

Adoptive immunotherapy for primary immunodeficiency disorders with virus-specific T lymphocytes

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Background: Viral infections are a leading fatal complication for patients with primary immunodeficiencies (PIDs) who require hematopoietic stem cell transplantation (HSCT). Use of

virus-specific T lymphocytes (VSTs) has been successful for the treatment and prevention of viral infections after HSCT for malignant and nonmalignant conditions. Here we describe the clinical use of VSTs in patients with PIDs at 4 centers.

Objective: We sought to evaluate the safety and efficacy of VSTs for treatment of viral infections in patients with PIDs.

Methods: Patients with PIDs who have received VST therapy on previous or current protocols were reviewed in aggregate.

Clinical information, including transplantation details, viral infections, and use of antiviral and immunosuppressive pharmacotherapy, were evaluated. Data regarding VST production, infusions, and adverse reactions were compared. **Results:** Thirty-six patients with 12 classes of PID diagnoses received 37 VST products before or after HSCT. Twenty-six (72%) patients had received a diagnosis of infection with cytomegalovirus, EBV, adenovirus, BK virus, and/or human herpesvirus 6. Two patients were treated before HSCT because of EBV-associated lymphoproliferative disease. Partial or complete responses against targeted viruses occurred in 81% of patients overall. Time to response varied from 2 weeks to 3 months (median, 28 days). Overall survival at 6 months after therapy was 80%. Four patients had graft-versus-host disease in the 45 days after VST infusion, which in most cases was therapy responsive. **Conclusion:** VSTs derived from either stem cell donors or third-party donors are likely safe and effective for the treatment of viral infections in patients with PIDs. (*J Allergy Clin Immunol* 2016;■■■■:■■■■-■■■■.)

Key words: Primary immunodeficiency, immunotherapy, cytotoxic T lymphocytes, antiviral therapy

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Primary immunodeficiencies (PIDs) are a large group of congenital defects of immunity and have many heterogeneous features, which can include abnormally frequent or severe illness from common organisms, opportunistic infections, autoimmune phenomena, and allergic disease. Among forms of PIDs with impaired or absent T-lymphocyte function, such as severe combined immunodeficiency (SCID), viral infections are frequent and often devastating.^{1,2} In children with SCID, cytomegalovirus (CMV), EBV, and adenovirus (ADV) are the most common cases of virus-associated mortality.³ Although antiviral pharmacotherapy exists, it is expensive and frequently complicated by toxicities and drug resistance.⁴ Response to pharmacotherapy is often incomplete without accompanying intact cellular immunity.

Abbreviations used

ADV:	Adenovirus
CMV:	Cytomegalovirus
EBV-LPD:	EBV-related lymphoproliferative disease
GVHD:	Graft-versus-host disease
HHV6:	Human herpesvirus 6
HSCT:	Hematopoietic stem cell transplantation
PID:	Primary immunodeficiency
SCID:	Severe combined immunodeficiency
VST:	Virus-specific T lymphocyte

Definitive treatment of SCID and many other forms of PIDs requires reconstitution of T-cell immunity, which can be accomplished by hematopoietic stem cell transplantation (HSCT) or gene therapy for certain monogenic disorders.^{5,6} Active viral infections have been shown to negatively affect survival during HSCT,^{1,7} and patients with PIDs remain extremely vulnerable to viral infections during and after HSCT, in part because of the 3 to 6 months required for T-cell engraftment to occur. This is of particular concern in recipients of stem cells from virus-naïve sources, such as umbilical cord blood, and in patients receiving HLA-mismatched stem cell products, which require depletion of mature T cells to prevent graft-versus host disease (GVHD).⁸ Unfortunately, clearance of many viral infections is difficult or impossible without T-cell reconstitution.

Virus-specific T lymphocytes (VSTs) have been used with great success in preventing and treating viral infections after HSCT.⁹⁻¹¹ Although methods have varied, the overall goal of VST therapy is to isolate donor T cells with activity against 1 or more viruses while excluding alloreactive T cells that might cause GVHD. To date, the majority of studies have produced VSTs by means of *ex vivo* culture and expansion of T lymphocytes after stimulation with viral antigens using donor-derived antigen-presenting cells. VST production previously required that a donor have existing immunity against the targeted viruses. However, new methods using specific cytokines have permitted culture of VSTs from virus-naïve cord blood and adult donors, and the use of overlapping peptide pools encompassing viral antigens has reduced culture time in virus-exposed donors to 10 to 12 days.¹²⁻¹⁴ VSTs can also be isolated directly from peripheral blood, either through selection of MHC multimer-binding T cells or through selection of T cells that secrete IFN- γ after stimulation with viral antigens.^{15,16} Selection technologies allow rapid production of VSTs (with <1 day of processing time) but requires large volumes of blood and can only be performed from donors with prior viral exposure.

Most prior studies using VSTs have focused on their administration after HSCT and used the stem cell donor as a source of VSTs.^{9,11} Alternately, partially matched VSTs that have been previously generated from healthy donors and banked (third-party VSTs) have been used successfully as “off-the-shelf” therapy for viral infections or prophylaxis.^{17,18} This approach eliminates the time and expense required for customized products.

Previous reports detailing the use of VSTs have not described their safety and efficacy exclusively in the PID setting. Thus an aggregate perspective regarding VSTs in patients with PIDs is not available to physicians faced with treatment decisions. Here we present the largest multicenter series to date of VST use for the prevention and treatment of viral diseases in the population with PIDs.

