41)	0.232	–	–	0.14 (0.03, 0.77)	0.024	0.16 (0.02, 1.27)	0.083
Without ATG	Reference				Reference		Reference	
Grade of aGVHD								
>II	13.47 (1.40, 129.77)	0.024	11.65 (1.00, 136.06)	0.050	5.19 (1.04, 25.92)	0.045	5.39 (0.75, 38.53)	0.095
≤II	Reference		Reference		Reference		Reference	

Abbreviations: AD, activity disease; aGVHD, acute graft-versus-host disease; ATG, anti-human thymocyte immunoglobulin; CI, confidence interval; CR, complete remission; EFS, event-free survival; HLH, hemophagocytic lymphohistiocytosis; HR, hazards ratio; HSCT, hematopoietic stem cell transplantation; OS, overall survival; PR, partial remission; –, not applicable.

in univariate analysis were entered into the multivariate model.

Univariate analysis of factors affecting OS showed that the variables related to death were the aGVHD grade (>II or ≤II) ($P = 0.024$) and age at onset ($P = 0.036$). Multivariate Cox regression analysis of factors with $P \leq 0.1$ in univariate analysis (age at onset, age at diagnosis, age at transplant, and aGVHD grade) found that aGVHD grade >II showed a tendency to be an independent risk factor for death (Hazards ratio: 11.65, 95% confidence interval: 1.00, 136.06; $P = 0.050$) (Table 3).

Univariate analysis of factors affecting EFS showed that the age at onset ($P = 0.016$), age at diagnosis ($P = 0.022$), age at transplantation ($P = 0.027$), conditioning regimen (with or without ATG) ($P = 0.024$), and aGVHD grade (>II or ≤II) ($P = 0.045$) were associated with EFS. Multivariate Cox regression analysis did not identify significant risk factors (all *P*-values were > 0.05) (Table 3).

DISCUSSION

Imashuku et al.[20] first successfully used sibling bone marrow transplantation with the MAC regimen to treat EBV-associated T lymphocyte mononuclear disease complicated with HLH in 1996. Since then, several reports have demonstrated that allo-HSCT is the only effective treatment method for CAEBV. Taketani et al.[21] successfully used allogeneic peripheral blood hematopoietic stem cell transplantation (allo-PBSCT) to treat CAEBV in children and completely eradicated EBV after 3 months, suggesting that allo-PBSCT may be also an effective therapeutic strategy. A prospective multicenter study showed a 15-year survival rate of 60.6% for EBV-associated T/NK lymphoproliferative disease treated with allo-HSCT compared with 25.7% in a group that did not receive allo-HSCT, suggesting that allo-HSCT is an independent factor in reducing mortality.[22] The effect of allo-HSCT is partly due to the substitution and reconstruction of hematopoiesis and the immune system, rather than just antiviral effects.[4–7]

The key to the success of allo-HSCT is the choice of the conditioning regimen. At present, there is no unified conditioning regimen for the treatment of CAEBV. The reduced-intensity conditioning (RIC) regimen and MAC regimen for allo-HSCT have been reported to successfully treat CAEBV. Early studies showed that the effect of MAC-HSCT in progressive CAEBV was better than that

of RIC-HSCT.[23] Kawa et al.[24] evaluated the therapeutic effect of allo-HSCT in 29 patients with CAEBV and found that the 3-year OS was higher in the RIC-HSCT group than the MAC-HSCT group (95.0% ± 4.9% *vs*. 54.5% ± 15.0%, $P = 0.016$), but there was no significant difference in the 3-year EFS (85.0% ± 8.0% *vs*. 54.5% ± 15.0%, $P = 0.239$); however, the median time from onset to treatment was shorter in the RIC-HSCT group (14.5 months) than the MAC-HSCT group (3 years), which may have conferred a survival advantage to the RIC-HSCT group.[25] Gotoh et al.[26] reported that the MAC-HSCT group had a higher incidence of transplant-related mortality (3/5) than the RIC-HSCT group (1/10), but the RIC-HSCT group had a higher recurrence rate than the MAC-HSCT group (30% *vs.* 11%). We used a modified MAC regimen. Considering the long-term adverse effects of total body irradiation, our conditioning regimen did not include total body irradiation. To reduce the adverse effects of Bu and Cy, the duration of Bu administration was reduced from 4 to 3 days, and the dose of Cy was reduced from 200 $mg \cdot kg^{-1}$ to 120 $mg \cdot kg^{-1}$. A total Flu dose of 150 $mg \cdot m^{-2}$ was added to the regimen to compensate for the decreased immunosuppression. VP-16, which selectively removes activated T/NK lymphocytes and macrophages and inhibits cytokine production, was added to the regimen at a total dose of 900 $mg \cdot m^{-2}$. A total dose of 10 $mg \cdot kg^{-1}$ of ATG was retained in MMRD-HSCT and MUD-HSCT to remove T lymphocytes *in vivo* to overcome the HLA mismatch, decrease the rate of GVHD, and enhance engraftment. In addition, ATG inhibits and eliminates proliferating T cells, playing a role in the treatment of primary diseases. Patients with MSD transplantation who failed to achieve CR were administered a total dose of 5 $mg \cdot kg^{-1}$ of ATG to control the primary disease. In our study, the 3-year OS and EFS rates were 88.8% ± 5.4% and 85.0% ± 5.7%, respectively. This suggests that modified MAC-HSCT had a high OS and low recurrence rate, and was safe and effective for treating CAEBV in children.

