VOLUME 35 · NUMBER 31 · NOVEMBER 1, 2017

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Off-the-Shelf Virus-Specific T Cells to Treat BK Virus, Human Herpesvirus 6, Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections After Allogeneic Hematopoietic Stem-Cell Transplantation

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Published at jco.org on August 7, 2017.

Clinical trial information: NCT02108522.

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0732-183X/17/3531w-3547w/$20.00

ABSTRACT

Purpose
Improvement of cure rates for patients treated with allogeneic hematopoietic stem-cell transplantation (HSCT) will require efforts to decrease treatment-related mortality from severe viral infections. Adoptively transferred virus-specific T cells (VSTs) generated from eligible, third-party donors could provide broad antiviral protection to recipients of HSCT as an immediately available off-the-shelf product.

Patient and Methods
We generated a bank of VSTs that recognized five common viral pathogens: Epstein-Barr virus (EBV), adenovirus (AdV), cytomegalovirus (CMV), BK virus (BKV), and human herpesvirus 6 (HHV-6). The VSTs were administered to 38 patients with 45 infections in a phase II clinical trial.

Results
A single infusion produced a cumulative complete or partial response rate of 92% (95% CI, 78.1% to 98.3%) overall and the following rates by virus: 100% for BKV (n = 16), 94% for CMV (n = 17), 71% for AdV (n = 7), 100% for EBV (n = 2), and 67% for HHV-6 (n = 3). Clinical benefit was achieved in 31 patients treated for one infection and in seven patients treated for multiple coincident infections. Thirteen of 14 patients treated for BKV-associated hemorrhagic cystitis experienced complete resolution of gross hematuria by week 6. Infusions were safe, and only two occurrences of de novo graft-versus host disease (grade 1) were observed. VST tracking by epitope profiling revealed persistence of functional VSTs of third-party origin for up to 12 weeks.

Conclusion
The use of banked VSTs is a feasible, safe, and effective approach to treat severe and drug-refractory infections after HSCT, including infections from two viruses (BKV and HHV-6) that had never been targeted previously with an off-the-shelf product. Furthermore, the multispecificity of the VSTs ensures extensive antiviral coverage, which facilitates the treatment of patients with multiple infections.

J Clin Oncol 35:3547-3557. © 2017 by American Society of Clinical Oncology

INTRODUCTION

Viral infections remain a major cause of post-transplantation morbidity and mortality in recipients of allogeneic hematopoietic stem-cell transplantation (HSCT), which adds substantially to the clinical and financial burden of transplantation.[1-6] Though pharmacologic agents are available for some clinically problematic viruses, they are not always effective and can result in significant adverse effects. In contrast, the adoptive transfer of stem-cell donor-derived virus-specific T cells (VSTs) has shown efficacy for the treatment of viral pathogens.[7-18] However, broader implementation of this therapeutic approach is limited by (1) the cost and complexity of individualized product manufacture, (2) the time needed for custom manufacturing, which may preclude the immediate availability of VSTs for urgent medical need, and (3) the requirement for seropositive donors—an issue of growing importance given

ASSOCIATED CONTENT

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Appendix
DOI: https://doi.org/10.1200/JCO.2017.73.0655

DOI: https://doi.org/10.1200/JCO.2017.73.0655

the increasing use of younger, virus-naïve donors and cord blood as a source of stem cells.

One way to overcome these limitations and to supply antiviral protection to recipients of allogeneic HSCT would be to prepare and cryopreserve banks of VST lines from healthy seropositive donors, which would be available for immediate use as an off-the-shelf product. Promising results with this approach were first achieved with Epstein-Barr virus (EBV)–specific T cells for the treatment of EBV post-transplantation proliferative disorder[19-21]; our group and others extended the viral target range to include cytomegalovirus (CMV) and adenovirus (AdV).[22,23] However, it was unknown whether banked VSTs would be effective against human herpesvirus 6 (HHV-6) and BK virus (BKV)—both frequent causes of morbidity and mortality that lack effective therapies.[24] It was also unknown whether additional T-cell specificities for these two viruses could be incorporated into a multiple-virus–specific cell product. Therefore, we generated banks of pentavalent T-cell lines specific for 12 viral antigens from EBV, CMV, AdV, HHV-6, and BKV and administered them to 38 recipients of allogeneic HSCT with drug-refractory infections or diseases associated with all five viruses in a phase II clinical trial.

PATIENTS AND METHODS

Third-Party VST Bank

A total of 59 VST lines were manufactured and characterized by flow cytometry and virus specificity by interferon gamma (IFNγ) enzyme-linked immunospot (ELIspot) assay, as previously described.[13] Lines were specific for the viral antigens hexon and penton (for AdV); IE1 and pp65 (for CMV); EBNA1, LMP2, and BZLF1 (for EBV); VP1 and large T (for BKV); and U11, U14 and U90 (for HHV-6). The selection of VST lines for infusion was based on the specificity of the line for the target virus through shared HLA alleles and the overall level of HLA match; the specificity through shared HLA alleles criterion took precedence.

Clinical Trial Design

The phase II study was approved by the US Food and Drug Administration and the Baylor College of Medicine institutional review board. Patients initially gave their consent to search for a suitable VST line. If a line was available, on the basis of the selection criteria (Appendix Fig A1, online only), and if patients met eligibility criteria (Appendix Table A1, online only), they could consent to treatment and receive a single intravenous infusion of 2×10^7 partially HLA-matched VSTs/m^2 with the option to receive a second infusion after 4 weeks and additional infusions at biweekly intervals thereafter. Therapy with standard antiviral medications could be continued at the discretion of the treating physician.

Safety End Points

Safety end points included acute grade 3 to 4 graft-versus-host disease (GVHD) within 42 days of the last dose of VSTs, infusion-related toxicities within 24 hours of infusion, and grade 3 to 5 nonhematologic adverse events related to the VSTs within 30 days of the last VST dose. Patients were also monitored for chronic GVHD for 12 months. Toxicities were graded by the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Clinical and Virologic End Points

Viral loads were monitored by quantitative polymerase chain reaction in laboratories approved by the Clinical Laboratory Improvement Amendments program. Clinical and virologic responses were assigned at week 6 (Appendix Table A2, online only). Clinical responses in individuals treated for BKV hemorrhagic cystitis (HC) were evaluated on the basis of clinical and laboratory documentation by three independent HSCT physicians (two were blinded) according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0, and also were graded on the grading system proposed by Bedi et al[25] (Appendix Table A3, online only).

Immune Monitoring

IFNγ ELIspot analysis was used to determine the frequency of circulating T cells specific for viral antigens and peptides.[13] When available, individual HLA-restricted epitope peptides (Genemed, San Antonio, TX) were used to track donor-derived VSTs postinfusion.[26-36]

Statistical Analysis

Descriptive statistics were calculated to summarize clinical characteristics. Comparisons were made between groups by using the nonparametric Wilcoxon rank sum test or Kruskal-Wallis exact test for continuous variables and the Fisher's exact test for categoric variables.

RESULTS

Patients

We screened 56 recipients of allogeneic HSCT and identified a suitable VST line for 54 patients (96.6%). Of these, 16 did not receive VSTs, either because intervention was considered not required (n = 4) or because the patients were ineligible (n = 12; Appendix Table A4, online only). Of the 38 patients who were treated (Table 1), 31 received cells to treat a single virus, and seven patients were treated for two viral infections (Table 2). A total of 23 patients received a single infusion, 11 patients had two infusions, and four patients had three infusions of VSTs.

VST Line Characteristics

From the bank of 59 VST lines, 23 were administered to one to eight patients, with matching at one of eight to seven of eight HLA alleles (Appendix Table A5, online only). The infused cells were almost exclusively $CD3^+$ T cells (mean ± SEM, 97.3% ± 0.4%). A mixture of $CD4^+$ (60.4% ± 3.9%) and $CD8^+$ (34.2% ± 3.6%) subsets expressed both central ($CD45RA^-/62L^+/CCR7^+$: 39.1% ± 5.5%) and effector ($CD45RA^-/62L^-/CCR7^-$: 11.8% ± 1.6%) memory markers and recognized the target viruses.

Clinical Responses

The cumulative clinical response rate in 37 evaluable patients was 91.9% (95% CI, 78.1% to 98.3%) after one VST infusion by week 6 (Fig 1A). Of 18 patients who were screened but did not receive VST therapy, 12 developed progressive disease (Appendix Table A4).