METHODS

A retrospective review was performed to collect clinical information regarding patients with PID diagnoses who received VST therapy between 2003 and 2014 at 4 institutions (Table I).^{12,19-23} Patients received VST infusions in the context of 7 clinical protocols (3 closed and 4 current) or off study through compassionate use agreements. Ethics board approval was received at each institution for the administration of these novel T-cell therapies (either on existing protocols or through dedicated emergency protocols for compassionate use), as well as for the collection of the aggregate PID data presented here.

Twenty-three of the evaluated patients were previously reported within the larger publication of their study protocol, and details were updated for this study.^{20,21,23-25} The remaining patients were unpublished. Demographics, immunologic and genetic phenotype (if available), transplantation details, and the specifics of VST production and dosing were recorded, as was information on viral infections both before and after VST therapy. Antiviral responses to VST therapy were classified as complete (resolution of viremia, visceral symptoms, or both), partial (≥ 1 log decrease in viral copy number and/or improvement in visceral symptoms without complete resolution or transient resolution with subsequent return of viremia and/or symptoms within 3 months of infusion), or no (no discernable change in viral copy number or clinical symptoms) response.

RESULTS**Patient demographics**

Thirty-six patients with PIDs received 1 or more infusions of VSTs during the 10-year study period (Table II).^{20,21,23-25} The patients had 12 classes of underlying diagnoses (Fig 1). SCID and X-linked lymphoproliferative disease/related disorders were the most common disorder types. Age at transplantation ranged from 2 months to 19 years, whereas age at VST infusion ranged from 5 months to 19 years. HSCT donors included matched related donors (n = 4), mismatched related (haploidentical) donors (n = 5), unrelated donors (n = 22), and cord blood (n = 4).

Two of the patients received VST infusions before planned HSCT. For those receiving HSCT, the time between HSCT and VST infusion varied from 8 days to 27 months. Twenty-six patients were receiving immunosuppressive medications at the time of VST infusion, either for prophylaxis or treatment for GVHD, including calcineurin inhibitors (n = 20), corticosteroids (n = 16), mycophenolate mofetil (n = 6), infliximab (n = 1), and extracorporeal photopheresis (n = 1). All patients receiving corticosteroids were weaned to 0.5 mg/kg/d or less (prednisone equivalents) before VST infusion.

VST production and infusion

VSTs were produced by using previously described protocols from HSCT donors, cord blood, or third-party donors.^{12,23-25} Thirty-five patients received VSTs produced through culture methods (Table I and see Fig E1 in this article's Online Repository at www.jacionline.org), and 1 patient was treated with streptamer-selected VSTs.¹⁵ VSTs were used that targeted 1 virus (19 products), 2 viruses (1 product), 3 viruses (14 products), or 5 viruses (3 products). In all cases VSTs were tested for sterility, as well viral specificity, by using the IFN- γ ELISpot. Most lines (excepting some dedicated third-party VST lines¹⁷) were tested for lack of alloreactivity by using mismatched PHA blasts or lymphoblastoid cell lines as targets in cytotoxicity assays (with either ⁵¹Cr release or flow cytometry-based methods²⁵). Patients received VSTs through intravenous

TABLE I. VST studies and participating institutions

Study	Specificity	VST stimulation	Manufacturing institution	Treating institution
ETNA NCT00058812	EBV	LCL ¹⁹	BCM	BCM
VICTA NCT00078533	EBV/CMV/ADV	Dendritic cells and LCLs transduced with Ad5/35 vector encoding pp65 ²⁰	BCM	BCM
LYPTAIST NCT00590083	EBV/ADV	Dendritic cells and LCLs transduced with Ad5/35 vector ²¹	BCM	BCM
ACTCAT NCT00880789	EBV/CMV/ADV	Dendritic cells and LCLs transduced with Ad5/35 vector encoding pp65 ^{12,22}	BCM	BCM
ARMS NCT01570283	EBV/ADV/CMV/BK/HHV6	PBMCs pulsed with overlapping peptide ²³	BCM	BCM
GOS EIND	EBV	LCLs	BCM	GOSH
MUSTAT NCT01945814	EBV/CMV/ADV	PBMCs pulsed with overlapping peptide	CNMC	CNMC
TREPID	EBV/CMV/ADV	PBMCs pulsed with overlapping peptide	CNMC	CNMC
CNMC EIND	EBV/CMV/ADV	PBMCs pulsed with overlapping peptide	CNMC	CNMC
NCL EIND	EBV	LCLs	Aberdeen	NCL

BCM, Baylor College of Medicine; GOSH, Great Ormond Street Hospital; CNMC, Children's National Medical Center; NCL, Newcastle Hospital; LCL, lymphoblastoid cell lines.

infusion at doses ranging from 5×10^6 to 1.35×10^8 cells/m² based on individual protocol details (Table II).

Viral infections and response

Of the patients evaluated, 26 (72%) had been given a diagnosis of CMV, EBV, ADV, human herpesvirus 6 (HHV6), and/or BK virus for which they were treated. Twenty (56%) had active infection with 1 or more viruses, many of which were resistant to antiviral pharmacotherapy, at the time of VST infusion (Table II). Two patients had multiple viral infections at the time of infusion. Eight patients had been given a diagnosis of EBV-related lymphoproliferative disease (EBV-LPD), and of these, 4 had ongoing EBV-LPD at the time of VST infusion. The remaining 16 (44%) patients were treated prophylactically and had no active viral infections at the time of infusion but were considered at high risk for infection. Of these patients, 13 (81%) remained free of detectable CMV, EBV, and ADV after infusion, whereas the remaining 3 (19%) patients had reactivation of CMV or EBV, all of which resolved.

