



# Biology of Blood and Marrow Transplantation

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## Unexpected High Incidence of Human Herpesvirus-6 Encephalitis after Naive T Cell–Depleted Graft of Haploidentical Stem Cell Transplantation in Pediatric Patients

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### A B S T R A C T

The CD45RA T cell depletion (TCD) method has been used to deplete naive T cells, preventing graft-versus-host disease (GVHD) but preserving memory cells, providing immediate functional T cells with anti-infection, antileukemia, and antirejection effects. We describe a series of 25 consecutive high-risk patients with leukemia who received haploidentical hematopoietic stem cell transplantation (haplo-HSCT) with CD45RA TCD. Each patient received 2 cell products: 1 created by CD34 positive selection and the other through CD45RA depletion from the CD34 negative fraction by a CliniMACS device. CD45RA-depleted haplo-HSCT was well tolerated, with rapid engraftment and low risk of severe acute GVHD and chronic GVHD. Although this treatment achieved a good control of viral reactivations, such as cytomegalovirus and adenovirus, we observed an unexpectedly high rate of limbic encephalitis due to human herpesvirus-6 (HHV-6; 8 cases). Characteristically, the infection appeared early in almost all patients, just after the engraftment. Although no patient died from encephalitis, 1 patient showed neuropsychological sequelae, and another experienced secondary graft failure just after the HHV-6 reactivation.

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### INTRODUCTION

Graft-versus host-disease (GVHD) remains a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT) [1]. The incidence of GVHD varies between 30% and 70% in terms of donor–recipient HLA compatibility [2–5]. Donor T cells in the transplanted graft contribute to the development of GVHD [1,3]; thus, T cell–depleted grafts constitute a strategy to overcome GVHD in HSCT [6].

Mobilized peripheral blood from a haploidentical relative constitutes an alternative source when a matched HLA donor

is not available [7,8]. Haploidentical transplantation constitutes the most common scenario for developing severe GVHD because of the strong T cell alloreactivity; therefore, performing T cell depletion (TCD) is mandatory. However, in vivo (serotherapy) and ex vivo full T cell–depleted grafts (CD34 selection or CD3CD19 depletion) have been associated with a high rate of graft failure and life-threatening opportunistic infections related to an important delay in immune reconstitution [9–22]. Attempts to improve immune function recovery and reduce nonrelapse mortality from infectious complications without increasing GVHD have focused on a partial T cell–depleted graft, such as  $\alpha,\beta$  TCD [23,24]. This graft retains a large number of effector cells, such as TcR $\gamma\delta$  T cells and natural killer (NK) cells. However, delayed immune reconstitution and subsequent infections such as cytomegalovirus (CMV) and/or

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adenovirus diseases are not unusual and still constitute major causes of death [7,11]. A novel partial TCD strategy such as depleted naive T cells (CD45RA<sup>+</sup> T cells) could enhance the recovery of immune function after haploidentical HSCT because donor pathogen memory T cells from the donor are retained [25–28].

In this study CD45RA depletion was performed to deplete the naive T cells responsible for GVHD [29] while preserving memory T cells, which provide immediate functional T cells with anti-infection, antileukemia, and antirejection effects. The study by Teschner et al. [30] had shown that CD45RA depletion of leukapheresis products using immunomagnetic beads is feasible and results in >3-log reduction of CD45RA<sup>+</sup> T cells.

Previous experiences with a naive T cell-depleted graft, both in a match-related setting and a haploidentical setting, reported universal corticoids responders to GVHD with a very low incidence of virus reactivation [31–33]. Although reactivation of human herpesvirus-6 (HHV-6) is common after HSCT, it has not yet been reported in this type of haploidentical transplantation [34–37].

Herein, we describe our experience with CD45RA<sup>+</sup> cell depleted haploidentical grafts in pediatric patients with high-risk acute leukemia and the outcomes. We found a high rate of engraftment with low rate of GVHD but a high incidence of early HHV-6 encephalitis.

## METHODS

### Patient Selection

Patients were enrolled in the study from January 2015 to June 2017. Four centers participated in the study: La Paz University Hospital in Madrid and Sant Pau Hospital, Sant Joan de Déu Hospital and Vall d'Hebron Hospital in Barcelona. We enrolled pediatric patients diagnosed with high-risk hematologic malignancies requiring an allogeneic transplantation and lacking an available suitable HLA-matched donor. The only exclusion criterion was a poor clinical condition, defined as a Lansky score < 60%. The study protocol was approved by the local ethics committees, and informed consent was obtained from the patients or their legal guardians. The patient and transplantation characteristics are shown in Table 1. The first 25 consecutive treated patients are presented in this study.

