Review Article

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Role of Virus-Specific T Cell Therapy for Cytomegalovirus and BK Infections in Kidney Transplant Recipients

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Abstract

Cytomegalovirus (CMV) and BK virus (BKV) are common viral infections after kidney transplant. Their negative effects on patient and graft outcomes have been well described. However, despite improvement in screening and prophylaxis strategies, CMV and BKV continue to negatively affect both short- and long-term graft survival. Adequate cell-mediated immunity is essential for the control and prevention of opportunistic viral infections, such as CMV and BKV. Therefore, immune reconstitution, in particular T cell recovery, is a key factor in antiviral control after kidney transplantation. Cell-based immunotherapy offers an attractive alternative approach to traditional interventions. Adoptive T cell transfer, via infusions of allogeneic virus-specific T lymphocytes is capable of restoring virus-specific T cell immunity, and are safe and effective in the treatment of viral infections after hematopoietic stem cell transplantation. In this article, we review the emerging role of virus-specific T cell therapy in the management of CMV and BKV after kidney transplantation. On the basis of the available data, virus-specific T cell therapy may be a promising addition to the antiviral treatment armamentarium after kidney transplantation. Future studies are needed to more clearly define the efficacy and risks of virus-specific T cell therapy in the kidney transplant population.

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Introduction

Cytomegalovirus (CMV) and BK virus (BKV) are common viral infections associated with significant morbidity and mortality after kidney transplantation (1–3). Despite an improvement in screening and prophylaxis strategies, CMV and BKV continue to negatively affect both short- and long-term graft survival (4,5). Adequate cell-mediated immunity is essential for the control and prevention of these opportunistic viral infections (6). Therefore, immune reconstitution after kidney transplantation, particularly T cell recovery, is paramount. However, the degree of cell-mediated immunity is inversely correlated to the intensity of the immunosuppressive regimen used to prevent allograft rejection, with lymphocyte depletion playing a key role in delayed reconstitution and viral risk (7). In this article, we discuss the potential role of allogeneic virus-specific T cell therapy (VST) for the management of CMV and BKV in kidney transplant recipients.

CMV after Kidney Transplantation

CMV is a ubiquitous herpes virus present in 40%–70% of the population (8). It is not associated with disease in the immunocompetent host; however, in the setting of immunosuppression, such as after kidney transplant, CMV can cause systemic illness. Risk of CMV infection is dependent on serostatus at transplant, and is highest in the first year after transplant. Although reactivation can lead to disease, patients without previous exposure (seronegative, R-) who receive allografts from exposed donors (seropositive, D+) are at the highest risk of severe CMV infectious sequelae due to primary infection. Primary infection has been associated with a myriad of negative infectious outcomes, including persistence, recurrence, and resistance, which in turn affect long-term graft survival (9). These negative outcomes are thought to be related to host inability to mount sufficient CMV-specific T cell immunity to adequately control viral replication (10). The drug of choice for CMV prevention and treatment is intravenous ganciclovir and its oral prodrug, valganciclovir (11). The market availability of valganciclovir improved the prophylaxis of CMV, given enhanced bioavailability over oral ganciclovir (12). Additionally, the extension of valganciclovir prophylaxis from 100 to 200 days after kidney transplant has been shown to reduce late-onset CMV

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disease in patients who are high risk. However, valganciclovir is associated with fairly significant myelosuppressive effects, which may be counterproductive to immune reconstitution, and cost can be a barrier to its use (13). Additionally, postprophylaxis late-onset CMV remains a common complication in the 3–6 months after antiviral drug withdrawal, particularly when potent induction and intense immunosuppression is used in a high-risk individual (14). Indeed, the literature suggests CMV serologic mismatch is still negatively affecting patient and graft survival after kidney transplant, even in the era of modern prophylaxis schemes (15). Of additional concern is the emergence of ganciclovir resistance in CMV, which is associated with significant morbidity and mortality. After ganciclovir-resistant CMV disease, the literature describes rates of virologic failure and recurrence range from 20% to 30%, along with 50% rejection incidence, 27% graft loss, and 20%–30% mortality (16,17). Although there are some antiviral agents in the drug pipeline, such as brincidofovir, a prodrug of cidofovir, and maribavir, a UL97 inhibitor, overall these have been plagued by efficacy concerns (17). Indeed, after phase 3 trials of brincidofovir failed to meet goal end points in hematopoietic stem cell transplantation (HSCT) recipients, concurrent phase 3 trials in renal transplant recipients were halted (18). Additionally, maribavir was shown to be inferior to ganciclovir for the prevention of CMV disease after liver transplantation, although this was thought to be due to suboptimal dosing (19). When maribavir was specifically studied as a treatment for refractory and resistant CMV infection in a phase 2 trial, including 120 solid organ transplant and HSCT recipients, results were favorable; however, the incidence of disease recurrence and adverse effects were high (20). These unapproved agents are also difficult to obtain for clinical use, with significant restrictions on compassionate use. The most recently available agent, letermovir, a 3,4-dihydro-quinazoline-4-yl-acetic acid derivative that inhibits viral terminase complex inhibitor encoded by UL56 is FDA approved for CMV prophylaxis in allogeneic HSCT recipients (21). However, its role in treatment and prophylaxis of CMV infection in solid organ transplant recipients has not been completely elucidated, and is still under active investigation (22).