CMV. A total of 17 patients received VSTs for persistent CMV (Table 2), which in eight patients was confirmed by CMV gene sequencing to be resistant to conventional antiviral drugs. A total of 16 patients responded to VSTs with six complete responses (CRs) and 10 partial responses (PRs); the cumulative response rate was 94.1% (95% CI, 71.3% to 99.9%; Fig 1B) by week 6. Figure 2A summarizes the outcomes of all patients treated for CMV as assessed by sequential viral load measurement. Clinical benefit was

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	No. (%) of Infused Patients (N = 38)
Male sex	18 (47)
Race/ethnicity	
Hispanic	5 (13.1)
Black or African American	3 (7.9)
White	29 (76.3)
Other	6 (15.8)
Mean age at infusion, years (SD)	35.6 (21.6)
Transplantation type	
Matched related donor	4 (10.5)
Matched unrelated donor	19 (50)
Mismatched unrelated donor	3 (7.9)
Haploidentical	5 (13.1)
Umbilical cord blood	7 (18.4)
Reason for transplantation	
AML/MDS	20 (52.6)
ALL	9 (23.7)
Lymphoma/MM	3 (7.9)
Nonmalignant disorders	6 (15.8)
Infections treated (n = 45)	
CMV	17 (44.7)
EBV	2 (5.3)
AdV	7 (18.4)
BKV	16 (42.1)
HHV-6	3 (7.9)
No. of HLA matches in recipient of eight possibilities to first VST line (n = 38)	
1	1 (2.6)
2	7 (18.4)
3	9 (23.7)
4	11 (28.9)
5	5 (13.1)
6	2 (5.3)
7	3(7.9)
No. of infusions	
1	23 (60.5)
2	11 (29)
3	4 (10.5)

Abbreviations: AdV, adenovirus; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BKV, BK virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV-6, human herpesvirus 6; MDS, myelodysplastic syndrome; MM, multiple myeloma; SD, standard deviation; VST, virus-specific T cell.

achieved both in patients with refractory infections and in individuals with biopsy-proven CMV colitis (n = 3). Overall, of the 16 patients who responded to VST treatment, nine had a concomitant increase in circulating CMV-reactive T cells and a five-fold mean increase in spot-forming cells (SFCs) from 73 (± 59) SFCs/5 × 10^5 peripheral blood mononuclear cells (PBMCs) before infusion to 399 (± 141) SFCs/5 × 10^5 PBMCs after infusion (Fig 3A). For example, patient 2936 had refractory CMV that was resistant to ganciclovir, as confirmed by CMV antiviral resistance sequencing. Within 6 weeks of VST therapy, the virus was undetectable, which coincided with an increase in the frequency of circulating CMV-specific T cells (Appendix Fig A2A, online only). Patient 3840 had refractory CMV that was resistant to foscarnet and ganciclovir, as confirmed by CMV antiviral resistance sequencing, and had CMV inclusions evident on numerous ulcers detected in the ileum and colon (Appendix Fig A2B). Despite 3 weeks of treatment with cidofovir, the CMV titers of this patient continued to increase, which caused worsened abdominal cramping that required high-dose opioid treatment. After VST infusion, the viral titers decreased (Fig 3A) coincident with rapid symptomatic improvement and complete resolution of abdominal cramping without the need for additional narcotics by postinfusion week 4.

EBV. Both patients treated for EBV achieved a virologic CR by week 6 (Table 2; Fig 2B). The CR in one patient was associated with a concomitant increase in circulating EBV-specific T cells (Fig 3B).

AdV. Seven patients received VSTs for persistent AdV (Table 2), which resulted in four CRs, one PR, two nonresponses, and a cumulative response rate of 71.4% (Fig 1C and Fig 2C). Clinical benefit was associated with an increase in the frequency of circulating AdV-specific T cells in four patients (Fig 3C). Responders included one patient (patient 4002) with AdV pneumonitis and HC who experienced a PR—a 93% reduction in viral load after an initial infusion of VSTs followed by complete virologic and clinical remission after a second infusion of the same VST line, with a corresponding increase in AdV-specific T cells (Appendix Fig A3, online only).

HHV-6. VSTs were infused to three patients to treat HHV-6 reactivations (Table 2), and the response rate was 67% (PRs [n = 2] and not evaluable [n = 1]). Patient 4084 had elevated viral titers along with fevers and symptoms of bone marrow suppression, including neutropenia. After treatment, symptoms resolved and the viral load decreased, which was associated with the detection of HHV-6–specific T cells in the peripheral blood (Fig 2D and Fig 3D). Patient 4057, who presented with decreased alertness and responsiveness, was infused with VSTs to treat refractory HHV-6 encephalitis and BKV HC. Within 24 hours, the patient exhibited improved alertness and sustained normalization of mental status within 1 week, which correlated with a decrease in HHV-6 viral titers from 51,500 to 1,200 copies/mL (Fig 2D). Finally, Patient 3854 received VSTs to treat persistently elevated HHV-6 levels in the absence of clinical symptoms. Though the viral load decreased postinfusion, it was not eliminated, and subsequent investigation confirmed the presence of chromosomally integrated HHV-6, which is associated with indefinitely elevated HHV-6 titers.[37] Therefore, given a lack of a clinical or virologic end point, this patient was excluded from additional analysis.

BKV. A total of 16 patients with tissue disease (BKV-associated HC [n = 14] and BKV-associated nephritis [n = 2]) were treated with VSTs; all 16 patients (100%; 95% CI, 79.4% to 100%; Fig 1D) achieved clinical benefit (Table 2) associated with a median decrease of 85.5% in urinary viral load by week 6 and of 96% by week 12 postinfusion. Both patients treated for biopsy-proven nephritis responded virologically to VSTs; in one patient, response was associated with a decrease in creatinine from 1.7 mg/dL before infusion to 1.25 mg/dL at week 6 postinfusion and sustained improved renal function. In addition, 13 of the 14 patients with HC had complete resolution of gross hematuria by week 6 postinfusion, but symptomatic improvement was notably more rapid as captured with the National Cancer Institute cystitis grading scale. Before VSTs, these patients with HC exhibited moderate (grade 2, n = 3) to severe (grade 3, n = 11) symptoms and an average cystitis grade of 2.8 ± 0.1. However, within 2 weeks, the symptomatic severity had decreased by at least one grade in 12 of these 14 patients and continued to decline thereafter (Figs 4A and 4B). Seven of the responding patients had a concomitant increase in the frequency of circulating BKV-specific T cells (mean increase,