### Conditioning and Immunosuppression Regimen

Recipients received a preparative regimen that consisted of 8 Gy total lymphoid irradiation over 4 equal fractions, 150 mg/m<sup>2</sup> fludarabine over 5 days, thiopeta 10 mg/kg for 1 day, and melphalan 140 mg/m<sup>2</sup> over 2 days. Eight patients received total body irradiation 4 Gy on 2 fractions instead of total lymphoid irradiation. One patient received a different conditioning regimen based on thiopeta, fludarabine, and busulfan. On day 0 patients received their first hematopoietic progenitor cell graft, which was CD34<sup>+</sup> enriched. On the same day or the next day they received a second hematopoietic progenitor cell graft that was CD45RA depleted. On day +7 granulocyte colony-stimulating factor was begun. The GVHD pharmacologic prophylaxis regimen consisted of cyclosporine A (n = 3) at a dose of 3 mg/kg/day, administered i.v. from day –1 or mycophenolate mofetil (n = 21) at a dose of 45 mg/kg/day starting on day +1; 1 patient also received methotrexate 10 mg/m<sup>2</sup>/day (on days +1, +3, and +6) apart from cyclosporine A. The immunosuppression was tapered as soon as possible, typically before day 30 if GVHD was absent (Table 1).

### Donor Selection

Donor evaluation included eligibility for mobilization, cross-match testing, ABO donor–recipient compatibility, CMV serologic status, a killer cell immunoglobulin-like receptor genotype, and the presence of killer cell immunoglobulin-like receptor and HLA mismatches.

### Graft Preparation

Donor peripheral blood stem cells were mobilized by administering granulocyte colony-stimulating factor (10 µg/kg per day for 4 days) and were harvested on day +5 by 1 to 2 leukapheresis procedures. The first hematopoietic progenitor cell product was T cell–depleted using the CliniMACS device and CD34 Microbead (Miltenyi Biotec, Bergisch Gladbach, Germany). The minimum cell dose required for the CD34<sup>+</sup>-enriched progenitor cell graft was 4 × 10<sup>6</sup> CD34<sup>+</sup> cells/kg. The maximum CD3<sup>+</sup> dose allowed for the CD34<sup>+</sup>-enriched hematopoietic progenitor cell graft was 1 × 10<sup>5</sup> CD3<sup>+</sup> cells/kg. The CD34<sup>+</sup> fraction was processed for CD45RA<sup>+</sup> cell depletion using the CliniMACS

**Table 1**

Patients and Transplant Characteristics (N = 25)

Variable	Value
Median age, yr (range)	12 (2–17)
Gender	
Female	15
Male	10
Disease	
B-ALL	10
T-ALL	6
AML	7
Biphenotypic	2
Disease status	
CR1	12
CR2	9
Advanced stage	4
Haploidentical donor	
Mother	16
Father	8
Brother	1
CMV status (donor–recipient)	
Positive–positive	15
Positive–negative	7
Negative–positive	0
Negative–negative	3
ABO compatibility	
Compatible	18
Incompatible	7
Killer cell immunoglobulin-like receptor mismatch	
Yes	5
No	14
Data not available	6
GVHD prophylaxis	
Cyclosporine	3
Cyclosporine + methotrexate	1
Mycophenolate mofetil	21
Infusion data, median (range)	
First product (CD34 <sup>+</sup> selection)	
CD34/kg × 10 <sup>6</sup>	6.29 (4.04–18.1)
CD3/kg × 10 <sup>3</sup>	5.4 (1–490)
Second product (CD45RA depleted)	
CD45RA <sup>+</sup> /kg × 10 <sup>3</sup>	5.3 (0–14.6)
CD45RO/kg × 10 <sup>7</sup>	10.6 (3.8–102)
log10 depletion of CD45RA±	4.75 (2.2–6.37)

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; AL, acute leukemia; CR, complete remission.

device and its Depletion 3.1 software (Miltenyi Biotec). The maximum CD3<sup>+</sup>CD45RA<sup>+</sup> dose allowed was 1 × 10<sup>5</sup> CD3<sup>+</sup>CD45RA<sup>+</sup> cells/kg and a ≥2.5 log<sub>10</sub> depletion of CD45RA<sup>+</sup> cells. The second fraction was infused after the CD34<sup>+</sup> fraction, on the same day or the day after. Both cell products were infused fresh.

### Supportive Care and Early Post-Transplantation Evaluation

Outcomes were censored on September 30, 2017. PCR screening for CMV, adenovirus, and Epstein-Barr virus was performed weekly until day +100. A galactomannan test was performed twice weekly until day +100. All patients received prophylactic acyclovir, micafungin, and trimethoprim-sulfamethoxazole. The preemptive treatment of CMV reactivation consisted of ganciclovir 5 mg/kg per dose i.v. twice a day or foscarnet 90 mg/kg per dose i.v. twice a day. PCR for HHV-6 was not routinely tested. Empiric treatment of invasive fungal infection consisted of liposomal amphotericin B (3 to 5 mg/kg i.v. once a day). Complete blood counts were performed daily until neutrophil engraftment. Chimerism studies were performed on peripheral blood since engraftment (variable number tandem repeat analysis). Flow cytometry quantification of lymphocyte subsets was performed on days +30, +60, +90, +150, and +210.