Complete or partial antiviral responses were seen in 86% of patients with CMV ($n = 7$), 76% of patients with EBV ($n = 16$), and all patients with ADV ($n = 2$) and HHV6 ($n = 1$). Six patients had reactivation of CMV ($n = 2$) or EBV ($n = 4$) after VST infusion. All reactivation infections resolved within 1 to 2 months, and in 4 patients no antiviral pharmacotherapy was required after VST infusion. One of the 2 patients treated for multiple viral infections (patients 16 and 29) had clearance of all viruses after VST infusion. Patient 16 had clearance of EBV and HHV6 viremia but had no improvement in BK viral load, and retrospectively, the used VST line was shown to lack specificity against BK virus, likely because of lack of donor exposure to the virus.

Decreases in viral copy numbers in blood were seen at 2 weeks to 3 months after infusion (Fig 2), with a median response time of 1 month. There was no correlation between VST dose and likelihood of antiviral response (reduction in viral load) or timing of response (data not shown). Antiviral efficacy was seen in most cases despite the use of immunosuppressive medications, including calcineurin inhibitors, mycophenolate mofetil, and low-dose corticosteroids (<0.5 mg/kg/d prednisone). In some

cases the use of immunosuppressive medications during or after initial VST infusion warranted additional doses of VST. In patients 26 and 29, second VST infusions resulted in partial or complete resolution of CMV and EBV.

Of note, 2 patients (patients 25 and 33) received third-party VST infusions before HSCT, both for EBV-LPD. Patient 33 received rituximab, cyclophosphamide, vincristine, and prednisone for treatment of EBV-LPD. She received 2 doses of well-matched third-party VSTs at day -21 and day -14 before unrelated cord blood transplantation, which was successful without further viral disease (Fig 2). Patient 25 had no response to a single dose of third-party VSTs for both CMV and EBV and was found to have monomorphic EBV-associated lymphoma, which was refractory to chemotherapy.

Overall survival and adverse events after VST infusion

Overall survival in treated patients was 80% at 6 months after VST infusion. Most early deaths occurred within 3 months of therapy and were due to existing infections, including 4 patients who died from progressive EBV-LPD and 1 patient who died of progressive CMV and disseminated BCG. One patient died from central nervous system complications of EBV-LPD at day $+26$ after VST infusion.

Adverse reactions after VST infusion were generally mild in all patients. Four (11%) patients had GVHD after VST infusion, which was often associated with weaning of immunosuppression and suspected to be transplant-associated rather than attributable to VST infusion. In 3 of the cases, GVHD was therapy responsive without notable viral reactivation. Patient 28 had a sterile pericardial effusion at 1 month after VST infusion, which resolved after placement of a pericardial window.

DISCUSSION

Viral infections and reactivations are a significant cause of morbidity and mortality in patients with moderate-to-severe forms of PIDs and account for as much as one third of transplant-related mortality.^{2,3,7} Adoptive immunotherapy with VSTs has been highly successful for the prophylaxis and