Table 2. Outcomes After Treatment With Banked VSTs

Patient	Infection	Duration of Standard Antiviral Therapy Failure	Duration of Standard Therapy After Infusion	Tissue Disease	Line Infused	HLA Matching (of eight lines)	No. of Infusions	Best Response by 6 Weeks	Outcome
2936	CMV*	No standard treatment	No standard treatment	No	C5998	5	3	CR	Sustained CR after third infusion
3784	CMV*	No standard treatment	3 weeks	No	C5404	4	1	CR	Sustained CR
3809	CMV* (AdV)	17 days	No standard treatment	No	C5404	7	1	CMV PR AdV CR	Died at 14 days postinfusion as a result of fungal pneumonia
3830	CMV*	11 weeks	No standard treatment	No	C5381	4	1	CR	Sustained CR
3840	CMV*	2 weeks	3 weeks	CMV colitis	C5404	3	1	PR	Resolution of colitis, CR after day 42
3848	CMV*	6 weeks	No standard treatment	CMV colitis	C5404 C5678	3 4	3	PR	PR with recurrence at 4 weeks
3843	CMV	3 months	3 weeks	CMV colitis	C6378	4	1	NR	Died 27 days postinfusion as a result of bacterial sepsis
3868	CMV	6 weeks	No standard treatment	No	C5667	3	1	PR	Died 29 days postinfusion as a result of relapsed AML
3904	CMV (BKV)	22 days	3 weeks	BKV HC	C5757	3	1	CMV CR	Sustained CR for CMV, BKV HC symptom resolution
3924	CMV*	6 weeks	6 weeks	No	C6323	7	2	PR	PR with recurrence
3827	CMV	16 days	11 weeks	No	C6391	1	2	PR	Sustained CR after second infusion
3357	CMV	3 weeks	4 weeks	No	C5678 C6323	4 5	2	PR	Sustained CR
3921	CMV	2 weeks	6 weeks	No	C5682	2	1	PR	Sustained CR
3967	CMV*	23 days	14 weeks	No	C5557	3	1	PR	Sustained CR after day 42
4056	CMV	7 weeks	No standard treatment	No	C5404	2	2	PR	Sustained CR after second infusion
4076	CMV AdV	2 weeks	2 weeks	No	C6209 C6611	6 3	2	CMV CR AdV CR	Sustained CR for CMV; recurrence of AdV with sustained CR after second infusion
4126	CMV BKV	3 weeks	No standard treatment	BKV HC	C5557	7	1	CMV CR BKV virologic CR, clinical CR	Sustained CR for CMV, BKV HC symptom resolution
3750	EBV	3 weeks	No standard treatment	PTLD	C5435	3	1	virologic CR	CR, died day 41 as a result of fungal and bacterial infections
3755	EBV BKV	No standard treatment	No standard treatment	No	C5602 C5624	5 2	2	EBV CR BKV PR	Sustained CR for EBV PR for BKV with stable renal function
3809	AdV CMV*	17 days	No standard treatment	No	C5404	7	1	AdV CR CMV PR	Died at 14 days postinfusion as a result of fungal pneumonia
3869	AdV	11 weeks	3 weeks	Enteritis	C6323	5	1	NR	Died day 23 postinfusion as a result of progressive AdV infection
4021	AdV	8 weeks	7 weeks	No	C6250 C6323	5 5	3	NR	PR after third infusion, but recurrence; died as a result of refractory AdV
4002	AdV	No standard treatment	2 weeks	Pneumonia, HC	C5442	4	2	PR	CR after second Infusion
4076	AdV CMV	Standard treatment held	2 weeks	No	C6209 C6611	6 3	2	AdV CR CMV CR	Sustained CR for CMV; recurrence of AdV with sustained CR after second infusion
4134	AdV	7 weeks	No standard treatment	URTI, enteritis	C6498	6	1	CR	Sustained CR for AdV
4157	AdV BKV	No standard treatment	No standard treatment	BKV and AdV HC	C6250	4	1	AdV CR BKV virologic PR, clinical PR	Sustained CR for AdV BKV HC symptom resolution

(continued on following page)

Table 2. Outcomes After Treatment With Banked VSTs (continued)

Patient	Infection	Duration of Standard Antiviral Therapy Failure	Duration of Standard Therapy After Infusion	Tissue Disease	Line Infused	HLA Matching (of eight lines)	No. of Infusions	Best Response by 6 Weeks	Outcome
3854	HHV-6	3 months	5 weeks	Bone marrow	C6378	4	2	NE	HHV-6 genomic integration confirmed, no clinical symptoms
4057	HHV-6 (BKV)	5 weeks	5 weeks	CSF	C7626	2	2	PR, BKV virologic PR, clinical PR	Complete resolution of encephalitis; resolution of HC after second infusion
4084	HHV-6	1 week	Intermittent treatment	No	C5757	3	1	PR	Resolution of symptoms
3755	BKV EBV	No standard treatment	No standard treatment	Nephritis	C5602 C5624	5 2	2	BKV virologic PR EBV CR	PR for BKV with unchanged renal function; sustained CR for EBV
3796	BKV	No standard treatment	No standard treatment	BKV HC	C5497	4	1	Virologic PR Clinical CR	Resolution of HC
3810	BKV	3 weeks	No standard treatment	BKV HC	C5497	4	1	Virologic PR Clinical CR	Resolution of HC
3859	BKV	12 weeks	6 months	Nephritis	C6509 C6323	4 3	2	Virologic PR	Sustained improvement of renal failure
3870	BKV	2 weeks	10 weeks	BKV HC	C6323	3	1	Virologic PR Clinical CR	Resolution of HC
3902	BKV	4 weeks	4 weeks	BKV HC	C5757	2	1	Virologic PR Clinical CR	Resolution of HC
3904	BKV CMV	3 weeks	3 weeks	BKV HC	C5757	3	1	BKV Virologic PR, clinical CR CMV CR	Resolution of HC Sustained CR for CMV
3908	BKV	No standard treatment	No standard treatment	BKV HC	C6250	4	1	Virologic PR Clinical PR	Resolution of HC
3864	BKV	No standard treatment	No standard treatment	BKV HC	C6323	2	1	Virologic PR Clinical CR	Resolution of HC
3877	BKV	No standard treatment	No standard treatment	BKV HC	C6322 C5602	3/6 4	3	Virologic PR Clinical PR	Resolution of HC after third infusion
3899	BKV	No standard treatment	No standard treatment	BKV HC	C6726 C5497	4 3	2	Virologic PR Clinical PR	Resolution of HC after second infusion
3929	BKV	No standard treatment	No standard treatment	BKV HC	C6323	5	1	Virologic PR Clinical PR	Resolution of HC
4057	BKV HHV-6	5 weeks	No standard treatment	BKV HC	C7626	2	2	Virologic PR Clinical PR	Resolution of HC after second infusion CR for HHV-6 on basis of symptoms
4108	BKV	No standard treatment	No standard treatment	BKV HC	C6323	2	1	Virologic PR Clinical PR	Resolution of HC
4126	CMV BKV	3 weeks	No standard treatment	BKV HC	C5557	7	1	CMV CR BKV virologic CR Clinical CR	Resolution of HC Sustained CR for CMV
4157	AdV BKV	No standard treatment	No standard treatment	BKV and AdV HC	C6250	4	1	AdV CR BKV virologic PR, clinical PR	Resolution of HC Sustained CR for AdV

Abbreviations: AdV, adenovirus; AML, acute myeloid leukemia; BKV, BK virus; CMV, cytomegalovirus; CR, complete response; EBV, Epstein-Barr virus; HC, hemorrhagic cystitis; HHV-6, human herpesvirus 6; NE, not evaluable; NR, no response; PR, partial response; PTLD, post-transplantation lymphoproliferative disorder; URTI, upper respiratory tract infection; VST, virus-specific T cell.
*Confirmed resistance of CMV strain to standard antiviral medications.

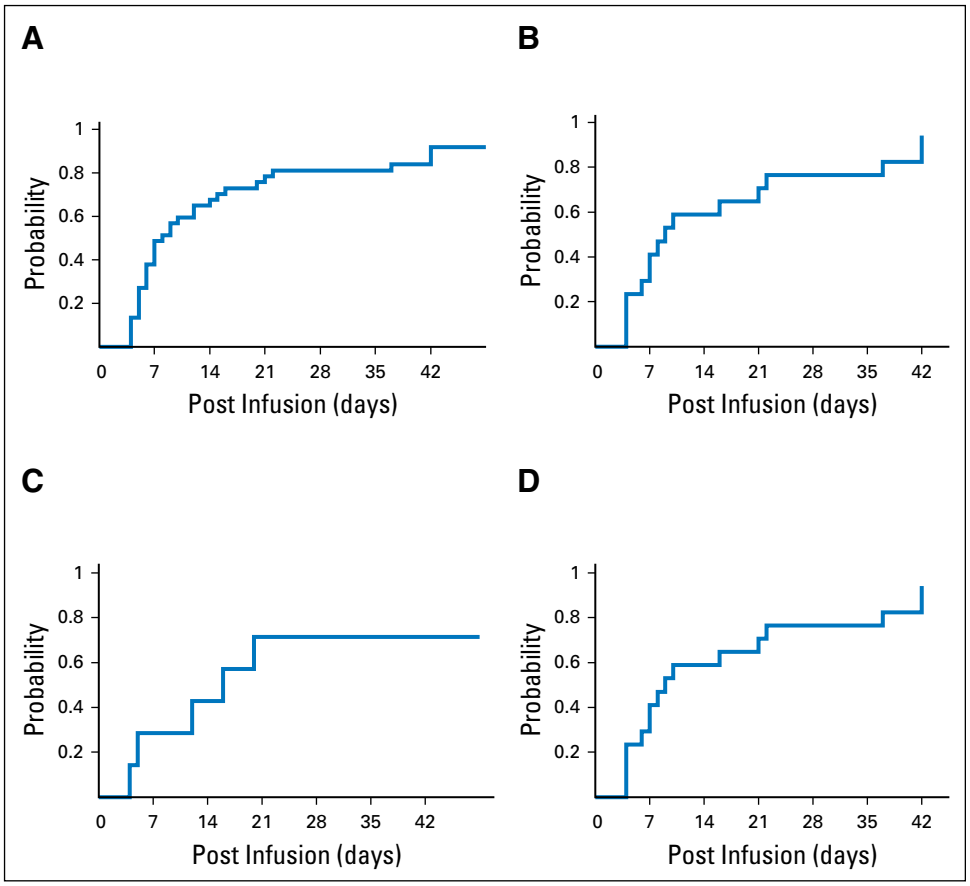

Fig 1. Cumulative incidence response rate that is based on time to first complete response and/or partial response in (A) overall patients (n = 37); one patient with nonevaluable data was excluded; (B) patients with cytomegalovirus infections (n = 17); (C) patients with adenovirus infections (n = 7), and (D) patients with BK virus infections (n = 16).

from 13 ± 10 to 245 ± 114 SFCs/5 × 10^5 PBMCs; Fig 3E). One example is patient 3899, who was in secondary graft failure after HSCT and BKV HC, which precluded him from advancement to a second HSCT because of profound hematuria with significant transfusion requirements. The patient had developed a large bladder clot and was unable to tolerate standard antiviral treatment (Fig 4C, left panel). After VST infusion, his hematuria resolved, the bladder clot decreased by 98% within 3 weeks (reduction, from 66.1 to 1.4 cm^3; Fig 4C, right panel), and he successfully proceeded to transplantation.

