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A Phase I Study Evaluating Safety and Tolerability of Viral-Specific T Cells Against BK-Virus in Adult Kidney Transplant Recipients

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Keywords: BK polyomavirus | BKPyV nephropathy | T-cell adoptive immunotherapy

ABSTRACT

BK polyomavirus (BKPyV) poses a significant threat to kidney transplant recipients (KTR). Current management primarily involves reducing immunosuppression, which increases the risk of rejection. Cell-based immunotherapy with virus-specific T cells (VST) has emerged as an alternative approach for treating BKPyV in KTRs. This single-center phase I, open-label trial enrolled KTRs with persistent BKPyV-DNAemia and BKPyV nephropathy (BKPyVAN) (NCT05042076) despite being on lower immunosuppression. BK-specific T cells were isolated from leukapheresis products from compatible donors. Patients were treated with VST therapy and followed for 52 weeks. Safety and tolerability were the primary focus of this trial. Three patients completed the trial. No grade III or IV adverse events, acute rejections, or graft versus host disease were reported. All patients tolerated the therapy well, with no significant safety concerns observed during the follow-up period. BK-VST demonstrated promising safety and tolerability profiles in this small cohort of kidney transplant recipients with severe BK infections. These findings suggest that VST therapy may offer a safe adjunctive treatment option for BKPyV infections post-transplantation. Larger studies are needed to confirm these preliminary results and assess long-term efficacy in treating BKPyV infections and preserving graft function in kidney transplant recipients.

Abbreviations: AdV, adenovirus; BKPyV, BK polyomavirus; BKPyVAN, BK nephropathy; BK-VST, BK virus-specific T cells; CCS, cytokine capture system; CMV, cytomegalovirus; EBV, Epstein-Barr virus; FACT, Foundation for the Accreditation of Cellular Therapy; GVHD, graft versus host disease; HHV-6, Human herpes virus 6; HSA, human serum albumin; HSCT, hematopoietic stem cell transplantation; IFN-γ, interferon gamma; IMP, investigational medicinal product; IVIG, Intravenous Immunoglobulin; JCV, JC Virus; KTR, Kidney transplant recipient; NHW, Non-Hispanic White; PACT, Program for Advanced Cellular Therapy; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PTLD, post-transplant lymphoproliferative disorder; VSTs, virus-specific T cells; WBC, white Blood Cells.

1 | Introduction

BK polyomavirus (BKPyV) is a ubiquitous human polyomavirus, considered a major viral pathogen after kidney transplantation. Immunocompetent individuals are typically infected at a young age, with only minor upper respiratory tract symptoms [1]. However, after kidney transplantation, the virus has a propensity for reactivation [2]. Suggested risk factors for BKPyV include male sex, age ≥ 50 years at the time of kidney transplant, lack of immunity against BKPyV, ischemic reperfusion injury, and a high degree of HLA mismatch. However, the intensity of overall immunosuppression is the only widely accepted risk factor [3]. Kidney transplant recipients (KTRs) are at risk of morbidity and mortality due to BKPyV infection. Infection can range from asymptomatic DNAemia to, infrequently, overt life-threatening diseases including pneumonia, encephalitis, colitis, and hepatitis [4, 5]. More commonly, BKPyV causes polyomavirus-associated nephropathy (BKPyVAN), placing kidney transplant recipients at risk of allograft failure [6]. Despite the prevalence of BKPyV and its clinical implications, there are no effective antiviral therapies, making BKPyV a formidable challenge in transplant medicine [7, 8].

BKPyV reactivation can manifest as viruria in 30%–40%, DNAemia in 10-20%, and BKPyVAN in 1%–10% of KTRs [3, 6]. BKPyVAN is an important complication [6]. BKPyVAN occurs when the virus is reactivated and replicates in renal tubular epithelial cells, progressively affecting graft function and increasing the risk of graft failure to 10%–90% [6]. BKPyVAN most commonly occurs within the first post-transplant year but can occur any time after transplantation [9]. It has been suggested that BKPyVAN is as nephrotoxic as rejection, which is regarded as the prominent threat to graft loss [9]. The introduction of more potent immunosuppressive agents to combat allograft rejection has been associated with an increase in the prevalence of BKPyV infection making it an increasing threat for KTRs [4]. Management of BKPyV after kidney transplant is challenging as decreasing immunosuppression can increase the risk of rejection.

Effective management of BKPyV infection after transplant is crucial due to its potential to compromise graft survival with the mainstay of treatment being a reduction in immunosuppression [10, 11]. Kharel et al. reported that even after initial and stepwise reduction in immunosuppression, only 53% were able to clear viremia without major complications within 2 years post-transplant [12]. This underscores the clinical importance of effectively managing BKPyV and BKPyVAN, especially given the lack of specific medications for prophylaxis or treatment [7]. 2%–8% of KTRs develop BKPyVAN, and 10%–80% KTRs develop graft failure prematurely, which highlights the urgent need for the development of effective therapeutics [6, 10]. Also, early clearance of BKPyV-DNAemia is associated with better outcomes compared to prolonged DNAemia [13]. However, while decreasing immunosuppression can prevent progression to BKPyVAN, it increases rejection risk and it is not always effective.

