Biol Blood Marrow Transplant 25 (2019) 1363–1373

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant Cyclophosphamide for Primary Immunodeficiencies and Inherited Disorders in Children

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Article history:
Received 21 December 2018
Accepted 8 March 2019

Keywords:
Haploidentical hematopoietic stem cell transplantation
Post-transplant cyclophosphamide
Primary immunodeficiency
Immune reconstitution
Inherited disorder

A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for some inherited disorders, including selected primary immunodeficiencies (PIDs). In the absence of a well-matched donor, HSCT from a haploidentical family donor (HIFD) may be considered. In adult recipients high-dose post-transplant cyclophosphamide (PTCY) is increasingly used to mitigate the risks of graft failure and graft-versus-host disease (GVHD). However, data on the use of PTCY in children (and especially those with inherited disorders) are scarce. We reviewed the outcomes of 27 children transplanted with an HIFD and PTCY for a PID (n = 22) or osteopetrosis (n = 5) in a single center. The median age was 1.5 years (range, .2 to 17). HSCT with PTCY was a primary procedure (n = 21) or a rescue procedure after graft failure (n = 6). The conditioning regimen was myeloablative in most primary HSCTs and nonmyeloablative in rescue procedures. After a median follow-up of 25.6 months, 24 of 27 patients had engrafted. Twenty-one patients are alive and have been cured of the underlying disease. The 2-year overall survival rate was 77.7%. The cumulative incidences of acute GVHD grade ≥ II, chronic GVHD, and autoimmune disease were 45.8%, 24.2%, and 29.6%, respectively. There were 2 cases of grade III acute GVHD and no extensive cGVHD. The cumulative incidences of blood viral replication and life-threatening viral events were 58% and 15.6%, respectively. There was evidence of early T cell immune reconstitution. In the absence of an HLA-identical donor, HIFD HSCT with PTCY is a viable option for patients with life-threatening inherited disorders.

INTRODUCTION

Allogenic hematopoietic stem cell transplantation (HSCT) is a treatment option for children with various malignant or non-malignant diseases, including selected primary immunodeficiencies (PIDs) and inherited disorders. A graft from a healthy, HLA-genoidentical, matched sibling donor is the best option but is available in fewer than 25% of cases. Less than 70% of the remaining patients will have a suitable matched unrelated donor, a proportion that is even lower for patients belonging to ethnic groups poorly represented in donor registries [1]. Furthermore, the search for a matched unrelated donor may delay HSCT; this delay is not always compatible with the clinical status of the recipients. Another possibility is HSCT with an HLA-haploidentical family donor (HIFD), although this procedure is associated with an elevated risk of graft-versus-host disease (GVHD), graft failure, and thus elevated transplant-related morbidity and mortality rates [2]. Various graft manipulations have been developed to mitigate these risks [3].

Financial disclosure: See Acknowledgments on page 1372.
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https://doi.org/10.1016/j.bbmt.2019.03.009
1083-8791/

Historically, $CD34^+$ cell selection was the most frequently used method and was associated with a lower risk of GVHD but also delayed the patient's immune reconstitution and slowed viral clearance, which are problematic in the context of a PID [4]. Nonengraftment was also a significant risk with this procedure [2]. More recently, selective depletions have been developed as CD3/CD19 depletion and TCR$\alpha\beta$/CD19 depletion [5-7]. The latter method does not eliminate $\gamma\delta$T cells and natural killer (NK) cells from the graft and has been applied to the treatment of both malignant and nonmalignant diseases, especially in pediatric patients [8-10]. The engraftment rate is good, as long as myeloablative conditioning (MAC) is administrated. The incidence of GVHD reported in the literature varies from 15% to 47.8% [8-10]. Graft depletion of CD45RA naïve T cells (to prevent acute GVHD [aGVHD] and preserve memory T cells with potent anti-infective properties) has also been developed [11]. Adoptive immunotherapy with the injection of donor-derived antiviral T cells during the post-transplant period may also speed up the recovery of virus-specific immunity [12].

Over the last decade nonmanipulated haplo-HSCT strategies using post-transplant cyclophosphamide (PTCY) as GVHD prophylaxis have been pioneered by the Johns Hopkins group and have been increasingly used to treat adult patients with malignant diseases [13]. Cyclophosphamide is selectively toxic to proliferating lymphocytes. Administered within 72 hours of a nonmanipulated graft, it mostly depletes alloreactive T cells from donor and recipient, facilitating engraftment and preventing GVHD. In nonmalignant diseases this approach has been mainly used in patients with sickle cell disease, major thalassemia, and severe aplastic anemia [14,15]. However, data on PTCY in children with PIDs are scarce, with only a few case reports published [16-19]. Here, we report on clinical and immunologic outcomes in 27 pediatric patients after emergency HIFD HSCT with PTCY (indicated by a life-threatening PID or osteopetrosis [OP]) at a single center.

METHODS

Patients

From December 2014 to June 2017, 27 patients underwent HIFD HSCT with PTCY at the Immunohematology and Rheumatology Department at Necker Children's Hospital (Paris, France). Medical decision regarding HSCT with HIFD and strategy with PTCY was made and validated each time during multidisciplinary meetings. Written informed consent was obtained from patients and donors regarding HSCT, anonymous data collection, and analysis. This was in accordance with the Declaration of Helsinki and the French laws and regulations.

Twenty-one patients underwent primary HSCT (designated by "P" and a number), and 6 patients underwent a rescue procedure after the failure of a primary or secondary graft (designated by "R" and a number). The data, collected prospectively were censored as of February 1, 2019. Patient characteristics are summarized in Table 1. For patients undergoing primary HSCT, the median age at transplantation was .9 years (range, .2 to 12.2). The underlying diseases were OP (n = 4); hemophagocytic lymphohistiocytosis (HLH, n = 6); immunodysregulation, polyendocrinopathy and enteropathy, X-linked (IPEX, n = 3); combined immunodeficiency (CID, n = 7); and leukocyte adhesion deficiency (n = 1). Pre-HSCT infectious complications were frequent, and an active cytomegalovirus (CMV) infection (positive blood PCR assay and antiviral treatment in the 10 days before transplantation) was detected in 7 patients. Adenovirus (ADV) carriage of the digestive and/or upper respiratory tract was documented in 4 individuals. A norovirus carriage (positive PCR in feces) was detected in 1 patient and a BK virus infection (with a positive PCR assay for urine and blood samples) in another. Three patients had Epstein-Barr virus (EBV)-related lymphoproliferative disease but were in partial remission at the time of HSCT. One patient with IPEX had developed chronic renal impairment after a kidney transplant. The 6 patients with HLH were all in complete remission before HSCT due to a combination treatment with steroids, cyclosporine, and alemtuzumab.