Dual Infections. Seven patients received VSTs for two viral infections, and all treatments were able to control both viruses with a single infusion. CMV, AdV, and EBV were cleared in all cases, and all patients with BKV HC and the patient with HHV-6 encephalitis had clinical improvement or disease resolution.

Durability. Of the 34 patients who achieved a PR or CR after a single infusion of VSTs, seven had a subsequent recurrence (median, 10 weeks), which was successfully treated in six patients. One (n = 5) or two (n = 1) additional infusions provided durable benefit.

Repeat Infusions. Overall, 15 patients with no response (n = 1), PR (n = 7), or recurrence (n = 7) received a second infusion of the same (n = 8) or a different (n = 7) VST line at a median interval of 39 days (range, 21 to 214 days) after the initial infusion, which produced clinical benefit in 10 patients (77%; CR [n = 1] and PRs [n = 9]). Four patients received a third VST infusion, and three of the patients (75%) responded to therapy (CRs [n = 2], PR [n = 1], and no response [n = 1]).

In Vivo T-Cell Persistence

To evaluate the in vivo longevity of these partially HLA-matched VSTs, we interrogated the specificity of circulating T-cells postinfusion, and we discriminated between infused versus endogenous cells on the basis of peptide-epitope specificity in 16 clinical responders with adequate PBMC numbers and available reagents (Appendix Table A6, online only). In 11 patients (69%), we confirmed the persistence of VSTs from the infused line for up to 12 weeks (Figs 3F to 3K). For example, Figure 3F shows the longitudinal analysis of T-cell responses in patient 3929, who received VST line C6323 to treat persistent BKV HC. At 2 weeks post-infusion the dominant detectable specificities represented epitopes presented in the context of alleles that were either unique to the infused VST line or shared between the line and patient. Over time, the frequency of third-party–derived specificities declined, coincident with endogenous immune reconstitution. Figures 3G through 3K show similar patterns of activity in other VST responders.

In patient 3864, short tandem repeats analysis was performed in addition to epitope profiling (Fig 3H), which showed that third-party VSTs comprised 4% of all circulating T cells after peak expansion and persisted for a total of 12 weeks until BKV clearance.

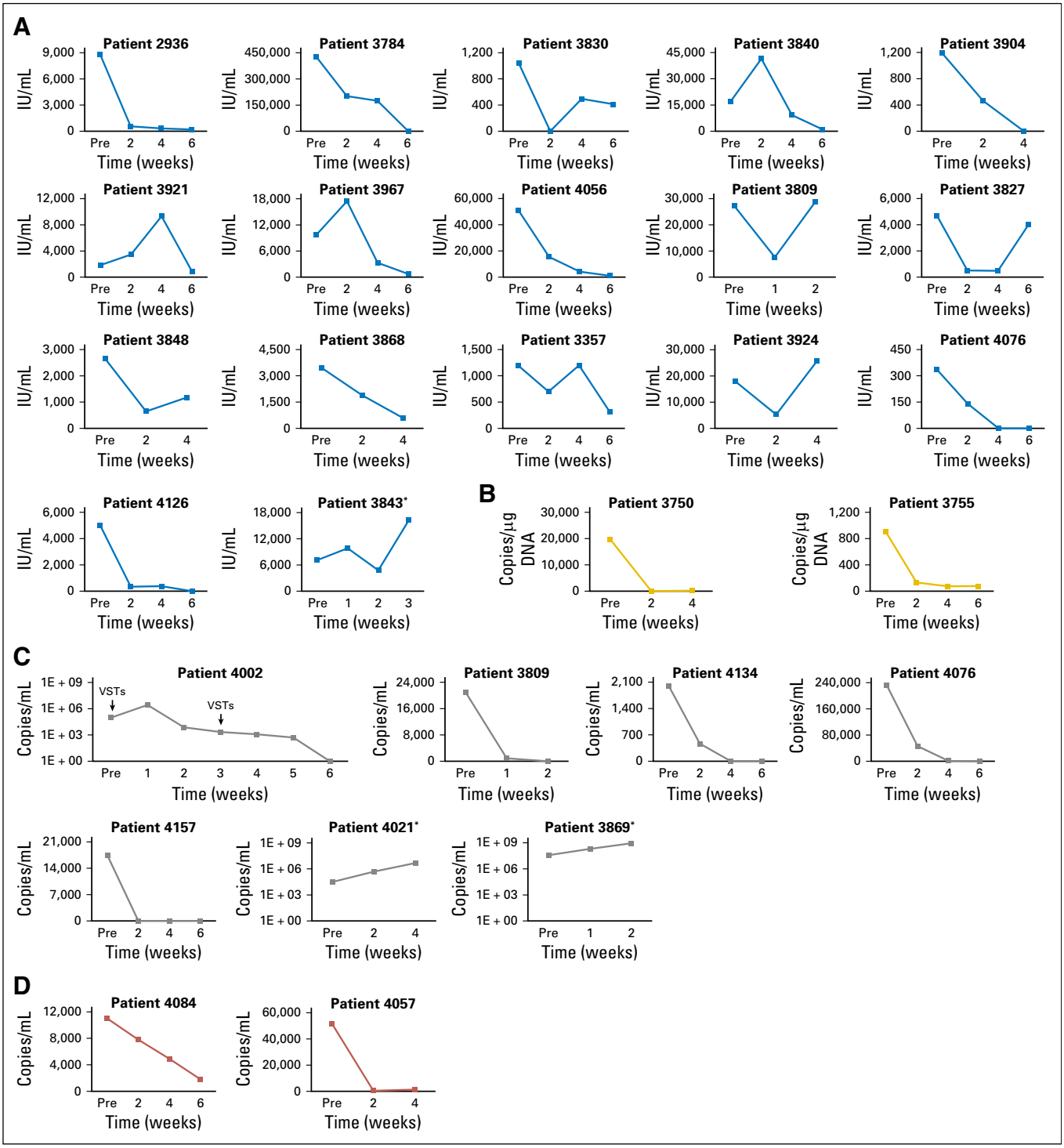

Fig 2. Treatment outcomes in patients infected with cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, and human herpesvirus 6 (HHV-6). Depiction of plasma viral load before (pre) and after (post) infusion of virus-specific T cells in all patients treated for (A) CMV infection, (B) EBV infection, (C) adenovirus infection, and (D) HHV-6 infection. (*) Nonresponders.

