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Virus-Specific T-Cell Therapy for the Management of Viral Infections in the Immunocompromised

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Abstract

Background: Immunocompromised individuals are at major risk for severe infectious complications. This is particularly relevant in the context of allogeneic hematopoietic stem cell transplantation (allo-HCT) – a treatment modality that has proven curative for a range of malignant and nonmalignant hematological diseases. However, transplant-associated immune suppression leaves patients susceptible to infectious complications from viruses such as cytomegalovirus (CMV), adenovirus (AdV), Epstein-Barr virus (EBV), and BK virus (BKV). While pharmacological agents are available to prevent and/or treat some of these viruses, they can be associated with significant toxicities and are often ineffective. To circumvent these issues, several groups have explored the clinical potential of adoptively transferred virus-specific T cells (VSTs) to prevent/treat virus-associated complications after allo-HCT or solid organ transplantation (SOT) and this review will provide an overview of these endeavors. ***Summary:*** This review will focus on the progress that has been made over the past 30 years in the field of nonengineered VST manufacturing technologies and will summarize the clinical experience with VSTs, primarily in the posttransplant setting. ***Key Messages:*** Over the last 3 decades, adoptively transferred VSTs – both HCT donor and third party-derived – have been tested in numerous single and multicenter clinical trials and have unequivocally proven to be safe and associated with clinical activity.

Introduction

While allogeneic hematopoietic stem cell transplant (allo-HCT) is increasingly available to treat and cure a number of malignant and nonmalignant hematological diseases, opportunistic viral infections post-HCT are a growing threat to patient health and remain responsible for significant morbidity and mortality [1]. This is due in part to more comprehensive patient screening using improved molecular detection methods, but also to the extension of HCT to higher-risk patients (i.e., individuals without a human leukocyte antigen [HLA]-matched sibling donor) who receive more extensively manipulated products (e.g., T cell-depleted grafts, cord blood units [CBUs]) and/or prolonged immunosuppression. Infection or reactivation of latent viruses including CMV, EBV, human herpes virus 6 (HHV-6), BKV, and JC virus (JCV) is frequent, whereas infections associated with an array of community-acquired respiratory viruses, such as AdV, influenza, parainfluenza, human metapneumovirus, respiratory syncytial virus (RSV), and severe acute

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respiratory syndrome coronavirus 2 (SARS-CoV-2) are increasingly reported [2–12]. Current standard of care for some of these infections involves antiviral drugs; however, they are associated with significant side effects (e.g., nephrotoxicity and hepatotoxicity), are not always effective, and can lead to the emergence of resistant viral strains.

In response to these challenges, T-cell therapies that restore virus-specific immunity in the HCT setting have been developed as a viable alternative. In the current review, we describe immunotherapeutic strategies with nonengineered virus-specific T cells (VSTs) that have been clinically used to prevent and/or treat viral infections in both adult and pediatric HCT patients. We also briefly touch upon the clinical application of VST therapy beyond the allo-HCT setting.

Donor Lymphocyte Infusion

The first immunotherapeutic approach utilized in the allo-HCT setting involved the adoptive transfer of unmanipulated donor lymphocytes – termed donor lymphocyte infusions (DLIs) [13, 14]. DLI therapy is based on the premise that unmanipulated lymphocytes isolated from HCT donors with prior exposure to clinically problematic viruses (i.e., seropositive donors) should contain VST populations capable of expanding in vivo (in patients) and providing antiviral protection. DLIs have proven effective in treating EBV-associated posttransplant lymphoproliferative disease (EBV-PTLD), AdV-associated hemorrhagic cystitis, CMV reactivation/infection, HHV-6 encephalitis, and persistent RSV pneumonia [15–19]. However, the efficacy of this approach is constrained by the low frequencies of memory VSTs in peripheral blood (particularly so in the case of lytic [acute] viruses) compared with the considerably higher frequency of alloreactive T cells that are present in the circulation and which can cause graft-versus-host disease (GvHD). In fact, up to 35% of patients infused with DLIs at doses ranging from 2.2 to 7.6 $\times$ 10^8 mononuclear cells/kg have been reported to develop GvHD grade ≥3, thereby precluding broad implementation of this approach to mediate antiviral effects [13, 20–24]. Nevertheless, these proof-of-concept studies demonstrated the potential of adoptively transferred VSTs as a treatment strategy.

Several approaches have been investigated in order to preserve antiviral memory T-cell activity while reducing the risk of GvHD induction. Given that the naive T-cell subset has been identified as the primary driver of GvHD, selective depletion of naive T cells (via immunomagnetic removal of CD45RA positive cells) from DLIs has been implemented as a means of limiting alloreactive T-cell responses. Indeed, the safety and feasibility of administering naive T-cell depleted DLIs following allo-HCT have been demonstrated by numerous groups in both the HLA-matched [25–27] and haploidentical [28–30] HCT setting. Thus far, infusion of CD45RA-depleted, memory T cell-enriched DLIs has been associated with a low incidence of acute and chronic GvHD in treated patients, with preliminary indications of improved antiviral protection [28–30].

In the same aim of preserving the benefits and minimizing the risks associated with DLI products, investigators have developed additional strategies to directly select and/or selectively expand virus-reactive T-cell populations so as to achieve VST enrichment, with a consequent reduction in alloreactive T cells. These strategies will be the primary focus of the current review.

Direct Isolation of Virus-Specific T Cells

To maximize clinical benefits while minimizing the risk of GvHD, a number of groups have sought to directly isolate circulating VSTs for immediate infusion to patients as a strategy to provide antiviral protection post-HCT. To date, two direct isolation methods have been tested clinically: multimer selection and IFNγ capture (Fig. 1).

Multimer Selection

Multimers are complexes of peptide-loaded HLA molecules that are fluorescently or magnetically labeled in order to allow for specific selection of T cells with reactivity against the presented peptide. The first clinical application of this approach was by Cobbold et al. [31] for the treatment of post-HCT CMV reactivation. Using a panel of CD8+ tetramers (complexes of four peptide-loaded HLA molecules) directed against pp65 and IE-1 peptides and restricted to HLA-A*0101, HLA-A*0201, HLA-B*0702, HLA-B*0801, and HLA-B*3502, they selected CMV-reactive T cells from stem cell donor (SCD) peripheral blood and infused them to nine HCT recipients within 4 h of selection. Despite the low numbers of selected CD8+ cells (median 8.6 $\times$ 10^3/kg) infused, there was an impressive in vivo expansion of CMV-specific T cells of up to 250-fold, leading to viral clearance in eight of nine cases with no infusion-related toxicity or de novo GvHD. Subsequently, Uhlin et al. [32] used higher avidity pentamers (complexes of five HLA molecules) to isolate EBV-, CMV-, and AdV-specific CD8+ VSTs from frozen donor grafts (n = 2) or haploidentical (haplo) third-party peripheral blood (n = 6). The selected cells (0.8–24.6 $\times$ 10^4/kg) were safely infused to 8 HCT patients with EBV-PTLD (n = 1), CMV reactivation (n = 6), or AdV infection (n = 1), with clinical improvement seen within 2 weeks in both patients who received SCD-derived cells and in 4 of the 6 patients treated with third-party cells.

