Allogeneic, off-the-shelf, SARS-CoV-2-specific T cells (ALVR109) for the treatment of COVID-19 in high-risk patients

Spyridoula Vasileiou,[*] LaQuisa Hill,[*] Manik Kuvalekar, Aster G. Workineh, Ayumi Watanabe, Yovana Velazquez, Suhasini Lulla, Kimberly Mooney, Natalia Lapteva, Bambi J. Grilley, Helen E. Heslop, Cliona M. Rooney, Malcolm K. Brenner, Todd N. Eagar, George Carrum, Kevin A. Grimes, Ann M. Leen[#] and Premal Lulla[#]

Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital and Houston Methodist Hospital, Houston, TX, USA

[*]*SV and LH contributed equally as co-first authors.*
[#]*AML and PL contributed equally as co-senior authors.*

Correspondence: S. Vasileiou
sxvasile@texaschildrens.org

Received: August 16, 2022.
Accepted: October 31, 2022.
Prepublished: November 10, 2022.

https://doi.org/10.3324/haematol.2022.281946

Abstract

Defects in T-cell immunity to SARS-CoV-2 have been linked to an increased risk of severe COVID-19 (even after vaccination), persistent viral shedding and the emergence of more virulent viral variants. To address this T-cell deficit, we sought to prepare and cryopreserve banks of virus-specific T cells, which would be available as a partially HLA-matched, off-the-shelf product for immediate therapeutic use. By interrogating the peripheral blood of healthy convalescent donors, we identified immunodominant and protective T-cell target antigens, and generated and characterized polyclonal virus-specific T-cell lines with activity against multiple clinically important SARS-CoV-2 variants (including 'delta' and 'omicron'). The feasibility of making and safely utilizing such virus-specific T cells clinically was assessed by administering partially HLA-matched, third-party, cryopreserved SARS-CoV-2-specific T cells (ALVR109) in combination with other antiviral agents to four individuals who were hospitalized with COVID-19. This study establishes the feasibility of preparing and delivering off-the-shelf, SARS-CoV-2-directed, virus-specific T cells to patients with COVID-19 and supports the clinical use of these products outside of the profoundly immune compromised setting (ClinicalTrials.gov number, NCT04401410).

Introduction

The impact of coronavirus disease 2019 (COVID-19) has been profound with more than 625,000,000 confirmed cases worldwide and emerging variants continuing to be a cause of global concern. Although substantial efforts have been made to develop preventative vaccines that induce protective humoral immunity, defects in the cellular arm of the immune response, including dysregulated and diminished T-cell function and trafficking, have been implicated as risks for severe illness despite vaccination.[1-13] Furthermore, immunodeficiency has been identified as a risk factor for infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and severe disease, and has also been linked to persistent viral shedding, which has been shown to select for "fitter" viral variants.[14-17]

One way to prevent severe disease in those at highest risk of COVID-19 would be to prepare and cryopreserve banks of virus-specific T-cell (VST) lines from convalescent healthy donors[18-24] which would be available as a partially HLA-matched product for immediate use. Our group has demonstrated the feasibility, safety and efficacy of "off-the-shelf", third-party VST reactive against otherwise resistant Epstein-Barr virus, cytomegalovirus, adenovirus, BK virus and human herpes virus-6 in patients who are profoundly immunocompromised after an allogeneic hematopoietic stem cell transplant.[25-27] Our phase II trial showed that partially HLA-matched VST adoptively transferred to patients with infection or reactivation of these viruses achieved a 92% response rate.[26]

To explore the therapeutic potential of a SARS-CoV-2-targeted product we sought to identify immunogenic T-cell antigens to target with our VST by examining the peripheral blood of convalescent individuals. Of the 18 SARS-CoV-2 structural and non-structural/accessory proteins (NSP/AP) examined, we identified five that were immunodominant and that we advanced to clinical VST manufacturing. We now report on the profile of the *ex vivo*-expanded VST generated (ALVR109), their potential to target emerging viral variants (including delta and omicron), and on their clinical use in four hospitalized COVID-19 patients at our center to whom these cells were administered.