BKV after Kidney Transplantation

BKV is a ubiquitous human polyomavirus. Although it does not cause disease in immunocompetent individuals, it is an important viral pathogen after a kidney transplant (23). Unlike CMV viremia, BK viremia is usually not associated with systemic symptoms. The concern with BK viremia is progression to BK nephropathy (BKN) (24,25). In a study by Drachenberg *et al.* (26), a BKV level of more than 10,000 copies/ml was significantly associated with BKN. Similarly, a study by Korth *et al.* (27) found that a high-level BKV of >10,000 copies/ml was associated with worse graft function, rejection, and higher rates of BKN. BKN is estimated to occur in 2% to 10% of all kidney transplant recipients, and is as nephrotoxic as acute rejection (28,29). Graft survival after diagnosis ranges from 10% to 60%, making the prevention of BKN as important as the prevention of acute rejection and calcineurin-inhibitor nephrotoxicity (30). There are multiple risk factors for the development of BKN; however, the intensity of the immunosuppressive regimen is the most widely accepted risk factor (31). Currently, there is no effective antiviral therapy for prophylaxis or treatment of BK viremia. Although there is rationale to suggest a possible role of cidofovir, its lipid conjugate brincidofovir, leflunomide, and intravenous Ig, well-designed studies demonstrating efficacy of these therapies are lacking (32). Pipeline antiviral agents with activity against BK are in development; however, these are mostly in preclinical phases. The most advanced candidate, currently in phase 2, is MAU868 (Amplyx Pharmaceuticals), a mAb directed against VP1, the major viral capsid protein of BKV (ClinicalTrials.gov identifier NCT04294472). Consensus guidelines recommend the prevention of BKN by protocolized screening for BK viremia. The consensus on the management of BKV detected with a positive viral-screen PCR is to decrease immunosuppression to facilitate the clearance and prevent deleterious outcomes, such as BKN (32,33). This presents a real challenge in post-transplant management, because immunosuppression is indicated for the prevention of graft rejection (28). In our institutional experience, stepwise immunosuppression reduction after diagnosis of BK viremia was effective in inducing resolution of viremia only in around half of patients; approximately one quarter of the remaining progressed to detrimental outcomes of either severe BK viremia or BKN, and the remaining one quarter developed *de novo* donor-specific antibody against HLA or experienced acute rejection (34).

Cell-based Immunotherapy

Improvement in the treatment of CMV and BKV after kidney transplant is needed. Cell-based immunotherapy offers an attractive alternative approach to traditional interventions. The use of adoptive allogeneic T cell transfer is a therapeutic option capable of restoring virus-specific T cell immunity with infusions of VST from donor-derived VSTs (35). VSTs are safe and effective in the treatment of viral infections post-HSCT over the last two decades (36–39).

The isolation of VST is time and labor intensive, and has been limited to a few specialized centers with a Good Manufacturing Practice–grade cell manufacturing facility. The CliniMACS cytokine capture system, which has been available for several years, allows for rapid isolation of IFN-γ–producing antigen-specific T cells, but remains labor intensive and presents significant operator-dependent variability.