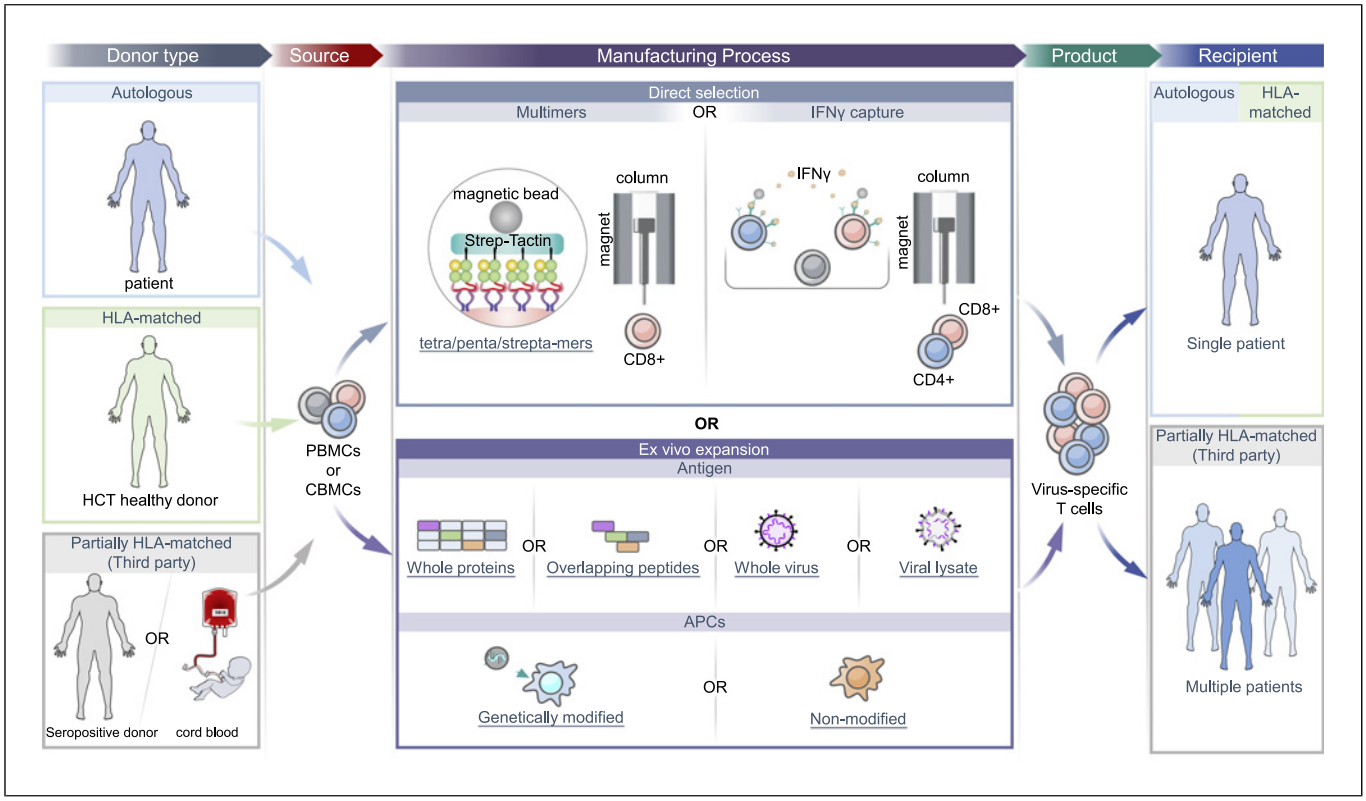

Fig. 1. Strategies for direct selection or ex vivo expansion of VSTs. There are four different types of blood donors suitable for the direct selection or ex vivo generation of VSTs. In an individualized approach, the patient or the patient's HLA-matched HCT donor can serve as donors. In a broader, off-the-shelf approach, seropositive healthy individuals or CBUs can serve as third-party donors. In all cases, isolated PBMCs or CBMCs constitute the source material for the VST products. PBMCs/CBMCs can be enriched for T cells with the desired antiviral specificities using different manufacturing strategies such as (i) direct VST isolation with multimers/streptamers or IFNγ capture and (ii) ex vivo VST expansion via stimulation with APCs (nonengineered or engineered) pulsed with various types of antigens (viral proteins/peptides, whole viruses, viral lysates). Depending on the donor type, the recipients of the VSTs can be a single patient (receiving an autologous or HLA-matched product – individualized setting) or multiple patients (receiving a partially HLA-matched product – third-party setting). HCT, hematopoietic stem cell transplant; PBMCs, peripheral blood mononuclear cells; CBMCs, cord blood mononuclear cells; HLA, human leukocyte antigen; APCs, antigen-presenting cells.

Despite these successful proof-of-concept studies, clinical concern has been raised around tetramer/pentamer use due to their irreversible binding to T cells that could potentially lead to unwanted immune responses or in vivo toxicity. In addition, several investigators have reported alterations in T-cell phenotype following multimer binding with the induction of tolerance or even apoptosis [33–35]. To address these issues, Knabel et al. [33] developed a next-generation multimeric complex – termed the streptamer. Streptamers are HLA-peptide complexes reversibly multimerized with magnetic beads and can thus be easily dissociated from T cells prior to patient administration, leaving the selected populations phenotypically and functionally identical to unmanipulated cells [36]. SCD-derived streptamer-selected CD8+ T cells were first administered by Schmitt et al. [37] to treat two adult-matched unrelated donor (MUD) HCT patients with refractory CMV infections; in both cases, the infusions (2.2×10^5/kg and 0.37×10^5/kg, respectively) were safe and resulted in prolonged viral clearance. Stemberger et al. [38] subsequently used the same approach to successfully treat two pediatric haplo-HCT recipients with refractory CMV, while Neuenhahn et al. [39] reported on the results of a prospective phase I/IIa trial using streptamers to select and administer (within 24 h of selection) CMV-reactive CD8+ T cells to 16 allo-HCT recipients with persistent CMV viremia and lack of endogenous CMV-specific immunity. The infused cells ($0.78–872 \times 10^3$/kg) were either SCD-derived ($n = 8$) or third-party donor-derived ($n = 8$), and infusions were well tolerated with minimal acute/chronic GvHD (2/16 patients over 6 months). However, while clinical responses were achieved with both SCD-derived (five CR, two PR) and third-party cells (four CR, one PR), the authors emphasized on the challenges in identifying appropriately partially HLA-matched third-party donors, resulting in delayed VST administration by an extra 42 days (median 18.5 vs. 60.5-day interval, stem vs. third-party donors).

Table 1. Overview of clinical studies using VSTs

Derivation	Manufacturing	Target	*N*	Prophylaxis or treatment	Clinical outcomes	Safety	Year	Reference
Donor	Ex vivo expansion	CMV	3	Treatment	3/3 PR	No adverse events	1992	Riddell et al. [41]
Donor	Ex vivo expansion	CMV	14	Prophylaxis	14/14 CR	3/14 GvHD grade I–II	1995	Walter et al. [42]
Donor	Ex vivo expansion	CMV	8	Treatment	6/8 CR, 1/8 PR, 1/8 NR	0/8 GvHD, no toxicities	2002	Einsele et al. [43]
Donor	Ex vivo expansion	CMV	16	Prophylaxis	14/12 CR, 2/16 reactivated	2/16 de novo GvHD grade I	2003	Peggs et al. [44]
Donor	Tetramer selection	CMV	9	Treatment	8/9 CR, 1/9 PR	0/9 de novo GvHD	2005	Cobbold et al. [31]
Donor	Ex vivo expansion	CMV	9	Prophylaxis	7/9 CR, 2/9 reactivated	0/9 de novo aGvHD, 2/9 cGvHD	2007	Micklethwaite et al. [45]
Donor	Ex vivo expansion	CMV	12	Prophylaxis	8/12 CR, 4/12 reactivated	4/12 GvHD grade II–III	2008	Micklethwaite et al. [46]
Donor	Ex vivo expansion	CMV	30	Prophylaxis	27/27 CR	7/30 aGvHD grade I, 4/30 aGvHD grade II–III, 12/28 cGvHD	2009	Peggs et al. [47]
Donor	IFNγ capture	CMV	18	Treatment	9/18 CR, 6/18 PR, 3/18 NR	0/16 de novo GvHD; 0/2 flare GvHD	2010	Feuchtinger et al. [48]
Donor	IFNγ capture	CMV	18	Prophylaxis/ treatment	Prophylaxis: 1/7 reactivated; treatment: 11/11 cleared CMV	3/18 aGvHD grade II–III, 3/18 extensive cGvHD	2011	Peggs et al. [49]
Donor	Streptamer selection	CMV	2	Treatment	2/2 CR	0/2 GvHD, no toxicities	2011	Schmitt et al. [37]
Donor	Ex vivo expansion	CMV	7	Treatment	4/7 CR, 2/7 PR, 1/7 NR	0/7 aGvHD, 1/7 cGvHD pre-infusion, no toxicities	2012	Bao et al. [50]
Donor or patient	IFNγ capture	CMV	6	Treatment	6/6 CR	0/6 GvHD, no toxicities	2012	Meij et al. [51]
Donor	Ex vivo expansion	CMV	50	Prophylaxis	26/50 reactivated-9/26 required antivirals, 5/26 reactivated post infusion	12/50 aGvHD grade II–IV, 1 CMV-related death	2013	Blyth et al. [52]
Donor	Streptamer selection	CMV	2	Treatment	2/2 CR	0/2 GvHD, no toxicities	2014	Stemberger et al. [38]
Donor	Ex vivo expansion	CMV	16	Treatment	13/16 CR, 1/16 PR, 2/16 NR	0/14 de novo GvHD; 0/2 flare GvHD No adverse events	2015	Koehne et al. [53]
Donor	Ex vivo expansion	CMV	32	Treatment	27/32 CR within 4 weeks post-infusion	1/32 GvHD grade II (grade I GvHD pre-infusion)	2017	Pei et al. [54]

Table 1 (continued)