Patients undergoing HIFD HSCT after 1 or more preliminary graft failure (s) had severe CID (SCID; n = 1), CID (n = 2), chronic granulomatous disease (n = 2), or OP (n = 1). The characteristics of the patients and their previous procedures are summarized in Table 1. The median age at the time of rescue HSCT was 1.8 years (range, .3 to 17.0), and the median time between the previous HSCT and the HIFD HSCT with PTCY was 45 days (range, 38 to 74). Five of 6 patients had active, life-threatening infections (CMV infection, ADV infection, bacterial sepsis, intracerebral abscesses, and pulmonary aspergillosis in 1 case each) at the time of transplantation.

Donors

Donor–recipient pairs were mismatched at $\geq$2 loci in all cases. The donor was the father in 19 cases and the mother in 8 cases. There was a sex mismatch between the donor and the recipient in 14 cases. All tested recipients (n = 11) were negative for donor-specific anti-HLA antibodies.

Conditioning Regimen and GVHD Prophylaxis

Twenty of 21 patients undergoing primary HSCT received a MAC regimen based on fludarabine (40 mg/m^2/day from days −6 to −2) and i.v. busulfan from days −8 to −5 guided by pharmacokinetic studies. Busulfan was administered in 4 daily doses while busulfan plasma concentrations were measured and pharmacokinetics calculated at dose 1, 9, and 13, allowing dose adjustment as previously reported [20]. Median area under the curve was 21,763 (range, 15,866 to 24,456 microM.min). Four patients received cyclophosphamide 14.5 mg/kg/day on days −3 and −2. One patient underwent nonmyeloablative (NMA) conditioning. All patients received up-front serotherapy, including rituximab 375 mg/m^2 on day −12 and alemtuzumab .4 to 1 mg/kg total dose (median, .5 mg/kg) (n = 20) or rabbit antithymocyte globulin 10 mg/kg total dose (n = 1) from days −11 to −10. Details of the conditioning regimens (all NMA) in patients with rescue HSCT are given in Table 1.

In all cases GVHD prophylaxis was based on PTCY (50 mg/kg/day) on days +3 and +4. Mycophenolate mofetil (45 mg/kg/day in 2 divided doses) and cyclosporine with a trough targeted level of 150 to 200 μg/L were started on day 5 and stopped in the absence of GVHD after 3 and 6 months, respectively.

Graft

The stem cells source was bone marrow in all cases. The graft composition is summarized in Supplementary Table 1. Median $CD34^+$ cells and CD3 T cells were, respectively, 13.8×10^6 /kg recipient body weight (range, 2.6 to 47.7) and 10.7×10^7 /kg (range, 2.6 to 30.2).

Supportive Care

All patients received intravenous immunoglobulin replacement therapy every 3 weeks until humoral immune reconstitution with normal B cell numbers in peripheral blood and normal IgM levels were reached. Viral screening consisted of quantitative EBV, CMV, and ADV PCR assays in blood and semi-quantitative PCR assays for respiratory (nasopharyngeal swabs) and enteric viruses (feces) weekly for the first 3 months post-HSCT, then every 2 other weeks until month 6, and then monthly until 12 months. Prophylaxis of veno-occlusive disease (VOD) with defibrotide was given to 17 high-risk patients from day −1 until day +28 or until resolution of VOD. VODe was defined according to the Baltimore criteria [21], and transplant microangiopathy was defined according to the Blood and Marrow Transplant Clinical Trials Network's criteria [22]. Patients received anti-infectious prophylaxis with trimethoprim-sulfamethoxazole started after engraftment, caspofungin, and acyclovir.

Endpoints

Neutrophil and platelets recovery were defined, respectively, as the first of 2 consecutive days with an absolute neutrophil count $\geq .5 \times 10^9$/L and as a platelet count $> 20 \times 10^9$/L without a platelet transfusion in the previous 7 days. Chimerism in peripheral blood was assessed at engraftment and at 3, 6 and 12 months using X/Y fluorescence in situ hybridization in cases of sex-mismatched HSCT or very short tandem repeat analysis. The percentage of donor-derived cells was >90% for full donor chimerism, between 10% and 90% for mixed chimerism, and <10% for graft failure. The grading of aGVHD and chronic GVHD (cGVHD) was based on established criteria [23,24]. Viral replication for CMV, ADV, and EBV was defined as a blood viral load $>1 \times 10^3$ copies/mL. Systemic viral replication with organ involvement or a persistent blood viral load despite treatment were considered to be life-threatening viral events.

Immune reconstitution over time was assessed using T cell, B cell, and NK cell immunophenotyping (at 1, 3, 4, 6, 9, 12, 18, and 24 months) once total lymphocyte counts had reached 300/μL. Naive and memory CD4 and CD8 populations were also assessed at 3 months post-HSCT and later. Whenever possible, anti-EBV, anti-CMV, and/or anti-ADV responses were documented. Detection of pathogen-specific $CD4^+$ and $CD8^+$ T cells was performed by intracellular IFN-γ staining after stimulation with CMV pp65 (Miltenyi Biotec, Bergisch Gladbach, Germany), pentameric staining (Pro-immune, Oxford, UK) for patients transplanted with HLA-A2 donors, or ELISpot to quantify IFN-γ–producing human CMV-specific T cell responses as previously described [25].

Table 1
Demographic, Patient, and Transplant Characteristics

First Haploidentical HSCT								
Patient	Sex	Diagnostic	Age (yr)	Immediate Pre-HSCT Morbidities	Donor	D/R CMV	CR	Serotherapy
P1	M	OP (*TCIRG1*)	8.5		Father	(-/+)	Bu/Flu150/ CY 29	Alemtuzumab (.5 mg)/rituximab
P2	F	OP (*TCIRG1*)	1.2	ADV (nose/stools)	Father	(+/+)	Bu/Flu150/ CY 29	Alemtuzumab (.6 mg)/rituximab
P3	M	OP (*TCIRG1*)	.8	ADV (nose/stools)	Father	(-/-)	Bu/Flu160 /CY 29	Alemtuzumab (.5 mg)/rituximab
P4	M	HLH (*PRF1*)	.4	CMV infection	Mother	(+/-)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P5	F	HLH (*PRF1*)	.3		Mother	(+/-)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P6	M	HLH (*PRF1*)	.2		Father	(-/-)	Bu/Flu160	Alemtuzumab (1 mg)/rituximab
P7	M	IPEX (*FOXP3*)	7.3	Renal transplant, CMV infection, BK virus (urine and blood), ADV (stools)	Father	(+/+)	RXT 2G/ Flu 150/CY 29	Alemtuzumab (.5 mg)/rituximab
P8	M	IPEX (*FOXP3*)	.4	Autoimmune enteropathy	Mother	(+/-)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P9	M	CID (*CARMIL 2*)	2.2	CMV infection	Father	(+/+)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P10	M	CID (*CORO1A*)	4.3	CMV infection, ADV stools carrier, EBV- LPD in PR	Mother	(+/+)	Bu/Flu160/CY 29	rATG (10 mg)
P11	M	CID (*CD70*)	10.9	EBV-LPD in PR	Father	(+/-)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P12	F	CID (*ITK*)	6.1	EBV-LPD in PR	Father	(+/-)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P13	F	LAD1 (*ITGB2*)	.3	CMV infection	Father	(+/+)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P14	M	CID (*WASP*)	3.0		Father	(-/-)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P15	M	HLH (*UNC13D*)	.4	CMV infection	Father	(+/+)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P16	F	HLH (*UNC13D*)	.5		Father	(-/-)	Bu/Flu160	Alemtuzumab (.4 mg)/rituximab
P17	M	CID (*IKBKG*)	.9	CMV infection, norovirus stools carriage, IBD	Mother	(+/+)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P18	M	HLH (*UNC13D*)	.3		Mother	(-/-)	Bu/Flu160	Alemtuzumab (.4 mg)/rituximab
P19	F	OP (CLCN7)	2.8	Norovirus stools carriage	Father	(+/+)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P20	M	IPEX (*FOXP3*)	.9	Autoimmune enteropathy	Father	(-/-)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab
P21	F	CID (*ITK*)	12.2		Father	(+/+)	Bu/Flu160	Alemtuzumab (.5 mg)/rituximab