Previous studies have found that the presence of concomitant HLH with CAEBV at the beginning of treatment may affect the outcome.[27] However, the 3-year OS and EFS rates did not significantly differ between the HLH and non-HLH groups in our study. Sawada et al.[5] reported that after receiving allo-HSCT, the 3-year OS was 87.3% ± 4.2% for patients with CAEBV with a stable state before allo-HSCT compared with 16.7% ± 10.8% for patients with an active state before allo-HSCT. Arai et al.[27] also found that the disease state before allo-HSCT affects the OS. However, we didn't find a significant difference in the 3-year OS and EFS in the patients with different disease states before allo-HSCT. This might be related to the use of stratified pre-transplant chemotherapy in accordance with the patient disease state, and the use of the modified MAC regimen, which may have better cleared activated T cells and better treated the primary disease.

As the main type of allo-HSCT in our study was MMRD-HSCT, the incidence of aGVHD was high (56.1%), although most patients had only grade I–II aGVHD. The incidence of cGVHD was 34.1% and was mostly mild, with no severe cGVHD. Except for one death, the GVHD was controlled by anti-GVHD therapy and supportive treatment. We found that grade III–IV aGVHD might be a risk factor for death, suggesting that reducing the incidence of grade III–IV aGVHD after transplantation may improve the outcome. However, large-scale, multicenter studies are required to further confirm these results.

In conclusion, allo-HSCT with the modified MAC regimen is safe and effective for treating CAEBV in children, with a high survival rate. Patients with concomitant HLH and patients with the active disease before allo-HSCT also benefit from modified MAC-HSCT. Post-transplantation grade III–IV aGVHD may be an independent prognostic factor for mortality.

CONFLICT OF INTEREST

Dr. Tianyou Wang is a member of the *Pediatric Investigation* editorial board.

REFERENCES

1. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from burkitt's lymphoma. *Lancet.* 1964;1:702-703. DOI: 10.1016/s0140-6736(64)91524-7
2. Nowalk A, Green M. Epstein-barr virus. *Microbiol Spectr.* 2016;4. DOI: 10.1128/microbiolspec.DMIH2-0011-2015
3. Arai A, Yamaguchi T, Komatsu H, Imadome K, Kurata M, Nagata K, et al. Infectious mononucleosis accompanied by clonal proliferation of EBV-infected cells and infection of CD8-positive cells. *Int J Hematol.* 2014;99:671-675. DOI: 10.1007/s12185-014-1548-4
4. Bollard CM, Cohen JI. How I treat T-cell chronic active Epstein-Barr virus disease. *Blood.* 2018;131:2899-2905. DOI: 10.1182/blood-2018-03-785931
5. Sawada A, Inoue M, Kawa K. How we treat chronic active Epstein-Barr virus infection. *Int J Hematol.* 2017;105:406-418. DOI: 10.1007/s12185-017-2192-6
6. Arai A. Chronic active epstein-barr virus infection: the elucidation of the pathophysiology and the development of therapeutic methods. *Microorganisms.* 2021;9:180. DOI: 10.3390/microorganisms9010180
7. Sawada A, Inoue M, Koyama-Sato M, Kondo O, Yamada K, Shimizu M, et al. 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. *Biol Blood Marrow Transplant.* 2014;20:214-221. DOI: 10.1016/j.bbmt.2013.10.026