Cell-based immunotherapy has emerged as a promising treatment for viral infections post-transplantation and offers an appealing alternative to traditional interventions. Adoptive transfer of virus-specific T cells (VSTs) is capable of restoring antiviral immunity without compromising overall immunosuppression [11, 14–16]. VST therapy has been explored as a treatment for viral infections following solid organ and hematopoietic stem cell transplantation (HSCT). Adoptive transfer of Epstein-Barr virus (EBV) specific T cells has demonstrated clinical benefit in managing EBV-related post-transplant lymphoproliferative disorder (PTLD) [17, 18]. Furthermore, VST therapy has shown promise in both prophylaxis and therapy for refractory EBV, cytomegalovirus (CMV), and adenovirus (AdV) infections post-HSCT [17, 19–26]. There is growing evidence supporting VST therapy as a targeted approach to managing viral diseases in transplant recipients. Safety and efficacy trials are needed to determine whether this approach reduces mortality, complications, and healthcare costs associated with severe viral infections.

Despite these recent advancements, the role of BKPyV-specific T-cell therapy in managing BKPyV infections post-kidney transplantation remains underexplored. This therapy offers a targeted approach to address BKPyV infections. This study aimed to evaluate the safety and tolerability of BKPyV-specific T-cell therapy in kidney transplant recipients with persistent BKPyV-DNAemia and BKPyVAN despite reduction in net immunosuppression, addressing a critical gap in current therapeutic options. Efficacy was not the main aim of this clinical trial.

2 | Methods

This clinical trial was a single-center phase I, open-label, non-randomized, non-placebo controlled, single-group assignment study to assess the safety and tolerability of transfer of BK-specific T cell isolated from a leukapheresis product. This study was funded by the University of Wisconsin-Madison and approved by the Institutional Review Board at the University of Wisconsin (UW IRB: 2021-1058, NCT05042076).

We defined BKPyV-DNAemia as detectable serum BKPyV by polymerase chain reaction (PCR) > 250 IU/mL and BKPyV nephropathy as positive SV-40 staining on kidney biopsy. Rejection was defined as biopsy-proven rejection based on the Banff criteria [27].

The main inclusion criteria included adult (age 18–75 years) KTRs who had persistent BKPyV-DNAemia despite being on lowered immunosuppression following kidney transplantation, along with biopsy-proven BKPyVAN and have an eligible donor. The main exclusion criteria included non-kidney organ transplant recipients, patients with acute rejection of the kidney allograft on biopsy, patients receiving high dose steroids, defined as > 0.5 mg/kg prednisone equivalents at the time of T-cell transfer, treatment with T-cell immunosuppressive monoclonal antibodies within 28 days before T-cell transfer, patients with extra-renal tissue invasive BKPyV infection, known HIV infection and female subjects that were pregnant or breastfeeding.

Donor eligibility included age 18 or older, available and capable of undergoing a single standard 2 blood volume leukapheresis, HLA compatible (original kidney donor or, no HLA DSA present and at least HLA haplomatched to recipient, for A, B and DR loci at the antigen level), BK IgG seropositive, and met criteria for donor selection, eligibility, and suitability defined in the UW Program for Advanced Cell Therapy Standard

Operating Policies and Procedures for Donor selection, eligibility, and suitability for the Donation of Immune Effector Cells, which complies with Foundation for the Accreditation of Cellular Therapy (FACT) standards for Immune Effector Cells. Written consent was obtained to explain the risks and benefits to both donor and recipient. Participants were followed for 52 weeks after treatment to assess any adverse events. Although not the primary focus of the trial, efficacy, and graft functions were assessed until the end of data analysis in 09/2024.

2.1 | BKPyV Monitoring

After kidney transplantation, most patients at our center receive induction followed by maintenance immunosuppression with tacrolimus, mycophenolic acid, and prednisone. Plasma BKPyV PCR was monitored every 2 weeks for the first 3 months post-transplant, monthly for 3–6 months post-transplant, and every 1–2 months from 6 to 24 months. Any positive serum BKPyV PCR was monitored every 2 weeks until negative three consecutive times. For BKPyV-DNAemia > 1000 IU/mL, stepwise reduction of immunosuppressive agents was performed, starting with a decrease in antimetabolite, reflecting current consensus guidelines [28].

BKPyV viral load in this study was measured by quantitative PCR at the screening on the day of an investigational medicinal product (IMP) infusion, as well as in follow-up Weeks 1, 2, 4, 8, and 12, along with 18, 26, and 52 weeks.