### Definitions

The diagnosis of acute and chronic GVHD was made clinically and confirmed by biopsy whenever possible. Grading was made according to the standard criteria (Seattle and National Institutes of Health criteria). The diagnosis of pre-engraftment and engraftment syndrome was made following the criteria by Spitzer [38]. Pre-engraftment syndrome was defined as noninfectious fever (≥38°C) and/ or fluid retention occurring before the day of

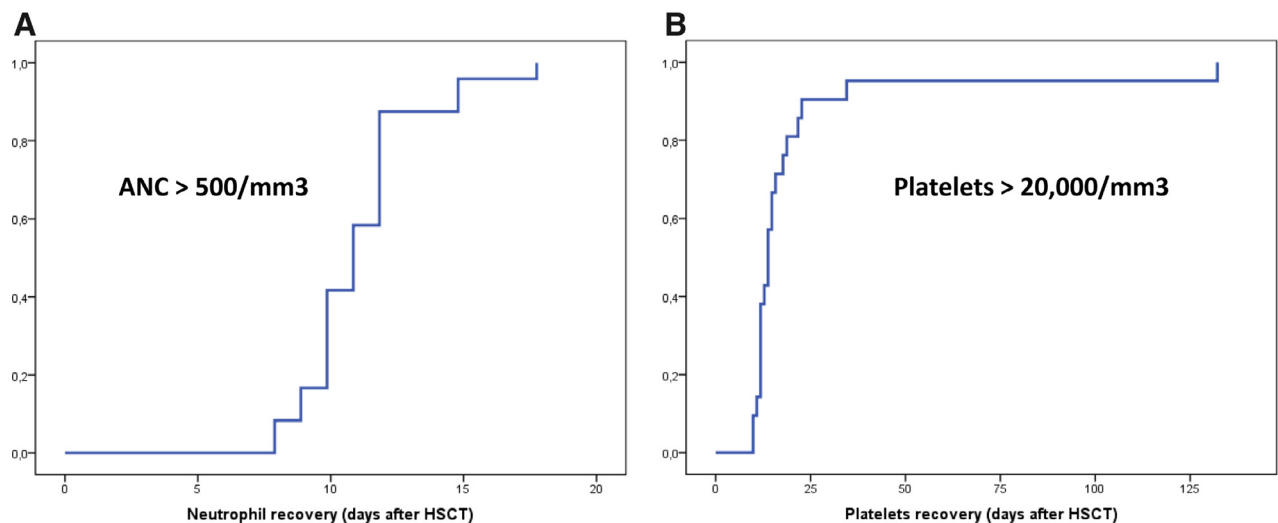


Figure 1. (A) Absolute neutrophil count recovery. (B) Platelet recovery.

neutrophil engraftment. Noninfectious fever was defined as a febrile episode associated with a negative blood culture. Relapse was defined as morphologic or clinical evidence of recurrence in the peripheral blood, bone marrow, or extramedullary sites. Overall survival (OS) was defined as survival from the time of transplantation. Disease-free survival was defined as survival from the time of transplantation without evidence of disease relapse. Transplant-related mortality (TRM) was defined as any cause of death related to transplantation other than disease.

#### Endpoints

The first endpoint of the study was to examine the feasibility for engraftment and for GVHD prophylaxis. The secondary endpoints were disease-free survival, cumulative relapse incidence, and TRM.

#### Statistical Analysis

OS and disease-free survival rates were calculated from the time of transplantation by means of the Kaplan-Meier product limit method. Cumulative incidence was used to estimate the GVHD, relapse, and nonrelapse mortality rates. Statistical analyses were performed with SPSS software (version 17.0; SPSS Inc., Chicago, IL). Competing-risks regression analysis has been performed using the Fine and Gray (1999) approach (stcrreg) by Stata software (release 11; StataCorp., College Station, TX).

## RESULTS

### Patients

Twenty-five consecutive patients with high-risk leukemia had received haploidentical transplantation and were at least 100 days post-transplantation at the time data were frozen for analyses (September 30, 2017). The median age at transplant was 12 years (range, 2 to 17). Ten patients were diagnosed with B cell acute lymphoblastic leukemia, 6 patients suffered from T cell acute lymphoblastic leukemia, 7 were diagnosed with acute myeloid leukemia, and 2 were diagnosed with biphenotypic leukemia. At the time of HSCT, 12 patients were in first complete remission (3 patients in morphologic remission but immunophenotypic or molecularly detectable disease), 9 were in second complete remission, and 4 had advanced disease or morphologically detectable disease. All patients received 2 haploidentical donor cell products: The first was a source of hematopoietic progenitor cells and the second was administered to adoptively transfer a large number of donor memory T cells.

### Graft Composition

The CD34<sup>+</sup>-enriched product provided a median CD34<sup>+</sup> and CD3<sup>+</sup> dose of  $6.29 \times 10^6$  cells/kg (range, 4.04 to 18.1) and  $5.4 \times 10^3$  cells/kg (range, 1 to 490), respectively.