Briefly, this method allows for the direct enrichment of virus-specific $CD4^+$ and $CD8^+$ T cells after incubation with the respective viral antigens (40). The method was first described in 1999 and exploits the natural mechanism that antigen-specific memory T cells produce IFN-γ on incubation with the specific antigen (41). In the first step of the selection process, unfractionated PBMCs from donors, typically collected by leukapheresis, are incubated with a peptide pool spanning defined viral protein antigens (MACS GMP PepTivator Peptide Pools). The peptides are presented by monocytes with APC competency part of PBMC mix, triggering the intracellular production of IFN-γ by memory lymphoid cells with peptide specificity. They are then labeled with two different IFN-γ–specific antibodies in a

stepwise procedure. The first binding step uses the CliniMACS IFN-γ Catchmatrix reagent. The second binding step uses the CliniMACS IFN-γ enrichment reagent. The Catchmatrix reagent forms a cytokine affinity matrix on the cell plasma membrane, which then will "trap" all cytokines subsequently produced by the cells on specific stimulation (40). The enrichment reagent then binds to the trapped cytokines, thus enhancing the signal. The enrichment antibody is conjugated to superparamagnetic particles and the final selection of the antibody/cell complexes is performed using the long-established magnetic-activated cell sorting (MACS) technology. The final product captured is predominantly constituted by IFN-γ^+ T cells (both CD4 and CD8), whereas IFN-γ–negative peptide unreactive T cells are discarded. The depletion of unreactive T cells is in itself of material importance because it allows removal of immunologically naïve T cells that may be theoretically causative of graft versus host disease (GVHD) when transfused in HLA-mismatched, immune-suppressed allogeneic recipients (42–45).

Clinical Use of Viral-Specific T Cell Therapy

The greatest degree of clinical experience with viral-specific T cell therapy has been with CMV among HSCT recipients and is summarized in Table 1 (46). However, VST has also been used in the HSCT population for several other opportunistic viral infections with limited alternative treatment options including adenovirus, Epstein-Barr virus (EBV), human herpesvirus 6, and BKV (47). On the basis of the relative safety and perceived efficacy of these therapies, expansion to the solid organ transplant population has been suggested as the next logical step. Although limited, accumulating evidence supports the potential use of virus-specific T cells after a solid organ transplant (Table 2) (48–54).

In kidney transplantation, there are few reported patients of adoptive allogeneic T cell immunotherapy for the treatment of EBV^+ post-transplant lymphoproliferative disorder (49) and CMV (48) infection. In the earlier report (49), a 60-year-old kidney transplant recipient with widespread drug-resistant non-Hodgkin's lymphoma 15 years after transplant failed allogeneic cytotoxic T lymphocytes therapy from a pool of healthy blood donors after six infusions. The second report described ganciclovir-resistant CMV disease in a renal transplant recipient, manifested by thrombotic microangiopathy and glomerulopathy, and adoptive T cell immunotherapy using CMV-specific T cells from a donor bank was safely used as salvage therapy (48). Finally, in a recent report by Nelson *et al.* describing the use of VST for BKV an "off-the-shelf" third-party VST was successful in clearing BK viremia <10,000 copies in two kidney transplant recipients who had failed cidofovir, leflunomide, and IV Ig therapy. One patient had a complete response after ten infusions, the second had a partial response after a single infusion (54). In aggregate, there have been no reported patients with acute rejection, GVHD, or death associated with VST in kidney transplantation. Together, these data present a proof-of-concept of the clinical and operational feasibility of this therapy in solid organ transplant recipients. There are few clinical trials registered on ClinicalTrials.gov that propose to study the role of VST against CMV in solid organ transplantation (Table 3). All these trials utilize VST from allogeneic, partially matched donors.

Potential Obstacles and Risks of VST in Kidney Transplant

GVHD. Although the T cells infused are selected to be antigen specific, a small amount of nonspecific alloreactive T cells may be infused, carrying a risk for increased GVHD. Also, cross-reactivity with other antigens, although unlikely, cannot be predicted. The resultant effect would be the development or worsening of GVHD. To date, multiple groups have reported the safety of infusion of virus-specific T cells in HSCT. There have been no infusion-related side effects, and GVHD reactivation has been reported in very few instances (55,56). However, this potential risk is met with significant trepidation in solid organ transplant, because unlike in HSCT, GVHD is almost universally fatal in this population (57).

T Cell Transfer Toxicity. There are toxicity concerns associated with the use of T cells to treat active infection, including the overstimulation of antigen-specific T cells that could potentially result in cytokine release syndrome (CRS) and tissue damage. CRS is a potential risk of the infusion of virus-specific, antigen-selected T cells. Most patients with CRS will see it occur within 48–72 hours of infusion, but it can occur later (58).