Rescue Haploidentical HSCT												
Patient	Sex	Diagnosis	Age (yr)	Previous Graft(s), Donor, and CR	Previous Graft(s) Depletion	Outcome of First HSCT	Immediate Pre-HSCT Morbidities	Donor	D/R CMV	Delay (d)*	CR	Serotherapy
R1	M	WA (*WASP*)	2.1	Haploid (mother) Bu/Flu160/ TT10/rATG10	$CD45RA^+$ depletion	Primary graft failure	CMV infection	Mother	(+/+)	39	Flu 150/CY 29	rATG 7.5 mg/kg
R2	F	MHC II (*RFXANK*)	1.5	MUD Bu/Flu160/rATG10	0	Primary graft failure	ADV infection	Father	(+/-)	59	Flu 150 /CY 30	rATG 7.5 mg/kg
R3	M	OP (*TCIRG1*)	1.5	Haploid (father) Bu/Flu160/ TT10/rATG10	$CD34^+$ selection	Primary graft failure	Bacterial sepsis	Father	(+/-)	48	Flu150/CY 29	alemtuzumab (.4 mg/kg)/ rituximab
				Haploid (mother) CY100/ alemtuzumab .6 mg/kg	$CD34^+$ selection	Primary graft failure						
R4	M	CGD (*CYBB*)	7.7	UCB Bu/Flu180/alemtuzumab .6 mg/kg	0	Primary graft failure	Active pulmonary aspergillosis	Mother	(+/+)	41	Flu 160	rATG (3 mg/kg)/ rituximab

(*continued*)

Table 1 (*Continued*)

Rescue Haploidentical HSCT										
Patient	Sex	Diagnosis	Age (yr)	Previous Graft(s), Donor, and CR	Previous Graft(s) Depletion	Outcome of First HSCT	Immediate Pre-HSCT Morbidities	Donor	D/R CMV	Delay (d)*	CR	Serotherapy
R5	F	Reticular dysgenesis†	.3	Haploid (father) Bu/Flu160/ rATG10	$CD34^+$ selection	Primary graft failure	Intracerebral abscess	Father	(-/-)	38	Flu 120	rATG 5 mg/kg
R6	M	CGD (*CYBB*)	17.0	MUD 10/10 Bu$/Flu180/ alemtuzumab .6 mg/kg	0	Secondary graft failure (6 mo)	Autoimmune aplas (relapse)	Father	(-/-)	74	RXT 2G/Flu 150 /CY 29	Alemtuzumab (.4 mg/kg)/ rituximab
				MUD 10/10 CY100/rATG	0							

D/R indicates donor/recipient; CR, conditioning regimen; LAD, leukocyte adhesion deficiency; WA: Wiskott-Aldrich syndrome; *TCIRC1*, T cell immune regulator 1; *PRF1*, perforin 1; *FOXP3*, Forkhead Box P3; *CARMIL2*, capping protein regulator and myosin 1 linker 2; *CORO1A*, coronin 1A; *ITK*, IL2-inducible T cell kinase; ITGB2, integrin, β2; *WASP*, WAS protein; *UNC13D*, UNC13, *C. elegans*, homolog of, D; *IKBKG*, inhibitor of kappa light polipetide gene enhancer in B cells kinase of, gamma; *CLCN7*, chloride channel, 7; EBV-LPD, EBV-related lymphoproliferative disease; PR, partial remission; IBD, inflammatory bowel disease; Bu, busulfan with myeloablative targeted busulfan 21,000-23,000 Mmol/min; flu, fludarabine in mg/m^2; CY, in mg/kg; RXT, radiotherapy; G, gray; rATG, rabbit antithymocyte globulin in mg/kg total dose; rituximab, 375 mg/m^2 total dose; *RFXANK*, regulatory factor X-ankyrin repeat-containing; CGD, chronic granulomatous disease; *CYBB*: cytochrome b, β subunit; UCB, unrelated cord blood; MUD, matched unrelated donor; Bu$, submyeloablative targeted busulfan between 14,000 and 16,000 Mmol/min; TT, thiotepa in mg/kg.

* Delay between rescue and the previous transplant.

† Not related to AK2 mutation.

Statistical Methods

Descriptive statistics were calculated using the frequency (percentage) for categorical variables and the median (range) for quantitative variables. The probability of overall survival (OS) after HSCT was estimated using the Kaplan-Meier method with R software and the survival library. Patients were censored at the time of death or at last follow-up. The probabilities of aGVHD, cGVHD, autoimmunity, viral reactivation, and life-threatening viral infection were summarized as the cumulative incidence, with death as a competing event. The 95% confidence interval (CI) was calculated using log transformation with R software. Immune reconstitution was analyzed in the subset of children who were alive at the corresponding time point.

RESULTS

Engraftment

Primary engraftment was confirmed in 25 of 27 patients (92.5%); 24 of these 25 had full donor chimerism, and 1 had mixed chimerism (Table 2). The median times to neutrophil and platelet recovery were, respectively, 19 days (range, 14 to 36) and 22 days (range, 0 to 123). Primary graft failure occurred in 2 patients (P9 and R5). P9 (suffering from CID) was negative for donor-specific anti-HLA antibodies, and the area under the curve of busulfan was within the myeloablative range. However, this nonengraftment might have been due to persistent CMV viremia at the time of transplantation. R5 (suffering from reticular dysgenesis) underwent rescue HIFD HSCT PTCY with the same donor; the patient died from the progression of intracerebral abscesses 62 days after the rescue HSCT. P7 transplanted for IPEX experienced secondary graft failure after initial mixed chimerism (38% of donor cells in whole peripheral blood) at engraftment. The patient received NMA conditioning because of his precarious clinical status. He died from multiple organ failure (precipitated by kidney failure) on day 81 post-HSCT. In all other patients the level of chimerism remained stable over time. Two patients (P16 and R6) received a boost of $CD34^+$ cells for persistent thrombocytopenia at 3 months post-HSCT, with a good outcome.