Clinical Safety

All infusions were well tolerated. Except for one patient who developed an isolated fever within 24 hours of infusion, no immediate toxicities were observed. None of the patients developed cytokine release syndrome. Nineteen patients (50%) had prior grade 2 to 4 GVHD (grade 2, n = 15; grade 3, n = 4), which was quiescent at the time of VST infusion (Appendix Table A7, online only). After infusion, one patient developed recurrent grade 3 GI GVHD after rapid corticosteroid taper, and five patients developed recurrent (n = 3) or de novo (n = 2) grade 1 to 2 skin

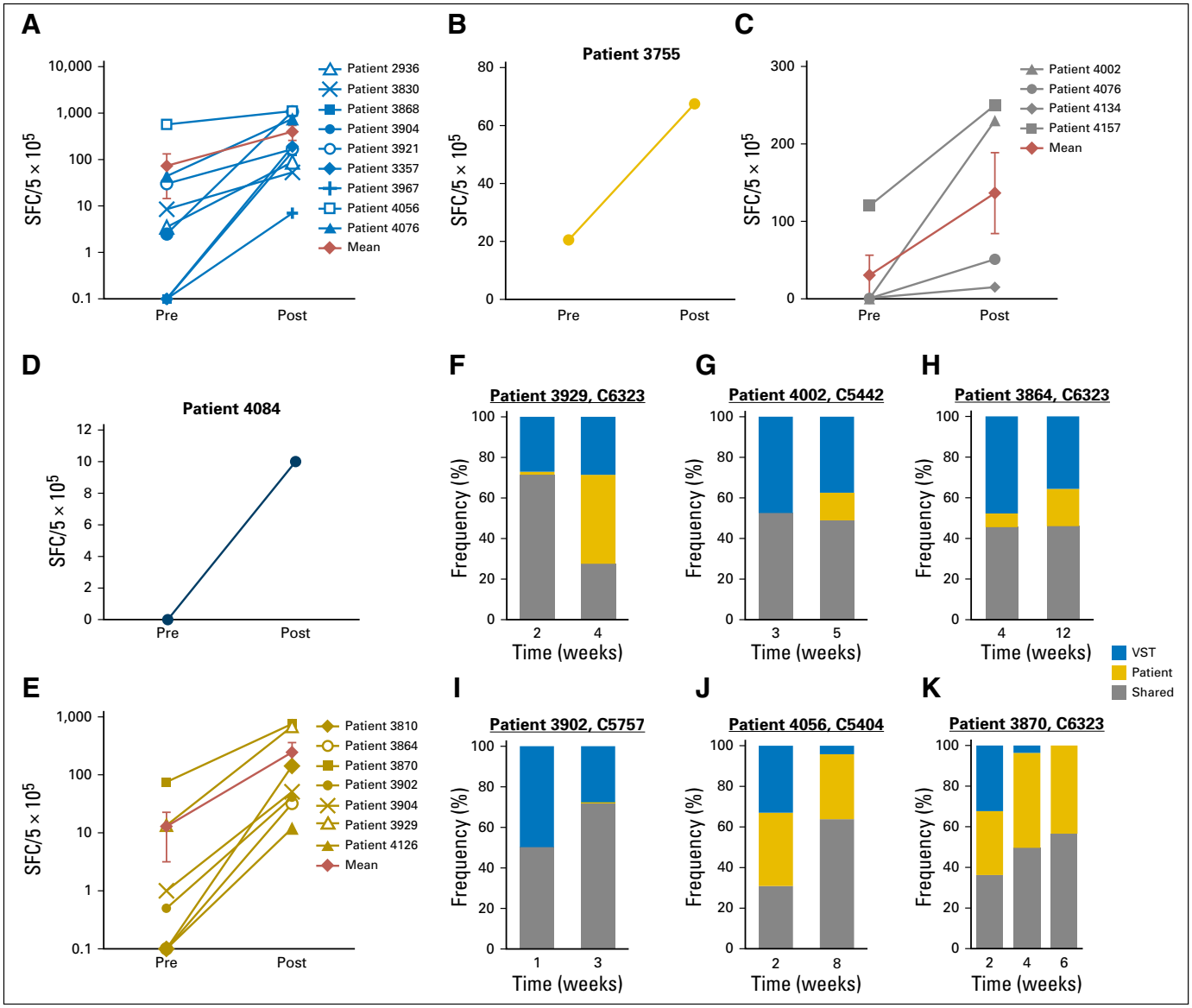

Fig 3. Frequency of viral-specific T cells (VSTs) in vivo in the peripheral blood before (pre) and after (post) infusion, as measured by interferon gamma enzyme-linked immunospot (ELIspot) assay after stimulation with viral pepmixes. Results are expressed as spot-forming cells (SFCs) per 5×10^5 input cells with specificity for patients with (A) cytomegalovirus, (B) Epstein-Barr virus, (C) adenovirus, (D) human herpesvirus 6, and (E) BK virus. (F-K) Frequency of T cells in peripheral blood as measured by interferon gamma ELIspot assay after stimulation with epitope-specific peptides with restriction to HLA antigens exclusive to the VST line or the recipient, or shared between the two.

GVHD, which resolved with the administration of topical treatments (n = 4) and reinitiation of corticosteroid treatment after a taper (n = 1). In long-term follow-up, two patients had a flare of upper-GI GVHD, which resolved after a brief corticosteroid course. Finally, one patient who received VSTs as treatment of BKV HC experienced transient hydronephrosis and a decrease in renal function associated with a concomitant bacterial urinary tract infection that resolved within 2 weeks.

DISCUSSION

In this phase II study, we administered third-party VSTs to recipients of allogeneic HSCT with drug-refractory infections associated with five of the most frequent infectious causes of post-transplantation morbidity and mortality. We could identify a suitable VST line for 54 of the 56 patients screened for study participation, of whom 38 were infused. Before study entry, the majority of these patients had experienced failure of at least two lines of conventional agents or were infected with drug-resistant viral variants; nevertheless, VSTs produced a 92% overall CR or PR rate (CMV, 94%; BKV, 100%; EBV, 100%; AdV, 71%; and HHV-6, 67%), which included responses in all seven patients who had multiple coexisting infections. To our knowledge, this trial is the first to extend the use of banked VSTs to five viruses, including BKV and HHV-6.

In recipients of HSCT, viral reactivations of BKV are frequent and are associated with severe pain, hematuria, and renal