TABLE II. Patients' characteristics and response to VST therapy

Center	Patient no.	Diagnosis	Age at HSCT	Donor	Time from HSCT to VSTs (mo)	Viral infections before VSTs	Viral status at time of VST infusion	Prior antiviral treatments	VST source and specificity
BCM	1	IL7RA-SCID	6 mo	6/6 UCB	4.5	None	NA	None	Cord blood, CMV/EBV/ADV
	2	IL2RG-SCID	8 mo	5/6 UCB	4.5	None	NA	None	Cord blood, CMV/EBV/ADV
	3	IL2RG-SCID	2 mo	5/6 UCB	3	None	NA	None	Cord blood, CMV/EBV/ADV
	4	IL2RG-SCID	2 mo	MMRD (maternal haploidentical)	9	None	NA	None	HSCT donor, CMV/EBV/ADV
	5	SCID, unknown gene	1 y	10/10 MUD	2	None	NA	None	HSCT donor, ADV
	6	MHC II deficiency	10 mo	10/10 MRD	1.5	CMV	CMV viremia, pneumonitis	Foscarnet, ganciclovir, Cytogam	HSCT donor, CMV/EBV/ADV
	7	WAS	8 mo	9/10 MMUD	5	None	NA	None	HSCT donor, CMV/EBV/ADV
	8	WAS	1.2 y	10/10 MUD	2.3	EBV	EBV viremia	Rituximab	Donor EBV
	9	NK defect/SCAEBV	12 y	7/10 MMUD	7	EBV	EBV viremia	Foscarnet for prior CMV	HSCT donor, EBV
	10	SCAEBV	6.9 y	MRD (syngeneic)	2.5	EBV	EBV viremia	None	Donor EBV
	11	SCAEBV	10.6 y	10/10 MUD	12	EBV-LPD	EBV resolved	Rituximab	Donor EBV
	12	XLP (SLAM mutation)	19 mo	8/8 MUD	3	EBV	EBV viremia	None	HSCT donor, EBV
	13	XLP and lymphoma	16 y	9/10 MMUD	2	None	NA	None	HSCT donor, EBV
	14	LAD1	6 mo	10/10 MRD	2	ADV	ADV viremia	Cidofovir	HSCT donor, CMV/EBV/ADV/HHV6/BK
	15	GATA2	18 y	9/10 MMUD	3	BK, CMV	BK viremia	Foscarnet, valganciclovir	HSCT donor, CMV/EBV/ADV/HHV6/BK
	16	HLH	19 y	10/10 - MUD	2	HHV6, BK	HHV6, BK viremia	None	HSCT donor, CMV/EBV/ADV/HHV6/BK
GOSH	17	CID/enterocolitis	5.9 y	10/10 MUD	5.3	EBV-LPD	EBV resolved	COP/rituximab	Donor EBV
	18	CID/enterocolitis	4.7 yr	10/10 MUD	3.7	None	NA	None	Donor EBV
	19	WAS	17.7 y	10/10 MUD	4.3	EBV-LPD	EBV resolved	Rituximab	Donor EBV
	20	XLP	12.4 y	9/10 MMUD	2.4	EBV	EBV resolved	Rituximab	Donor EBV
	21	XLP	11.8 y	9/10 MMUD	3.3	EBV	EBV viremia	None	Donor EBV
	22	XLP	8.7 y	9/10 MMUD	2.8	EBV	EBV resolved	Rituximab	Donor EBV
	23	XLP	11.8 y	9/10 MMUD	4	EBV-LPD	EBV viremia	COP/COPAD	Donor EBV
	24	XLP-like	5.4 y	9/10 MMUD	2.8	EBV	EBV resolved	Rituximab	Donor EBV
CNMC	25	ADA-SCID	NA	NA	Pretransplant (6 y old)	EBV, CMV	EBV-LPD	Rituximab, ganciclovir	Third party (MMUD, 5/10 match), CMV/EBV/ADV
	26	SCID, unknown gene	5 mo	MMRD (maternal 7/10)	1	CMV	CMV viremia	Ganciclovir, foscarnet, cidofovir	HSCT donor (7/10 match) CMV/EBV/ADV
	27	SCID, unknown gene	4 mo	MMRD (maternal 7/12)	1	CMV	CMV viremia	Foscarnet, cidofovir, Cytogam	HSCT donor, CMV/EBV/ADV
					2.5		CMV viremia and pneumonitis		Third-party (MMUD, 4/12 match), CMV/EBV/ADV
	28	MHC II deficiency	7 mo	MRD	2	None	NA	Acyclovir (ppx)	HSCT donor, CMV/EBV/ADV
	29	HLH (STXPB2)	4 y	8/10 MMUD	1	CMV, EBV	CMV, EBV viremia	Ganciclovir, rituximab	HSCT donor, CMV/EBV/ADV
	30	WAS	17 mo	9/10 MMUD	2	None	NA	None	HSCT donor, CMV/EBV/ADV
	31	IL-10R deficiency	10 mo	MMRD (maternal 6/8)	0.7	CMV, ADV, HBV	ADV viremia/pneumonia	Cidofovir, foscarnet	Third-party (paternal, 6/8 match), CMV/EBV/ADV
NCL	32	CGD	12 y	11/12 MMUD	27	BK, CMV, EBV (urine) HHV6, ADV (blood), EBV-LPD	EBV-LPD	Acyclovir, rituximab	Third-party (MMUD, 3/10 match), EBV
	33	CTPS1	15 mo	10/10 UCD	1 mo before HSCT	EBV (pretransplantation LPD), RSV, ADV	EBV-LPD	Acyclovir, rituximab	Third-party (MMUD, 9/10 match), EBV
	34	HLH	7 y	MUD	1.7	HHV6, EBV	EBV viremia	Foscarnet, acyclovir, rituximab	Third-party (MMUD, 5/10 match), EBV
	35	CID	3.7 y	9/10 MMUD (HLA-DQmm)	5	ADV HHV6, EBV-LPD	EBV-LPD	Acyclovir, cidofovir, rituximab, COP	Third-party (MMUD, 3/10 match) EBV
	36	CD4 lymphopenia	13 mo	MMRD (maternal 5/10)	8 d	Multidrug-resistant CMV	CMV-viremia	Ganciclovir, foscarnet, cidofovir	HSCT donor, CMV-selected cells

Rows corresponding to patients who were prophylactically treated with VSTs are shown in boldface.

aGVHD, Acute graft-versus-host disease; *BCG*, Bacillus-Calmette-Guerin; *BCM*, Baylor College of Medicine; *cGVHD*, chronic graft-versus-host disease; *CNMC*, Children's National Medical Center; *COP*, cyclophosphamide/vincristine/prednisone; *COPAD*, cyclophosphamide/vincristine/prednisolone/doxorubicin; *CR*, complete response; *CSA*, cyclosporin A; *CTPS1*, CTP synthase 1 deficiency; *ECP*, extracorporeal photopheresis; *FK*, tacrolimus; *GOSH*, Great Ormond Street Hospital; *IL-10R*, IL-10 receptor; *LPD*, lymphoproliferative disease; *MHC II*, MHC class II; *MMRD*, mismatched related donor; *MRD*, matched related donor; *MMF*, mycophenolate mofetil; *MMUD*, mismatched unrelated donor; *MUD*, matched unrelated donor; *NA*, not applicable; *NCL*, Newcastle Hospital; *NK*, natural killer cell; *NR*, no response; *PR*, partial response; *PTLD*, posttransplantation lymphoproliferative disease; *ppx*, prophylaxis; *RSV*, respiratory syncytial virus; *SCAEBV*, severe chronic active EBV; *SLAM*, signaling lymphocyte activation molecule; *UCB*, umbilical cord blood; *WAS*, Wiskott-Aldrich syndrome; *XLP*, X-linked lymphoproliferative disease.