2.2 | Viral-Specific T-Cell Therapy

The IMP of Viral-Specific T-cell therapy (VST) consists of naturally occurring, allogenic donor lymphocytes derived from a leukapheresis, enriched for BKPyV-specific CD4+ and CD8 + T cells suspended in approximately 7 mL of 0.9% NaCl with 2.5% human serum albumin (HSA) at a cell dose of ≥ 300 and ≤ 5000 BKPyV virus-specific CD3 + T cells/kg BW. It was administered as an IV bolus injection of IMP over approximately 2–4 min, resulting in an infusion rate of approximately 3 mL/min.

Manufacturing of the enriched antigen-stimulated interferon-gamma (IFNγ) + BKPyV specific CD4+ and CD8 + T-cells was performed within the GMP facility of the Program for Advanced Cellular Therapy (PACT) at the University of Wisconsin Hospitals and Clinics. The manufacturing process and quality control were performed according to qualified procedures and documented in compliance with full GMP requirements for phase I clinical trials. The individual, donor-derived white blood cells were incubated with PepTivator BKV LT & VP1 (Miltenyi Biotec, Cat. 170-076-138, 170-076-139). After incubation, BKPyV-specific cells were enriched using the CliniMACS Cytokine Capture System (IFNγ) (Miltenyi Biotec, Cat. 200-070-217). The entire preparation process was performed using the fully automated CliniMACS Prodigy.

BK-VST T cells were manufactured using a platform technology designed and engineered by Miltenyi Biotec (Bergisch Gladbach, Germany) using the Miltenyi Biotec CliniMAC Prodigy (Prodigy) automated instrument and CliniMACS Cytokine Capture System (CCS) process and reagents. The Prodigy combines cell isolation, stimulation, and selection in a closed system. All open manipulations were performed in a certified biological safety cabinet.

Donor leukapheresis was collected on the morning of cell manufacturing by a trained user at the UW Health Hospital (600 Highland Ave, Madison, WI) Apheresis Collection Facility and transported directly to the PACT GMP manufacturing facility. PACT personnel reviewed the accompanying documentation to confirm donor identification and visually inspected the product.

The concentration of the PBMC cell-enriched donor leukapheresis starting material cell count was determined by hemocytometer Trypan Blue (Corning, cat# 25-900-Cl) exclusion method. 1×10^9 viable WBC were removed from the leukapheresis bag and diluted in CliniMACS PBS/EDTA (Miltenyi Biotec, Cat# 200-070-022) buffer with 0.5% HSA (Shire, 25%, USP, Cat# 2G0012) under aseptic conditions.

Virus-specific T cell manufacturing process tubing set TS500 (Miltenyi Biotec, Cat# 200-073-609), BKV LT (Miltenyi Biotec, Cat# 170-076-139) and BKV VP1(Miltenyi Biotec, Cat# 170-076-138) GMP BK virus-specific PepTivators, TexMACS GMP medium (Miltenyi Biotec, Cat# 170-076-306), and the CliniMACS Cytokine Capture System (IFN-gamma) (Miltenyi Biotec, Cat# 200-070-217) were installed by qualified operators on the Prodigy as per the manufacturer's instructions.

The automated method allows for the direct enrichment of virus-specific $CD4^+$ and $CD8^+$ T cells after incubation with the respective viral antigens [24, 29]. The method was first described in 1999 and exploits the natural mechanism that antigen-specific memory T cells produce IFNγ upon incubation with the specific antigen [25, 30]. In the first step of the selection, process cells are incubated with specific viral antigens triggering the intracellular production of IFNγ (MACS GMP PepTivator Peptide Pools). They are then labeled with two different IFNγ-specific antibodies in a stepwise procedure. The first binding step uses the CliniMACS IFNγ Catchmatrix Reagent. The second binding step uses the CliniMACS IFNγ 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 upon specific stimulation [24, 29]. The Enrichment Reagent then binds to the trapped cytokines, thus enhancing the signal. The enrichment antibody is conjugated to super-paramagnetic particles and final selection of the antibody/cell complexes is performed using the long-established MACS technology (Magnetic-Activated Cell Sorting). Upon completion of the automated process, the Prodigy collects approximately 7 mL of BK-VST cell final drug product.

Quality control testing commenced immediately upon collection of the final product from the Prodigy. 1 mL of final product was removed from the collection bag. QC sampling was as follows: 200 μL: BD Bactec sterility (Becton-Dickinson, FX Blood Culture System), 100 μL: flow cytometry cell count, 400 μL: frequency panel, 200 μL: Gram stain. The remaining

volume was used for endotoxin testing as per a qualified method using the Charles River Laboratories (CRL), Endosafe nexgen-PTS. Samples were diluted 20-fold in LAL Reagent Water (CRL, Cat# W130) before loading an Endosafe PTS cartridge (CRL, Cat# PTS2005F). Gram stain analysis and BD Bactec testing was performed by the UW Health Clinical Laboratory by trained Medical Technologists.

