Clinical Infectious Diseases

BRIEF REPORT

Effectiveness and Safety of Autologous Virus-Specific T-Cell Therapy for Persistent COVID-19 in People With Immunocompromise: A Clinical Trial Study

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In this prospective study (N = 12), autologous virus-specific T cells (auto-VSTs) were successfully manufactured in 10 patients (83.3%). Three received VSTs, achieving viral clearance and symptom resolution without severe adverse events. Auto-VST therapy appears feasible and effective for managing persistent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection; however, manufacturing and timing challenges remain.

Keywords. COVID-19; SARS-CoV-2; T-lymphocytes, cytotoxic; immunocompromised host; adoptive transfer.

Persistent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, characterized by prolonged viral shedding accompanied by persistent symptoms such as pneumonia, are common in people with immunocompromise, particularly those with hematologic malignancies, with an incidence of 14%–30% [1, 2]. However, no established treatment exists for persistent coronavirus disease 2019 (COVID-19) [1–3].

Lymphocyte depletion, including $CD4^+$ T-cell and B-cell loss, is a key contributor to viral persistence in hematologic malignancy [4, 5]. While neutralizing antibodies and virus-specific T cells (VSTs) are essential for coordinating broader immune responses, both may be deficient in severely immunocompromised individuals. Thus, we investigated autologous VST (auto-VST) therapy as a potential treatment. While allogeneic virus-specific T cells (allo-VSTs) have been explored for severe COVID-19 and other viral infections, their therapeutic effect in persistent SARS-CoV-2 infections remains unclear [3, 6, 7]. Moreover, the potential for alloreactivity complicates interpretation of their therapeutic effects.

To address this, we evaluated autologous SARS-CoV-2–specific T cells, allowing a direct assessment of their therapeutic potential without allogeneic immune interactions. This study aimed to assess the feasibility of manufacturing SARS-CoV-2–specific auto-VSTs from people with prolonged COVID-19 and to evaluate their safety and virus-specific efficacy following infusion.

METHODS

Study Setting and Patient Selection

An investigator-initiated clinical study was conducted between June 2023 and October 2024. Adult participants (≥19 years) with hematologic malignancies and COVID-19 were prospectively monitored. Those with persistent symptoms and SARS-CoV-2 polymerase chain reaction (PCR) positivity beyond 4 weeks were screened for study eligibility. Inclusion criteria included persistent COVID-19 symptoms, pneumonia on chest computed tomography (CT), severe/critical classification, and unresponsiveness to antivirals or corticosteroids [1]. Exclusion criteria included mechanical ventilation use, T-cell suppressant use, predicted survival of less than 6 months, and high-dose steroid use (>0.5 mg/kg/d prednisolone). Steroids were discontinued post–VST therapy and minimized for non–COVID-19 conditions. Participants were re-evaluated 2 weeks after VST therapy, and a second dose was administered if PCR positivity or symptoms persisted.

Ethics

The study was approved by the Ministry of Health and Welfare, Republic of Korea (ARM-C2022131, Approval No. R-2-0007) and Institutional Review Board (KC23CISI0026) of Seoul St. Mary's Hospital. Informed consent was obtained in line with the Declaration of Helsinki. This study was registered with the Clinical Research Information Service, Republic of Korea, as part of the World Health Organization (WHO) Registry Network (no. KCT0008222).

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SARS-CoV-2 Testing

PCR testing was performed using nasopharyngeal swabs targeting the E gene and RdRp gene. Immunoglobulin (Ig) G (IgG) and IgM levels were assessed via enzyme-linked immunosorbent assay (ELISA; Abcam, Cambridge, UK), with cycle threshold values indicating viral RNA load.

VST Generation and Infusion

SARS-CoV-2–specific VSTs were generated from leukapheresis-derived mononuclear cells stimulated with overlapping peptide pools targeting spike, membrane, and nucleocapsid proteins (Peptivator; Miltenyi Biotec) and expanded for 21 days in interleukin (IL)-2–supplemented AIM-V medium with 5% human serum. The VSTs meeting predefined release criteria (viability, phenotype, sterility, endotoxin, mycoplasma, and adventitious viruses) were cryopreserved in liquid nitrogen. Prior to infusion, cells were thawed at bedside, diluted in 100 mL of 0.9% saline containing 5% human serum albumin, and administered intravenously at a dose of 1×10^7 cells/m^2 within 30 minutes. Detailed protocols are described in the Supplementary Methods.