Overall Survival

As of February 1, 2019 (after a median follow-up period of 25.6 months [range, 1.7 to 43.2]), 21 of 27 patients were alive and disease-free. The probability of OS at 2 years for the entire cohort was 77.7% ± 8% (95% CI, 57.1% to 89.3%). OS was better for patients with OP than for patients with HLH and other PIDs, (difference not statistically significant) (Figure 1A). There was no difference in OS between patients having an initial HSCT and those having a rescue HSCT. Six patients died after a median of 74 days (range, 51 to 192) post-HSCT. Two deaths (P9 and R5) were related to primary graft failure, 2 to transplant-related toxicities (VOD, pulmonary hemorrhage, and infection in P15; thrombotic microangiopathy, followed by multiple organ failure and infection in P7), and 2 primarily to GVHD followed by infections (in P10) and severe bacterial sepsis (in P16).

Toxicity

Early conditioning-related adverse events occurred in 7 patients, all of whom had received MAC. Despite defibrotide prophylaxis, 3 patients (P05, P15, and P16, all with an indication of HLH) developed VOD. These were all severe cases, requiring transfer to a pediatric intensive care unit for transient noninvasive ventilation support (n = 2) or invasive ventilation (n = 1). One patient (P16) recovered from VOD but developed transient pulmonary hypertension 3 months post-HSCT, which responded to specific treatment. Thrombotic microangiopathy occurred in 3 patients (P7, P10, and P20). The condition was transient and reversible in P10. P7 and P20

(both with an indication of IPEX) were treated with eculizumab, leading to remission in P20. Progression to renal failure occurred in P7 (a kidney transplant recipient). One patient (P8) experienced cardiogenic shock (possibly related to cyclophosphamide toxicity and/or fluid overload) on days 5 but recovered.

Graft-versus-Host Disease

aGVHD grade $\geq$ II was observed in 12 of 25 assessable patients (48%, not considering the 2 patients with primary graft failure) with a median time to onset of 22 days (range, 17 to 60) post-HSCT (Figure 1B). Most cases of aGVHD were mild (grade II, 10; grade III, 2). All affected patients responded to corticosteroids. A corticosteroid-sparing agent was required in 7 patients but led to resolution of GVHD.

cGVHD and/or autoimmunity was observed in 10 of 22 patients (45%) who survived for more than 6 months; the 2-year cumulative incidences of cGVHD and autoimmunity were, respectively, 24.2% $\pm$ 9.5% (95% CI, 10.8% to 49%) and 29.6% $\pm$ 10.3% (95% CI, 14.4% to 54.8%) (Figure 1C). cGVHD was documented in 5 patients and was limited in all cases (skin, 5; eyes, 1). At last follow-up all affected patients had achieved remission without systemic immunosuppression. Autoimmune manifestations occurred in 7 patients, with extensive vitiligo (n = 1), autoimmune hemolytic anemia (n = 3), extramembranous glomerulonephritis (n = 1), and thyroiditis (n = 2). Immunosuppressive treatments were withdrawn in 17 patients after a median of 9 months (range, 4 to 14). Four patients in remission from autoimmune hemolytic anemia (n = 3) or extramembranous glomerulonephritis (n = 1) were still taking immunosuppressants at last follow-up (21, 28, 37, and 16.3 months post-HSCT, respectively). None of the parameters (neither donor age nor sex, recipient age, graft composition, type of disease, pre-HSCT active infection, type and dose of serotherapy, nor the number of HSCTs per patient) was found to be significantly associated with the occurrence of aGVHD, cGVHD, or autoimmune disease.

Infections after Transplant

At the time of HSCT, 9 patients displayed systemic viremia (CMV, 8; ADV, 1) requiring antiviral therapy in the 10 days before transplant. Fifteen patients experienced a total of 18 systemic viral infections or reactivations post-transplant (CMV, 15; ADV, 2; EBV, 1). The cumulative post-transplant incidences of systemic CMV, ADV, and/or EBV replication and life-threatening viral events were 58% $\pm$ 9.8% (95% CI, 40.2% to 76.9%) and 15.6% $\pm$ 7.2%, (95% CI, 6.4% to 36.3%), respectively (Figure 1D).

The most frequent events were related to CMV infections; 15 episodes occurred in 13 of the 19 (68%) at-risk patients (ie, CMV-seropositive patients and recipients with a CMV-seropositive donor). Most of these events were CMV reactivations (n = 13, in 11 patients). Eleven episodes were successfully treated with preemptive antiviral agents (ganciclovir or foscavir) for a median of 20 days (range, 15 to 36), leading to persistent negative blood viral loads. In the remaining 2 episodes (in P9 and P15), the viral load remained stable or increased. Two patients displayed CMV disease with systemic replication and concomitant CMV retinitis, occurring at day 71 in P4 and day 18 in R2. Both were treated with 4 intraocular injections of foscavir and/or ganciclovir, together with systemic antiviral treatment. Furthermore, R2 received a CD45RA-depleted donor leukocyte infusion at day 42. The outcome was good without sequelae. Two of 8 patients (25%) who were positive for ADV (in feces and/or nasal swabs) before and/or after transplant displayed systemic ADV replication without organ involvement (R2 and P10). Both infections were successfully controlled with brincidofovir. Only 1 patient presented transient EBV reactivation, which did not require treatment. To date, no patient has developed EBV-related lymphoproliferative disease after transplant. Remarkably, none of the 3 patients with pre-HSCT, EBV-related lymphoproliferative disease in partial remission (with a detectable EBV viral load at the time of transplantation) relapsed; they all cleared the EBV rapidly after HSCT. Seven patients experienced BK virus cystitis; none of the cases was severe, and 2 had mild symptoms (microscopic hematuria and transient dysuria only).

One patient presented with *Pneumocystis jirovecii* pneumonia 9 months after transplant while he was off prophylaxis and was treated successfully. R6 was treated for a disseminated *Mycobacterium avium* infection diagnosed 9 months post-transplant. Prolonged immunosuppression in this patient with chronic granulomatous disease and who was rescued after 2 previous graft failures may have contributed to the emergence of this opportunistic infection. P16 developed severe bacterial sepsis related to the long-term use of a central venous access device at 6 months post-HSCT and died suddenly at home.

Immune Reconstitution

The time course of immune reconstitution is shown in Figure 2. The median times needed to reach CD3 T cell counts of 300 and 1000/μL were, respectively, 76 days (range, 21 to 222) and 157 days (range, 28 to 373), whereas the median times needed to reach $CD3^+CD4^+$ cell counts of 50 and 200/μL were, respectively, 53 days (range, 31 to 155) and 111 days (range, 22 to 227). As shown in Figure 2A, the absolute CD4 and CD8 T cell counts differed markedly from 1 patient to another. Factors found to contribute significantly to this variability were the use of serotherapy before HSCT and a history of viral reactivation; recipient age, the type of disease, graft composition, and the occurrence of aGVHD were not significant contributors. Indeed, patients with a CD3 count $<$ 1000/μL before HSCT and who received alemtuzumab at days -11 and -10 showed significantly slower T cell recovery during the first 4 months post-HSCT (Figure 2B). As shown in Figure 2C, patients with CMV or ADV reactivation displayed a significant expansion of CD8 T cells in the first few months post-HSCT. Although specific antiviral responses were not systematically assessed, 4 of 5 patients with systemic EBV, CMV, or ADV infections within 3 months of HSCT were found positive in ELISpot, pentamer, or intracellular IFN-γ staining (Figure 3A to D). Naive CD4 and CD8 T cells appeared between 4 and 6 months post-HSCT and increased between 6 and 12 months (Figure 2D). B cell count and IgM level both began to increase 6 months post-HSCT, as shown in Figure 4A. The median times to recover a CD19 B cell count $>$ 200/μL and an IgM titer $>$ .5 g/L were, respectively, 204 days (range, 67 to 580) and 256 days (range, 63 to 413) in the 20 assessable patients. Immunoglobulin replacement therapy was withdrawn after a median of 451 days (range, 251 to 750) post-HSCT in 17 patients after they started to be vaccinated (4 patients were still on immunoglobulin replacement at last follow-up). The recovery in the NK cell count is shown in Figure 4B.