Flow cytometry was performed on a MACSQuant Analzyer 10 (Miltenyi Biotec, Cat# 130-096-343) in the PACT facility by a qualified analyst. Data analysis was performed using MACSQuantify Software (Miltenyi Biotec, Cat# 130-094-559).

Eluted product (post-column positive fraction) was tested for viability, dose, purity, and potency by flow cytometry-based methods. The negative fraction (flow-through) and the original (pre-column) fraction were analyzed in parallel and served as controls. The following was performed and analyzed sequentially.

The absolute number of viable white blood cells was counted using a MACSQuant 10 volumetric flow cytometer and CD45-VioBlue antibody (Miltenyi Biotec, Cat# 130-113-122) with 7AAD viability dye (Miltenyi Biotec, Cat# 130-111-568).

Samples of each fraction were stained with the following cell surface markers: CD3-FITC (Miltenyi Biotec, Cat# 130-113-128), CD4-APC (Miltenyi Biotec, Cat# 130-113-250), CD8-APC-Vio770 (Miltenyi Biotec, Cat#130-113-155), CD14-VioGreen (Miltenyi Biotec, Cat# 130-113-153), CD20-VioGreen (Miltenyi Biotec, Cat#130-113-379), CD45-VioBlue (Miltenyi Biotec, Cat#130-113-122), Anti-IFN-ɣ-PE (Miltenyi Biotec, Cat# 130-113-493). Residual red blood cells were lysed with Ammonium Chloride Solution (StemCell Technologies, Cat# 07800) and 7AAD viability dye was added before sample acquisition.

The following sequential gating strategy was used: (1) Doublet exclusion; (2) %CD45$^+$ gating; (3) Debris exclusion and lymphocyte population identification; (4) %CD3$^+$ gating (purity criteria); (5) Viability gating; (6) Monocyte/B cell exclusion; (7) %CD4$^+$ and %CD8$^+$ populations identification among viable CD3$^+$/CD14$^-$/CD20$^-$ cells; (8) %IFNɣ expression by CD4$^+$ and CD8$^+$ populations (potency criteria).

The absolute number of CD3$^+$ cells in the product was then extrapolated from the two panels described above by multiplying the total number of white blood cells in the product and the percent viable CD3$^+$ cells. The infusion dose was then determined based on the patient's weight. Example flow results for the Original and Positive fractions are shown in Figure 1.

The final product was stored at refrigerated conditions until transport to the UW Health Hospital research clinic in a validated cooler on refrigerated cool packs with a temperature logger to verify controlled storage. The product was infused within approximately 6 h, calculated from the final collection of cells.

Patients were followed for a total of 52 weeks with primary endpoints of the incidence and severity of acute rejection of the kidney allograft and incidence and severity of Graft-versus-host-disease (GVHD). The safety endpoint was determined by infusion-related toxicity. Adverse events were monitored and graded according to Common Terminology Criteria for Adverse Events v5.0 US Dept Health and Human Services [31]. Grade 1 adverse events include conditions that are asymptomatic or