The CD34<sup>+</sup> fraction was processed for CD45RA<sup>+</sup> cell depletion. We achieved the target cell doses and log depletion in all patients but 1 that here we describe separately. The CD45RO<sup>+</sup> cell dose was a median of  $6.5 \times 10^7$  cells/kg (range, 2.9 to 10.1), and the CD45RA<sup>+</sup> cell dose was a median of  $.6 \times 10^4$  cells/kg (range, .2 to 1). The median log<sub>10</sub> depletion of CD45RA<sup>+</sup> cells was 4.75 (range, 3.5 to 6.3).

The patient, for whom we did not achieve the target cell depletion, received a median CD45RA<sup>+</sup> cells of  $4.98 \times 10^6$  and CD45RO<sup>+</sup> cells of  $51 \times 10^7$ , with a log<sub>10</sub> depletion of 2.21.

We performed lymphocyte subset count in 15 patients. The CD4<sup>+</sup>CD45RO<sup>+</sup> cell dose was a median of  $5.58 \times 10^6$ /kg (range, 2.04 to 9.12), the CD8<sup>+</sup>CD45RO<sup>+</sup> cell dose a median of  $.69 \times 10^6$ /kg (range, .3 to 1.1), the NK cell dose a median of  $.08 \times 10^6$ /kg (range, .01 to .16), and the CD19<sup>+</sup> cell dose a median of  $.02 \times 10^6$ /kg (range, .006-.037).

### Engraftment and Immune Recovery

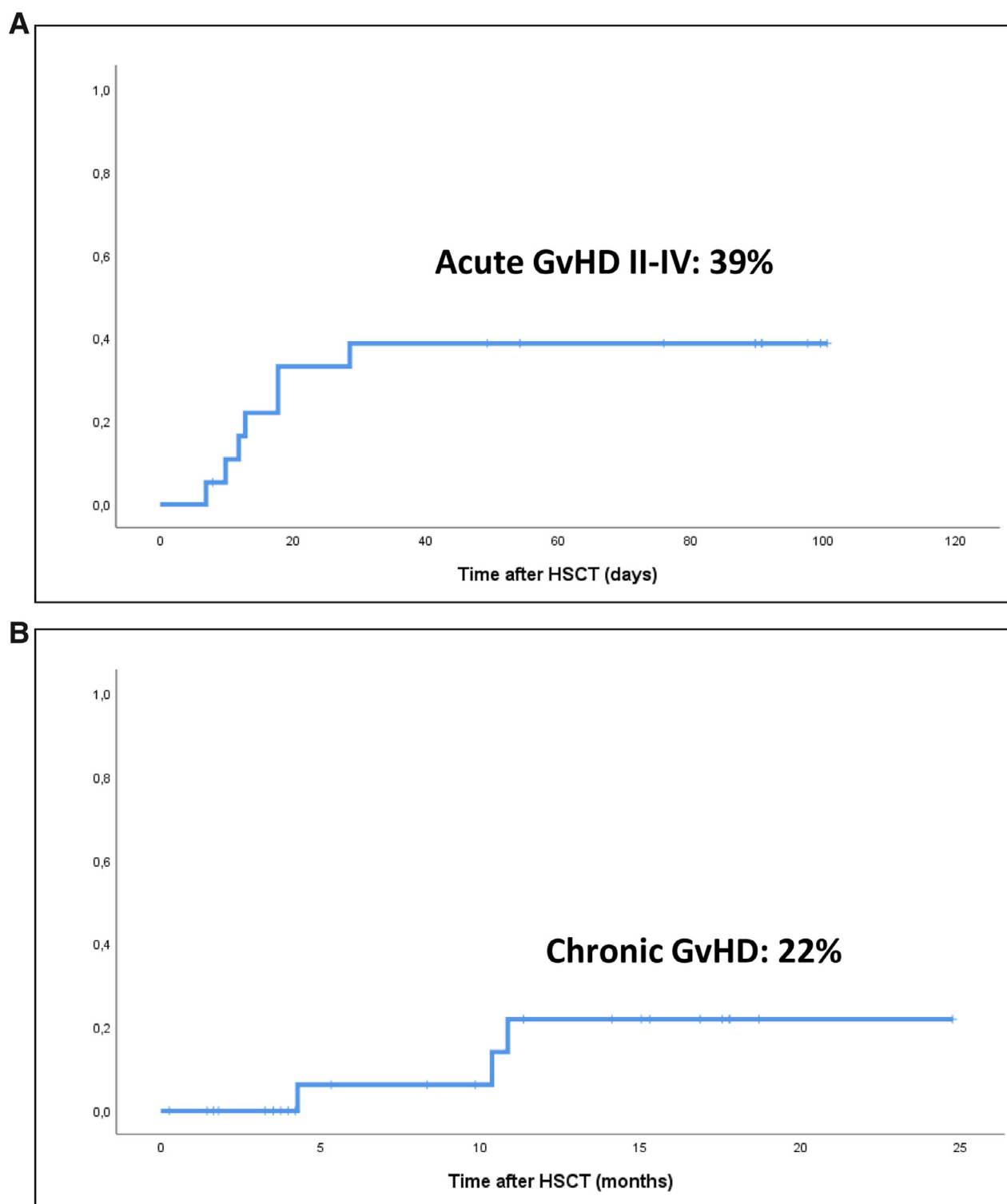
All patients achieved neutrophil engraftment at a median of 10 days (range, 8 to 18) post-transplant (Figure 1A). The median time to platelet engraftment > 20,000 was at day 14 (range, 10 to 151) post-transplant (Figure 1B). Two patients experienced a secondary graft rejection: in 1 patient due to de novo development of anti-HLA antibodies and in the second patient rejection followed viral reactivation (HHV-6). Both patients underwent a second HSCT.