Long-Term Follow-Up and Complications

At last follow-up, all 21 surviving patients were free of the disease that prompted the HSCT. All patients had a Lansky or Karnofsky score of 100%. One patient had persistent vitiligo, 1 suffered from ocular GVHD (being treated topically), and 4

Table 2
Patient Outcomes after HSCT with PTCY

Patient	Engraftment (PMN/Platelets)		Early Toxicity	Viral Infection Post-HSCT	aGVHD (Grade)	cGVHD	Auto immunity	Stop IS(mo)	Last Chimerim (%)	Follow-Up(mo)	Outcomes
First HSCT											
P1	16	25		CMV (infection), BK (microscopic cystitis)	II	Limited	0	14	100	38.0	Cured, AW
P2	19	41		CMV (infection), ADV (nose/stools carriage)	I	0	0	11	100	41.2	Cured, AW
P3	32	41			0	0	0	6	100	36.8	Cured, AW
P4	17	24		CMV (disease)	II	0	Vitiligo	4	100	38.0	Cured, vitiligo
P5	24	19	VOD		0	0	0	7	100	29.4	Cured, AW
P6	15	15			II	0	0	14	100	38.0	Cured, AW
P7	14	0	TMA	BK virus (cystitis) CMV (infection)	0	NA	NA	NA	2	2.7	Dead (toxicity, MOF)
P8	16	13	Cardio		0	Limited	AIHA	On going	95	37.8	Cured, IS for AIHA
P9	No engraftment			CMV (infection), RSV, ADV (stools carriage)	NA	NA	NA	NA	100	1.7	Dead (ARDS after second HSCT)
P10	18	45	TMA	CMV (infection), ADV (infection), BK virus (cystitis)	III	NA	NA	NA	100	6.1	Dead (GVHD, infection)
P11	22	22		CMV (infection), BK virus (cystitis), EBV (infection)	II	Limited	Thyroiditis	9	100	30.6	Cured, AW, hypothyroiditis
P12	18	20		BK virus (cystitis)	II	Limited	0	9	100	33.6	Cured, AW
P13	24	19		CMV (infection)	0	0	AIHA	On going	100	28.2	Cured, IS for AIHA
P14	25	12			0	0	0	10	100	25.6	Cured, AW
P15	20	12	VOD	CMV (disease)	0	NA	NA	NA	100	1.8	Dead (toxicity, infection)
P16	22	87	VOD/PAH		0	NA	NA	NA	99	6.4	Dead (sepsis)
P17	36	32		CMV (infection), norovirus (stools carriage)	II	0	0	12	100	24.4	Cured, AW
P18	18	9			I	0	0	4	95	19.8	Cured, AW
P19	20	14		CMV (infection), ADV (stools carriage)	I	0	0	6	100	14.0	Cured, AW
P20	18	17	TMA		II	0	Glomerulonephritis	On going	100	16.3	Cured, IS for glomerulonephritis
P21	25	23		CMV (infection), ADV (stools carriage)	II	0	Thyroiditis	9	100	19.4	Cured, AW, hypothyroiditis
Rescue HSCT											
R1	15	16		CMV (disease)	I	0	0	11	100	21.8	Cured, AW
R2	16	19		ADV (infection)	II	0	0	9	100	32.3	Cured, AW
R3	19	31			II	0	0	12	100	43.2	Cured, glomerulonephritis
R4	18	33		CMV (infection)	III	Limited	0	12	100	30.5	Cured, ocular cGVHD
R5	no engraft				NA	NA	NA	NA	NA	2.2	Dead, nonengraftment
R6	20	123		BK virus (cystitis)	0	0	0	Ongoing	100	21	Cured, mycobacterial infection, IS for AIHA

PMN indicates polymorphonuclear cells; TMA, thrombotic microangiopathy; Cardio, cardiogenic shock; PAH, pulmonary arterial hypertension; IS, immunosuppressive therapy; AW, alive and well; ARDS, acute respiratory distress syndrome; MOF, multiple organ failure; NA, nonassessed; AIHA, autoimmune hemolytic anemia.