Derivation	Manufacturing	Target	*N*	Prophylaxis or treatment	Clinical outcomes	Safety	Year	Reference
Donor	Ex vivo expansion/DC vaccine	CMV	4	Prophylaxis	3/4 CR	1/4 aGvHD grade III, 3/4 cGvHD	2018	Ma et al. [55]
Donor or third party	Streptamer selection	CMV	16	Treatment	9/16 CR, 3/16 PR, 4/16 NR	1/16 aGvHD; 1/16 cGvHD	2017	Neuenhahn et al. [39]
Third party	Ex vivo expansion	CMV	10	Treatment	9/10 CR, 1/10 PR	0/10 GvHD	2019	Tzannou et al. [56]
Third party	IFNγ capture	CMV	2	Treatment	2/2 CR	No adverse events	2019	Alonso et al. [57]
Third party	Ex vivo expansion	CMV	68	Treatment	20/59 CR, 18/59 PR	1/67 de novo GvHD grade III; 9/67 adverse events grade III–V	2023	Prockop et al. [58]
Donor	Ex vivo expansion	EBV	3	Treatment	3/3 CR	N/A	1995	Rooney et al. [59]
Donor	Ex vivo expansion	EBV	12	Treatment	6/6 increased anti-EBV activity, 3/3 increased EBV CTL precursors	N/A	1996	Heslop et al. [60]
Donor	Ex vivo expansion	EBV	39	Prophylaxis/ treatment	Prophylaxis: 37/37 CR; treatment: 2/ 2 CR	No adverse events	1998	Rooney et al. [61]
Donor	Ex vivo expansion	EBV	6	Treatment	5/6 PR, 1/6 NR	1/6 aGvHD	2000	Gustafsson et al. [62]
Third party	Ex vivo expansion	EBV	1	Treatment	1/1 CR	No adverse events	2001	Haque et al. [63]
Third party	Ex vivo expansion	EBV	5	Treatment	3/5 CR, 2/5 NR	0/5 GvHD	2002	Haque et al. [64]
Donor or third party	Ex vivo expansion	EBV	4	Treatment	2/4 CR, 1/4 PR, 1/ 4 PD	0/4 GvHD	2002	Sun et al. [65]
Patient	Ex vivo expansion	EBV	12	Prophylaxis/ treatment	Prophylaxis: 10/10 CR; treatment: 2/ 2 PR	No adverse events	2006	Savoldo et al. [66]
Donor	Ex vivo expansion	EBV	4	Treatment	4/4 CR	No adverse events	2007	Comoli et al. [67]
Third party	Ex vivo expansion	EBV	33	Treatment	14/33 CR, 3/33 PR, 16/33 NR	No adverse events	2007	Haque et al. [68]
Donor	Ex vivo expansion	EBV	6	Treatment	6/6 CR	0/6 GvHD, no adverse events	2008	Comoli et al. [69]
Third party	Ex vivo expansion	EBV	2	Treatment	2/2 CR	No adverse events	2010	Barker et al. [70]
Donor	IFNγ capture	EBV	6	Treatment	3/6 CR (early disease pre-infusion), 3/6 NR (advanced disease pre-infusion)	No adverse events	2010	Moosman et al. [71]

Table 1 (continued)

Derivation	Manufacturing	Target	*N*	Prophylaxis or treatment	Clinical outcomes	Safety	Year	Reference
Donor	Ex vivo expansion	EBV	19	Treatment	13/19 CR, 6/19 NR	0/19 de novo GvHD or flare GvHD	2012	Doubrovina et al. [72]
Donor	Ex vivo expansion	EBV	114	Prophylaxis/ treatment	Prophylaxis: 101/ 101 CR; treatment: 11/13 CR, 2/NR	8/114 aGvHD grade I–II, 13/ 114 cGvHD	2010	Heslop et al. [73]
Donor	IFNγ capture	EBV	10	Treatment	3/10 CR, 3/10 PR, 4/10 NR	1/10 aGvhD grade I–II	2012	Icheva et al. [74]
Third party	Ex vivo expansion	EBV	11	Treatment	3/11 CR, 1/11 PR, 7/11 NR	0/11 GvHD	2014	Gallot et al. [75]
Third party	Ex vivo expansion	EBV	11	Treatment	8/11 CR, 1/11 PR, 2/11 NR	1/11 GvHD grade I	2014	Vickers et al. [76]
Third party	Ex vivo expansion	EBV	10	Treatment	7/10 CR, 1/10 PR, 2/10 NR	0/10 GvHD	2018	Chiou et al. [77]
Third party	Ex vivo expansion	EBV	1	Treatment	1/1 CR	No adverse events	2018	Schultze-Florey et al. [78]
Third party	Ex vivo expansion	EBV	59	Treatment	HSCT: 8/28 CR, 5/ 28 PR, 15/28 NR; SOT: 10/20 CR, 5/ 20 PR, 5/20 NR; other: 5/11 CR, 2/ 11 PR, 4/11 NR	2/59 mild GvHD	2019	Kazi et al. [79]
Third party	Ex vivo expansion	EBV	46	Treatment	SOT: 2/13 CR, 5/13 PR, 1/13 SD, 5/13 PD; HCST: 19/33 CR, 3/33 PR, 1/33 SD, 9/33 PD	1/46 de novo GvHD grade I, no adverse events	2020	Prockop et al. [80]
Donor or third party	IFNγ capture	EBV	20	Treatment	Third party: 6/10 CR, 1/10 SD, 1/10 PD; donor: 9/10 CR, 1/10 PD	2/10 aGvHS grade I–II, 1/ 10 GvHD grade III	2023	Bonifacius et al. [81]
Third party	Ex vivo expansion	EBV	15	Treatment	Newly diagnosed: 4/10 CR, 3/10 PR, 3/10 NR; R/R: 1/5 SD, 4/5 PD	4/15 Grade I or II fever	2024	Wistinghausen et al. [82]
Third party	IFNγ capture	AdV	1	Treatment	1/1 CR	1/1 GvHD grade III–IV	2011	Qasim et al. [83]
Donor	Ex vivo expansion	AdV	2	Treatment	1/2 CR, 1/2 NR	1/2 GvHD grade IV	2014	Geyerreger et al. [84]
Donor	IFNγ capture	AdV	1	Treatment	1/1 CR	No adverse events	2014	Nardo et al. [85]
Donor	IFNγ capture	AdV	30	Treatment	18/30 CR, 3/30 PR, 8/30 NR	2/30 aGvHD grade I	2015	Feucht et al. [86]
Third party or cord blood	IFNγ capture	AdV	11	Treatment	10/11 CR	0/11 de novo GvHD	2017	Qian et al. [87]
Donor	Ex vivo expansion	AdV	8	Treatment	8/8 CR	1/8 aGvHD, 3/ 8 SAEs	2018	Ip et al. [88]
Donor	IFNγ capture	AdV	9	Treatment	4/6 CR, 1/6 PR, 1/ 6 NR	1/6 aGvHD grade II	2006	Feuchtinger et al. [89]

Table 1 (continued)

Derivation	Manufacturing	Target	*N*	Prophylaxis or treatment	Clinical outcomes	Safety	Year	Reference
Donor	Ex vivo expansion	JCV	1	Treatment	1/1 PR	No adverse events	2010	Balduzzi et al. [90]
Donor	IFNγ capture	JCV	1	Treatment	1/1 CR	No adverse events	2020	Steinhardt et al. [91]
Donor or patient	Ex vivo expansion	JCV	9	Treatment	5/9 long term survival, 3/9 died PML progression, 1/9 died VZV infection	No adverse events	2021	Berzero et al. [92]
Donor	Ex vivo expansion	CMV or AF	35	Treatment	AF: 9/10 CR; CMV: 18/25 no reactivation	1/35 aGvHD grade II	2005	Perruccio et al. [93]
Donor	Ex vivo expansion	CMV, EBV, AdV	11	Prophylaxis/ treatment	9/9 CR of patients with reactivations	0/11 GvHD, no adverse events	2006	Leen et al. [94]
Donor	Ex vivo expansion	CMV or EBV	3	Treatment	3/3 CR	1/3 aGvHD grade II	2010	Dong et al. [95]
Donor	Ex vivo expansion	EBV, AdV	13	Prophylaxis/ treatment	5/5 CR of patients with reactivations	0/13 GvHD, no adverse events	2009	Leen et al. [96]
Donor or third party	Pentamer selection	CMV or EBV or AdV	8	Treatment	5/8 CR, 1/8 PR, 2/8 NR	N/A	2012	Uhlin et al. [32]
Third party	Ex vivo expansion	CMV or EBV or AdV	50	Treatment	31/45 CR, 6/45 PR, 8/45 NR	2/50 de novo GvHD grade I	2013	Leen et al. [97]
Donor	Ex vivo expansion	CMV, EBV, AdV	10	Treatment	11 CR, 1 PR, 1 PD	1/10 GvHD grade I–II	2013	Gerdemann et al. [98]
Donor	Ex vivo expansion	CMV, EBV, AdV, VZV	10	Prophylaxis/ treatment	Prophylaxis: 18/18 no reactivation; treatment: 2/2 CR	No adverse events	2015	Ma et al. [99]
Donor	Ex vivo expansion	CMV, EBV, AdV, BK, HHV6	11	Prophylaxis/ treatment	15 CR, 2 PR, 1 NR	1/11 GvHD grade II	2014	Papadopoulou et al. [100]
Donor or third party or cord blood	Ex vivo expansion	CMV, EBV, AdV, BK, HHV6	36	Prophylaxis/ treatment	Prophylaxis: 20/20 no reactivation; treatment: 22/33 CR, 6/33 PR, 5/33 NR	3/36 aGvHD grade II–III, 1/36 cGvHD grade I	2016	Naik et al. [101]
Donor	IFNγ capture	CMV or AdV	15	Treatment	6/13 CR, 1/13 PR, 6/13 NR	1/13 GvHD grade III, 6/13 SAEs	2016	Creidy et al. [102]
Third party	Ex vivo expansion	CMV or EBV or AdV	30	Treatment	23/30 CR, 5/30 PR, 2/30 NR	2/30 aGvHD grade II–IV; 3/30 mild-moderate cGvHD, 2/30 severe cGvHD	2017	Withers et al. [103]
Third party	Ex vivo expansion	CMV, EBV, AdV, BKV, HHV6	38	Treatment	35 CR, 8 PR, 2 NR	3/38 de novo GvHD	2017	Tzannou et al. [104]
Donor	IFNγ capture	CMV or EBV or AdV	9	Treatment	11 CR, 1 PR	1/9 CRS, 0/9 de novo GvHD	2018	Kallay et al. [105]

Table 1 (continued)