We observed a rapid expansion of lymphocytes, reaching a median absolute lymphocyte count (ALC) at day +15 of 1200 cells/ $\mu$ L. Nevertheless, after this early and rapid increase we saw a slower recovery, with a median ALC at day +90 of 2200 cells/ $\mu$ L.

### Graft-versus-Host Disease

Thirteen patients (52%) developed acute GVHD: grade I, 5; grade II, 2; and grades III to IV, 6. The cumulative incidences of acute GVHD grades II to IV and III to IV were 39% and 33%, respectively. Only 1 patient required a second-line treatment extracorporeal photopheresis (ECP) (Figure 2A).

Three patients (12%) showed chronic GVHD (moderate in 2 patients and mild in the third). The cumulative incidence of chronic GVHD at 30 months of follow-up was 22% (Figure 2B). No patient died due to acute or chronic GVHD.



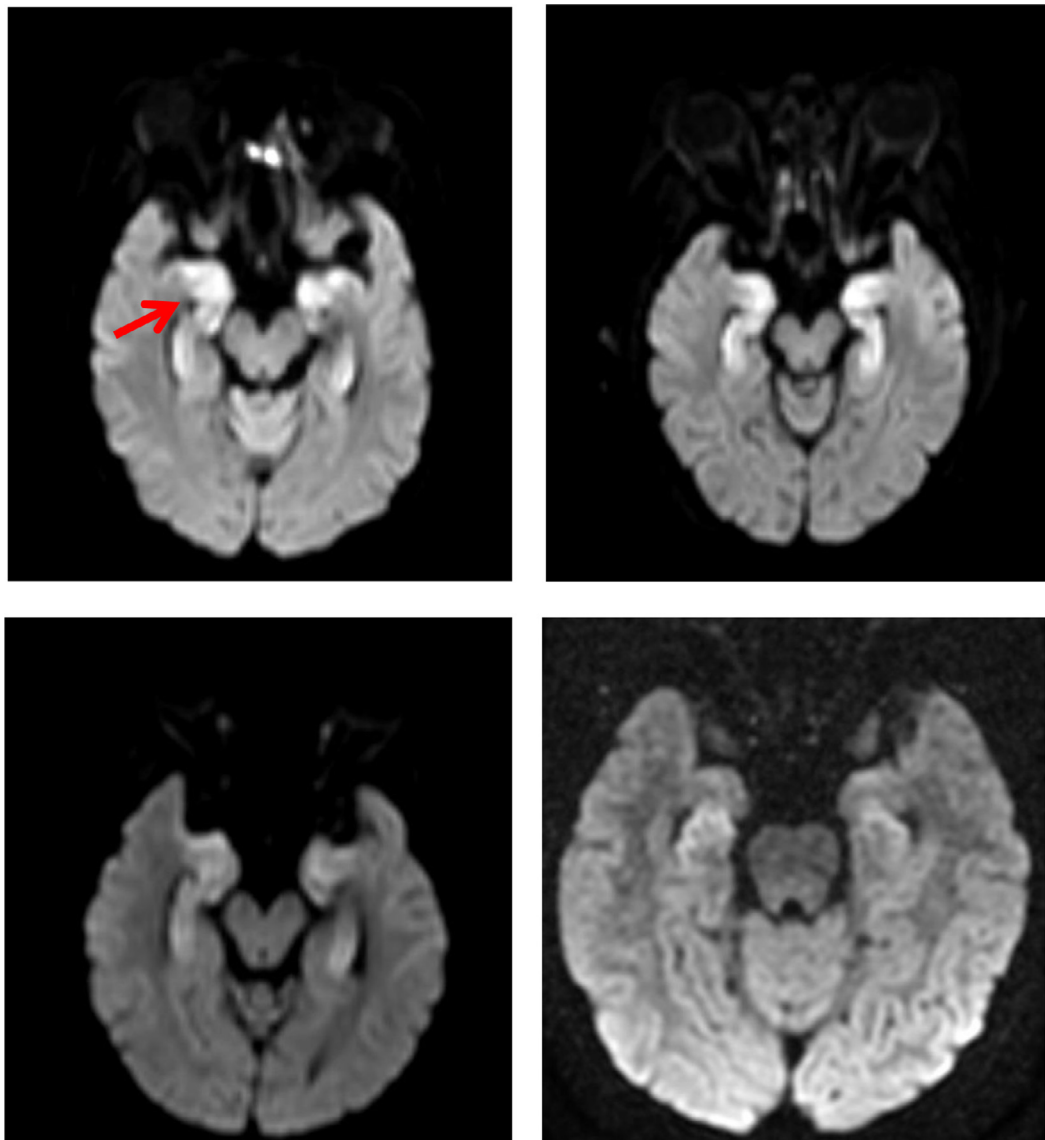
**Figure 2.** Cumulative incidence of acute GVHD grades II-IV (A) and chronic GVHD (B).