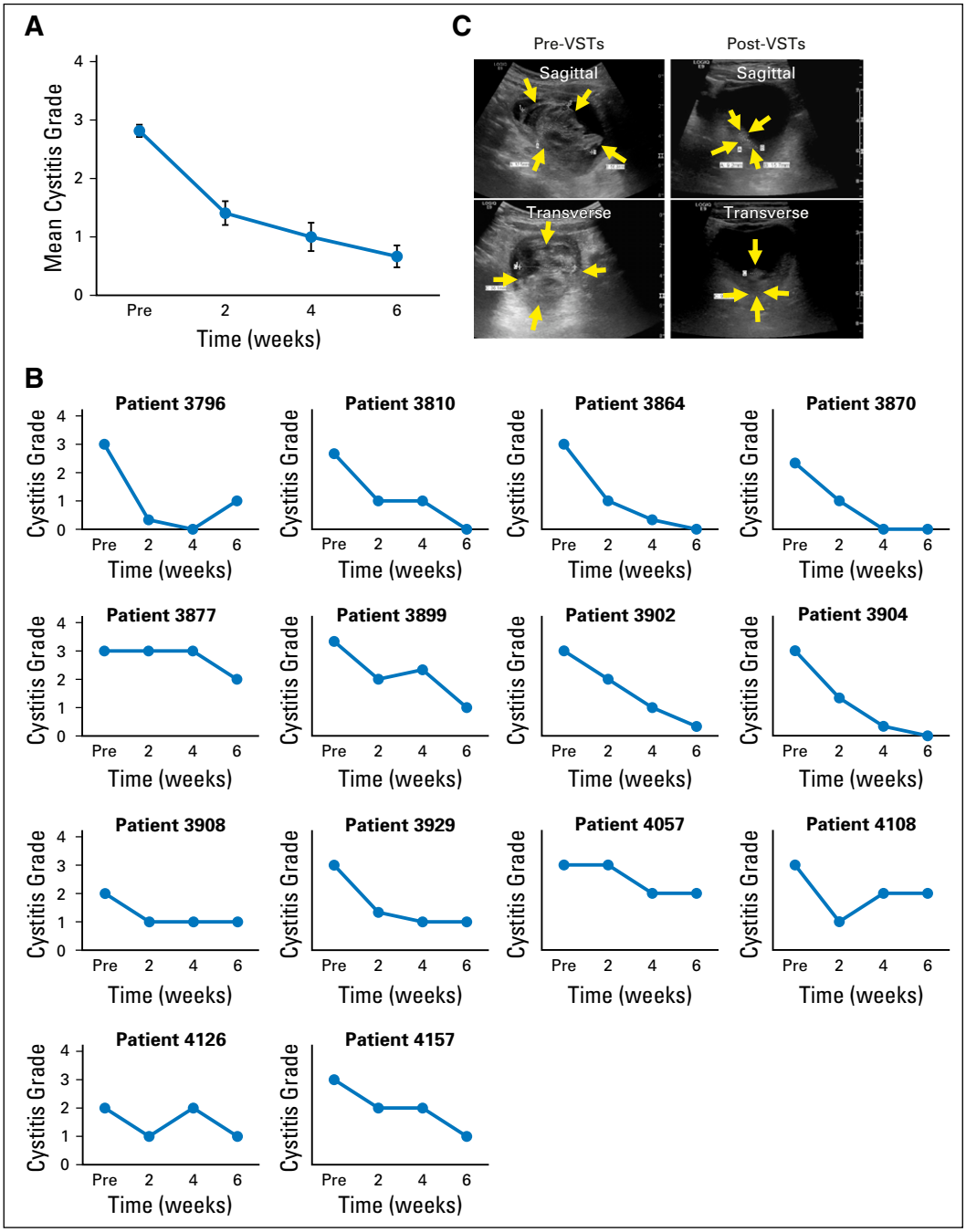

Fig 4. Treatment outcomes in BK virus–infected patients (n = 14). BK virus–associated hemorrhagic cystitis symptom score before infusion and at 2, 4, and 6 weeks after infusion of virus-specific T cells (VSTs); the grade is based on the National Cancer Institute hemorrhagic cystitis grading scale. Results are presented as (A) mean (± SEM) grade of all patients and (B) individually for each patient. (C) Example of a patient with BK virus–associated hemorrhagic cystitis. Ultrasound imaging depicts the blood clot in the bladder, shown (left) preinfusion and (right) 3 weeks after infusion.

disease.[4,38,39] No antiviral drugs for BKV have been approved or tested in randomized clinical trials. Cidofovir, which is most often administered to treat BK-associated HC, is associated with major drug-related adverse effects that include myelotoxicity and nephrotoxicity; cidofovir is frequently ineffective and offers a survival rate of just 14% in the third of patients whose disease fails to respond.[4,40] In this study, we treated 16 patients with BKV disease without significant treatment-related toxicities, and benefit—including complete resolution of gross hematuria in 13 of 14 patients with BKV-associated HC by week 6 postinfusion—was achieved in all patients. In contrast, four of five patients with HC who were screened for study participation but not treated with VSTs experienced disease progression. In some patients, symptomatic improvement of HC without reduction in BK viruria was observed, which is consistent with previous reports that virus titers in urine in patients with significant gross

hematuria show no clear association with symptoms[38] and that asymptomatic viremia or viruria is not necessarily clinically relevant.[41-43]

Both evaluable patients treated for HHV-6, one of whom had neurologic impairment caused by HHV-6 encephalitis, responded to VSTs. There was a decrease in HHV-6 levels and resolution of clinical symptoms in each case.

Incorporation of these additional specificities into multiple-virus VSTs did not compromise activity against CMV, AdV, or EBV. When antiviral activities of the new pentavalent and previous triple-virus lines were compared, the magnitude of the in vitro response for these viruses was similar. Not surprisingly, therefore, clinical benefit was maintained. Indeed, in our previous phase II study, response rates for CMV, AdV, and EBV were 74%, 78%, and 67%, respectively, in patients with similar disease characteristics; response rates were 94%, 71%, and 100%, respectively, in this study. We found no correlation between viral load reduction and HLA matching in patients who received low (one to three alleles matched) versus high (four to eight matching alleles) HLA-matched VST lines (P = .961). Indeed, a CR was achieved in a patient treated with a VST line matched at just a single HLA allele, with strong antiviral activity through this shared allele, which highlights the importance of careful VST line selection. In this study, the majority of responders had a detectable expansion in the frequency of circulating VSTs postinfusion. The epitope profiling method enabled us to track the presence of functional VSTs of third-party origin over time, which persisted for up to 12 weeks, in 11 patients. Third-party T cells predominated immediately post-infusion, but this early expansion did not come at the expense of increased alloreactivity; we observed only two occurrences of (mild) de novo GVHD. Thereafter, the numbers declined coincident both with resolution of viral infection and with endogenous T-cell recovery.

Although a randomized trial will be required to definitively assess the value of banked VSTs, this study strongly suggests that off-the-shelf multiple-virus–directed VSTs are a safe and effective broad-spectrum approach to treat severe viral infections after HSCT. These VSTs can be rapidly and cost-effectively produced in scalable quantities with excellent long-term stability, which facilitates the broad implementation of this therapy. More widespread and earlier use of this modality could minimize both drug-related and virus-associated complications and thereby decrease treatment-related mortality in recipients of allogeneic HSCT.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Support

Supported by a National Heart, Lung, and Blood Institute Production Assistance for Cellular Therapy grant (to B.O. for the clinical virus-specific T-cell manufacture of this investigator-initiated study); by a Conquer Cancer Foundation/American Society for Clinical Oncology grant (to B.O. for correlative studies); and by Dan L. Duncan Cancer Center Support Grant No. P30CA125123 (for shared resources). B.O. was supported by an educational National Institutes of Health K12 faculty fellowship grant at Texas Children's Hospital.

The funding sources had no role in trial design, data collection, interpretation of data, or decision to publish.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Off-the-Shelf Virus-Specific T Cells to Treat BK virus, Human Herpesvirus 6, Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections After Allogeneic Hematopoietic Stem-Cell Transplantation

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Acknowledgment

We thank all those who participated in this trial; the clinicians, their staff, and their patients: Amy Reyna for study coordination; Sara Richman and Deborah Lyon for quality assurance and quality control; Huimin Zhang and her team for cell processing; and Bridget Medina for regulatory assistance.

Appendix

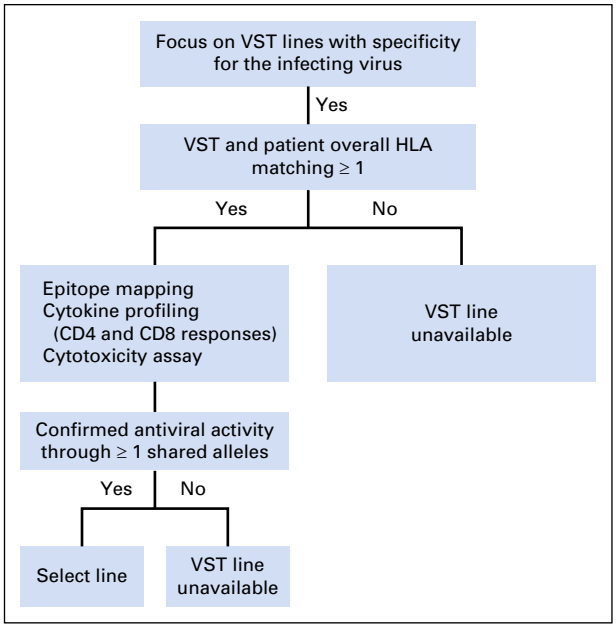

Fig A1. Decision algorithm for virus-specific T-cell (VST) selection.

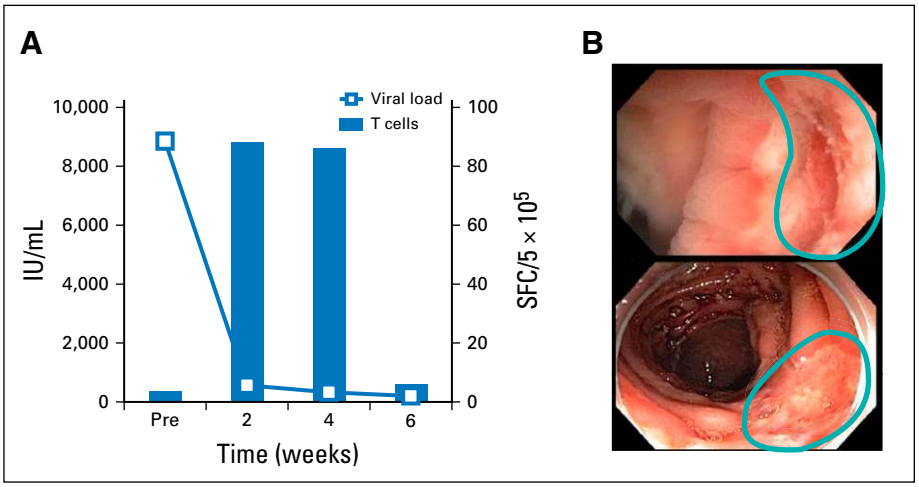

Fig A2. Examples of patients infected with cytomegalovirus (CMV): (A) Patient 2936 with CMV infection. Viral load (left *y*-axis) and frequency of CMV-directed T cells in peripheral blood (right *y*-axis) before infusion and at 2, 4, and 6 weeks after infusion as measured by interferon gamma enzyme-linked immunospot assay. (B) Endoscopic picture of patient 3840 with CMV colitis that depicts ulcers (in circled areas) in the ileum and colon before infusion. SFC, spot-forming cell.