Derivation	Manufacturing	Target	*N*	Prophylaxis or treatment	Clinical outcomes	Safety	Year	Reference
Cord blood	Ex vivo expansion	CMV, EBV, AdV	14	Prophylaxis/ treatment	6/7 CR (of patients with reactivations)	1/14 GvHD	2019	Abraham et al. [106]
Donor or third party	Ex vivo expansion	CMV, EBV, AdV, BKV	41	Treatment	SOT 2/2 CR; HSCT: 26/38 CR, 7/38 PR, 5/38 NR	1/41 aGvHD	2020	Nelson et al. [107]
Donor or third party	Ex vivo expansion	CMV, EBV, AdV, BK	26	Treatment	15/26 CR, 6/26 PR, 5/26 NR	1/26 GvHD grade II	2021	Rubinstein et al. [108]
Donor	Ex vivo expansion	CMV, EBV, AdV, BKV, VZV, AF, Influenza	11	Prophylaxis	53/57 CR	3/11 aGvHD grade I–II, 4/ 11 aGvHD grade III–IV, 2/ 11 cGvHD	2021	Gottllieb et al. [109]
Third party	CD137– selection/ex vivo expansion	CMV or EBV	30	Treatment	31/33 CR, 2/33 PR	2/30 aGvHD grade IV	2022	Jiang et al. [110]
Donor	Ex vivo expansion	CMV, EBV, AdV, BKV	23	Prophylaxis	18/23 CR, 5/23 NR	2/23 GvHD grade II–III	2022	Rubinstein et al. [111]
Third party	Ex vivo expansion	CMV, EBV, AdV, BKV, HHV6, JCV	58	Treatment	17 CR, 49 PR, 4 NR	2/58 aGvHD grade II, 1/58 aGvHD grade III	2023	Pfeiffer et al. [112]
Donor or third party or cord blood	Ex vivo expansion	CMV, EBV, BK, HHV6, HPIV3	12	Prophylaxis/ treatment	Prophylaxis: 6/7 CR; treatment: 4/5 CR, 1/5 PR	2/12 aGvHD grade I–II, 2/ 12 aGvHD grade III–IV, 4/ 12 cGvHD	2023	Kinoshita et al. [113]
Donor or third party	Ex vivo expansion	CMV, EBV, AdV, BK	145	Treatment	Third party: 63/96 CR in 77 patients, donor: 47/75 CR in 68 patients	6/145 aGvHD grade II–IV, 7/ 145 cGvHD	2023	Galletta et al. [114]
Donor	IFNγ capture	BKV	1	Treatment	1/1 CR	No adverse events	2017	Pello et al. [115]
Third party	Ex vivo expansion	BKV	3	Treatment	2/3 PR, 1/3 SD	No adverse events	2018	Muftuoglu et al. [116]
Third party	Ex vivo expansion	BKV	1	Treatment	1/1 CR	No adverse events	2020	Jahan et al. [117]
Third party	Ex vivo expansion	BKV	59	Treatment	34/49 CR; 6/49 PR; 9/49 NR	1/59 aGvHD grade II, 1/59 aGvHD grade III, 9/59 cGvHD	2021	Olson et al. [118]
Third party	IFNγ capture	BKV	2	Treatment	2/2 PR	N/A	2021	Hopfner et al. [119]
Third party	Ex vivo expansion	BKV	12	Treatment	5/12 CR, 2/11 PR, 5/11 NR	No adverse events	2021	Cortese et al. [120]
Third party	Ex vivo expansion	AdV	4	Treatment	1/4 CR, 3/4 PR	0/4 GvHD	2021	Rubinstein et al. [108]
Third party	Ex vivo expansion	BKV	1	Treatment	1/1 NR	1/1 cytokine release syndrome	2021	Holland et al. [121]

Table 1 (continued)

Derivation	Manufacturing	Target	*N*	Prophylaxis or treatment	Clinical outcomes	Safety	Year	Reference
Third party	Ex vivo expansion	SARS-CoV-2	1	Treatment	1/1 CR	No adverse events	2022	Martits-Chalangari et al. [122]
Third party	Ex vivo expansion	SARS-CoV-2	58	Treatment	SARS-CoV-2-STs: recovery rate 65%; SoC: recovery rate 38%	SARS-CoV-2-STs versus SoC: no increase in adverse events	2023	Papadopoulou et al. [123]
Third party	Memory T-cell selection	SARS-CoV-2	39	Treatment	SARS-CoV-2-STs: recovery rate 74%; SoC: recovery rate 47.5%	No adverse events	2024	Ferreras et al. [124]
Third party	Ex vivo expansion	SARS-CoV-2	4	Treatment	3/4 CR, 1/4 PR	No adverse events	2023	Vasileiou et al. [125]
Third party	Ex vivo expansion	SARS-CoV-2	6	Treatment	3/6 CR, 3/6 PR	No adverse events	2023	Haidar et al. [126]
Donor	Streptamer selection	CMV, EBV, ADV, TAA (PRAME, NY-ESO-1, WT1, RHAMM, proteinase 3)	24	Prophylaxis	CMV: 23/24 CR, 1/24 PR; EBV: 23/24 CR, 1/24 PR; ADV: 24/24 C; TAA: 5/24 PD	2/24 aGvHD grade I	2019	Roex et al. [40]
Donor	Ex vivo expansion	CMV, EBV, ADV, AF, or WT1, PRAME	10	Prophylaxis	7/10 CR	2/10 aGvHD, 2/10 cGvHD	2023	Jiang et al. [127]

AdV, adenovirus; AF, *Aspergillus fumigatus*; BKV, BK virus; CMV, cytomegalovirus; HHV6, human herpesvirus 6; JCV, JC virus; VZV, varicella-zoster virus; HPIV3, human parainfluenza virus type 3; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TAA, tumor-associated antigens; PRAME, preferentially expressed antigen of melanoma; WT1, Wilms tumor 1; RHAMM, receptor for hyaluronan-mediated motility; CR, complete response; PR, partial response; NR, no response; PD, progressive disease; SD, stable disease; SOT, solid organ transplantation; HSCT, hematopoietic stem cell transplantation; aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; SAE, serious adverse event; *N*, number of infused patients.

This highlights the importance of establishing an extensive donor registry to support rapid third-party VST preparation.

SCD-derived streptamer-selected cells have also been tested in the prophylactic setting by Roex et al. [40], who infused 24 recipients of T-cell depleted allo-HCT with isolated CD8+ T cells directed against CMV, EBV, and AdV in a phase I/II study aiming to reduce the risk of viral complications post-HCT. Infusions were safe (median 5.2×10^6 total cells infused) and well tolerated with no occurrence of GvHD. The infused cells provided antiviral protection, as only one-third (8 of 24) of patients experienced CMV and/or EBV reactivations, four of which were clinically relevant and two progressed to viral disease that ultimately resolved. Importantly, in the majority of patients, viral reactivations and subsequent clearance coincided with the expansion of SCD-derived VSTs.

Overall, these studies established the feasibility, efficacy, and safety of administering multimer/streptamer-selected VSTs of high purity to patients with refractory infections post allo-HCT (Table 1). It should be noted though that this approach can only be applied to immunologically well-characterized viruses with known antigenic epitopes and to individuals of particular HLA haplotypes; in addition, it has been restricted to CD8+ T cells, thereby limiting its wider applicability.

IFNγ Capture

The IFNγ capture approach represents an alternative selection method in which antigen-stimulated, IFNγ-producing cells can be isolated using magnetic beads and are then adoptively transferred to patients. The advantage of this approach over streptamer selection is that it enables the isolation of both CD4+ and CD8+ antigen-specific T cells if whole antigen sources (e.g.,

overlapping peptide libraries) are used as a stimulus and is thus not restricted to specific HLA haplotypes or peptides. Feuchtinger et al. [89] first utilized IFNγ-captured AdV-reactive T cells to treat nine pediatric HCT recipients, all of whom had systemic AdV infections (n = 2) or disease (n = 7). The infusions (ranging from 1.2 to 50 × 10^3 cells/kg) were well tolerated, with a single report of skin chronic GvHD aggravation, and led to control of infections/disease in 5 of the 6 evaluable patients. The same group later treated 18 HCT patients [48], both adult and pediatric, with refractory CMV infections (n = 10) or disease (n = 8) using pp65-directed IFNγ-captured T cells. The infused cells were well tolerated, expanded in vivo, and achieved clinical responses in 15 of 18 patients that either cleared or had significant reduction (>1 log) in their viral loads, including 2 patients with CMV encephalitis. Similar observations were made by Peggs et al. [49] who used CMV-captured T cells in a phase I/II clinical trial, where the cells provided antiviral protection in both the prophylactic (7 of 8 patients) and preemptive setting (9 of 11 patients).

EBV reactivations/disease have also been shown to be sensitive to this therapeutic modality. Moosmann et al. [71] utilized EBV-captured T cells (0.4–9.7 × 10^4/kg) from donor peripheral blood leukaphereses to treat six adults with biopsy-proven EBV-PTLD that was unresponsive to conventional treatment. Notably, clinical responses to the infused cells were directly associated with disease stage; in vivo expansion of the transferred cells and complete remissions were observed in 3 patients with early-stage disease showing relatively mild symptoms (including lymphadenopathy, fever, and tonsillitis), whereas no T-cell expansion and no clinical response was seen in 3 patients with late-stage PTLD and multiorgan dysfunction. This highlights the importance of an early, ideally prophylactic or preemptive immunotherapeutic intervention. Subsequently, Icheva et al. [74] infused EBNA-1-reactive IFNγ-captured T cells to 10 pediatric and adult patients with refractory EBV viremia or PTLD post-HCT, with virologic and clinical responses (i.e., >1 log decrease in viral load and resolution of PTLD) seen in 7 of 10 patients, accompanied by in vivo expansion of transferred cells. Interestingly, the authors observed a tendency toward improved outcomes in the patients (n = 3) who received a second T-cell infusion.