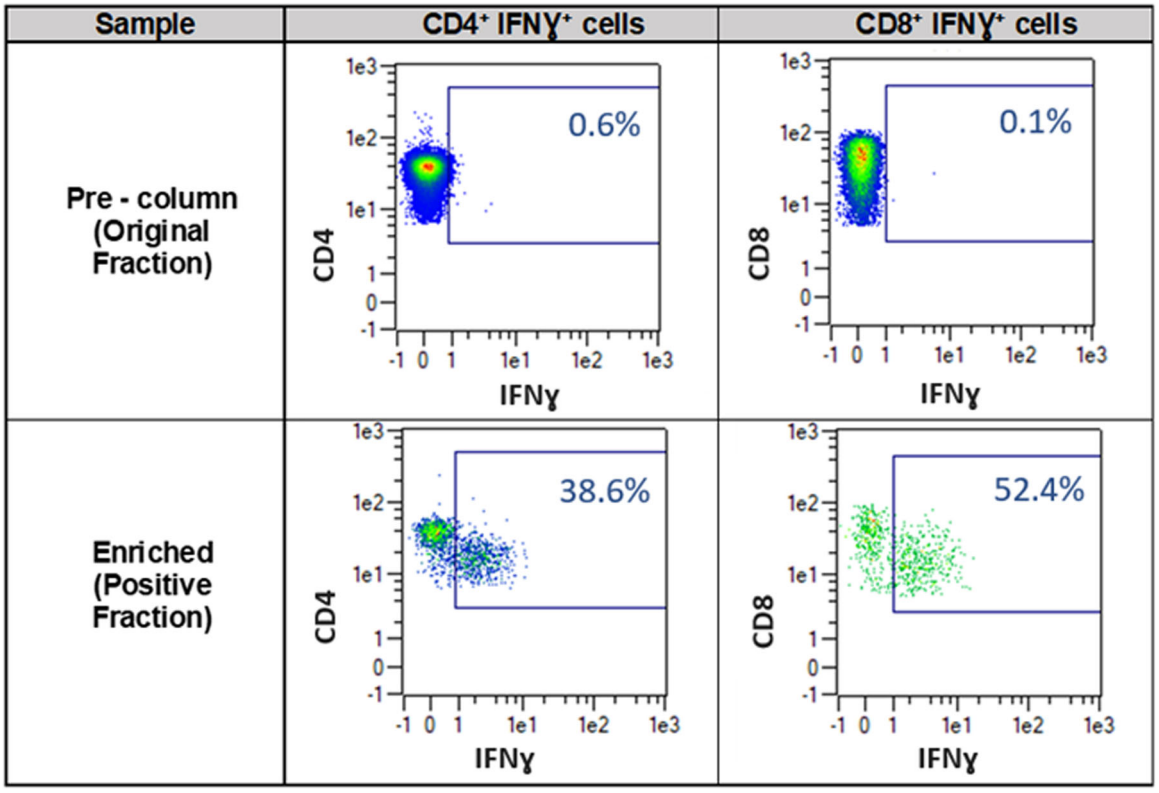

FIGURE 1 | Typical flow cytometry results showing Original and Positive fractions.

mildly symptomatic, with no intervention needed. Grade 4 adverse events would have life-threatening consequences requiring urgent interventions.

2.3 | Statistical Analysis

The time of occurrence of any adverse events was documented. Basic demographics were presented as mean for continuous variables and absolute numbers with percentages for the categorical variables. All primary outcomes of interest were within 52 weeks (1 year) post-VST infusion.

3 | Results

Twenty-seven patients were pre-screened for the study and of those, three passed screening and completed treatment. All three were non-Hispanic white (NHW) female recipients of living donor kidneys from NHW donors. Other baseline characteristics are summarized in Table 1. Quality control specifications and results for each final product are shown in Table 2A. One recipient had two eligible donors; details of BK antibody titer are presented in Table 2B.

Primary reasons for low enrollment were lack of an appropriate donor as well as patients' willingness to participate in a yearlong study requiring travel to our center. The study was able to successfully produce BK-VST from donors on an intent-to-treat basis. The drop-out rate for the study was zero. The average time from patient inclusion to administration of IMP was 5.6 days.

The mean interval from transplant to first BKPyV-DNAemia was 19.0 ± 29.9 months. Details of BKPyV-DNAemia and PCR and kidney graft functions are summarized in Table 3 along with Figure 2. All three recipients were maintained on a triple-drug immunosuppressive regimen at the time of transplant and later maintenance immunosuppressive agents were tapered down due to BKPyV-DNAemia and BKPyVAN. At the time of BK-VST infusion, two recipients were on tacrolimus and prednisone 5 mg daily, and one was on sirolimus and prednisone 5 mg daily. At time of last follow-up, all recipients were on different immunosuppressive regimens, with one maintained on tacrolimus, mycophenolic acid, and prednisone; one on tacrolimus and prednisone; and one on leflunomide and prednisone.

TABLE 1 | Baseline demographics.

Variables	
Total number	3
Mean age at transplant (Yrs, SD)	49.3 (22.8)
Cause of ESKD (%)	
Glomerulonephritis	2 (67)
Reflux nephropathy	1 (33)
Previous transplant (%)	1 (33)
Induction immunosuppression (%)	
Anti-thymocyte globulin	2 (67)
Basiliximab	1 (33)
cPRA > 20%	1 (33)
Recipient BMI (kg/m^2, SD)	27.0 (8.7)
Mean HLA mismatch (per 6, SD)	2.3 (0.6)
Patient # 1 HLA mismatches	A = 0, B = 1, DR = 1
Patient #2 HLA mismatches	A = 2, B = 1, DR = 0
Patient #3 HLA mismatches	A = 0, B = 0, DR = 2
Mean donor age (Yrs, SD)	46.3 (18.0)
Donor- female (%)	1 (33)
Donor BMI (Kg/m^2, SD)	22.7 (0.6)

In the first patient, serum tacrolimus levels at the time of the first BKPyV-DNAemia, BKPyVAN, BK-VST infusion, 1-month post-BK-VST, 3 months post-BK-VST, 6 months post-BK-VST, 12 months post-BK-VST and last follow up were 4.3 μg/L, 6 μg/L, 4.3 μg/L, 4.9 μg/L, 5.4 μg/L, 4.3 μg/L, 4.9 μg/L and 4.5 μg/L respectively. In the same patient, low dose mycophenolic acid at 180 mg PO twice a day, after clearance of BKPyV-DNAemia, and as this patient was considered at high risk for rejection due to a previous transplant. Similarly in the second patient, serum tacrolimus levels at the time of the first BKPyV-DNAemia and BKPyVAN were 7.3 μg/L and 7 μg/L respectively. This patient was on sirolimus at the time of the BK-VST infusion with a sirolimus level of 7 ng/mL. 1-month post-BK-VST, 3 months post-BK-VST, and 6 months post-BK-VST were 11, 12 and 10 ng/mL respectively. Around 11 months post-BK-VST infusion, sirolimus was discontinued and leflunomide was started with a leflunomide level of 74.3 μg/mL at 12 months post-BK-VST and 47.6 μg/mL at the last follow up. In the third patient, serum tacrolimus levels at the time of the first BKPyV-DNAemia, BKPyVAN, BK-VST infusion, 1-month post-BK-VST, 3 months post-BK-VST, 6 months post-BK-VST, 12 months post-BK-VST 5.9 μg/L,5 μg/L, 6 μg/L, 4.5 μg/L, 4.8 μg/L, 4.0 μg/L and 3.0 μg/L respectively