Lack of Efficacy Related to Ongoing Immunosuppression. Although HSCT and kidney transplant recipients are at risk for many of the same opportunistic viral infections, the etiology of their immunosuppressed state is inherently different. In the setting of HSCT, the duration of immunocompromise is not anticipated to be lifelong. Additionally, beyond the conditioning regimen, immunosuppressive therapies are reserved for complications such as GVHD. Conversely, patients who have had a kidney transplant are maintained lifelong on immunosuppressive drugs. These regimens typically include potent T cell inhibitors, such as tacrolimus and mycophenolate. The effect of iatrogenic immunosuppression on the efficacy of VST has not been completely elucidated (59). There are limited studies in HSCT that report a lack of impairment of VST efficacy in the setting of glucocorticoid doses of 1 mg/kg, used to treat GVHD in HSCT (39,60). However, the negative effect of T cell–depleting antibodies on VST expansion would be anticipated to be similar to its profound effects on host cell–mediated immunity to viral infection (59,61). Furthermore, the role of steroids in the absence of T cell–specific immunosuppression in impairment of T cell–mediated immunity to viral infections is thought to be fairly minimal (61,62). With standard transplant immunosuppressive potency falling somewhere between T cell depletion and glucocorticoid monotherapy, the anticipated effect on VST efficacy is unknown.

Generation of calcineurin inhibitor–resistant VST by gene-transfer technology has been explored (63,64). This would allow coadministration with these drugs and increase potential utility of VST in the solid organ transplant population if clinical studies demonstrate impaired *in vivo* VST expansion in the setting of immunosuppressive therapy. Further clinical studies are needed to better explore the effect of iatrogenic immunosuppression on the *in vivo* efficacy of VSTs.

Table 1. Clinical studies of donor-derived cytomegalovirus-specific T cells in patients after hematopoietic stem cell transplantation

Cell Therapy	No. of Patients	Date of Study	Activation	Acute Graft Versus Host Disease	Dose	Cytomegalovirus-related Outcome	Ref[a]
CD8 cell clones	14	1995	Autologous fibroblasts pulsed with CMV virion proteins	3 grade 1–2 GVHD	Intrapatient dose escalation: range $33 \times 10^6/m^2$ to $1 \times 10^9/m^2$	Reconstitution CMV immunity in 14/14	(36)
Polyclonal T cell lines	8	2002	CMV lysate	None	1×10^7 T cells/m2	5/8 cleared after dose 1; 1/8 cleared after dose 2; 1/8 nonevaluable	(68)
Polyclonal T cell lines	16	2003	CMV Ag-pulsed DCs	3 Grade 1 skin GVHD	1×10^5/kg	8/16 patients did not require antiviral drugs; further reactivation in 2/16 only	(38)
CD8 cells (multimer selected)	9	2005	NLV-HLA-A02 pentamers	2 Grade 1–2 GVHD	1.23×10^3/kg to 3.3×10^4/kg	8/9 cleared viremia	(69)
CD4 T cell clones	25	2005	CMV Ag	1 Grade 2 GVHD	1×10^5/kg to 1×10^6/kg	7/25 reactivated CMV; 5/25 disease (2 died)	(70)
Polyclonal T cell lines	9	2007	NLVPMVATV (HLA-A2 restricted) pulsed DCs	3 Grade 3 GVHD (fatal in 1)	Target dose $2 \times 10^7/m^2$	Transient detection CMV-specific T cells by NLV-tetramer staining; 2/9 reactivated CMV without requirement antiviral drugs	(71)
Polyclonal T cell lines	12	2008	DCs transduced with Ad5f35pp65 adenoviral vector encoding CMV-pp65	2 Grade 3; 2 Grade 2 GVHD	$2 \times 10^7/m^2$	Reconstitution CMV immunity in 12/12; no requirement for antiviral drugs	(72)
Polyclonal CD4 and CD8 T cells (gamma catch)	18	2010	CMV-pp65 protein	1 possible GVHD	Mean dose 21×10^3/kg –65-specific T cells	Partial or complete viral clearance in 15/18	(56)
Polyclonal CD4 and CD8 T cells (gamma catch)	18 (11 preemptive, 7 prophylactic)	2011	Recombinant pp65 or overlapping pp65 peptide pool	5 Grade 1; 2 Grade 2; 1 Grade 3 GVHD	Target dose 1×10^4/CD3 T cells/kg	CMV-reactive T cells in 11/11 preemptively treated patients; 7/7 patients treated prophylactically did not reactivate CMV	(73)
CD8 T cells (multimer selected)	2	2011	Peptides derived from pp65	None	0.37×10^5 to 2.2×10^5 CMV-pp65-CTL/kg	2/2 complete responses	(74)
Polyclonal T cell lines	7	2012	Peptides derived from IE-1 and pp65	None	2.5×10^5 to 5×10^5 CD3+CMV CTL/kg	5/7 developed CMV-specific CTL activity in the blood; 2/7 no response	(75)
Polyclonal CD4 and CD8 T cells (gamma catch)	6	2012	Two CMV-pp65 peptides	None	0.6×10^6 to 17×10^6 T cells (comprising 54%–96% CMV-pp65-specific CD8 T cells	6/6 cleared viremia	(76)
CD8 T cells (multimer selected)	2	2012	NLV-containing HLA-A02 pentamers	None	0.8×10^4 to 10.8×10^4 cells/kg	2/2 complete responses	(77)
Polyclonal T cell lines	16	2015	15-mer peptides spanning pp65	None	5×10^5/kg (x 1 dose) to 1×10^6/kg (×3 weekly doses)	14/16 cleared viremia (including 2 with CMV disease)	(78)