Methods

Donors and cell lines

Peripheral blood mononuclear cells (PBMC) were obtained from convalescent healthy volunteers with a history of SARS-CoV-2 infection (confirmed by polymerase chain reaction analysis) following informed consent using Baylor College of Medicine (BCM) Institutional Review Board-approved protocols (H-7666, H-45118) and were used to generate phytohemagglutinin-activated blasts and SARS-CoV-2-VST. The phytohemagglutinin-activated blasts were generated as previously reported and cultured in T-cell medium (45% RPMI 1640 [HyClone Laboratories, Logan, UT, USA], 45% Click medium [Irvine Scientific, Santa Ana, CA, USA], 2 mM GlutaMAX TM-I [Life Technologies, Grand Island, NY, USA], and 10% human AB serum [Valley Biomedical, Winchester, VA, USA]) supplemented with 100 U/mL interleukin 2 (IL2; Proleukin® [aldesleukin], TCH, Houston, TX, USA), which was replenished every 2 days.

Generation of SARS-CoV-2 virus-specific T cells

Pepmixes

For generation and immunodominance studies of VST, pepmixes (15mers overlapping by 11 amino acids) spanning SARS-CoV-2-derived structural (S, M, N, E), accessory (7A, 7B, 8) (JPT Peptide Technologies, Berlin, Germany) and non-structural proteins (NSP 1, 3, 4, 5, 6, 10, 12, 13, 14, 15, and 16) (Genemed Synthesis, San Antonio, TX, USA) were synthesized. Lyophilized pepmixes were reconstituted in dimethylsulfoxide (DMSO) (Sigma-Aldrich) and stored at -80°C. For SARS-CoV-2 variant studies pepmixes spanning S from each variant (alpha, beta, gamma, delta, epsilon, kappa and omicron variants) or peptides (15mers overlapping by 11 amino acids) spanning individual mutated sequences and their wildtype equivalents (D614G, 69/70del, P681H, K417N, K417T, E484K, E484Q, N501Y, P681R, L452R) (Genemed Synthesis) were generated.

Generation of virus-specific T cells

For preclinical studies SARS-CoV-2-VST were generated by culturing PBMC ($1.25x10^7$) in a G-Rex5 (Wilson Wolf Manufacturing Corporation, St. Paul, MN, USA) with 50 mL of VST medium (90% TexMACS™ GMP medium [Miltenyi Biotec, GmbH], 2 mM GlutaMAX, and 10% human AB serum supplemented with IL7 [20 ng/mL], IL4 [800 U/mL] [R&D Systems, Minneapolis, MN, USA]) and pepmixes (2 ng/peptide/mL) and cultured for 10-16 days at 37°C in 5% CO_2. For clinical production, VST received a second stimulation with irradiated, autologous, pepmix-pulsed PBMC as antigen-presenting cells (4:1 APC:VST) and were cultured in IL-2-supplemented medium (100 U/mL). The VST lines were checked for identity, phenotype and sterility, and cryopreserved prior to administration. All cell culture manipulations were carried out in the Center for Cell and Gene Therapy GMP facility using current standard operating procedures. Products that met study-specific release criteria were released for clinical use.

Full details on VST phenotypic and functional characterization can be found in the *Online Supplementary Materials*.

Clinical trial

Patients hospitalized with COVID-19 (proven by polymerase chain reaction analysis), and with at least two Center for Disease Control and Prevention-defined risk factors for progression to severe COVID-19 disease were eligible to participate in a protocol that was conducted under an application for an investigational new drug cleared by the Food and Drug Administration with approval from the Baylor College of Medicine Institutional Review Board (H-47739, NCT 04401410). Key risk factors were: age ≥60 years, obesity (body mass index ≥30), after hematopoietic stem cell transplantation or solid organ transplantation, diabetes, and cancer diagnosis on active treatment (within 3 months of last therapy). Additional details on the clinical trial design can be found in the *Online Supplementary Materials*.