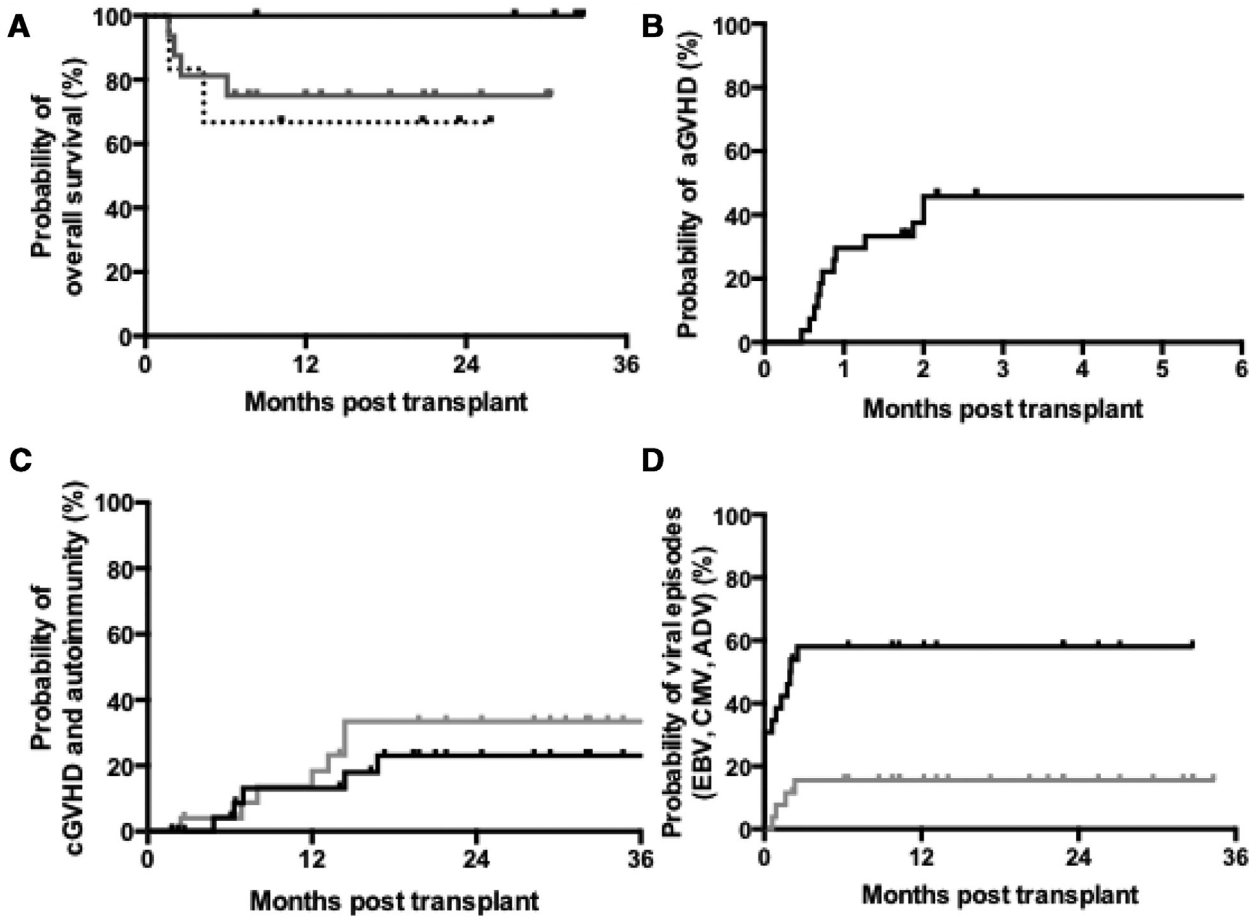

Figure 1. OS, occurrence of GVHD, autoimmune disease, and viral infections after HSCT. (A) The overall probability of survival as a function of the underlying disease: cases of OP (n = 5) are represented in black, cases of HLH (n = 6) as represented as a black dashed line, and other PIDs (n = 15) are represented in gray. (B) The overall probability of aGVHD after HSCT. (C) The overall probability of cGVHD (in black) and autoimmunity (in gray) after HSCT. (D) The overall probability of viral replication (EBV, CMV, and ADV) (in black) and life-threatening viral events (in gray) after HSCT.

were being weaned off immunosuppressants (for autoimmune hemolytic anemia in remission in 3 and glomerulonephritis in 1). The 2 patients with hypothyroiditis were receiving hormonal replacement therapy.

DISCUSSION

Although HIFD HSCT with PTCY is widely used in adult patients with malignant diseases, data on its application in pediatric patients (and especially those with nonmalignant diseases, such as PIDs) are scarce. Here, we reported on our experience of HIFD HSCT with PTCY in 27 consecutive patients suffering from a broad range of PIDs and inherited disorders (such as OP) and showed a 2-year OS rate of 77.7%; most diseases treated here have worse outcomes after conventional haploidentical HSCT, as shown by Gennery et al. [2], who reported that the OS rate for non-SCID recipients of HIFT HSCT after $CD34^+$ selection was 47% [18,26]. These poor results were mainly due to the high risk of rejection and/or infectious complications. It should be noted that the patients in our series had major risk factors for a poor post-HSCT outcome, such as active viral infections just before transplant (n = 9), EBV-related lymphoproliferation in partial remission (n = 3), previous kidney transplant (n = 1), and a second HSCT (n = 6).

The risk of graft rejection was overcome in most patients, including those with OP, a group of patients with a high risk of nonengraftment, especially in haploidentical settings [26]. Of the 20 patients undergoing primary HSCT after a MAC and upfront serotherapy, only 1 developed primary graft failure; the engraftment rate was similar to that obtained after TCRab/CD19 depletion (provided that 2 alkylating agents had been used). Conditioning-related adverse events were frequent (26%) and were responsible for 2 deaths. It is noteworthy that VOD occurred mainly in patients with HLH, a high-risk group for this type of complication [27]. Severe thrombotic microangiopathy occurred in 2 patients, both with IPEX. Strategies for minimizing toxicity are thus required. The high engraftment rate with PTCY approaches in other settings (including after reduced-intensity and NMA conditioning) [15,28,29] may allow a reduction in the intensity of conditioning. A sub-MAC regimen with targeted busulfan or low-toxicity combinations should be prospectively evaluated in the context of PIDs [30-32].

Although the incidence of grades II to III aGVHD was relatively high (48%), the cases were mild. In view of the underlying proinflammatory conditions (such as active viral infection at the time of HSCT, rescue HSCT, HLH, etc.), the affected patients were considered to be at a high risk of severe aGVHD. The incidence of GVHD in our series is higher than that (22%) in previous reports on haploidentical HSCT and PTCY in pediatric patients with malignant diseases [17,33]. Interestingly, the incidence in the present study is in the same range as that reported by Shah et al. [8] for TCR$\alpha\beta$/CD19-depleted graft in a similar (but not identical) population of pediatric patients with