Immunological Analyses

Blood samples for immune profiling were collected before infusion and at each follow-up. All immunological assays were performed according to previously reported methods [8]. Immune subsets were characterized via flow cytometry with fluorescence-conjugated antibodies targeting predefined markers. The VST frequency in peripheral blood was determined by pentamer staining. Antigen-specific activity was assessed using an interferon-gamma (IFN-γ) ELISPOT assay, in which peripheral blood mononuclear cells were stimulated with SARS-CoV-2 antigens and IFN-γ–secreting cells were quantified.

Outcomes

The primary safety outcome was the occurrence of adverse events (AEs) or severe AEs (SAEs) post-VST administration [9]. Composite efficacy was measured by significant symptom improvement defined by a 2-point or greater reduction on the WHO Ordinal Scale or National Early Warning Score 2 (NEWS2) score sustained for 4 weeks, SARS-CoV-2 PCR negativity, and CT severity score improvements. The CT severity scores were measured by 1 thoracic radiologist using a standardized system [10]. Exploratory outcomes included ELISpot results, immune subset profiles, VST-specific T-cell expansion, and cytokine levels.

RESULTS

Baseline Characteristics of Study Participants

Twelve participants completed baseline evaluations and VST production. The median age was 63 years (22–81 years), and 7 patients (58.3%) were male. Most had lymphoma (9/12; 75%), with 1 case each of multiple myeloma, acute myeloid leukemia, and myelodysplastic syndrome. All enrolled individuals who underwent VST production had baseline cycle threshold values less than 30 and SARS-CoV-2–specific IgG was detected in 3 individuals (25%). The median time from COVID-19 diagnosis to enrollment was 45 days (range: 17–315 days). At baseline, 8 of 12 individuals (66.7%) exhibited hypogammaglobulinemia. CD4+ and CD8+ T-cell counts were evaluated (see Supplementary Table 1).

Virus-specific T cells were administered to 3 participants who achieved PCR negativity and clinical recovery. Nine participants did not receive VST therapy due to PCR negativity or clinical improvement (n = 2), COVID-19 progression (n = 2), voluntary withdrawal (n = 3), or unsuccessful VST production (n = 2) (Table 1).

Characteristics of Final Product

Ten successfully generated VST products exhibited variability in cellular composition (see Supplementary Figure 1). Key components included CD3$^+$ and CD8$^+$ T cells, CD4$^+$ effector memory T cells, and CD3$^+$ CD56$^+$ natural killer (NK) T cells. One product showed expanded CD3$^-$ CD56$^+$ NK cells, while others had minimal NK cells. Functional assays confirmed strong immune activity, particularly against SARS-CoV-2 membrane and nucleocapsid peptides.

Clinical and Immunological Improvement

The 3 individuals had prolonged infections, with symptom durations of 31, 45, and 46 days before enrollment, indicating clinically persistent and treatment-refractory disease courses. All 3 patients received 2 VST doses (1×10^7/m^2). All showed clinical improvement, SARS-CoV-2 PCR negativity within 2–8 weeks, and CT severity score reduction by week 4 (15.33 vs 7.67; $P = .049$) (see Supplementary Tables 2 and 3). Their courses varied: patient S006 had rapid symptom resolution and viral clearance within 4 weeks; S007 recovered more slowly, achieving improvement by 2 months; and S011 showed steady recovery within 1 month.

Six-month follow-up revealed sustained PCR negativity and stable CT findings. SARS-CoV-2–specific immunity improved in all cases, with significant responses to membrane and nucleocapsid peptides. Pentamer-specific T cells targeting SARS-CoV-2 epitopes were detected within 1 week to 6 months post-infusion. No notable shifts in major immune subsets were observed, suggesting effective antigen-specific immune reconstitution (Figure 1; see Supplementary Figures 2 and 3).