Two patients had functional grafts by the end of the data analysis, and one died due to metastatic bladder cancer more than 1-year post-VST. All three recipients had achieved transient BKPyV-DNAemia clearance at some point within 52 weeks or by the end of the data analysis as summarized in Figure 2. Among the two surviving recipients, one achieved persistent viral clearance, and the other transient clearance with a significantly lower absolute BKPyV-viral load at the last follow-up.

3.1 | Endpoints

All three subjects completed the study. There were zero grade IV, zero grade III, seven grade II, and 27 grade I adverse events. (Table 4). There were no SAEs. Additionally, no subjects experienced any incidence of kidney allograft rejection or GVHD. Also, doses of viable CD3+ cells/Kg bodyweight did not correlate with adverse events or treatment efficacy. All three recipients had adverse events of urinary tract infections, arthralgia, and dizziness. Two patients had nausea, nasal congestion, and ear pain, along with various other minor adverse events.

4 | Discussion

In this single-center phase I, open-label, non-randomized, non-placebo controlled, single-group assignment study (NCT05042076)

TABLE 2A | Quality control specifications and results.

Release test	Release Criteria	Specifications	Results		
			Pt#1	Pt #2	Pt #3
Viability	% viable $CD3^+$ cells	≥ 70%	77%	72%	85%
Product characterization	Purity[1]	≥ 20%	56%	32%	54%
	Expression of IFNγ[2]	≥ 10%	79%	78%	91%
Sterility	Gram stain	Negative	Neg	Neg	Neg
	14-Day Bactec Culture[3]	No growth	No growth	No growth	No growth
Endotoxin	Endotoxin, EU/kg BW	< 5EU/kg BW	< 0.007	< 0.006	< 0.09
Infusion dose (Viable CD3+ cells/kg BW)	Min. infusion dose	≥ 300	1160	300	1850
	Max. infusion dose	$\leq 5 \times 10^3$			
Visual appearance	No visible aggregates, discoloration, or cloudiness	No visible aggregates, discoloration, or cloudiness	Pass	Pass	Pass

[1]Percent CD3 + T cells by flow cytometry.
[2]Sum of % $CD4^+$ IFN^+ and $CD8^+$ IFN^+ by flow cytometry.
[3]Data available 14 days postinfusion by BD Bactec method.

TABLE 2B | BK virus antibody titer ELISA test for donor eligibility.

Patient #	Donor relationship	BKV antibody titer (IU/mL)
1	Son	10,240
2	Mother	10,240
	Father*	40,960
3	Kidney donor	40,960

Note: ELISA titer test was performed by Eurofins Viracor (Lenexa, KS). The test result is reported as an antibody titer which is the inverse of the specimen dilution that produces a signal greater than the assay background level of 1:2560. Based on the current body of scientific literature, an individual is considered seropositive for BKV IgG antibodies when the titer is ≥ 2,560.
*Donor.

to assess the safety and tolerability of transfer of BK-specific T cell isolated from a leukapheresis product, three kidney transplant recipients were treated with the investigational product and followed for 52 weeks. All three patients completed the study. BKV-specific T-cell therapy was well-tolerated with no reported cases of acute rejection or GVHD. This finding aligns with previous research indicating that adoptive transfer of VSTs is generally safe in immunocompromised patients [19, 32, 33]. The absence of severe adverse events underscores the potential of VST therapy as a safe adjunctive treatment in BKPyV management post-transplant.

The introduction of VST therapy represents a promising advancement in personalized medicine for transplant recipients, offering a targeted approach to controlling BKPyV without compromising graft function [11, 34]. Several other trials have been conducted recently to examine the clinical utility of VST for BKPyV infection [35]. Tzannou et al. completed a study that showed clinical benefit following VST therapy in 16 HSCT recipients who developed BKPyV infection [22]. In another study involving 59 HSCT patients with BK hemorrhagic cystitis who received VST therapy, 81.6% mounted an enduring response by Day 45 [36]. VST has been more widely utilized in HSCT recipients but conceptually should have application in the management of viral infections in solid organ transplant recipients. Several proof-of-concept studies show promise for T-cell therapy as a treatment of BKPyV after transplantation [35]. A recent case report demonstrated clinical stabilization after infusion of third-party donor BKPyV-VST in a kidney transplant recipient who developed progressive multifocal encephalopathy [28]. A few case reports have documented the use of VST in kidney transplant recipients with BKPyV infection, with no instances of acute rejection, GVHD, or mortality reported [37, 38].