### Infections

To date, 8 patients received antiviral treatment (ganciclovir or foscarnet) for CMV reactivation. None has progressed to CMV disease. No donor lymphocyte infusion was required to clear viremia. One patient received antiviral treatment (cidofovir) for adenoviral viremia; the same patient developed hemorrhagic cystitis for BK polyomavirus. No patient developed

post-transplant lymphoproliferative disease or received treatment for Epstein-Barr virus reactivation.

We observed an unexpectedly high rate of encephalitis due to HHV-6 (8 cases) (Table 2). Figure 3 shows magnetic resonance imaging (MRI) in 4 patients with encephalitis. The cumulative incidence of HHV-6 encephalitis was 34% (Figure 4A). Recalculating by adding death as a competitive



**Figure 3.** MRI images of 4 patients affected by HHV-6 limbic encephalitis.

risk, a cumulative incidence of 31% was reached. Median onset was 35 days (range, 13 to 56) after transplantation. The symptoms included seizures, confusion, encephalopathy, short-term memory loss, hypothermia, and hyponatremia. The encephalitis was proven by demonstration of HHV-6 viral DNA in cerebrospinal fluid samples in all but 1 patient. The patients also showed HHV-6 positivity in plasma (no viral load is available). HHV-6 type B viral load was only detected by the RealStar HHV-6 PCR kit (Altona Diagnostic, Hamburg, Germany) and a CFX 96 real-time PCR detection system (BioRad, Hercules, CA). Typical images of limbic encephalitis by MRI were found in all the patients (Figure 3). At the time of HHV-6 infection, all patients were receiving acyclovir as prophylaxis. The treatment was based on foscarnet (90 mg/kg per dose twice a day) in 3 patients, ganciclovir (5 mg/kg per dose twice a day) in 1 patient, and a combination of foscarnet and ganciclovir in 4 patients.

Seven patients received intravenous immune globulin (.4 g/kg) weekly during the infection. Two patients died, although not directly for the encephalitis but in the context of bacterial sepsis. A third patient died because of leukemia relapse. One

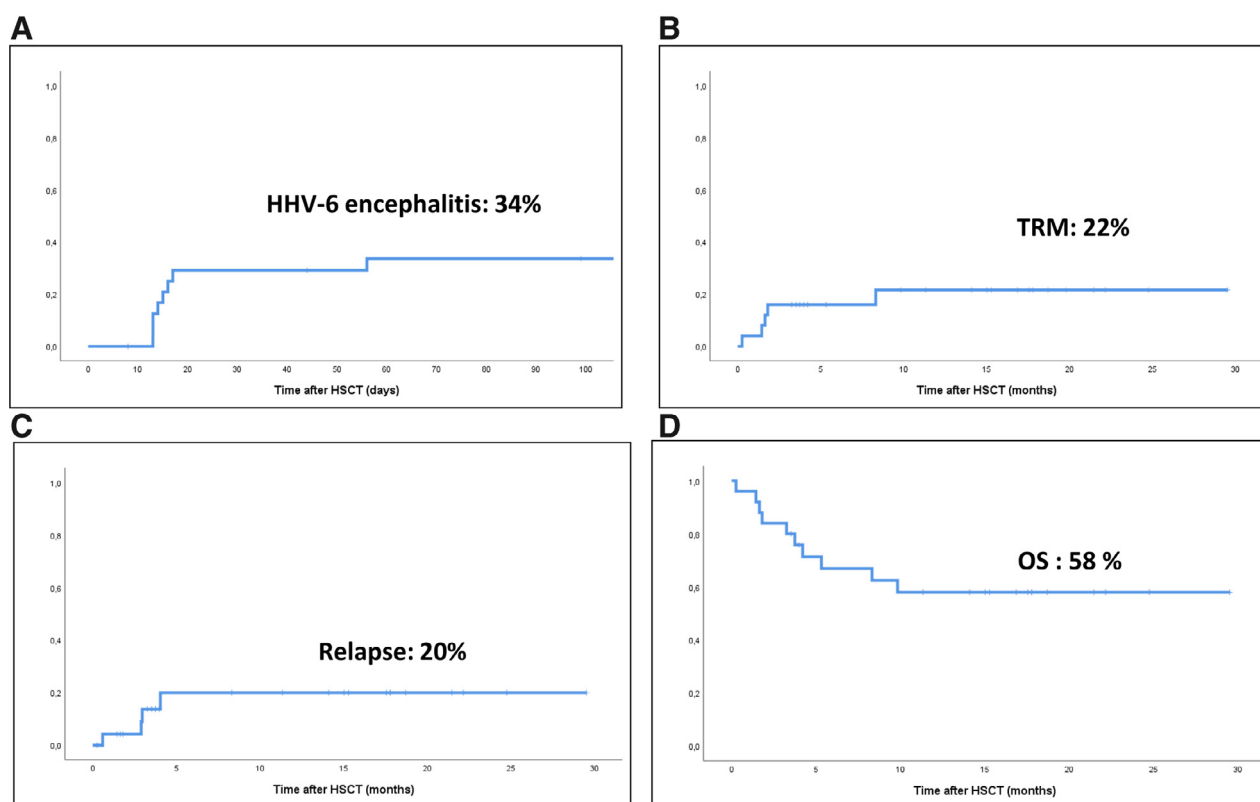
patient showed neuropsychological sequelae and hyponatremia that persisted 1.5 years post-infection. One other patient showed HHV-6 reactivation in blood and neurologic symptoms (3 consecutive episodes of seizures); however, it was not possible to demonstrate the encephalitis by MRI. This patient experienced secondary graft failure just after the HHV-6 reactivation.