8. Okano M, Kawa K, Kimura H, Yachie A, Wakiguchi H, Maeda A, et al. Proposed guidelines for diagnosing chronic active Epstein-Barr virus infection. *Am J Hematol.* 2005;80:64-69. DOI: 10.1002/ajh.20398
9. Ohshima K, Kimura H, Yoshino T, Kim CW, Ko YH, Lee SS, et al. Proposed categorization of pathological states of EBV-associated T/natural killer-cell lymphoproliferative disorder (LPD) in children and young adults: overlap with chronic active EBV infection and infantile fulminant EBV T-LPD. *Pathol Int.* 2008;58:209-217. DOI: 10.1111/j.1440-1827.2008.02213.x
10. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2007;48:124-131. DOI: 10.1002/pbc.21039
11. Henter JI, Aricò M, Egeler RM, Elinder G, Favara BE, Filipovich AH, et al. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. HLH study Group of the Histiocyte Society. *Med Pediatr Oncol.* 1997;28:342-347. DOI: 10.1002/(sici)1096-911x(199705)28:5⟨342::aid-mpo3⟩3.0.co;2-h
12. Ma H, Zhang L, Wei A, Yang J, Wang D, Zhang Q, et al. Outcome of L-DEP regimen for treatment of pediatric chronic active Epstein-Barr virus infection. *Orphanet J Rare Dis.* 2021;16:269. DOI: 10.1186/s13023-021-01909-y
13. Luo YH, Yang J, Wei A, Zhu GH, Wang B, Zhang R, et al. Haploidentical hematopoietic stem cell transplantation for pediatric patients with chronic active Epstein-Barr virus infection: a retrospective analysis of a single center. *World J Pediatr.* 2021;17:626-636. DOI: 10.1007/s12519-021-00470-9
14. Zhang XH, Chen J, Han MZ, Huang H, Jiang EL, Jiang M, et al. The consensus from The Chinese Society of Hematology on indications, conditioning regimens and donor selection for allogeneic hematopoietic stem cell transplantation: 2021 update. *J Hematol Oncol.* 2021;14:145. DOI: 10.1186/s13045-021-01159-2
15. Hamidieh AA, Pourpak Z, Hosseinzadeh M, Fazlollahi MR, Alimoghaddam K, Movahedi M, et al. Reduced-intensity conditioning hematopoietic SCT for pediatric patients with LAD-1: clinical efficacy and importance of chimerism. *Bone Marrow Transplant.* 2012;47:646-650. DOI: 10.1038/bmt.2011.140
16. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai Acute GVHD International Consortium. *Biol Blood Marrow Transplant.* 2016;22:4-10. DOI: 10.1016/j.bbmt.2015.09.001
17. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National institutes of health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: i. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant.* 2015;21:389-401.e1. DOI: 10.1016/j.bbmt.2014.12.001
18. Bonifazi F, Barbato F, Ravaioli F, Sessa M, Defrancesco I, Arpinati M, et al. Diagnosis and treatment of VOD/SOS after allogeneic hematopoietic stem cell transplantation. *Front Immunol.* 2020;11:489. DOI: 10.3389/fimmu.2020.00489
19. Cho BS, Yahng SA, Lee SE, Eom KS, Kim YJ, Kim HJ, et al. Validation of recently proposed consensus criteria for thrombotic microangiopathy after allogeneic hematopoietic stem-cell transplantation. *Transplantation.* 2010;90:918-926. DOI: 10.1097/TP.0b013e3181f24e8d
20. Imashuku S, Naya M, Yamori M, Nakabayashi Y, Hojo M, Kihara A, et al. Bone marrow transplantation for Epstein-Barr virus-related clonal T cell proliferation associated with hemophagocytosis. *Bone Marrow Transplant.* 1997;19:1059-1060. DOI: 10.1038/sj.bmt.1700776
21. Taketani T, Kikuchi A, Inatomi J, Hanada R, Kawaguchi H, Ida K, et al. Chronic active Epstein-Barr virus infection (CAEBV) successfully treated with allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant.* 2002;29:531-533. DOI: 10.1038/sj.bmt.1703392
22. Kimura H, Ito Y, Kawabe S, Gotoh K, Takahashi Y, Kojima S, et al. EBV-associated T/NK-cell lymphoproliferative diseases in nonimmunocompromised hosts: prospective analysis of 108 cases. *Blood.* 2012;119:673-686. DOI: 10.1182/blood-2011-10-381921
23. Kakinoki Y, Matsuoka S, Hashiguchi J, Chiba K, Miyake T. Successful treatment of immediate allogeneic myeloablative hematopoietic stem cell transplantation from a HLA-mismatched sibling donor for active systemic Epstein-Barr virus-positive T-cell lymphoproliferative disease of childhood following primary acute epstein-barr virus infection. *Clin Case Rep.* 2015;3:231-236. DOI: 10.1002/ccr3.204
24. Kawa K, Sawada A, Sato M, Okamura T, Sakata N, Kondo O, et al. Excellent outcome of allogeneic hematopoietic SCT with reduced-intensity conditioning for the treatment of chronic active EBV infection. *Bone Marrow Transplant.* 2011;46:77-83. DOI: 10.1038/bmt.2010.122
25. Sawada A, Inoue M. Hematopoietic stem cell transplantation for the treatment of Epstein-Barr virus-associated T- or NK-Cell lymphoproliferative diseases and associated disorders. *Front Pediatr.* 2018;6:334. DOI: 10.3389/fped.2018.00334
26. Gotoh K, Ito Y, Shibata-Watanabe Y, Kawada J, Takahashi Y, Yagasaki H, et al. Clinical and virological characteristics of 15 patients with chronic active Epstein-Barr virus infection treated with hematopoietic stem cell transplantation. *Clin Infect Dis.* 2008;46:1525-1534. DOI: 10.1086/587671
27. Arai A, Sakashita C, Hirose C, Imadome KI, Yamamoto M, Jinta M, et al. Hematopoietic stem cell transplantation for adults with EBV-positive T- or NK-cell lymphoproliferative disorders: efficacy and predictive markers. *Bone Marrow Transplant.* 2016;51:879-882. DOI: 10.1038/bmt.2016.3

SUPPORTING INFORMATION

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