Similar to these initial studies, several groups [51, 57, 81, 83, 85–87, 102, 105] have successfully used IFNγ-captured T cells isolated from either stem-cell or third-party donors (related or unrelated) to prevent or treat AdV, CMV, and EBV infections/disease post-HCT. In recent years, the clinical utility of adoptively transferred IFNγ-selected VSTs has been extended to additional viruses. Pello et al. [115] successfully treated an HCT patient with severe BKV-associated hemorrhagic cystitis using BKV-captured T cells (0.34 × 10^4/kg), while Steinhardt et al. [91] reported on a patient with progressive multifocal leukoencephalopathy (PML) post allo-HCT who received SCD-derived JCV-reactive T cells (2 × 10^4 in total) and achieved clinical remission, with containment of PML lesions.

Taken together, these studies demonstrated the clinical potential of utilizing IFNγ capture to rapidly isolate VSTs for the treatment of post-HCT infections associated with a range of common viruses (Table 1). However (and similar to streptamer technology), this method can only be applied to viruses with substantial frequencies of circulating specific T cells and to viruses for which immunogenic antigens have been identified. In addition, the requirement for large blood volumes (typically a leukapheresis collection) may add another layer of complexity to the process.

Ex vivo Expansion of Virus-Specific T Cells

An alternative strategy to overcome the limitations of direct selection protocols is to amplify virus-reactive populations by repetitive in vitro stimulation with antigen-loaded antigen-presenting cells (APCs) (Fig. 1).

Cytomegalovirus

CMV was the first virus to be immunotherapeutically targeted with ex vivo expanded T cells. This approach was pioneered by Riddell et al. from Fred Hutchinson Cancer Research Center [41], who demonstrated the feasibility of preparing SCD-derived, in vitro propagated CMV-specific CD8+ T cells using autologous CMV-infected fibroblasts as APCs and then adoptively transferring them (4 weekly escalating doses ranging from 3.3 × 10^7 to 1 × 10^9/m^2) to 3 immunodeficient HCT recipients. Subsequently [42], the same group used their VST generation platform to prepare and prophylactically infuse 14 HCT patients using the same treatment regimen, with no infusion-related toxicities and only mild (grade I/II) GvHD reported in just 3 patients. Notably, none of the infused patients developed CMV viremia or disease and all 14 patients had reconstitution of their cellular immunity, as demonstrated by an increase in the circulating frequency of CMV-specific T cells post-infusion. However, the frequency of circulating CD8+ CMV-specific T cell numbers rapidly declined in patients deficient in endogenous CMV-specific CD4+ T cells, highlighting the importance of helper T cells for sustaining the transferred CD8+ effector T cells. Subsequently, Einsele et al. [43] utilized an alternative approach to generate SCD-derived polyclonal CMV-directed VSTs by using CMV lysate as a source of antigen. After multiple stimulation cycles, the final product was enriched in CD4+ and CD8+ CMV-specific T cells and, importantly, lacked alloreactivity (as

assessed by mixed lymphocyte reaction assay) even when donor and recipient were mismatched on 3 HLA alleles. The VSTs were administered to eight recipients of matched related donor (MRD), mismatched related donor (MMRD), MUD, or mismatched unrelated donor (MMUD) HCT with drug-resistant CMV infections at a single dose of 10^7 cells/m^2, with no infusion-related adverse events and no GvHD. Five of 7 evaluable patients had significant (>1 log) and durable reductions in CMV viral load, while a second infusion was sufficient to induce viral clearance in a patient who initially had a transient decrease in CMV-DNA.

Following these initial studies demonstrating the safety and clinical efficacy of ex vivo expanded CMV-directed VSTs, numerous investigators have prepared polyclonal, CMV-reactive T-cell populations for both prophylactic and therapeutic use; in all cases, similar favorable safety profiles were observed. For instance, Peggs et al. [44, 47] reported on the results from a phase II study in which CMV-specific T cells (generated by 14- to 21-day ex vivo culture of donor lymphocytes with donor monocyte-derived dendritic cells [DCs] pulsed with CMV lysate) were prophylactically or preemptively administered to 30 high-risk HCT recipients. Infusions appeared to be protective in those treated prophylactically since only 2 of 14 evaluable patients (14%) required treatment for further reactivation episodes, as opposed to 63% of contemporary and historical controls. Furthermore, in patients who were infused preemptively, the administered cells expanded in vivo (as assessed by de novo detection of tetramer-binding CD8+ T cells post-infusion) with a resultant decrease in viral load. Blyth et al. [52] conducted a phase II trial where 50 recipients of allo-HCT, both pediatric and adults, were prophylactically infused with a single dose of $2 \times 10^7/m^2$ SCD-derived CMV-directed T cells, which were generated using either pp65/HLA-A2-restricted NLV peptide-pulsed DCs (n = 10) or DCs transfected with a pp65-expressing adenoviral vector (n = 40). Although infusions did not seem to have any effect on the frequency of CMV reactivations, the proportion of patients requiring antiviral drugs and the duration of treatment were decreased in the study population compared to a historical control cohort (17 vs. 36% and 3.4 vs. 8.9 days, respectively). Thus, they concluded that adoptively transferred cells could expand in response to viral antigen, limit viral replication, and prevent progression to clinically significant disease.

Several groups [45, 46, 50, 53–55, 93] (Table 1) have reported similar results using donor-derived CMV-reactive T cells post-HCT either as prophylaxis or as the treatment of refractory infections. The infusions were generally safe, without exacerbations of GvHD, and were associated with clinical benefit that coincided with CMV-specific immune reconstitution. Overall, the data from these studies supported the utility of CMV-directed VSTs in mediating direct antiviral effects, thus reducing the requirement for antiviral drug treatment and the associated morbidity, along with a reduction in transplant costs.

Epstein-Barr Virus

EBV was first targeted immunotherapeutically by our group at Baylor College of Medicine (BCM) using SCD-derived, polyclonal (CD4+ and CD8+) VSTs expanded using autologous EBV-transformed B lymphoblastoid cell lines (LCLs), which express the same antigens that are present on EBV+ malignancies [128]. One hundred and fourteen HCT recipients (age 0.5–38 years) have been infused with EBV-STs to prevent (n = 101; n = 90 T-cell-depleted HCT; n = 11 diagnosis associated with a high risk of lymphoproliferative disease) or treat biopsy-proven or probable EBV-PTLD (n = 13). In the initial dose-escalation study, cell doses ranging from 1×10^7 to $1.2 \times 10^8/m^2$ were administered to MRD, MMRD, MUD, MMUD, or haplo-HCT recipients [59–61, 73].

In general, EBV-ST infusions were well tolerated, with no immediate adverse reactions and no de novo GvHD. Eight patients had a recurrence of acute GvHD (grade I/II) and 13/108 evaluable patients developed chronic GvHD, which was extensive in 2 cases. None of the patients infused prophylactically developed EBV-PTLD, compared with a rate of 11% in a historical control cohort. Of the 13 patients with proven or probable disease at the time of EBV-ST administration, 11 had complete and sustained clinical responses [59–61, 73]. Since all cell doses administered proved to be safe and effective, a fixed dose of 2×10^7 cells/m^2 was used thereafter. Notably, one of the nonresponding patients was later found to harbor an EBV strain in the tumor with a deletion in the EBNA-3B gene, which essentially removed the immunodominant HLA-A11 epitopes that were found to be exclusively targeted by the infused line. Therefore, this case highlighted the importance of infusing polyclonal T cells reactive against multiple antigens/epitopes expressed by the endogenous tumor in order to ensure clinical benefit [129].

The first 26 patients infused in these studies received EBV-STs that were genetically marked with a retroviral vector encoding the neomycin resistance gene to facilitate in vivo tracking. In 2010 [73], a long-term follow-up study reported persistence of these gene-modified SCD-derived cells for up to 9 years post-infusion. Additionally, these gene-marked cells could be expanded ex vivo following stimulation with EBV-LCLs, yielding CD4+ and CD8+ populations that expressed memory markers. Hence, the transferred SCD-derived VSTs could survive long-term and expand upon antigen rechallenge, akin to natural endogenous memory EBV-specific T cells.

Since then, these results demonstrating the safety and efficacy of donor-derived, adoptively transferred EBV-STs have been recapitulated at numerous other centers including Memorial Sloan Kettering Cancer Center (MSKCC) [72], the Karolinska Institute [62], and the University of Pavia [67, 69] (Table 1).

Multivirus-Specific T Cells

With the aim of targeting not one but multiple clinically problematic viruses simultaneously, our group developed a strategy to generate bi- and tri-virus-directed VSTs with specificities for AdV+EBV (from CMV-seronegative donors) or AdV+EBV+CMV (from CMV-seropositive donors). Multivirus-specific T cells (multiVSTs) were activated using monocytes transduced with a chimeric Ad5f35-null vector (AdV) or Ad5f35-pp65 (AdV and CMV antigens) followed by weekly stimulations with autologous EBV-LCLs transduced with the same vector. The bi- and tri-virus lines were polyclonal (CD4+ and CD8+ T cells), with activity against each of the target viruses as assessed by characterization assays such as the IFNγ ELISpot and cytotoxicity assays; of note though, the CMV-specific component was dominant in the tri-virus products.