CMV, cytomegalovirus; GVHD, graft versus host disease; Ag, antigen; DC, dendritic cell; NLV, NLVPMVATV; CTL, cytotoxic T lymphocytes; VST, virus-specific T lymphocyte.
aSome cited studies incorporate results of other published studies.

Table 2. Clinical studies of virus-specific T cells in solid organ transplantation

Cell Therapy	Indication	*N*	Date Study	Solid organ transplant	Virus-Specific T Cell Donor	Activation	Dose	Graft Versus Host Disease	Acute Rejection	Adverse Events	Disease-related Outcome
Autologous polyclonal EBV-specific Cytotoxic T cells	EBV^+ PTLD	1	1999	Lung	Autologous	EBV infected lymphoblastoid cell lines	35×10^6 T cells$\times2 + 60\times10^6$ T cells$\times2$	0	0	Death (pulmonary vein invasion with necrosis and hemorrhage)	PTLD near resolution
Allogeneic polyclonal EBV-specific cytotoxic T cells	EBV^+ PTLD	5	2002	Liver (4), kidney (1) small bowel (3)	Frozen bank of CTLs derived from healthy blood donors	EBV infected lymphoblastoid cell lines	10^6/kg x one to six times	0	0	0	3/5 complete remission 2/5 no response
Autologous CMV-specific $CD4^+$ and $CD8^+$ cells	CMV	1	2009	Lung	Autologous	Overlapping IE-1/pp65 peptide pools	10^7 T cells/m^2 x 2	0	1	Death (rejection)	CMV resolution
Autologous CMV-specific $CD4^+$ and $CD8^+$ cells	CMV	1	2015	Lung	Autologous	Autologous PBMC coated with HLA class I restricted CMV peptide epitopes	Four infusions (total of 12×10^7 cells)	0	0	0	Persistent negative CMV PCR
Allogeneic Polyclonal CMV-specific $CD4^+$ and $CD8^+$ cells	CMV	1	2015	Kidney	3/6 HLA matched third-party donor	Overlapping peptides covering pp65	1.6×10^7 T cells/m^2	0	0	Mild fever post VST infusion	At 1 yr CMV viral load declined from 5.5 M to 73 copies/ml
Allogenic BK-specific VST	BKV	3	2017	Kidney (1) kidney + heart (1) heart (1)	Adult volunteers	IFN-γ production in response to repeat stimulation with BKV pepmixes	5×10^7T cells/m^2	0	0	0	BKV cleared (1) BK partial response or reduced (2)

EBV, Epstein-Barr virus; PTLD, post-transplant lymphoproliferative disorder; CTL, cytotoxic T lymphocytes; CMV, cytomegalovirus; BKV, BK virus.

Table 3. Registered clinical trials of virus-specific T cells in solid organ transplantation

ID	Cell Therapy	Targets	Indication	*N*	Solid organ transplant	Virus-specific T Lymphocyte Donor	Primary Endpoint	Site
NCT02779439	CTL	CMV, EBV, ADV	Resistance to Rx >2 wk	25	Any	Allogeneic	Infusion related safety at 1 wk	Sydney
NCT02532452	CTL	EBV, CMV, ADV, or BKV	Any Infection	100	Any	Allogeneic	Infusion related toxicity	Cincinnati
NCT03010332	CTL	CMV	Intolerant to or failed Rx	N/A	Any	Allogeneic (ATA230)	N/A	Atara Biotherapeutics
NCT03950414	CTL	CMV	Intolerant to or failed Rx	20	Kidney	Allogenic	Safety and tolerability	Madison, Wisconsin

CTL, cytotoxic T lymphocytes; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ADV, adenovirus.