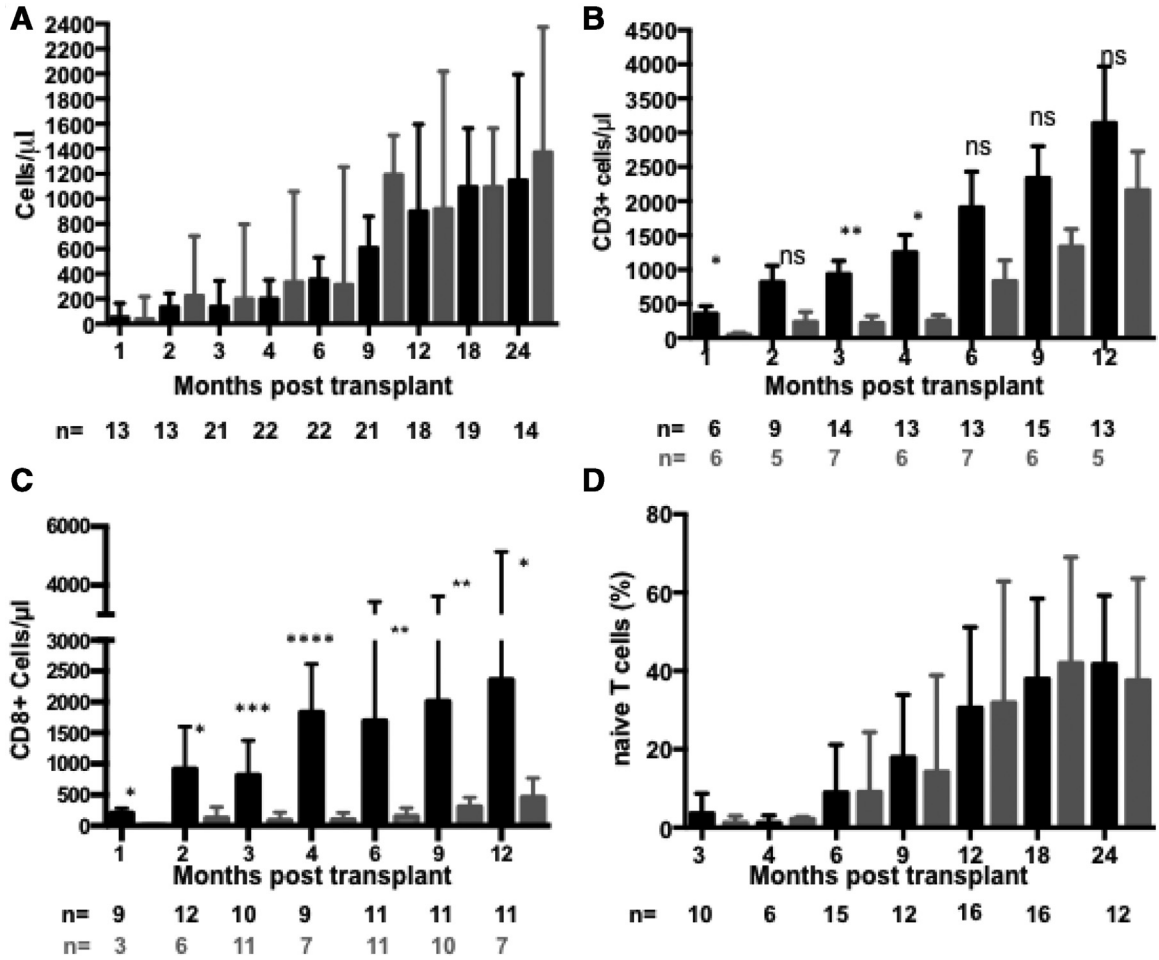

Figure 2. T cell immune reconstitution after transplantation. (A) Changes over time in the mean ± SEM absolute $CD3^+CD4^+$ count (in black) and absolute $CD3^+CD8^+$ count (in gray). (B) Changes over time in the mean ± SEM absolute $CD3^+$ count as a function of pretransplant serotherapy. Mean ± SEM $CD3^+$ counts for patients with a pretransplant lymphocyte count < 1000/µL and the use of alemtuzumab at days −11 and −10 are shown in gray, and mean ± SEM counts for other patients are shown in black. The absolute counts in each group are compared at each time point. NS indicates not significant. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$. (C) Changes over time in the mean ± SEM absolute $CD8^+$ count as a function of viral reactivation. Patients with EBV, CMV, or ADV reactivation/disease are shown in black, and those without viral reactivation/disease are shown in gray. The absolute counts in each group are compared at each time point. (D) CD4 and CD8 naïve T cell recovery: changes over time in the mean ± SEM percentage of naïve CD4+ cells (in black, defined as $CD3^+$ $CD4^+$ $CD31^+$ $CD45RA^+$) and naïve CD8 cells (in gray, defined as $CD3^+$ $CD4^+$ $CCR7^+$ $CD45RA^+$).

PID. Unsurprisingly, cGVHD was observed more frequently than after conventional haploidentical T cell–deplete HSCT, but most cases were mild and outcomes favorable. Autoimmune disease with a significant clinical impact is a concern and occurred in 4 cases; similarly, Shah et al. [8] reported the occurrence of autoimmune cytopenia in 4 of their 26 patients transplanted with TCRαβ/CD19-depleted grafts. The recipients' young age might also account for the increased risk of GVHD, as reported previously in this context of HIFD HSCT with PTCY [34]. It is known that younger patients show interindividual variability in cyclophosphamide pharmacokinetics, metabolism, and clearance [34]. Pharmacokinetic studies of cyclophosphamide will thus be needed to define the best dosage for optimizing GVHD prophylaxis while reducing early toxicity.

The frequent occurrence of viral infection in this high-risk population is of note, although standard antiviral treatment led to favorable outcomes in most cases. This result is in line with literature reports in adults [35,36] and pediatric patients [17,33] having undergone HIFD HSCT with PTCY for malignant diseases. The incidence of systemic viral reactivation reported by Shah et al. for TCRαβ/CD19-deplete transplantation in patients with PID was in the same range. However, Shah et al. reported a higher incidence of life-threatening viral infections (defined as systemic viral replication with organ involvement or a persistent blood viral load despite treatment as in this report) of 59%, versus 15% in this report. Even though we cannot rule out interstudy differences in populations and risk factors, this might reflect differences in the time course of immune reconstitution, a key issue in patients with PID and active infections at the time of HSCT. The low observed frequency of ADV viremia (despite a high proportion of patients with viral carriage) and the absence of EBV lymphoproliferative disease recurrence contrast with observations in other settings of haploidentical HSCT. This may reflect earlier T cell reconstitution [37,38].

Immune reconstitution in the early post-HSCT phase (ie, before thymic output) relies on the peripheral expansion of graft-infused T cells. The impact of PTCY on this expansion has not been extensively studied. Kanakry et al. [39] found that early immune reconstitution in patients receiving PTCY alone as GVHD prophylaxis (ie, in the absence of other immunosuppressants) mainly relied on the expansion of effector memory