*Viral infections/reactivations that occurred after VST infusion.

†As of December 31, 2014.

TABLE II. (Continued)

Immunosuppression at time of VST therapy	VST dose	Antiviral response	Adverse events	Follow-up time (y after HSCT) [†]	Current status	Study no./reference
CSA, prednisone	One dose of $1.5 \times 10E7/m^2$	No detectable EBV, ADV, CMV	None	2 y, 7 mo	Alive and well	NCT00880789/unpublished
CSA/prednisone	One dose of $2.5 \times 10E7/m^2$	No detectable EBV, ADV, CMV	None	2 y, 4 mo	Alive and well	NCT00880789/unpublished
MMF/prednisone	One dose of $1 \times 10E7/m^2$	No detectable EBV, ADV, CMV	None	3 y, 2 mo	Alive and well	NCT00880789/unpublished
None	One dose of $1 \times 10E7/m^2$	No detectable EBV, ADV, CMV	None	4 yr, 4 mo	Alive and well	NCT00078533/Leen et al ²⁰
CSA, prednisone	One dose of $1.35 \times 10E8/m^2$	No detectable ADV	None	11 mo	Alive and well	NCT00590083/Leen et al ²¹
CSA	One dose of $1 \times 10E7/m^2$	CMV, CR	None	2 y, 10 mo	Alive and well	NCT00078533/Leen et al ²⁰
None	One dose of $1 \times 10E7/m^2$	No detectable EBV, ADV, CMV	None	5 y, 3 mo	Alive and well	NCT00078533/Leen et al ²⁰
CSA	One dose of $2 \times 10E7/m^2$	EBV, CR	None	3 y, 9 mo	Alive and well	NCT00058812/Heslop et al ²⁴
None	One dose of $1 \times 10E8/m^2$	EBV, CR	None	4 y	Alive and well	NCT00058812/Heslop et al ²⁴
None	One dose of $2 \times 10E7/m^2$	EBV, PR	None	4 y, 6 mo	Died, progressive T-cell lymphoma	NCT00058812/Heslop et al ²⁴
None	One dose of $2 \times 10E7/m^2$	No further EBV reactivation	None	10 y, 3 mo	Alive and well	NCT00058812/Heslop et al ²⁴
CSA, prednisone	One dose of $2 \times 10E7/m^2$	EBV, CR	None	9 y, 5 mo	Alive and well	NCT00058812/Heslop et al ²⁴
CSA	One dose of $2 \times 10E7/m^2$	No detectable EBV	None	1 y, 6 mo	Alive and well	NCT00058812/Heslop et al ²⁴
FK	One dose of $2 \times 10E7/m^2$	ADV, CR	None	1 y, 7 mo	Alive and well	NCT01570283/Papadopolou et al ²³
FK/prednisone	One dose of $2 \times 10E7/m^2$	BK, CR; EBV, CR*	None	1 y, 11 mo	Alive and well	NCT01570283/Papadopolou et al ²³
MMF/prednisone	One dose of $1 \times 10E7/m^2$	HHV6, CR; BK, NR; EBV, CR*	None	1 y, 10 mo	Alive and well	NCT01570283/Papadopolou et al ²³
Prednisolone	One dose of $2.5 \times 10E7/m^2$	No further EBV reactivation	None	13 y, 1 mo	Alive and well	EIND/Heslop et al ²⁴
Methylprednisolone	One dose of $2.5 \times 10E7/m^2$	No detectable EBV	None	11 y, 2 mo	Alive and well	EIND/Heslop et al ²³
None	One dose of $2.5 \times 10E7/m^2$	No further EBV reactivation	None	13 y, 2 mo	Alive and well	EIND/Heslop et al ²⁴
CSA, MMF	One dose of $2.0 \times 10E7/m^2$	No further EBV reactivation	None	10 y, 6 mo	Alive and well	EIND/Heslop et al ²⁴
CSA	One dose of $2.0 \times 10E7/m^2$	EBV, PR	None	11 y	Alive and well	EIND/Heslop et al ²⁴
CSA	One dose of $2.0 \times 10E7/m^2$	EBV, CR*	None	10 y, 1 mo	Alive and well	EIND/Heslop et al ²⁴
CSA	One dose of $2.0 \times 10E7/m^2$	EBV, PR	None	9 y, 2 mo	Alive and well	EIND/Heslop et al ²⁴
CSA, prednisolone	One dose of $2.0 \times 10E7/m^2$	No further EBV reactivation	None	10 y, 1 mo	Alive and well	EIND/Heslop et al ²⁴
None	One dose of $5 \times 10E6/m^2$	EBV, NR	None	NA	Died, EBV lymphoma, D+110	NCT02510404/unpublished
MMF	Two doses of $5 \times 10E6/m^2$	CMV, PR	None	1 y	Alive and well	NCT01945814/unpublished
None	One dose of $1 \times 10E7/m^2$	CMV, NR	None	NA	Died, refractory CMV and disseminated BCG, D+92	NCT01945814/unpublished
None	One dose of $2 \times 10E7/m^2$	NA	NA	<2 wk		
CSA/prednisone	One dose of $5 \times 10E6/m^2$	CMV, CR*	aGVHD grade II, pericardial effusion	10 mo	Alive and well	NCT01945814/unpublished
FK, prednisone	Two doses of $1 \times 10E7/m^2$	CMV, CR; EBV, CR	Grade I cGVHD	4 mo	Alive, grade I cGVHD	NCT01945814/unpublished
FK/prednisone	One dose of $2 \times 10E7/m^2$	EBV, CR*	None	2 mo	Alive and well	NCT01945814/unpublished
None	One dose of $2 \times 10E7/m^2$	ADV, CR; CMV, CR*	Acute GVHD, grade II gut	5 mo	Alive, ongoing GVHD	EIND/unpublished
Methylprednisolone	Four doses of $2 \times 10E6/kg$ ($5.9 \times 10E7/m^2$)	EBV, NR	None	0.3	Died, PTLT	EIND/Vickers et al ²⁵
None, though received COP before VSTs*	Two doses of $2 \times 10E6/kg$ ($3.9 \times 10E7/m^2$)	EBV, CR	None	2.5 y	Alive and well	EIND/Vickers et al ²⁵
CSA/MMF/methylprednisolone/infliximab	Three doses of $2 \times 10E6/kg$ ($6.2 \times 10E7/m^2$)	EBV, PR	Cerebral edema	Died 26 d after first VST dose	Died, EBV-PTLD with cerebral edema, D+76	EIND/unpublished
Methylprednisolone/FK/infliximab/ECP	Four doses of $2 \times 10E6/kg$ ($4.6 \times 10E7/m^2$)	EBV, NR	None	NA	Died, PTLT	EIND/unpublished
CSA/MMF	One dose of $2 \times 10E6/kg$ ($3.8 \times 10E7/m^2$)	CMV, PR	GVHD, grade III skin	D+280	Died of fungal pneumonia, D+280	EIND/unpublished