Table 4. Blood Donor Eligibility Test data summary of 16 cytomegalovirus seropositive donors and 14 BK seropositive donors

	Cytomegalovirus Donor Pre-test Summary (Mean of n=16 donors)		BK Donor Pre-test Summary (Mean of n=14 donors)	
Marker %	Negative Control (n=1/analysis)	CMV PepTivator-stimulated Mean (n=3/analysis)	Negative Control (n=1/analysis)	BK PepTivator-stimulated Mean (n=3/analysis)
%CD3$^+$IFNγ^+	0.08	0.42	0.08	0.07
%CD4$^+$IFNγ^+	0.07	0.22	0.09	0.08
%CD8$^+$IFNγ^+	0.13	0.69	0.08	0.07

Methods and Challenges in The Preparation of VST

FDA-Compliant VST Manufacturing within Academic Health Centers

VSTs are regulated as more-than-minimally manipulated products, as defined by Public Health Service 351, and require an investigational new drug license issued by the FDA Center for Biologics Evaluation and Research for use as investigational cell pharmaceuticals in human clinical trials before marketing approval. Both CMV and BKV VST manufactured by the CliniMACS Prodigy present similar challenges for the accurate determination of the viability of the final cell collection. VST cells are purified by the cytokine capture system and eluted from the MACS column by an automated process. The intrinsic nature of the automated processes favors the coelution of VST cells and nonviable cells, rendering the determination of the final product by the hemocytometer–trypan blue enumeration method inadequate. In addition, the concentration of cells in the final cell elution is low and often does not meet standard system suitability of ≥10 cells/hemocytometer quadrant, resulting in an inaccurate determination of both the live and nonviable cell count. In addition, the presence of visual cellular debris results in method interference, confounding the specificity of cell product identity. As a result, cell count and percent viability are determined by flow cytometry using CD45 as a marker.

As per the FDA guidance for investigational new drug chemistry, manufacturing, and control for human somatic cell therapy, ≥70% is the generally accepted viability criteria (65). A lower viability limit must accompany data demonstrating nonviable cells do not influence the safe administration of the