Currently, the primary approach to managing severe BKPyV)or BKPyVAN involves reducing immunosuppression [39]. This is due to the lack of safe and effective adjuvant therapies for controlling BKPyV replication, as rigorous studies evaluating the efficacy of treatments such as leflunomide, cidofovir, intravenous immunoglobulin (IVIG), switching from tacrolimus to cyclosporine or mycophenolic acid to mTOR inhibitors, and the use of fluoroquinolones remain insufficient [40, 41]. Typically management begins with reducing or discontinuing the antimetabolite, followed by adjusting calcineurin inhibitor trough levels. However, many of these treatment strategies are costly and carry a significant risk of rejection and graft loss, further increasing healthcare expenses.

There is a clear need for improved BKPyV treatment options following kidney transplantation. Cell-based immunotherapy presents a promising alternative to conventional interventions. The most extensive clinical experience with virus-specific T-cell (VST) therapy has been in the management of CMV infections in HSCT recipients [42]. Additionally, VST therapy has been explored for treating other opportunistic infections in HSCT patients, including AdV, EBV, human herpes virus 6 (HHV-6), and BK virus [43]. However, data on VST therapy in solid organ transplant recipients, particularly for BKPyV, remain limited [44–50].

TABLE 3 | BKPyV-DNAemia PCR and graft function trends.

Outcomes	
Mean Interval from transplant to first BKPyV-DNAemia (mo) (SD)	19.0 (29.9)
Mean level of first BK PCR (IU/mL, (log10))	31,676 (39,211); 4.17 (0.72)
Mean serum creatine (mg/dl) and eGFR at the time of first BK	1.55 (0.13); 39.3 (3.8)
Mean Interval from transplant to BK nephropathy (mo)	20.7 (29.5)
Mean level of BK PCR (IU/ml, (log10)) at time of nephropathy	1,735,580 (2,548,727); 5.82 (0.74)
Mean serum creatine and eGFR (ml/min/1.73 m^2) at the time of nephropathy	2.08 (0.49); 38.7 (12.5)
Mean Interval from transplant to BK-VST infusion (mo, (SD))	39.5 (22.7)
Mean level of BK PCR (IU/ml, (log10)) at BK-VST infusion	77,756 (131,065); 3.89 (1.34)
Mean serum creatine and eGFR at the time of BK-VST infusion (mg/dL, (mL/min/1.73 m^2))	1.59 (0.71); 43.3 (14.8)
Mean level of BK PCR (IU/ml, (log10)) 1 month post BK VST	119,903 (203,445); 4.02 (1.37)
Mean serum creatine and eGFR 1 month post BK VST (mg/dL, (mL/min/1.73 m^2))	1.59 (0.71); 43.0 (14.2)
Mean level of BK PCR (IU/ml, (log10)) 3 months post BK VST	130,508 (225,592); 2.77 (2.80)
Mean serum creatine and eGFR 3 months post BK VST (mg/dL, (mL/min/1.73 m^2))	1.87 (1.09); 37.7 (17.4)
Mean level of BK PCR (IU/mL, (log10)) 6 months post BK VST	129,451 (223,045); 2.91 (2.80)
Mean serum creatine and eGFR 6 months post BK VST (mg/dL, (mL/min/1.73 m^2))	1.95 (1.20); 37.3 (18.2)
Mean level of BK PCR (IU/mL, (log10)) 12 months post BK VST	579 (541); 1.95 (1.69)
Mean serum creatine and eGFR 12 months post BK VST (mg/dL, (mL/min/1.73 m^2))	1.88 (1.0); 37.3 (14.3)
Mean level of BK PCR (IU/mL, (log10)) at last follow-up	898 (937); 2.92 (1.79)
Mean serum creatine and eGFR at the last follow-up (mg/dL, (mL/min/1.73 m^2))	2.09 (1.14); 33.5 (16.3)
Mean interval from BK-VST to last follow-up (mo)	23.9 (6.7)

Note: BK PCR in IU/mL, Serum creatinine mg/dL, eGFR mL/min/1.73 m^2.