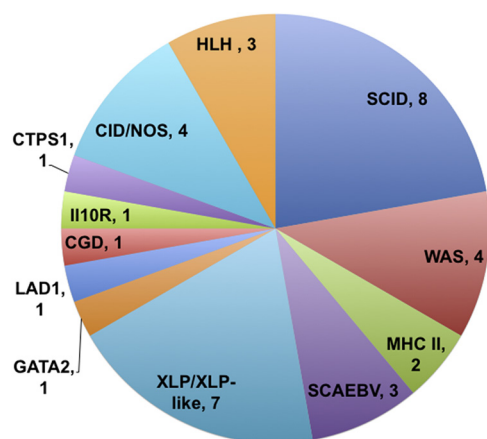


FIG 1. Underlying forms of PIDs in patients receiving VST therapy. *CGD*, Chronic granulomatous disease; *CID/NOS*, combined immunodeficiency, not otherwise specified; *CTPS1*, CTP synthase 1 deficiency; *HLH*, hemophagocytic lymphohistiocytosis; *LAD1*, leukocyte adhesion deficiency 1; *MHC II*, MHC class II; *SCAEBV*, severe chronic active EBV; *WAS*, Wiskott-Aldrich syndrome; *XLP*, X-linked lymphoproliferative disease.

treatment of CMV, EBV, and ADV infections after HSCT in more than 300 patients with both malignant and nonmalignant conditions treated in Europe, Australasia, and the United States. Although previously limited by the costs and specialization required for production, new protocols have reduced these barriers to VST production while widening the available donor pool for this therapy. Although early diagnosis of SCID and related T-cell immunodeficiencies through newborn screening has been very successful in permitting earlier HSCT and definitive treatment before development of many infections,²⁶ the effect of viral infections on survival even after HSCT remains substantial. Furthermore, newborn screening is not available universally and does not detect forms of PIDs that lack T-cell lymphocytopenia.

Here we describe the first and largest series of patients with PIDs who were treated with VST for the prevention or treatment of viral infections. Viral reduction or clearance was seen in the majority of patients treated, in spite of the failure of antiviral pharmacotherapy in many cases. Clinical benefit was seen in spite of low VST doses because the cells have been shown in these and other studies to be capable of expansion *in vivo* in the setting of viral infections, which has been demonstrable in prior studies using IFN- γ ELISpot analysis of peripheral blood of patients after VST infusion.^{20,27,28} Time to effect varied from 2 weeks to 3 months, with most responses seen within 1 month. Although this study was inadequately powered to evaluate the effect of cell dose on the likelihood or timing of antiviral efficacy, prior phase I trials have established that a VST dose of 2×10^6 /m² is typically well tolerated and effective.^{17,28,29} Clinical benefit was seen with VSTs derived from a variety of donors, including HSCT donors, cord blood donors, and third-party donors. VST therapy was successful in a patient with CTPS1 deficiency and EBV-LPD before transplantation (patient 33) who had clearance of disease after third-party VST therapy, after which he underwent successful umbilical cord blood transplantation. One other patient has been previously reported who was similarly treated successfully in the pretransplantation period,³⁰ suggesting that this might be a viable bridge therapy for patients with PIDs with refractory viral infections before HSCT. Recent studies of third party-derived VSTs after HSCT have demonstrated that

even partially matched VST lines are clinically effective, as long as antiviral activity is mediated through a shared MHC allele.^{17,18,31}

Most VST treatment failures occurred in patients with advanced viral disease, particularly in those with treatment-refractory EBV-LPD. Previous studies have shown that *in vivo* expansion is required for antiviral efficacy, which likely lessens the potential effect of VSTs in patients who are critically ill. Nonetheless, patient 31 had a rapid response to VST therapy in spite of advanced adenoviral lung disease requiring intensive care. Patient 27 did not improve after maternally derived VST therapy in spite of *in vitro* confirmation of antiviral restriction through shared MHC alleles. Viral escape in this case might have been due to variability within viral epitopes, or epitope recognition through nonshared MHC alleles.