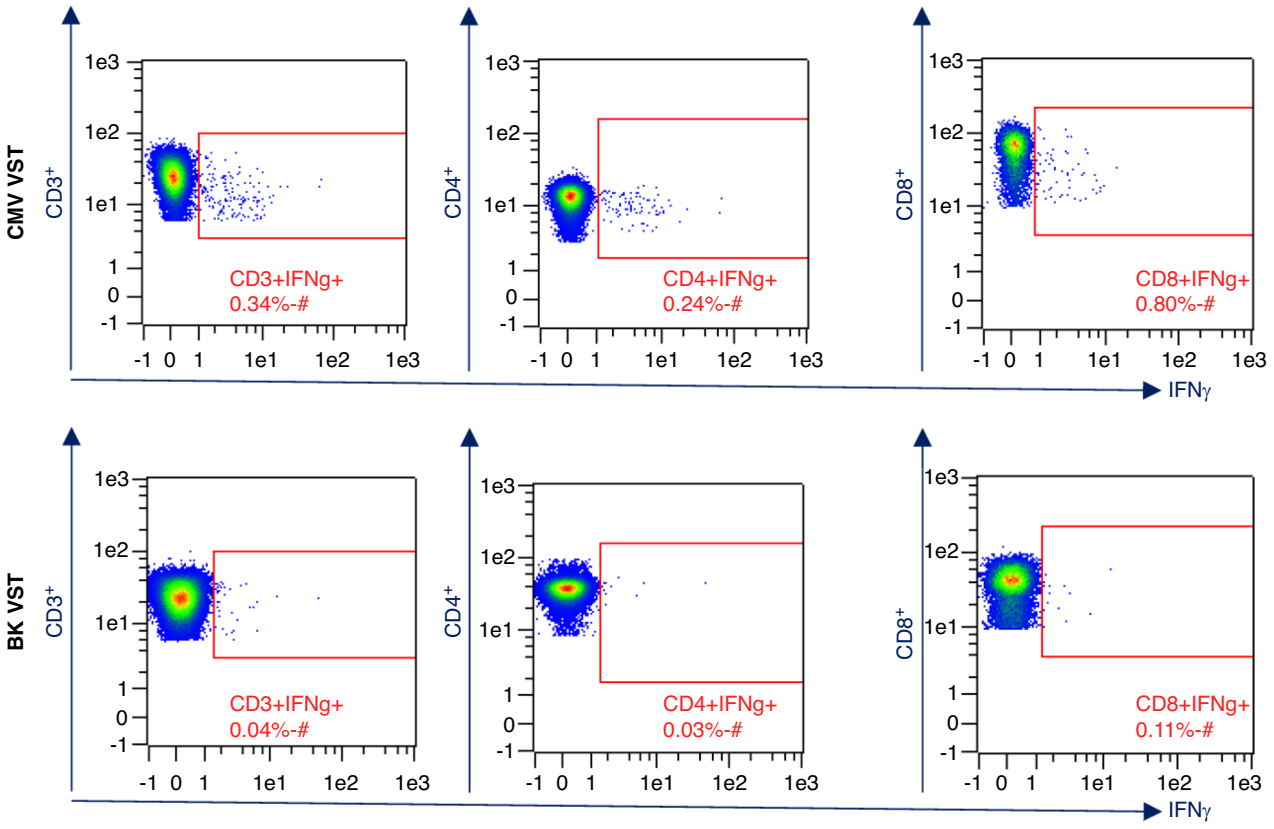

Figure 1. | Example Blood Donor Eligibility Test flow cytometry data analysis of Ficoll-isolated PBMCs after 4-hour stimulation with virus-specific peptides. Frequency comparison of CMV versus BK virus–specific INFγ+ T cells. CMV, cytomegalovirus; BKV, BK virus; VST, virus-specific T lymphocytes.