Twenty-four patients (age 1–63 years) were safely infused, either prophylactically or therapeutically, with 5×10^6–1.5×10^8 SCD-derived multiVSTs/m^2 between days 35 and 150 post MRD, MMRD, MUD, or haplo-HCT. The transferred multiVSTs were associated with the induction of partial or complete responses in 3 patients with CMV reactivations (including 1 case that was refractory to antiviral drugs), 6 patients with EBV reactivations (including 1 patient with EBV-PTLD), and 5 patients with AdV infections [94, 96]. Notably, none of the treated patients developed de novo AdV infection, as opposed to an incidence of 68% in a historical cohort of pediatric HCT recipients in the absence of VST therapy [130]. These data suggest that the infused cells have "memory" features and can therefore persist, expand following stimulation, and provide protection against viral disease.

Though safe and associated with broad activity, the initial manufacturing strategy was subject to complexities due to the time required for cell line generation, the need for infectious virus material, and the production cost of clinical-grade adenoviral vectors. In an attempt to reduce manufacturing time and avoid the use of live virus (i.e., EBV-LCLs) or viral vectors (i.e., Ad5f35), our group explored alternative manufacturing approaches including the use of DCs nucleofected with plasmids encoding immunogenic EBV (EBNA1, LMP2, BZLF1), CMV (IE1, pp65), and AdV (Hexon, Penton) antigens to stimulate multiVSTs. In a total of 17 days (compared to the 8–12 weeks of culture required with the previous protocol), the resulting VSTs were polyclonal, had all antigenic specificities (based on IFNγ ELISpot assay), and exhibited functional activity (assessed by intracellular cytokine staining, chromium release assay, and proliferation assay) similar to the VST lines generated using the adenoviral vector [131]. These multiVSTs were safe when administered at doses of $0.5–2 \times 10^7$ cells/m^2 to 10 patients with active CMV (n = 5), EBV (n = 4), and AdV (n = 5) infections and produced complete responses in 80% of cases, including in all 4 patients that presented with dual infections [98].

In recent years, we extended the spectrum of viruses targeted using a single VST cell line to include not only EBV, CMV, and AdV but also BKV and HHV-6. For this purpose, the manufacturing process was further simplified by substituting plasmids for clinical-grade pepmixes (15mer peptides overlapping by 11 amino acids spanning the entire sequence of immunogenic antigens). To expand reactive cells, PBMCs were directly stimulated with pepmixes spanning immunodominant viral antigens (12 in total: EBV – EBNA1, LMP2, BZLF1; CMV – IE1, pp65; AdV – Hexon, Penton; BKV – LP, VP1; HHV-6 – U11, U14, U90) followed by ~2 weeks of ex vivo culture in a G-Rex culture device in medium supplemented with cytokines (IL4 and IL7) to support the selective activation and expansion of virus-reactive T cells and preservation of multivirus specificity and polyclonality [132, 133]. Eleven SCD-derived multiVST lines were administered to recipients of MRD (n = 5), MUD (n = 3), MMUD (n = 2), or haplo (n = 1) HCT at doses ranging from 0.5 to 2×10^7 cells/m^2 either as prophylaxis (n = 3) or as treatment of patients with one or more (up to 4 viruses) viral infections (n = 8). Despite receiving just a single in vitro stimulation (and thus potentially containing residual alloreactive cell populations), the infused VSTs exhibited a safety profile that was similar to more extensively expanded VST products. Moreover, the transferred cells induced clinical responses in all patients with EBV (n = 5), CMV (n = 3), HHV-6 (n = 2), and AdV (n = 1) infections/reactivations, while 6 of 7 patients with BKV infections had a complete or partial response (defined as a reduction of >50% in the baseline viral load with improvement of clinical signs/symptoms). These responses included all 4 patients with symptomatic tissue involvement (EBV-PTLD [n = 1] and BKV-associated hemorrhagic cystitis [n = 3]) [100]. These data showcase the feasibility of generating VST lines that simultaneously target multiple viruses and the potential of such a multivirus-specific cell therapy to provide broad antiviral protection.

Similar to our studies, these results demonstrating the safety and clinical utility of donor-derived multivirus-directed T cells have been reproduced by several other institutions including Cincinnati Children's Hospital (Cincinnati, OH) [111], Children's National Hospital (Washington, DC, USA) [113], the University of Sydney [99, 109, 127], the University College of London [88], the

Medical University of Vienna and the Institute of Pathology of Berlin [84], the University of Milan [90], and the Institute of Hematology of Shanghai [95] (Table 1). Notably, the array of targeted viruses has been extended to include varicella-zoster virus as prophylaxis for the control of herpes zoster episodes and/or disseminated varicella infection after cessation of prophylactic antiviral treatment [99, 109].

Ex vivo VST Generation from Virus-Naïve Donors

The preparation of T-cell products enriched for antiviral specificities, either by direct selection or by selective expansion of virus-reactive T-cell populations, is a relatively simple process when starting from seropositive donors who have previously been exposed to the targeted pathogens and thus have preexisting immunity. However, the isolation of such cells from antigen-seronegative donors, such as younger individuals, or CBUs is substantially more challenging given the naïve phenotype of precursor virus-reactive cells and their low circulating frequency. Therefore, professional APCs are required in order to activate and expand these naïve T cells in vitro in the presence of potent Th1-polarizing cytokines, such as IL7, IL15, and IL12 [134, 135]. Utilizing this type of methodology, investigators from BCM [136] demonstrated that is feasible to generate tri-virus VSTs (specific for CMV, EBV, and AdV) in a single culture using the 20% fraction of a CBU as the starting material. These cells were safely administered to seven CB transplant recipients at doses ranging from 0.5 to 2.5 $\times$ 10^7 cells/m^2 and provided antiviral protection to all 5 patients who were treated prophylactically. The 2 remaining patients had a CMV and AdV infection, respectively, and were both able to clear the viruses (although a second VST infusion was required for the CMV infection [137]).

These preliminary findings in favor of the safety and clinical efficacy of CBU-derived VSTs have been reproduced at Children's National Hospital [138, 139]. Specifically, Abraham et al. [106] safely infused CBU-derived VSTs targeting CMV, EBV, and AdV to 14 CB-HCT recipients, with no infusion-associated acute grade 3–4 GvHD. None of the patients (n = 7) who were treated prophylactically developed virus-related end-organ disease, while out of the 7 patients who were treated therapeutically, only 1 patient developed end-organ viral disease which, of note, was in an immune privileged site (CMV retinitis) and occurred after steroid treatment for GvHD. In addition, the authors demonstrated the long-term persistence of the transferred cells, further supporting the potential of these CBU-derived VSTs to enhance immune reconstitution after CB transplant.

Allogeneic VST Therapy (Third-Party Banks)

Despite the excellent safety profile and antiviral control associated with adoptively transferred VSTs, this modality is still restricted to select academic centers with specialized GMP infrastructure and expertise. In addition, the requirement for individualized products is problematic when the transplant donor is seronegative for target viruses or in cases of urgent need, where the time required to generate (~2 weeks) and release (7–10 days) a clinical-grade product may preclude the implementation of such a therapy. An alternative approach that bypasses these complexities is the prospective preparation and banking of VST lines from healthy donors with diverse HLA haplotypes, which would be available for use as an "off-the-shelf" partially HLA-matched product to treat patients with viral infections/disease. In this regard, several institutions, including the Hannover Medical School and the University of Barcelona [81, 140], have been working toward the establishment of third-party donor registries in order to facilitate patient access to VST therapy in a timely manner.

Importantly though, while this type of therapy would be immediately available, one needs to consider the potentially greater risk of GvHD associated with the infusion of lines that are mismatched at one or more HLA loci. The proof-of-concept studies addressing these issues were performed by Haque et al. [63, 64, 68], who generated and tested the activity of third-party EBV-STs in 31 SOT and two HCT recipients with refractory, biopsy-proven EBV-PTLD. The lines for infusion were chosen on the basis of HLA matching and in vitro cytotoxic activity against EBV. Patients received products that were matched at two to five HLA alleles (considering HLA-A, B, and DRB1), in four weekly doses of 2 $\times$ 10^6 cells/kg. The infusions were well tolerated in these refractory patients and resulted in clinical responses in 64% at 5 weeks, which were sustained in 52% at 6 months. In a follow-up study, Vickers et al. [76] administered lines matched at 3/10 to 9/10 alleles to 11 patients with EBV-PTLD post SOT (n = 5) or HCT (n = 6). Eight of 10 evaluable patients achieved complete remission, including 4 patients with central nervous system (CNS) disease. The group at MSKCC also reported similar safety and efficacy results from 7 patients with EBV-PTLD who were treated with repeated doses of 0.2–1 $\times$ 10^6/kg third-party EBV-ST lines matched at 4/6 to 5/6 HLA alleles; 6 of the patients achieved complete responses [70, 72].