Other significant viral infections included 1 case of parvovirus infection and 1 case of influenza A. All patients but 1 experienced an infection before day 30; 1 patient showed CMV reactivation later, around day +200, and this same patient thereafter developed chronic GVHD. Four patients died from bacterial sepsis.

#### **Transplant-Related Mortality**

The cumulative incidence of TRM was 22% at 30 months after transplantation (Figure 4B). The TRM before day +100 was 16% (4 patients). The cause of death was a bacterial sepsis in all cases. One more patient died after day +100 because of cardiogenic shock.





**Figure 4.** Cumulative incidence of HHV-6 encephalitis (A), TRM (B), relapse (C), and OS (D).

### Relapse

Three patients (12%) relapsed and subsequently died with progressive disease. Of these, 2 had active disease at the time of the transplant.

The cumulative incidence of relapse was 20% at 30 months (Figure 4C). Recalculating by adding death as a competitive risk, a cumulative incidence of 16.6% was reached.

### Survival

Fifteen patients (60%) were alive at a median of 365 days (range, 8 to 898) post-transplant. Two patients who experienced graft failure received a second allotransplant, and both died: 1 from systemic toxoplasmosis and 1 from disease progression.

The OS was 58% at 30 months of follow-up (Figure 4D).

### DISCUSSION

In this study we report our results using CD45RA depletion in a haploidentical setting for high-risk acute leukemia in pediatric patients who lack an available suitable donor. The method was feasible, and the procedure was well tolerated by the patients. We observed no toxicity related to the procedure, reflecting the experience previously described by other groups [31–33]. We observed a high rate of engraftment with a rapid recovery of neutrophils and platelets, with acceptable rate of severe acute GVHD and chronic GVHD.

The rate of relapse was acceptable, mostly if we consider the high risk of leukemia types in our series and the fact that 2 of 3 patients who experienced relapse had active disease before the transplant. TRM was low, and the OS was comparable with the rates described in conventional transplantation from unrelated donors [39].

The CD45RA TCD method could have the advantage of preserving the memory T cells useful for controlling viral infections. We have observed that the rapid immune recovery allowed for good control of viral reactivations such as CMV and adenovirus, with the rate of adenovirus extremely low. Unexpectedly, we observed a high rate of HHV-6 encephalitis (8/25 patients experienced the infection). HHV-6 appeared early, just after engraftment in 7 patients (median time of 14 days post-transplant); only in 1 patient did HHV-6 appear later (day +56). Characteristically, the infection followed a similar pattern in almost all cases: Clinical signs compatible with a strong “pre-engraftment syndrome” (fluid retention, erythematous rash, fever) preceded the start of neurologic symptoms. None of the patients who did not develop HHV-6 showed pre-engraftment syndrome or, at most, only mild signs (such as fever) appeared. This correlation between the “pre-engraftment immune reaction” and the onset of encephalitis was also reported by Mori et al [34], in the cord blood setting. A high level of serum IL-6 has been correlated with the subsequent development of HHV-6 encephalitis [35]. Recently, Miyashita et al. [36] demonstrated that mycophenolate mofetil for GVHD prophylaxis is also a significant risk factor for the disease.

In our series the median ALC was  $665 \times 10^9$  cells/L when the encephalitis began; however, we cannot find any association to explain why the infection as the median ALC at day +14 (the median time for HHV-6 encephalitis) was similarly as high ( $830 \times 10^9$  cells/L) as the patients who did not develop the infection.

We did not find any factors associated with a statistically significant increased risk of HHV-6 encephalitis among the variable that were studied (sex, pre-engraftment syndrome, CMV donor–receptor compatibility, ABO donor–receptor compatibility, leukemia phenotype, ethnicity, CD34 and CD45RO count).