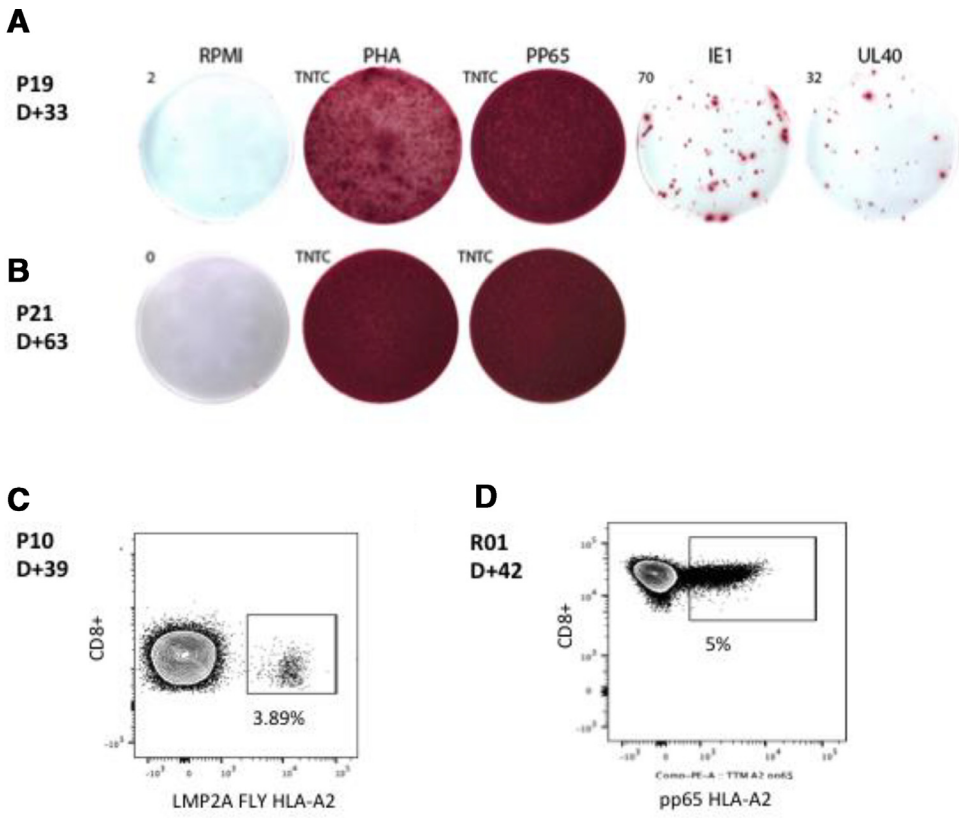

Figure 3. Antiviral responses. (A) Anti-CMV responses in P19, measured at day 33 post-HSCT during a CMV infection. Peripheral blood mononuclear cells were incubated overnight with culture media (the negative control), phytohemagglutinin (the positive control), and PP65, IEA, and UL40 peptides. TNTC indicates too numerous to count; PHA, phytohemaglutinin. (B) Anti-CMV responses in P21, measured at days 61 post-HSCT during a CMV infection. Peripheral blood mononuclear cells were incubated overnight with culture media (the negative control), phytohemagglutinin (the positive control) and PP65. (C) Detection of EBV-specific $CD8^+$ T lymphocytes in P10 using pentamers restricted to MHC HLA-A2 and the LMP2 antigen (FLYALALLL peptide) after HSCT (day +39). (D) Detection of CMV-specific $CD8^+$ T lymphocytes in patient R02 using pentamers restricted to MHC HLA-A2 and the pp65 antigen (NLVPMVATV peptide) after HSCT (day +42).

T cells with a narrow repertoire (partially driven by CMV exposure), as observed in some cases in our series. In Kanakry et al.'s study, aGVHD was associated with lower naïve T cell counts 1 year after transplant. The latter association was not observed in our series, possibly due to limited statistical power or because the GVHD was generally mild. The pretransplant use of serotherapy has also a strong impact on immune reconstitution by inducing in vivo T cell depletion of the graft. It has been shown that the absolute lymphocyte counts before antithymocyte globulin and alemtuzumab infusions and the day on which serotherapy is initiated (relative to transplantation) influence early immune reconstitution [40-42]. Indeed,

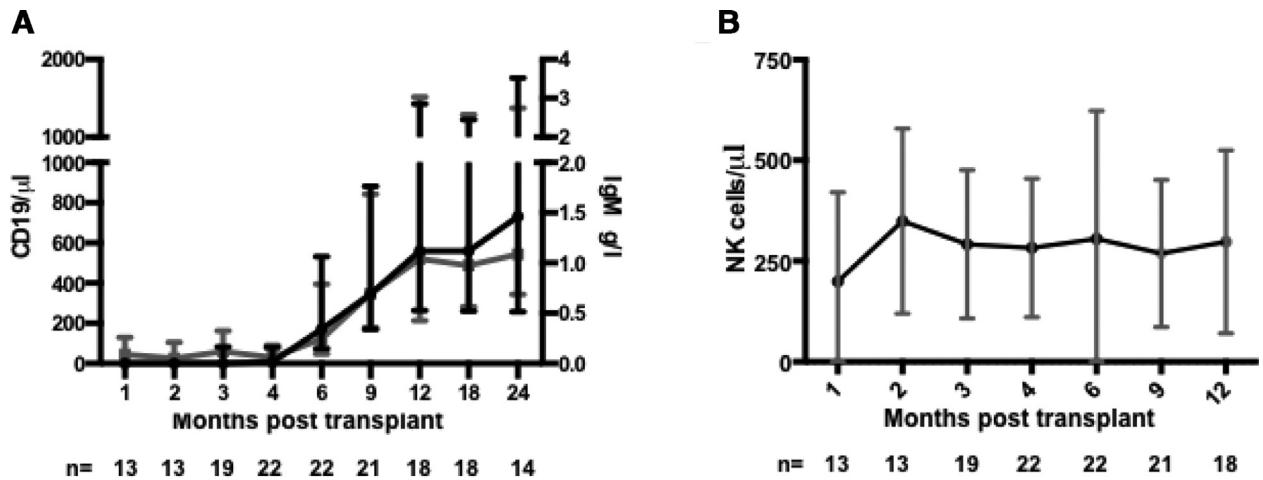

Figure 4. Humoral and NK cell immune reconstitution after transplantation. (A) Changes over time in the mean ± SEM $CD19^+$ cell count (in black) and IgM levels (in gray) after HSCT. (B) Changes over time in the mean ± SEM NK cell count after HSCT.

we observed a similar impact of alemtuzumab in patients with lymphopenia before HSCT. Performing alemtuzumab pharmacokinetic studies will be of prime value in the future to individualize serotherapy regimens, thus limiting the serotherapy's impact on T cell development.

In the context of PIDs and inherited disorders, comparisons with other haploidentical HSCT approaches (such as TCRαβ/CD19 graft depletion) are necessary but problematic because of the great variability in the patients' underlying diseases, comorbidities, and appropriate conditioning regimens. Ideally, the 2 approaches should be compared in a prospective study. Both approaches lead to robust engraftment [8-10]. The incidence of aGVHD with TCRαβ/CD19 depletion varies, although the frequency of visceral GVHD is usually lower than the value reported here. The time course of early immune reconstitution and the latter's correlation with the viral disease control should also be prospectively assessed.

Use of mismatched unrelated cord blood graft is another option that may be considered in the absence of an HLA identical donor. In case of infection threat, omitting serotherapy may allow a faster immune reconstitution but increase the risk of GVHD [43]. Reduced-intensity conditioning including upfront high-dose alemtuzumab has been proposed in patients with nonmalignant diseases transplanted with an unrelated cord blood graft allowing good engraftment but slower immune reconstitution [44]. These options might be considered and balanced depending on patient comorbidities.

In conclusion, haploidentical HSCT provides an opportunity for nearly all patients to benefit from HSCT. We found that T cell–replete HSCT with PTCY (for reducing the incidence of GVHD while sparing other T cells) is a valid option in high-risk patients with PIDs or OP and who lack a conventional donor. Reduction of conditioning intensity to decrease conditioning-related toxicity needs to be prospectively assessed.

ACKNOWLEDGMENTS

The authors thank the patients and their families and are grateful to Elvira Duchene for help in coordinating the HSCT activities, to Sophie Camilleri and Peter Van Endert for HLA typing, and to Marianne Leruez for viral monitoring.

Financial disclosure: The authors have nothing to disclose.

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

Authorship statement: B.N. and J.S.D. performed HSCT, monitored patients, collected data, and wrote the manuscript. M.C., M.L.F., B.C., M.B., G.M., R.L., A.F., D.M., and S.B. performed HSCT, monitored patients, and helped collect data. J.R. and C.P. performed the genetic diagnosis of patients with PID and assessed immune reconstitution. M.V. participated in statistical analysis. A.M. and E.M. coordinated and performed graft preparation. F.T., B.M., and L.C. assessed antiviral responses. P.B. studied the pharmacokinetics of busulfan. All authors critically read, commented on, and approved the final version of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.03.009.

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