Our group has utilized banks of multiVSTs with specificity for CMV, EBV, and AdV to treat patients with refractory CMV, AdV, and/or EBV infections/disease post-HCT. In a multicenter clinical trial [97], 82 HCT recipients were screened for study participation and a suitable VST line (based on the partial HLA match

between the VST line, patient, and their HCT donor) was identified for 74 individuals. The presence of T-cell activity against the infecting virus through a shared HLA allele was considered, but was not mandatory for study participation. A total of 18 VST lines were infused to 50 patients with severe or refractory infections (CMV [n = 23], AdV [n = 18], and EBV-PTLD [n = 9]) after bone marrow (n = 14), peripheral blood (n = 21), or CB (n = 15) HCT at a fixed dose of 2×10^7 cells/m^2. Despite the administration of VST lines matching from 1/6 to 4/6 alleles (considering HLA-A, B, and DRB1), de novo GvHD occurred only in 2 patients and was grade 1 in both cases. Clinical benefit was achieved with responses to all 3 targeted viruses with a cumulative response rate at 6 weeks post-infusion of 74% (74%, 78%, and 67% for CMV, AdV, and EBV, respectively), the majority of which were durable.

Subsequently, we utilized VSTs generated with the simplified pepmix manufacturing process targeting either one virus (CMV; CMV-ST) or five viruses (CMV, AdV, EBV, BKV, HHV-6; multiVSTs) to treat refractory infections. In a single-center trial targeting CMV infections [56], CMV-STs (matched at 1/8 to 6/8 alleles, considering HLA-A, B, DRB1, and DQB1) were infused (fixed dose–2×10^7 cells/m^2) to 10 patients (pediatric: n = 7, adult: n = 3, MUD: n = 4, MMUD: n = 1, CB: n = 2, haplo: n = 1, MRD: n = 2). Of note, infused lines were chosen solely based on partial HLA match. Despite the HLA mismatch between the patient, their HCT donor cells, and the infused CMV-STs, none of the patients developed recurrent or de novo acute or chronic GvHD posttreatment. Furthermore, the infused cells were associated with clinical benefit with a cumulative response rate of 100% (95% CI, 69.2–100) and confirmed persistence for up to 12 weeks, as evaluated by IFNγ ELISpot assay using individual HLA-restricted epitope peptides to track donor-derived VSTs.

Our group also assessed the activity of multiVSTs targeting AdV, BKV, CMV, EBV, and HHV-6 (Posoleucel), which were administered (fixed dose–2×10^7/m^2, 1–3 infusions) to 58 pediatric and adult HCT recipients (MUD-48%, MMUD-16%, CB-16%, haplo-10%, MRD-10%) to treat 70 infections (patients with 1 infection [n = 46]; 2 infections [n = 11]; 3 infections [n = 1]), the majority of which were caused by BKV (n = 27, 39%) and CMV (n = 24, 34%). Lines for infusion matched at 1/8–7/8 alleles (considering HLA-A, B, DRB1, and DQB1) and proved safe with just 4 cases of de novo GvHD, which resolved. Overall, 55 of 58 patients (95%; 95% CI, 85.6%–98.9%) experienced a PR (defined as at least 50% decrease in viral load or 50% improvement of clinical signs and symptoms) or CR (defined as return to normal range) by week 6 post-infusion. Of the 46 patients with a single-virus infection, 45 (98%) were reported to have either a PR or CR by the week 6 assessment, while 1 patient was reported to have progressive disease. Of the 12 patients with >1 infections, 10 (83%) were reported to have either a PR or CR for all evaluable target viruses by week 6 post-infusion [104, 112]. Of note, using epitope profiling we confirmed that VST responses were of third-party donor origin in 11 cases and the transferred cells were shown to persist in vivo for up to 12 weeks.

The safety and therapeutic potential of VST therapy administered as a third-party product in both pediatric and adult HCT recipients have been repeatedly demonstrated by several other institutions including Cincinnati Children's Hospital [108], Children's National Hospital [113], MSKCC [58, 80], MD Anderson Cancer Center [118], and the University of Sydney [103, 110]. Notably, the spectrum of targeted viruses has been extended to include JCV owing to its genetic similarity to BKV (a highly homologous polyoma virus), which allows VSTs directed against BKV to be utilized for the treatment of JCV infections. Indeed, repeated doses of up to 5×10^7/m^2 allogeneic BKV-reactive VSTs matched at 2/10 to 5/10 HLA alleles have been safely administered to HCT recipients with JCV-induced PML and were associated with clinical improvement, while third-party cells were shown to persist for at least 250 days [116, 141]. In addition, our group has developed an allogeneic multiVST product targeting four problematic community-acquired respiratory viruses (Influenza, RSV, human metapneumovirus, and parainfluenza) for the treatment of respiratory infections in vulnerable populations [142–145]. In fact, a placebo-controlled, randomized phase I/II clinical trial was conducted evaluating the safety and activity of these multiVSTs in HCT recipients as an off-the-shelf T-cell therapy (NCT04933968); results are anticipated.

Finally, Galletta et al. [114] recently reported the results of a retrospective study aiming to compare clinical efficacy and safety outcomes of SCD-derived versus third-party VST administration to recipients of allo-HCT for the treatment of AdV, BKV, CMV, and/or EBV infections. The study cohort included 145 pediatric and young adult HCT patients who had received 1–8 infusions of up to 5×10^7/m^2 SCD-derived (n = 77) or third-party (n = 68) VSTs manufactured under identical conditions. The primary study outcome was clinical response to VSTs, defined as a decrease in viral load or resolution of symptoms without the need for additional antiviral medication. Indeed, there was no statistically significant difference in clinical response rates between the two groups (SCD-derived – 65.6%; third-party – 62.7%, p = 0.747), while the safety profile was similarly favorable in both cohorts. This is a noteworthy finding that highlights the importance of third-party VST therapy as a viable alternative for centers that lack the capabilities for individualized VST manufacturing.

Broadening Implementation of VST Therapy beyond HCT

The extensive clinical experience gained so far has clearly demonstrated the safety and therapeutic benefit of VST infusions in the management of viral complications in pediatric and adult recipients of allo-HCT. Hence, these results provided the rationale to extend implementation of adoptive T-cell therapy in additional immunocompromised patient populations, outside the post-HCT setting, who could potentially benefit from an immunotherapeutic approach aiming to support virus-directed immune reconstitution.

Solid Organ Transplantation

The high level of immunosuppression required to prevent allograft rejection renders SOT patients particularly vulnerable to viral reactivations/infections; for instance, EBV-PTLD is a common infectious complication posttransplant. Adoptive T-cell transfer has long been utilized for the treatment of EBV-PTLD in SOT recipients. As previously mentioned, Haque et al. from the University of Edinburgh [63, 64, 68] pioneered the proof-of-concept studies that demonstrated the safety and feasibility of this approach in the SOT setting (kidney: n = 15, liver: n = 12, liver/small bowel: n = 6, heart: n = 2, lung: n = 2, heart/lung: n = 1) and provided a preliminary proof of clinical efficacy. Since then, several centers including MSKCC, Children's National Hospital, BCM [66], the University of Alabama [65], INSERM (France) [75], Hannover Medical School [78], Birmingham Women's and Children's Hospital [77], and the University of Aberdeen have reported on the successful use of third-party EBV-STs in SOT patients. For instance, Kazi et al. [79] infused 20 SOT recipients (the majority of which were kidney: n = 10, liver: n = 3, and heart: n = 2) with allogeneic EBV-STs for the treatment of rituximab-resistant EBV-PTLD. Favorable response and survival rates were observed (75% and 60%, respectively) even in the setting of CNS disease, while the cells were well tolerated with only 2 cases of mild cutaneous GvHD observed. Subsequently, Prockop et al. [80] safely administered third-party EBV-STs to 13 SOT recipients (kidney: n = 5, heart: n = 3, liver: n = 2, heart/liver: n = 1, lung: n = 1, small bowel: n = 1) with established EBV-PTLD who had failed rituximab therapy, with CR/sustained PR achieved in 54% of patients. Similarly, Bonifacius et al. [81] successfully treated 5 SOT patients with allogeneic EBV-STs for refractory or high-risk EBV-PTLD; 4 patients exhibited complete responses, while the fifth patient had disease stabilization accompanied by a complete metabolic response, as evaluated by PET/CT. Finally, Wistinghausen et al. [82] very recently reported on the results of a study evaluating the use of third-party EBV-STs in pediatric SOT recipients (n = 15) who were either newly diagnosed (n = 10) or relapsed/refractory (R/R) disease (n = 5). The infused cells were generally well tolerated, with one episode of grade 3 cytokine release syndrome occurring in an R/R patient who was taken off the protocol. Interestingly, clinical outcomes were associated with disease severity, with an overall response rate of 70% (7/10) for the newly diagnosed cohort and 20% (1/5) for the R/R cohort.