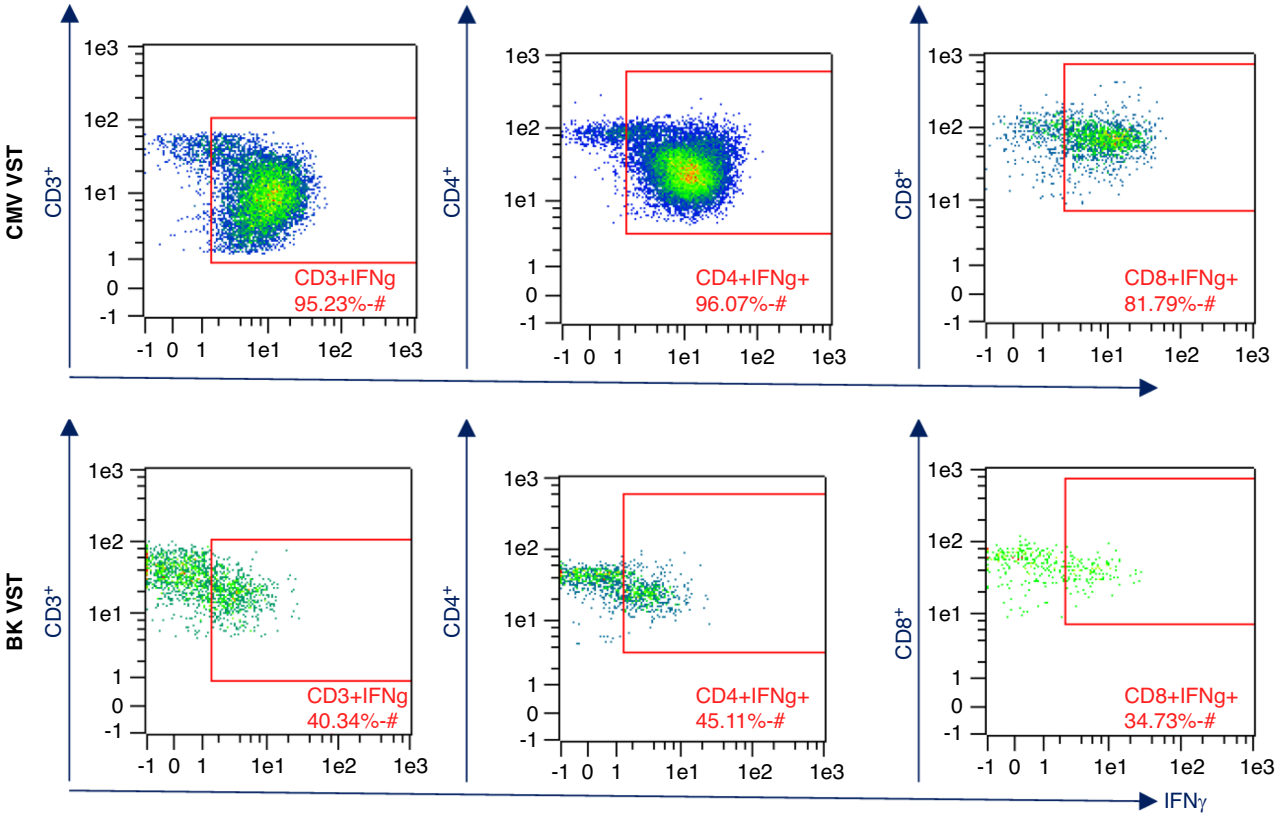

Figure 2. | **Cell therapy product release data example.** Flow cytometry analysis of enriched virus-specific T cells post automated processing by CliniMACS Prodigy. Comparison of frequency of CMV versus BKV-specific INFγ^+ T cells.

product and/or the therapeutic effect. The CliniMACS Prodigy procedure is an automated process. However, once the instrument has eluted cells from the MACS column, the procedure for quality control (QC) testing and preparation of the final product is a continuous process without interruption to deliver the product to the clinic within a 6-hour time limit from collection to infusion. As a result, a high level of risk management, readiness, and coordinated, sequential events must be precisely executed. Time management is critical for the resolution of QC test system suitability issues, final product preparation, and quality assurance, and medical director authorization for infusion. Quality assurance must be present throughout the entire QC testing and final product preparation process for real-time review of in-process, check-point procedures, and accompanying documentation, and approval/rejection of product specification results.

Cell collection from the CliniMACS Prodigy gives an approximate final volume of 7 ml, and from that volume, 1 ml is removed for QC testing purposes, leaving approximately 6 ml available for the clinic. From the 1 ml QC sample, only minimum volumes are available to complete endotoxin, gram stain, sterility, and flow cytometry test methods. There is little or no extra final product for repeat testing, if necessary and justified. The 1 ml volume is removed from the bag and tested and approved as a drug substance. The final product is prepared from the drug substance by drawing the remaining drug substance into a syringe and sealing it with a sterile cap.

Donor Selection

The Miltenyi Biotec Blood Donor Eligibility Test is a flow cytometry–based method designed, manufactured, and marketed specifically for the purpose of VST cell donor eligibility (66). PBMCs are incubated with either CMV or BKV Miltenyi Biotec virus-specific PepTivators. Negative controls are incubated in the absence of PepTivators, and positive controls are prepared and analyzed for system suitability purposes. For our CMV clinical trials, leukapheresis donor selection for CMV VST cell manufacturing is on the basis of a percent of $CD3^+IFN\gamma^+$, $CD4^+INF\gamma^+$, and $CD8^+IFN\gamma^+$ positive response. Table 4 summarizes the results of 16 CMV seropositive donors and 14 BKV seropositive donors that were screened for process development and donor selection at our institution. These data support the published findings that BKV VST cells are a rare cell type (67). For this reason, BKV VST leukapheresis donors are selected On the basis of BKV seropositivity only. QC testing flow cytometry results from CliniMACS Prodigy manufactured VST cells support the donor pretest data collected from blood donor eligibility tests. Figures 1 and 2 show example results comparing CMV and BKV VST blood donor eligibility test and manufactured VSTs.

Summary. In summary, CMV and BK are common viral infections in kidney transplant recipients with imperfect treatment strategies, and are associated with negative patient and graft outcomes. VST may be a promising novel therapeutic approach for these viral infections in kidney transplant

recipients. With emerging data mainly from HSCT and very limited studies in the solid organ transplant population, more evidence is needed exploring the therapeutic efficacy and potential risks of VST in kidney transplant recipients, taking into account the unique aspects of this population over HSCT recipients and bespoke FDA-compliant cell manufacturing outcomes.

Disclosures

A. Djamali reports having consultancy agreements with CSL and CareDx; reports receiving research funding from CareDx and Takeda; reports receiving honoraria from CSL and CareDx; and reports being a scientific advisor or member of CSL and CareDx. S. Parajuli reports receiving research funding from Veloxis. All remaining authors have nothing to disclose.

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Author Contributions

R.O. Meyers was responsible for the validation; S. Parajuli wrote the original draft; and all authors reviewed and edited the manuscript.

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