**Table 2**  
Characteristics of Patients with HHV-6 Encephalitis

Patient No.	Age (yr)/ Sex	Ethnicity	Diagnosis/ Status at HSCT	Pre-engraftment Syndrome(onset day)	Immunosuppression at the Time of Infection	Acute GVHD (grade)	Day of Onset HHV-6 Encephalitis	Latency PES-HHV-6 Encephalitis	ALC at the Time of Infection ( $\times 10^9/L$ )	Status at Follow-Up	Cause of Death
1	16/F	Hispanic	T-ALL/CR1	Yes (+7)	MMF + prednisone 2 mg/kg/day	No	+15	8 days	830	Alive	—
2	8/F	Hispanic	B-ALL/CR2	Yes (+8)	MMF + prednisone 2 mg/kg/day	No	+13	5 days	90	Dead	Sepsis
3	16/F	Hispanic	AML/refractory	Yes (+7)	MMF + prednisone 2 mg/kg/day	No	+17	10 days	720	Dead	Sepsis
4	10/F	Hispanic	T-ALL/CR1	No	MMF	Yes (II)	+13		400	Alive	—
5	13/M	Hispanic	AML/CR1	No	Cyclosporine	No	+15		230	Alive	—
6	4/F	Hispanic	B-ALL/CR1	Yes (+12)	MMF	No	+14	2 days	500	Alive	—
7	10/M	Chinese	T-ALL/CR2	Yes (+8)	MMF	Yes (III-IV)	+13	5 days	490	Dead	Relapse
8	8/M	White	T-ALL/CR1	Yes (+8)	MMF + prednisone 2 mg/kg/day + ECP	Yes (II)	+56	48 days	300	Alive	—

PES indicates pre-engraftment syndrome; MMF, mycophenolate mofetil; ECP, extracorporeal photopheresis.

We hypothesize that the HHV-6 could have been transferred by donor lymphocytes. The inflammation induced by the engraftment could have given the conditions for viral reactivation considering also the high amount of CD4<sup>+</sup> cells (reservoir of the virus) in the graft that is much higher than in other transplant procedures. We also believe that the lack of NK cells in the grafts could have played a role in the development of the infection. We did not perform an extra infusion of NK cells at day +6 as reported by the St. Jude's group [32]. Nevertheless, the same group reported an increased incidence of graft rejection in the context of HHV-6 reactivation. A pre-emptive treatment based on ganciclovir or foscarnet was started in any patient showing erythematous rash and fever before the engraftment as a strategy to prevent HHV-6 reactivation [40]. We have considered the possibility of chromosomally integrated HHV-6, but it is a rare condition reported in less than 1% of control subjects in the United States and the United Kingdom [41] so it would hardly explain the high rates we observed.

Interestingly, 7 patients who showed HHV-6 infection originated from outside Spain (6 patients from South America and 1 patient from China). Genetic polymorphism has been reported to possibly be responsible for individual viral susceptibility [42]. Leite et al. [43] reported that the presence of proline at codon 72 of the *TP53* gene increased the risk for HHV-6 infection 5-fold in the Brazilian population. The fact that p53 plays a critical role in cell cycle control, promoting apoptosis in HHV-6-infected cells and the functional assay, reveals that the p.P72R variant induces apoptosis markedly better than does the variant p.P72 [44,45]. A better understanding of the role played by this polymorphism in viral infections in the transplant setting would be useful for screening patients for a higher risk of post-transplant infections, thus identifying individuals who could benefit from preventive treatment.

Despite HHV-6 reactivation being common, reported in 30% to 70% of patients at 2 to 4 weeks after HSCT, the incidence of HHV-6 encephalitis is low, varying from .95% to 11.6%, and is higher in the cord blood setting [34,36,46] in the context of delayed immune reconstitution. The 34% cumulative incidence we observed in our series has not been previously reported.

For haploidentical transplantations there are fewer reports. In adult patients a higher incidence of acute GVHD and graft failure that could be triggered by the virus has been reported [47]. Therefore, despite HHV-6 infection usually being self-limiting, patients who experienced symptomatic HHV-6 infections showed poorer long-term survival [48]. Immune reconstitution has been considered 1 of the most important factors for controlling HHV-6 reactivation and infection, with a better OS in patients with CD3<sup>+</sup>  $\geq 200$  cells/ $\mu L$  at the time of infection [49].

The interaction between HHV-6 and the immune system is not well known; however, 2 major issues have been already proposed. First, upon infection, NK cells act to control the dissemination of the HHV-6, lysing autologous HHV-6-infected peripheral blood mononuclear cells [50]. Second, CD4<sup>+</sup> T cells co-expressing CD134 (OX40) contain significantly greater numbers of HHV-6B copies than CD4<sup>+</sup> T cells without CD134 expression [51]. These 2 recent assessments would support our hypothesis.

In conclusion, haploidentical transplantation using the CD45RA TCD method is feasible and shows high rates of engraftment with acceptable rate of severe GVHD. Our series is too small for firm conclusions regarding the antileukemic effect of this method. We observed a very high incidence of HHV-6 encephalitis, and further studies are needed to reveal the associated mechanisms. Strict control of HHV-6 viremia

and preemptive treatment appear to be mandatory in the context of the new haploidentical strategies.

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