Safety Outcome

No acute adverse reactions were observed post-VST infusion. One participant experienced transient grade 1 leukocytosis, unrelated to VST. No additional AEs or SAEs occurred.

DISCUSSION

This study is the first to demonstrate the feasibility, safety, and preliminary efficacy of auto-VST therapy for persistent COVID-19 in

Table 1. Baseline Characteristics of Study Participants

Participant ID	Age, y	Sex	Underlying Diseases	Disease Status at COVID-19	Last Chemotherapy	Days From Last Treatment to COVID-19 Infection	Days From COVID-19 Infection to Enrollment	Variant Type	COVID-19 Treatment Before VST Therapy	Baseline SARS-CoV-2–Specific IgG	IP Infusion	Withdrawal Reason	Baseline WHO OS	Baseline NEWS2 Score	Outcome at 6 Months
S001	69	F	FL	CMR	Rituximab/ bendamustine	71	315	XBB.2.3.7	Remdesivir, dexamethasone, prednisolone	Negative	None	IP fail	3	3	Discharged to hospice care
S002	67	F	AML	Relapse	Decitabine/ venetoclax	61	37	BA5.1	Remdesivir, prednisolone	Positive	None	ARDS with shock	3	0	Survived
S003	22	F	DLBCL	PD	R-DHAP	11	20	XBB.2.3.7	Remdesivir	Negative	None	ARDS	3	0	Death
S004	46	M	DLBCL	CR	Bispecific antibody	0	85	EG.1	Paxlovid, remdesivir, prednisolone	Positive	None	Self-withdrawal	2	0	Survived
S005	46	M	DLBCL	CR	Bispecific antibody	0	90	EG.1	Paxlovid, remdesivir, prednisolone	Positive	None	Self-withdrawal	2	0	Survived
S006	81	F	FL	CMR	Rituximab maintenance	334	31	XBB.2.3	Remdesivir, dexamethasone, prednisolone	Negative	Infusion	…	4	3	Survived
S007	79	F	FL	CMR	Rituximab/ lenalidomide	12	46	XBB.2.3	Remdesivir, dexamethasone, prednisolone	Negative	Infusion	…	2	0	Survived
S008	38	M	MDS	CR, post-PBSCT	HID-PBSCT	898	17	FL.4	Remdesivir, dexamethasone, prednisolone	Positive	None	SARS-CoV-2 PCR-negative conversion	3	4	Survived
S009	48	M	FL	CMR	Rituximab maintenance	57	55	N/A	Remdesivir, prednisolone	Negative	None	IP fail	2	0	Survived
S010	66	M	DLBCL	CMR	CAR-T	38	33	N/A	Paxlovid, remdesivir, prednisolone	Negative	None	SARS-CoV-2 PCR-negative conversion	2	0	Survived
S011	59	M	FL	PR	Tazemetostat, clinical trial	14	45	JN.1.4	Remdesivir, dexamethasone, prednisolone	Negative	Infusion	…	2	4	Survived
S012	63	M	FL	PD	R-CHOP	60	122	JN.1.4.5	Remdesivir, methylprednisolone, prednisolone	Negative	None	Self-withdrawal	3	2	Death
S013	75	M	MM	VGPR	Pomalidomide, dexamethasone	39	43	KP.1.1.1	Remdesivir, dexamethasone, prednisolone	Negative	None	Self-withdrawal	3	2	Survived

Abbreviations: AML, acute myeloid leukemia; ARDS, acute respiratory distress syndrome; CAR-T, chimeric antigen receptor T-cell therapy; CMR, complete metabolic response; COVID-19, coronavirus disease 2019; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; F, female; FL, follicular lymphoma; HID-PBSCT, haploidentical peripheral blood stem cell transplantation; ID, identification; IP, investigational product; IgG, immunoglobulin G; M, male; MDS, myelodysplastic syndrome; MM, multiple myeloma; N/A, not available; NEWS2, National Early Warning Score 2; OS, Ordinal Scale; PBSCT, peripheral blood stem cell transplantation; PCR, polymerase chain reaction; PD, progressive disease; PR, partial response; R-CHOP, rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone; R-DHAP, rituximab, dexamethasone, high-dose cytarabine, cisplatin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VGPR, very good partial response; VST, virus-specific T cells; WHO, World Health Organization.