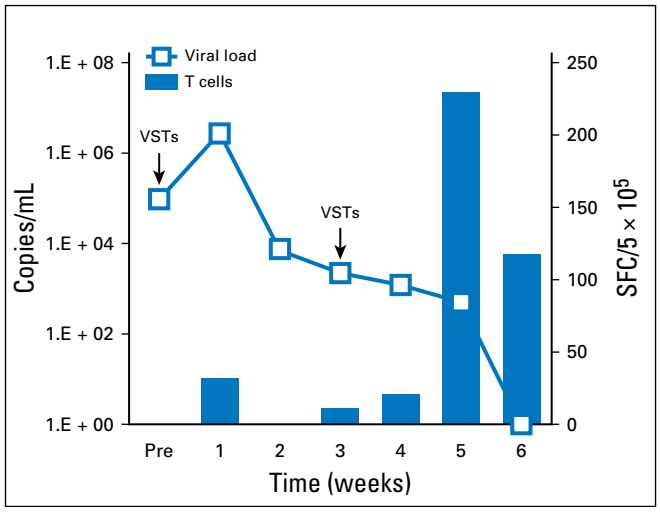

Fig A3. Example of a patient infected with adenovirus; patient 4002 with adenovirus respiratory tract infection; plasma viral load (left *y*-axis) and frequency of virus-specific T cells (VSTs; right *y*-axis) in peripheral blood over time. SFC, spot-forming cell.

Table A1. Eligibility Criteria

Inclusion Criteria	Exclusion Criteria
Received prior myeloablative or nonmyeloablative allogeneic hematopoietic stem-cell transplantation with bone marrow, single/double cord blood, or PBSC	Received ATG, alemtuzumab, or other T-cell immunosuppressive monoclonal antibodies in the last 28 days
For treatment of relapsed or persistent reactivation or infection for EBV, CMV, AdV, HHV-6, and/or BKV despite standard therapy (multiple infections eligible to enroll)	Patients with other uncontrolled infections
For early treatment of single or multiple infections with EBV, CMV, AdV, HHV-6, and/or BKV after experiencing treatment failure or unable to tolerate standard therapy	Patients who are fewer than 28 days removed from their allogeneic hematopoietic stem-cell transplantation or who have received donor lymphocyte infusions within 28 days
For treatment of progressive or persistent JC virus infection	Evidence of grade 2 or greater GVHD
Corticosteroids (prednisone $\leq$ 0.5 mg/kg/d)	Active and uncontrolled relapse of malignancy
HgB $>$ 8.0 (can be transfused)	
Pulse oximetry of $>$ 90% on room air	
Available VSTs	
Negative pregnancy test (if woman of childbearing potential after reduced intensity conditioning)	
Patient or parent/guardian capable of providing informed consent	

Abbreviations: ATG, anti-thymocyte globulin; AdV, adenovirus; BKV, BK virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; HgB, hemoglobin; HHV-6, human herpesvirus 6; JC virus, John Cunningham virus; PBSC, peripheral blood stem cell; VST, virus-specific T cell.

Table A2. Definitions of Response

Response	Definition
Complete response	Return to normal range, as defined by specific assay (quantitative PCR in all reported patients) used and clinical signs and symptoms
Partial response	Decrease in viral load by quantitative PCR of at least 50% from baseline or 50% improvement of clinical signs and symptoms
Stable disease	Changes insufficient to qualify as partial response or progression (combined with progressive disease as no response in text)
Progressive disease	Increase in viral load by quantitative PCR of at least 50% from baseline or dissemination to other sites of disease (combined with stable disease as no response in text)

Abbreviation: PCR, polymerase chain reaction.

Table A3. Cystitis Grading Scale

Cystitis Grade	Symptoms
1	Microscopic hematuria; minimal increase in frequency, urgency, dysuria, or nocturia; new onset of incontinence
2	Moderate hematuria; moderate increase in frequency, urgency, dysuria, nocturia, or incontinence; urinary catheter placement or bladder irrigation indicated; limits instrumental activities of daily living
3	Gross hematuria; transfusion, intravenous medications, or hospitalization indicated; elective endoscopic, radiologic, or operative intervention indicated
4	Life-threatening consequences; urgent radiologic or operative intervention indicated

NOTE. Grading scale used was the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Table A4. Outcome of Patients Who Did Not Receive VSTs

Patient	Virus	Reason Not Treated	Outcome
16	CMV	Ineligible because of O_2 requirement	Died as a result of pneumonia
35	CMV	Ineligible because of O_2 requirement	Died as a result of rapidly progressive CMV pneumonitis
44	CMV	Ineligible because of O_2 requirement	Died as a result of respiratory failure (unknown organism)
5	EBV	In remission at time of screening visit	Remained in remission
8	EBV	Unable to transfer for treatment	Died as a result of PTLD
10	EBV	Concomitant cryptococcal infection	Died as a result of PTLD and cryptococcal infection
2	AdV	Ineligible because of O_2 requirement	Died as a result of progressive AdV infection
7	BKV	Enrolled on other investigational therapy	BKV hemorrhagic cystitis improved
11	BKV	Improving on standard treatment	Ongoing response to standard treatment with cidofovir
14	BKV	Ineligible because of O_2 requirement	Progressive BK hemorrhagic cystitis requiring nephrostomy tubes
17	BKV	Ineligible because of O_2 requirement	Progressive BKV disease; developed ESRD
21	BKV	Asymptomatic BK viruria only	Remained asymptomatic
23	BKV	Primary disease relapse	Improved on standard treatment
24	BKV	Ineligible because of grade 2 GVHD	Patient with persistent BKV infection; developed ESRD and required dialysis
30	BKV	Ineligible because of O_2 requirement	Progressive BK cystitis, died as a result of multiorgan failure within 2 months
45	BKV	Asymptomatic BK viruria only	Remained asymptomatic
31	HHV-6	No suitable VST line available for infusion	Developed progressive HHV-6 infection refractory to treatment
50	AdV	No suitable VST line available for infusion	Died as a result of progressive AdV infection

Abbreviations: AdV, adenovirus; BKV, BK virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ESRD, end-stage renal disease; GVHD, graft-versus-host disease; HHV-6, human herpesvirus 6; PTLD, post-transplantation lymphoproliferative disorder; VST, virus-specific T cell.