Focusing on the kidney transplant (KT) setting, KT recipients are highly susceptible to polyoma virus (BKV/JCV) reactivation leading to persistent viruria and viremia in up to 30% and 12% of patients, respectively, with BKV being the primary etiologic agent. These symptoms almost invariably precede the development of BKV-associated nephropathy in up to 10% of patients, causing renal dysfunction and potentially resulting in graft loss [146–148]. Since there are no approved BKV-targeted therapies, the standard of care remains reduction of immunosuppression; however, it is not universally effective and, importantly, it substantially increases the risk of allograft rejection. This unmet medical need has prompted investigators to explore the use of adoptively transferred, BKV-targeted VSTs (BKV-STs) for the control of BKV viremia in SOT recipients. Nelson et al. [107] administered third-party BKV-STs to 3 SOT patients (1 KT, 1 heart transplant, 1 both KT and heart transplant) with no associated toxicity or graft rejection; 1 patient had complete clearance of BK viremia, while the other 2 had partial responses. Jahan et al. [117] also reported on the case of a KT recipient who received allogeneic BKV-STs for the treatment of severe BKV-associated nephropathy. Although the infused cells significantly reduced BK viral load, the extensive fibrosis and tubular atrophy ultimately led to graft failure, thus highlighting the importance of early intervention with T-cell therapy in order to improve efficacy.

Of note, our multiVST product targeting AdV, BKV, CMV, EBV, and HHV-6 (Posoleucel) was assessed in a randomized, double-blind, placebo-controlled phase II clinical trial (NCT04605484) studying the safety and effectiveness of third-party multiVSTs in KT recipients with BK viremia. Infusions proved safe, with rates of treatment-related adverse events similar between experimental (n = 42) and placebo (n = 19) groups and no graft rejection events attributed to the infused product. Importantly, participants at high risk for disease progression that received cells had clinically meaningful reductions in plasma BK viral load that were coincident with improved BKV functional immunity [149]. Hence, these encouraging results support further investigation of VST therapy for the treatment of BK viremia and/or disease in KT recipients.

Progressive Multifocal Leukoencephalopathy

PML is a rare CNS disease caused by the polyoma virus JCV occurring in immunosuppressed individuals and is typically fatal in the absence of any immune intervention. Hence, adoptive T-cell transfer approaches have been applied for the treatment of PML in HCT recipients (as described earlier) by utilizing either JCV-directed or BKV-directed VSTs that are capable of cross-recognizing JCV antigens due to their high amino-acid sequence homology (>70%).

The first report of T-cell therapy for PML occurring outside the HCT setting appeared in 2018, when Muftuoglu et al. [116] reported on 3 patients with PML who received third-party BKV-STs; patient 1 was a CB-HCT recipient, patient 2 had a myeloproliferative neoplasm, and patient 3 had acquired immunodeficiency syndrome (AIDS). All 3 patients had reduction in JC viral loads, with complete viral clearance in the cerebrospinal fluid and alleviation of the clinical signs and imaging features of PML in patients 1 and 3. Patient 2 had disease stabilization that persisted until her death.

Following this initial study, several other groups reported on the utilization of BKV- or JCV-directed T cells for the treatment of PML in immunocompromised patients. Berzero et al. [92] infused 9 hematological patients (four of which had not undergone HCT) with autologous or allogeneic JCV-STs to treat clinically deteriorating PML. Infusions were well tolerated and led to disease control in 6 of 9 patients, with 5 of them alive and in good clinical condition at their last follow-up. Soon after this, Hopfner et al. [119] reported on 2 patients (a bilateral lung transplant recipient and a patient with dermatomyositis) who developed PML and were administered allogeneic BKV-STs. Both patients saw improvement of their symptoms within weeks, with a decrease in cerebrospinal fluid viral loads and an improvement of their imaging features, along with signs of endogenous BKV- and JCV-specific immune reconstitution. Finally, Cortese et al. [120] reported the results of a pilot study in which allogeneic BKV-STs were used to treat PML in 12 patients, the majority of whom had lymphoproliferative disease (n = 6), systemic autoimmune disease (n = 2), and primary immunodeficiency (n = 2). Infusions were well tolerated and no serious treatment-related adverse events were seen. Although the study was not powered to assess efficacy, indications of clinical efficacy (i.e., prolonged survival) were observed. Overall, these studies provide preliminary evidence in favor of the clinical potential of BKV-STs or JCV-STs as a therapeutic option for PML that should be further investigated in clinical trials.

Coronavirus Disease

Coronavirus disease (COVID-19) has had a profound impact with >770,000,000 confirmed cases worldwide (2019–2024) and emerging variants still causing global concern. Defects in cellular immunity (i.e., dysregulated and/or reduced T-cell function and trafficking) confer individuals at increased risk for developing severe disease from SARS-CoV-2, thus placing the immune compromised population in danger of serious illness [11, 150, 151]. This prompted several institutions, including ours, to explore the potential of an adoptive T-cell therapy approach for the prevention of severe disease in susceptible patients.

In order to be immediately poised to intervene in symptomatic patients, we prepared a bank of SARS-CoV-2-targeted VST lines from convalescent healthy donors that would be available as a partially HLA-matched product for immediate use. When this work was initiated, protective immunity to SARS-CoV-2 was not well understood. Hence, we first characterized the cellular T-cell immune response to 18 structural and nonstructural proteins encoded by the virus and established a hierarchy of immunodominance based on the profile of T-cell activity detected in 16 healthy convalescent individuals [125, 152]. Based on these studies, we identified three structural (S, M, and N) and two nonstructural (NSP4 and AP7a) antigens that were advanced to clinical VST manufacturing. Ultimately, we prepared a bank of 15 VST lines for clinical use that were available for administration to hospitalized patients in a phase I clinical trial but we also provided access to external physicians via a compassionate access program. In total, these cells were infused to 4 patients internally – all of whom were at high risk for disease progression due to preexisting malignancy (lymphoma: n = 2 and chronic myeloid leukemia: n = 1) or other risk factors (old age and hypertension: n = 1). The VSTs were generally well tolerated and associated with clinical benefit in 3 of 4 patients who achieved complete resolution of infection; the fourth patient had transient disease improvement, followed by COVID-19 progression and death approximately 5 weeks post-VSTs. We also observed expansion of SARS-CoV-2-reactive T cells post-infusion and persistence of the infused cells for up to 6 months, based on TCR deep sequencing analysis [125]. Additionally, 15 patients outside BCM have received these SARS-CoV-2-VSTs under emergency investigational new drug applications – the results from 7 of these patients have been published, with similar reports of clinical benefit (heart transplant: n = 1 [122]; lung transplant: n = 2 [126]; lymphoma: n = 4 [126]).

Notably, randomized trials utilizing adoptively transferred T cells to treat high-risk patients with COVID-19 have also been conducted. Ferreras et al. [124] reported on the outcomes of a randomized (1:1), phase II multicenter study evaluating the safety and efficacy of

allogeneic, memory (CD45RA-depleted) T cells from convalescent donors plus the standard of care (SoC) versus SoC alone. Eighty-one patients with COVID-19 pneumonia and/or lymphopenia were enrolled, with no infusion-related adverse events seen in the T cell-treated arm (n = 39). Overall, infusion of these off-the-shelf memory T cells was well tolerated, and those who received the cells had accelerated lymphocyte recovery in comparison with patients who received SoC only (median lymphocyte count on day 3 post-infusion – 1.4×10^6/μL vs. 0.9×10^6/μL, respectively; p = 0.004). Finally, Papadopoulou et al. [123] published the results of a phase I/II, randomized (2:1) trial assessing the safety and efficacy of the administration of ex vivo expanded, partially HLA-matched SARS-CoV-2-VSTs in addition to SoC in 57 patients with severe COVID-19 as compared to 30 patients who received SoC alone. The add-on treatment with VSTs was well tolerated and increased the likelihood of recovery by day 30 (VSTs+SoC vs. SoC, 65% vs. 38%, p = 0.017), shortened the time to recovery (median time from day 0 to 11 days vs. not reached, p = 0.052), and lowered the risk of mortality by 51% compared with SoC alone, suggesting that the off-the-shelf immunotherapy with SARS-CoV-2-VSTs can serve as a safe and effective treatment for patients at risk.

Conclusions

Three decades of clinical experience have demonstrated beyond doubt that adoptive T-cell therapy is safe and associated with the reconstitution of antiviral immunity and viral control in immunocompromised patients. During this time, manufacturing methods have evolved to become more streamlined and efficient, supporting the generation of both individualized, SCD-derived and third-party VST products, while the VST platform has developed into a broad spectrum therapeutic that can accommodate multiple antiviral specificities. Furthermore, the extension of VST therapy beyond the HCT setting opens new opportunities for therapeutic interventions in immunosuppressed patient populations with limited treatment options. This is particularly relevant to the SOT field, where promising data have already emerged.

Ultimately, the broad implementation of VST therapy in the armamentarium of antiviral treatment options will rely on the generation of strong clinical evidence supporting the safety and clinical utility of adoptive T-cell therapy. This type of data can only be collected through large, randomized, placebo-controlled clinical trials that will be powered to assess the efficacy of the infused cells in conjunction with the novel pharmacological agents that have become SoC for the majority of centers (e.g., letermovir for prevention of CMV reactivation post-HCT). In this respect, the results from Trace ("Transfer of Adenovirus, Cytomegalovirus and Epstein-Barr virus-specific T cells" – NCT04832607), which is the first multinational phase III trial sponsored by the European Commission, are highly anticipated.

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Conflict of Interest Statement

S.V. is a former consultant to AlloVir. A.M.L. is a co-founder and equity holder in AlloVir and Marker Therapeutics and a consultant to AlloVir. K.K. and P.G.P. have no conflict of interest to disclose.

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Author Contributions

K.K. and P.G.P. collected data and wrote the manuscript. A.M.L. edited the manuscript. S.V. supervised and wrote the manuscript.

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