Previous studies have demonstrated that different classes of immunosuppressive agents differentially affect T-cell proliferation and cytotoxicity. Calcineurin inhibitors have been shown to reduce proliferation but not cytotoxicity *in vitro*.³² This has been confirmed in clinical studies because VSTs have been successfully used to treat EBV-LPD after solid organ transplantation in spite of the use of calcineurin inhibitors.³³ Conversely, corticosteroids do affect T-cell function, and most VST protocols to date have excluded patients taking moderate-to-high steroid doses.

Adverse reactions to VST infusion were generally mild, which is in agreement with most prior studies. Four cases of GVHD were described, of which 3 were therapy responsive and more likely caused by HSCT than VST therapy. A recent review of adverse reactions to VST therapy showed that most toxicity was attributable to premedication (acetaminophen and diphenhydramine) rather than cellular therapy itself.³⁴ The central nervous system complication in patient 34, although highly concerning, was not definitively tied to VST therapy by histology or CSF cytology. One prior case of systemic inflammatory response syndrome was described in a patient with cancer with bulky tumor burden treated with EBV-VST.³⁵ One report of grade III “bystander” GVHD has been described after third-party VST therapy in a patient with adenoviremia,³⁶ but no similar events have occurred in any subsequent trials, including those using only partially HLA-matched VST.^{17,18} Nonetheless, caution is prudent when considering VST therapy for patients with advanced viral disease.

This study is subject to the many limitations of retrospective analyses. Although it represents the largest collection to date of patients with PIDs treated with VST therapy, it remains underpowered and heterogeneous, making it difficult to draw conclusions on optimal VST dosing or on the relative effectiveness of VST therapy versus antiviral pharmacotherapy. Additionally, previous studies of VST therapy have not used control groups to evaluate the effect on overall survival, although given the clear benefits of this therapy in prior trials, withholding therapy in an eligible patient would be ethically questionable.

VSTs represent a novel therapy for the prevention and treatment of viral infections for patients with PIDs before and after HSCT. Rapid advancements in production techniques promise to increase the availability of this treatment option in the coming years, whereas preclinical studies to target additional viruses, including respiratory syncytial virus and influenza, might further extend the usefulness of VST therapy.³⁷ Given the results of this study, a phase II trial of VST therapy for PIDs would be invaluable to determine whether this treatment affects long-term survival and antiviral pharmacotherapy needs. In time, both