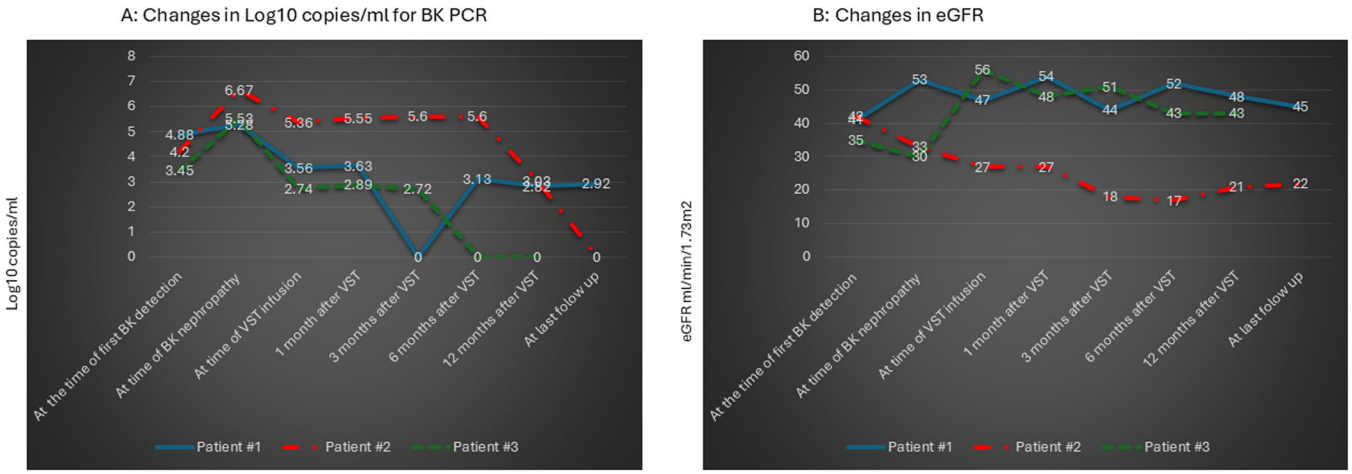

FIGURE 2 | Changes in Log10 copies/mL for BK PCR and eGFR.

Despite the promising safety outcomes presented in this study, it was limited by its small sample size, homogenous patients' population and single-center design, which may affect the generalizability of results. While future availability of "off-the-shelf products" may address some of the issues, our analysis of the BK-VST product revealed that BK-VSTs are a rare cell population within the total lymphocyte pool. Given this finding, it was challenging to enrich and manufacture these cells for therapeutic use. This finding may reflect a limited natural immune response to BKPyV in immunocompromised individuals, which could limit mainstream use of patient specific products. Also, we used different cell manufacturing methods of Cytokine Capture System, compared to the invitro expansion technique used by the recent study published by Chandraker et al. [51] Future studies with larger cohorts are warranted to validate these findings and further establish the efficacy of BKPyV-specific T-cell therapy. Additionally, long-term follow-up beyond 52 weeks would be valuable to assess sustained virological control and impact on graft function. Also, recently, in one phase 2, double-blind study, kidney transplant

TABLE 4 | Adverse events.

Adverse event	Grade 1	Grade 2
Gastrointestinal disorders		
Abdominal pain	1	
Nausea	2	
Vomiting (Occasional)	1	
Hemorrhoid	1	
Diarrhea	1	
Infections and infestations		
Urinary tract infection		3
COVID-19 infection	1	1
Respiratory, thoracic, and mediastinal disorders		
Sore Throat	1	
Nasal Congestion	2	
Rhinorrhea	1	
Cough	1	
Acute Bronchitis	1	
Musculoskeletal and connective tissue disorders		
Flank Pain		1
Arthralgia	3	
Ear and labyrinth disorders		
Ear Pain	2	
Injury, poisoning and procedural complications		
Shingles vaccine reaction	1	
Right knee abrasion (bruise) due to fall	1	
Nervous system disorders		
Dizziness	3	
Headache	1	
Blood and lymphatic system disorders		
Worsening of anemia	1	
Skin and subcutaneous tissue disorders		
Rash maculo-papular	1	
Neoplasm of uncertain behavior	1	
Immune system disorders		
Graves Disease		1
Reproductive system and breast disorders		
Abnormal pap smear		1
Total	27	7
Percent of total	79%	21%

recipients with BKPyV-DNAemia off-the-shelf, allogeneic, multi-virus-specific T-cell investigational therapy targeting BK virus, as well as five other opportunistic viruses: AdV, CMV, EBC, HHV-6, and JC Virus (JCV), was found to be safer, well tolerated and associated with large reduction of BKPyV-DNAemia [51].

In summary, BK-specific T-cell therapy shows potential for being a safe strategy for managing BKPyV infections post-kidney transplantation to address an unmet need in transplant medicine. While preliminary findings are encouraging, further research is needed to evaluate efficacy, optimize treatment protocols and generation of patient-specific products, evaluate long-term outcomes, and establish guidelines for clinical application.

Author Contributions

Lucy Ptak: initial draft, data collection, analysis. **Ross O. Meyers:** editing. **Radko-Ganz:** editing. **Kimberly A. McDowell:** editing. **Margaret Jorgenson:** editing. **Maggie Chilsen:** editing, patient screening. **Didier Mandelbrot:** editing. **Jacques Galipeau:** editing. **Sandesh Parajuli:** original concept, manuscript preparation analysis, editing.

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Disclosure

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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