Table A5. Characteristics of Infused Lines

	HLA Type				Specificity					Phenotype							Treatment	
VST No.	A	B	DRB1	DQB1	AdV	CMV	EBV	BKV	HHV-6	CD3	CD4	CD8	$CD45RA^-/CD62L^+/CCR7^+$	$CD45RA^-/CD62L^-$	$CD3^+/CD56^+$	$CD3^-/CD56^+$	No. of Patients	Infections
C6323	02,02	08,15	03,04	02,03	**388**	**553**	38	**334**	3	99.5	82	17.5	26	6.96	0.32	0.11	8	AdV, BKV, CMV
C5404	02,02	35,39	08,14	03,04	**1820**	**5,355**	**2,729**	**313**	**303**	98.2	45.5	46.3	59.4	13.4	1.3	0.66	5	CMV, AdV
C5497	01,02	15,40	12,12	03,03	**602**	3.5	**285**	**1,279**	**287**	97.8	42.7	49.0	60.2	6.57	1.86	1.58	3	BKV
C6378	24,24	38,40	04,15	03,05	**385**	**448.5**	**1014**	**251**	**400**	96.9	47.4	50.5	54.9	10.7	0.98	1.07	2	CMV, BKV
5602	11,03	27,14	04,04	03,03	**314**	**268.5**	**550**	**760**	**55**	98.7	54.3	32.9	68.0	5.96	2.01	0.25	2	BKV
5757	01,03	07,08	01,03	02,05	**985**	**1,273**	**169**	**427**	**95**	93.6	38.8	52.9	74.1	12.6	3.36	6.12	3	BKV, CMV, HHV-6
5678	01,24	08,44	03,15	02,06	**626**	**536**	13	1	2	94.6	42.7	49.7	72.4	26.1	0.66	4.52	2	CMV
6726	11,13	14,15	01,14	25,05	**510**	**721**	**342**	**427**	**669**	97.8	54.3	34.4	7.78	4.96	0.23	1.11	2	BKV, HHV-6
5381	66,66	41,52	03,08	02,04	**950**	**3,334**	**1337**	**303**	**248**	98.2	51.9	39.4	73.3	9.32	1.22	1.02	1	CMV
5435	02,80	15,44	13,16	03,06	**2,700**	**812**	**329**	**534**	**115**	98.6	75.7	18.7	55.4	6.15	0.42	0.42	1	EBV
5667	11,68	35,58	01,11	02,05	**928**	**606**	**313**	12	5	97.5	62.1	30.6	58.7	28.7	1.71	2.14	1	CMV
5998	02,31	27,44	11,13	03,06	13	**1,502**	33	6	17	97.1	69.8	28.6	5.98	15.2	4.47	0.12	1	CMV
6322	03,11	07,55	01,04	03,05	**517**	5.5	**266**	**80**	**75**	99.4	89.4	10.4	10.5	5.58	0.33	0.14	1	BKV
6391	24,30	44,81	03,12	04,05	**1,006**	**807**	**176**	20	4	98.9	66.8	30.6	18.9	9.5	0.64	0.43	1	CMV
6250	01,02	15,37	04,11	03,03	**598**	**931.5**	**326**	**275**	**278**	95.7	62.8	34.3	13.2	10.3	1.26	0.41	1	BKV
6509	02,68	14,57	01,01	05,05	**434**	6	**130**	**218**	28	95.3	78.3	20.3	9.29	4.27	0.22	0.19	1	BKV
5624	23,26	13,59	04,11	03,03	**160**	**59**	**634**	**1,147**	352	98.3	58.8	31.2	66.2	5.44	3.18	0.3	1	BKV, EBV
5682	02,33	07,14	01,15	05,06	**2,174**	**5,847**	36	**270**	**145**	98.6	43.6	52.4	76.9	18.9	21.19	0.72	1	CMV
5557	03,03	07,35	15,15	06,06	**620**	**2,642**	**602**	48.5	23	98.2	9.6	84.9	15.3	29.5	4.03	0.28	1	AdV, CMV
5442	03,03	35,35	01,04	03,05	**280**	**3,049**	**329**	**289**	**114**	93.5	74.5	13.7	31.2	20.6	2.84	3.96	1	AdV
6209	03,30	13,44	04,04	03,03	**301**	**250**	1	4	5	99.1	71.3	28.5	13.6	8.59	0.57	0.39	1	CMV
6498	01,24	08,58	03,03	02,02	**380**	**2,824**	44	41	0	93.7	95.6	1.83	13	11.5	8.01	0.42	1	AdV
6611	01,68	08,40	03,04	02,03	**464**	1.5	**417**	**65**	9	98.7	71.2	27.1	15.08	0.71	4.92	0.15	1	AdV

NOTE. Only viral specificities with numbers in bold were considered for treatment of the respective virus(es) by the indicated VST line.
Abbreviations: AdV, adenovirus; BKV, BK virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV-6, human herpesvirus 6.

Table A6. VST Tracking Studies in Patients With and Without Detectable Third-Party VSTs

Patient	Infection	HLA Matching	No. of Infusions	Best Response by Week 6	Time Points Tested (weeks)	Duration of VST Detection (weeks)
3864	BKV	2	1	PR	4, 6, 12	12
3870	BKV	3	1	PR	2, 3, 4, 12	4
3902	BKV	3	1	PR	1, 3	3
3929	BKV	5	1	PR	1, 2, 4	4
3908	BKV	4	1	PR	2	2
3840	CMV	3	1	PR	3	3
3921	CMV	3	1	PR	2, 3, 6	6
4056	CMV	2	2	PR	Infusion 1: 1, 2, 6, 10, 12; infusion 2: 6	12 6
4157	AdV BKV	4	1	AdV CR BKV PR	2, 3, 6	6
4002	AdV	4	2	PR	Infusion 1: 1, 2, 3; infusion 2: 1, 2, 3	3 3
4084	HHV-6	3	1	PR	1, 12	12
3830	CMV	4	1	CR	2, 4	Not detected
3357	CMV	Infusion 1: 4; infusion 2: 5	2	PR	Infusion 1: 1, 2, 6; infusion 2: 2, 3, 9	Not detected
3904	CMV BKV	3	1	CMV CR BKV PR	3	Not detected
4076	AdV CMV	Infusion 1: 6; infusion 2: 3	2	AdV CR CMV CR	Infusion 1: NA; infusion 2: 3, 4	Not detected
4134	AdV	6	1	CR	4, 6	Not detected

Abbreviations: AdV, adenovirus; BKV, BK virus; CMV, cytomegalovirus; CR, completely response; HHV-6, human herpesvirus 6; NA, not applicable; PR, partial response; VST, virus-specific T cell.

Table A7. GVHD Before and After Infusion

Patient	Prior GVHD	GVHD at Infusion	GVHD Prophylaxis or Treatment at Infusion	aGVHD by Week 6 (treatment; outcome)	cGVHD Within 1 Year (treatment; outcome)
2936	Grade 1 (skin)	No	None	No	No
3357	Grade 1 (skin)	Active grade 1 (skin)	Topical corticosteroids	Grade 1 skin (topical corticosteroids; resolved)	No
3750	No	No	None	No	NA
3755	Grade 2 aGVHD (skin/UGI/LGI), extensive chronic GVHD (skin)	Extensive chronic GVHD (quiescent)	Prednisone, sirolimus, triamcinolone	No	Quiescent chronic GVHD
3784	No	No	None	Grade 1 skin (topical corticosteroids; resolved)	NA
3796	Grade 2 (skin)	Quiescent	Prednisone, tacrolimus	Grade 2 skin flare (prednisone plus tacrolimus; resolved)	NA
3809	No	No	Cyclosporine	No	NA
3810	No	No	None	No	NA
3827	No	No	Tacrolimus	No	No
3830	No	No	Tacrolimus	No	No
3840	Grade 3 (LGI/skin)	Quiescent	Tacrolimus	No	No
3843	Grade 2 (LGI/skin)	Quiescent	Prednisone	No	NA
3848	Grade 2 (LGI/UGI)	Quiescent	Tacrolimus, prednisone, budesonide	No	NA
3854	Grade 2 (LGI/skin)	Quiescent	None	No	No
3859	Grade 3 (LGI/skin)	Quiescent	Tacrolimus	No	No
3864	No	No	Tacrolimus	No	No
3868	Grade 2 (skin)	Quiescent	Prednisone, tacrolimus	No	NA
3869	No	No	None	No	NA
3870	Grade 2 (skin/UGI)	Quiescent	Triamcinolone, tacrolimus, budesonide	No	Flare of UGI GVHD after taper of budesonide (prednisone; resolved)
3877	Grade 2 (skin)	Quiescent	Topical steroids	Grade 1 skin (topical corticosteroids; resolved)	No
3899	No	No	None	No	NA
3902	Grade 2 (LGI/UGI)	No	Prednisone, budesonide	No	Flare of UGI GVHD after stopping budesonide (prednisone; resolved)
3904	Grade 2 (UGI)	Quiescent	Tacrolimus, budesonide	No	No
3908	Grade 2 (UGI)	Quiescent	Tacrolimus	No	No
3921	No	No	Tacrolimus	No	No
3924	Grade 2 (LGI)	Quiescent	Tacrolimus	No	No
3929	No	No	Tacrolimus	No	No
3967	Grade 2 (LGI/UGI)	Quiescent	Prednisone, tacrolimus	No	NA
4002	No	No	Tacrolimus	Grade 1 skin (topical corticosteroids; resolved)	NA
4021	Grade 2 (UGI)	Quiescent	Tacrolimus	No	NA
4056	Grade 2 (LGI/skin)	Quiescent	None	Grade 1 skin (topical corticosteroids; resolved)	No
4057	No	No	Tacrolimus	No	NA
4076	No	No	None	No	No
4084	No	No	Tacrolimus	No	No
4108	No	No	None	No	No
4126	No	No	Tacrolimus	No	No
4134	Grade 3 (LGI)	Quiescent	Prednisone, cyclosporine	Flare of LGI aGVHD after rapid corticosteroid tapering (prednisone; responded)	No
4157	Grade 3 (skin/LGI)	Quiescent	Tacrolimus	No	No

Abbreviations: aGVHD, acute GVHD; cGVHD, chronic GVHD; GVHD, graft-versus-host disease; LGI, lower GI; NA, not applicable; UGI, upper GI.