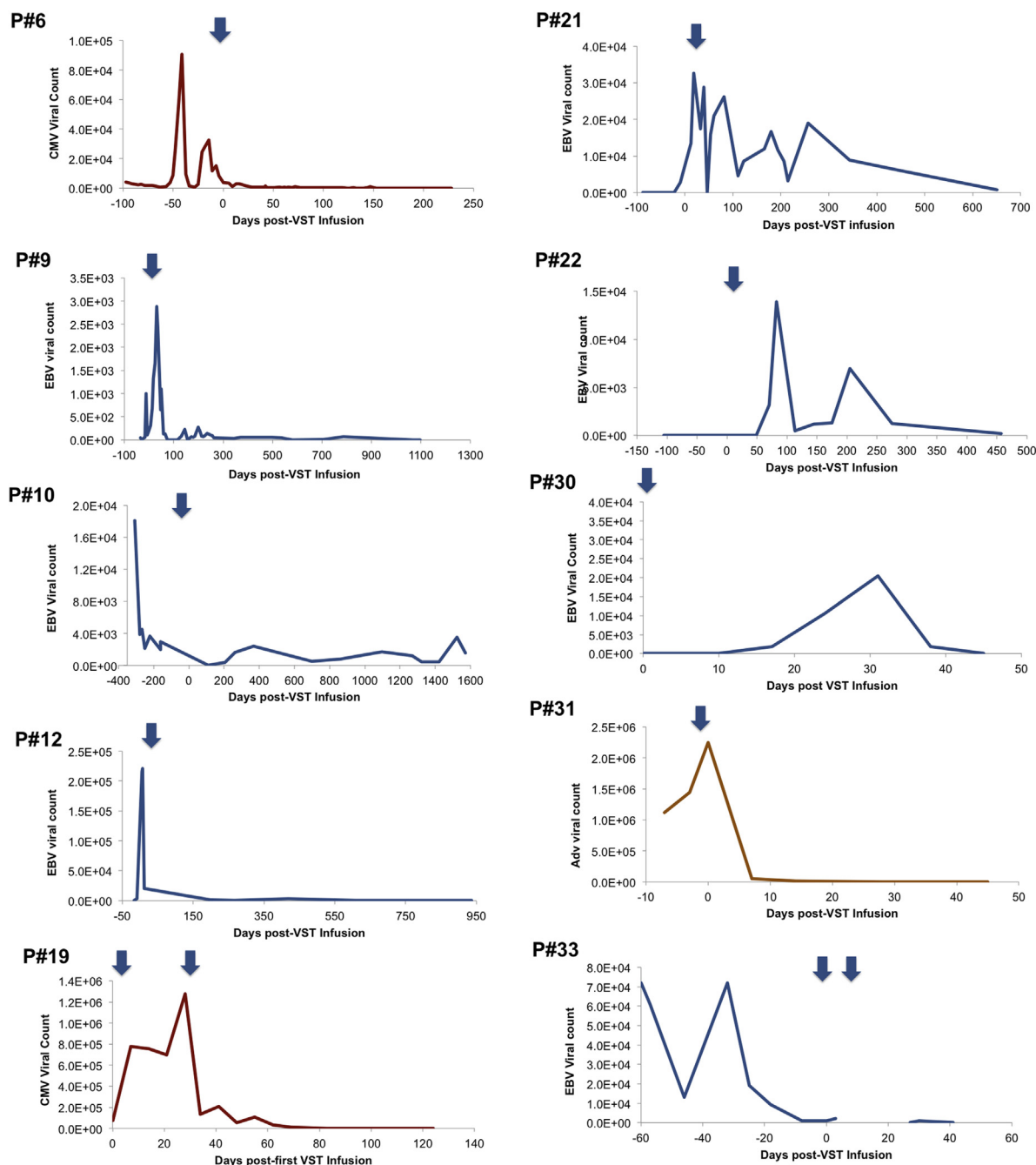


FIG 2. Antiviral responses to VST therapy. Representative plots of viral copy numbers (red, CMV; blue, EBV; and orange, ADV) over time are shown before and after VST infusions (noted as blue arrows).

HSCT donor-derived and “off-the-shelf” VST therapy might become the standard of care alongside antiviral pharmacotherapy for patients with PIDs.

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Clinical implications: Therapy with VSTs is associated with control or prevention of targeted viruses, has minimal associated toxicity in patients with PIDs, and could become a standard of care in patients with viral complications before or after HSCT.

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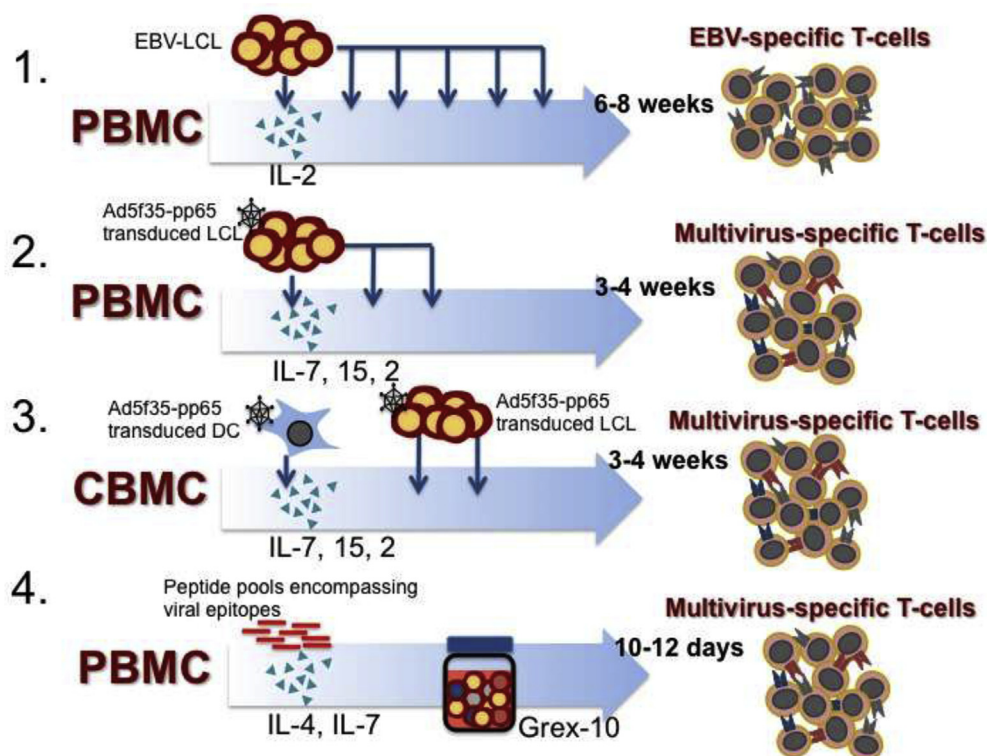


FIG E1. Manufacturing methods of virus-directed T cells. 1, EBV-specific T cells were produced by means of serial stimulation of PBMCs with donor-derived irradiated EBV-LCLs over 5 to 6 stimulations. 2, Multivirus-specific T cells were produced with EBV-LCLs that were virally transduced with a Ad5f35-pp65 vector for 3 stimulations. 3, Multivirus-specific T cells were produced from cord blood mononuclear cells (CBMC) using donor-derived dendritic cells (DC) that were virally transduced with a Ad5f35-pp65 vector, followed by 2 stimulations with Ad5f35-pp65-transduced irradiated EBV-LCLs. 4, Multivirus-specific T-cells were produced by using a rapid protocol with stimulation of PBMCs with peptide pools encompassing viral epitopes (against CMV, EBV, ADV, $\pm$ HHV6 and BK virus) in a single stimulation, followed by 10 to 12 days of culture in a G-Rex10 culture device (Wilson Wolf Manufacturing, New Brighton, Minn).