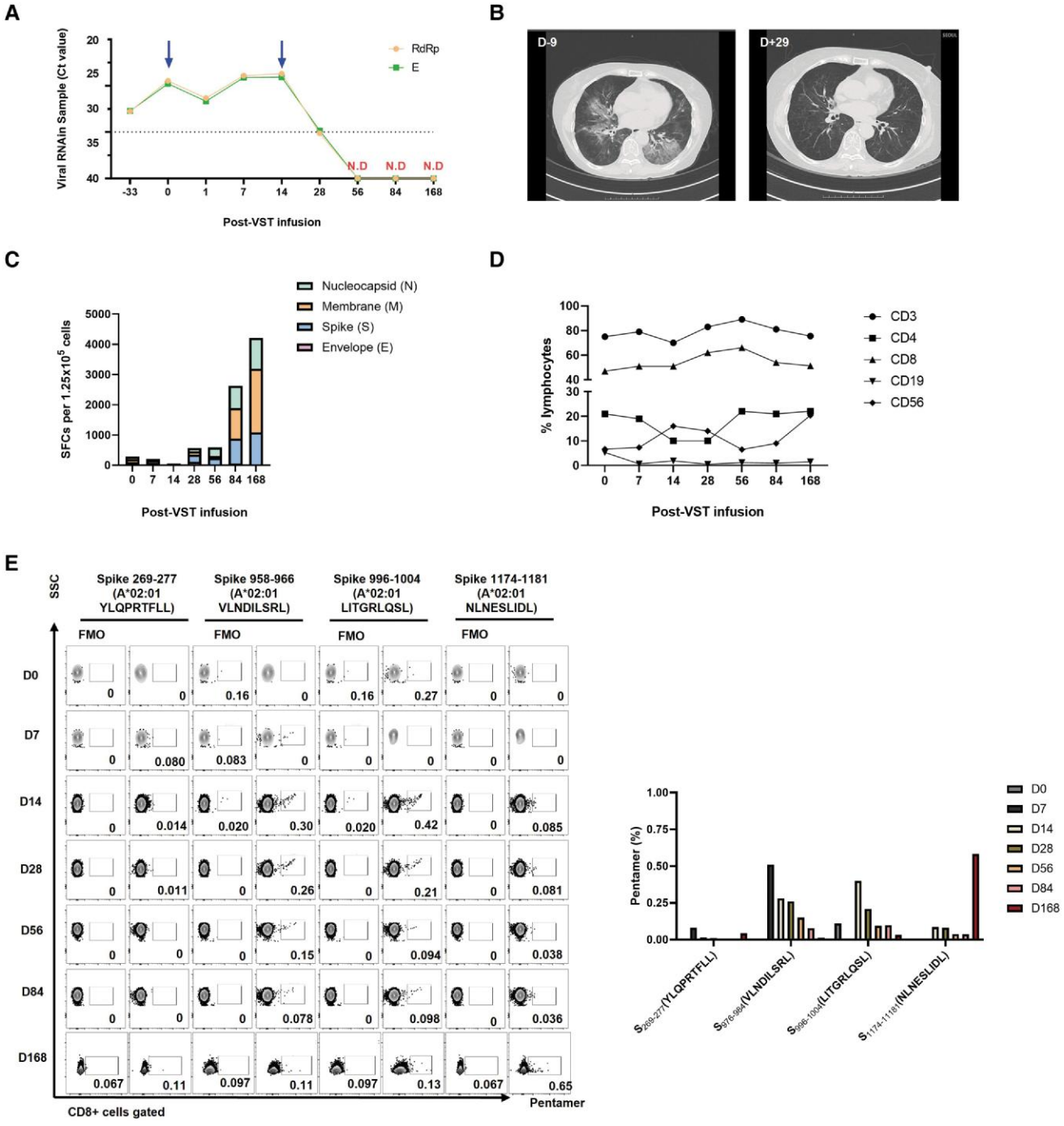

Figure 1. Representative clinical and immunological outcomes for individual S007. *A*, Viral RNA load assessed via real-time PCR, with arrows indicating SARS-CoV-2–specific T-cell (VST) infusions. *B*, Computed tomography (CT) images of the lungs before and after VST infusion. *C*, Interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISPOT) assay results post-infusion, showing spot-forming cells per 1.25×10^5 cells. Experiments were conducted in triplicate. *D*, Flow cytometry analysis of major immune subsets at each follow-up, with cell percentages gated from lymphocytes. *E*, Pentamer assay results, analyzed by flow cytometry, showing $CD3^+$ $CD19^-$ $CD8^+$ T cells gated from live lymphocytes. Fluorescence-minus-one (FMO) control was used. The bar graph on the right illustrates the percentages of pentamer-specific T cells. Abbreviations: Ct, cycle threshold; D, day; N.D., no detection; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFC, spot-forming cells; SSC, side scatter; VST, virus-specific T cell.

people with immunocompromise. While auto-VSTs were successfully expanded in most cases, 3 individuals ultimately received treatment. Despite this limited number, all treated participants achieved viral clearance and clinical recovery, with no SAEs. These results reflect both the promise and practical limitations of auto-VST therapy in real-world clinical settings.

Virus-specific T cells were successfully expanded in 10 of 12 participants (83.3%), eliciting robust immune responses against membrane and nucleocapsid proteins. Despite using wild-type peptides for VST production, this restored immune responses against conserved viral proteins and facilitated the complete recovery of all 3 treated individuals, even though they were infected with the Omicron variant. Importantly, the robust responses to membrane and nucleocapsid antigens further support the role of vaccine-independent, virus-targeted T-cell immunity.

Furthermore, pentamer assays confirmed the post-infusion expansion of VSTs, which correlated with clinical improvement. These T cells gradually declined as viral loads became undetectable but remained present at low levels, suggesting sustained immune protection. Notably, 1 participant experienced spontaneous reinfection resolution, which was accompanied by a transient increase in VST count. Six-month immune profiling revealed stable immune markers, reinforcing the durability of immunological recovery [11]. Our findings provide timely and relevant evidence for addressing a critical therapeutic gap for people with prolonged COVID-19, including transplant recipients, people with hematologic malignancies, and older adults.

This study revealed important limitations. Two participants failed to meet cell expansion criteria and 4 could not receive infusions due to disease progression or spontaneous improvement during the manufacturing period. These cases highlight the practical challenges of auto-VST therapy, including long production timelines and variable expansion success. Although auto-VSTs offer a personalized, low-risk option without risks such as graft-versus-host diseases or cytokine release syndrome, these logistical hurdles may hinder broad clinical application [3, 5, 7, 12]. Given these limitations, optimizing culture conditions to accelerate expansion and initiating auto-VST production before severe lymphocyte depletion could help mitigate these challenges. Efforts are currently underway to refine expansion protocols with the goal of shortening the production period from 21 days to approximately 14 days, thereby improving clinical applicability. For high-risk individuals, preemptive cell banking or use of allo-VSTs ("off-the-shelf" product) could enable timely intervention. Furthermore, future strategies integrating VST therapy with contemporary antivirals and immunomodulators may further enhance outcomes in this population. Finally, excluding people on high-dose corticosteroids may limit generalizability, as steroid-induced T-cell dysfunction remains a barrier to efficacy.

In conclusion, this study suggests that auto-VSTs can be generated and administered in select persistent SARS-CoV-2 infection cases, with promising clinical and immunological responses. These findings highlight the potential of VST therapy and underscore the need for further investigations into allogeneic approaches to enhance accessibility and scalability.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. R. L., N. K., C. P., S.-G. C., and D.-G. L. conceptualized and R. L., W.-B. K., and C. P. coordinated this study. R. L., N. K., W.-B. K., K.-I. I., D. N., S.-Y. C., K. S. B., G. H. L., and I. L. performed data analysis, and W.-B. K., N. K., K.-I. I., S.-Y. C., C. P., K. S. B., G. H. L., and I. L. interpreted the data. R. L., N. K., W.-B. K., and K. S. B. drafted the manuscript, and all authors thoroughly reviewed the manuscript. All authors approve the manuscript's content and conclusion.

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Data availability statement. Complete data are available upon request from the corresponding author.

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