Statistical analysis

Descriptive statistics were calculated to summarize preclinical data and clinical characteristics. Where applicable, statistical significance was evaluated by a two-tailed paired *t* test ($P<0.05$). Details can be found in the *Online Supplementary Materials*.

Results

Immunogenicity of SARS-CoV-2-derived antigens

To characterize the cellular immune response to SARS-CoV-2, we examined the T-cell response of infected healthy individuals (confirmed by polymerase chain reaction from a nasopharyngeal swab) who had cleared the virus without requiring hospitalization. In these individuals we assessed T-cell activity directed against all four structural proteins (spike [S], membrane [M], envelope [E], nucleocapsid [N]) and 14 NSP/AP (1, 3, 4, 5, 6, 7a, 7b, 8, 10, 12, 13, 14, 15 and 16). This was done by exposing PBMC from 16 donors to pepmixes (15mer peptides overlapping by 11 amino acids) spanning each of the individual target antigens and evaluating the frequency of IFNγ-producing antigen-specific T cells in their PBMC by ELIspot assay.

While most donors responded to S (n=16; median: 127.5; range, 26-602 spot-forming cells [SFC]/$5x10^5$ PBMC), M (n=14; median: 82; range, 8-319) and N (n=15; median: 58; range, 6-328), activity to E and the NSP/AP was weak/undetectable, as summarized in *Online Supplementary Table*

S1 (left panel) and Figure 1A. To investigate whether the paucity of T cells reactive with E and NSP/AP in peripheral blood was due to the limited immunogenicity of the antigens or simply reflected a frequency of circulating T cells below the ELIspot detection threshold, we performed a single *in vitro* stimulation designed to selectively amplify SARS-CoV-2-specific T cells. Thus, we exposed PBMC to a mastermix of the SARS-CoV-2 peptide libraries followed by an expansion period of 10-16 days. Subsequently, we repeated our IFNγ ELIspot and, as shown in Figure 1B, we detected increased activity, allowing us to establish a hierarchy of immunodominance based on the frequency of responding donors and magnitude of reactive cells (*Online Supplementary Table S1*, right panel). Overall, all donors recognized at least three antigens and 87.5% recognized five or more antigens with S, N, M, AP7a and NSP4 identified as immunodominant and hence advanced for clinical VST manufacturing.

SARS-CoV-2-specific T cells are polyclonal

To generate VST that were enriched for activity against our immunodominant target antigens, we exposed donor PBMC to a mastermix of pepmixes spanning S, N, M, AP7a and NSP4 followed by expansion for 10-16 days (Figure 1C). This resulted in a mean 7.3±0.8-fold increase in total cell numbers (Figure 1D), which were enriched for T cells reactive against the stimulating antigens (Figure 1E). One of the objectives of our approach was to generate a VST product that was polyclonal, representing broad T-cell receptor (TCR) diversity. We first examined the phenotypic profile of the expanded cells, which were predominantly $CD3^+$ T cells (95.5±0.7%), representing a mixture of helper cells ($CD4^+$; 77.5±3.0%) and cytotoxic cells ($CD8^+$; 17.5±2.4%), expressing central memory markers ($CD45RO^+/CD62L^+$; 57.2±5.0%) and effector memory markers ($CD45RO^+/CD62L^-$; 25.3±5.0%); and were activated based on upregulation of CD28 and CD69 (65.0±6.0% and 26.3±4.3%, respectively) (Figure 1F). We further confirmed the TCR diversity present in our VST by assessing the TCR vβ repertoire using a flow cytometric panel that detects more than 70% of all available vβ chains. As shown in Figure 1G (representative donor [left] and summary data [right]) all measurable vβ families were present in these *ex vivo*-expanded cells.

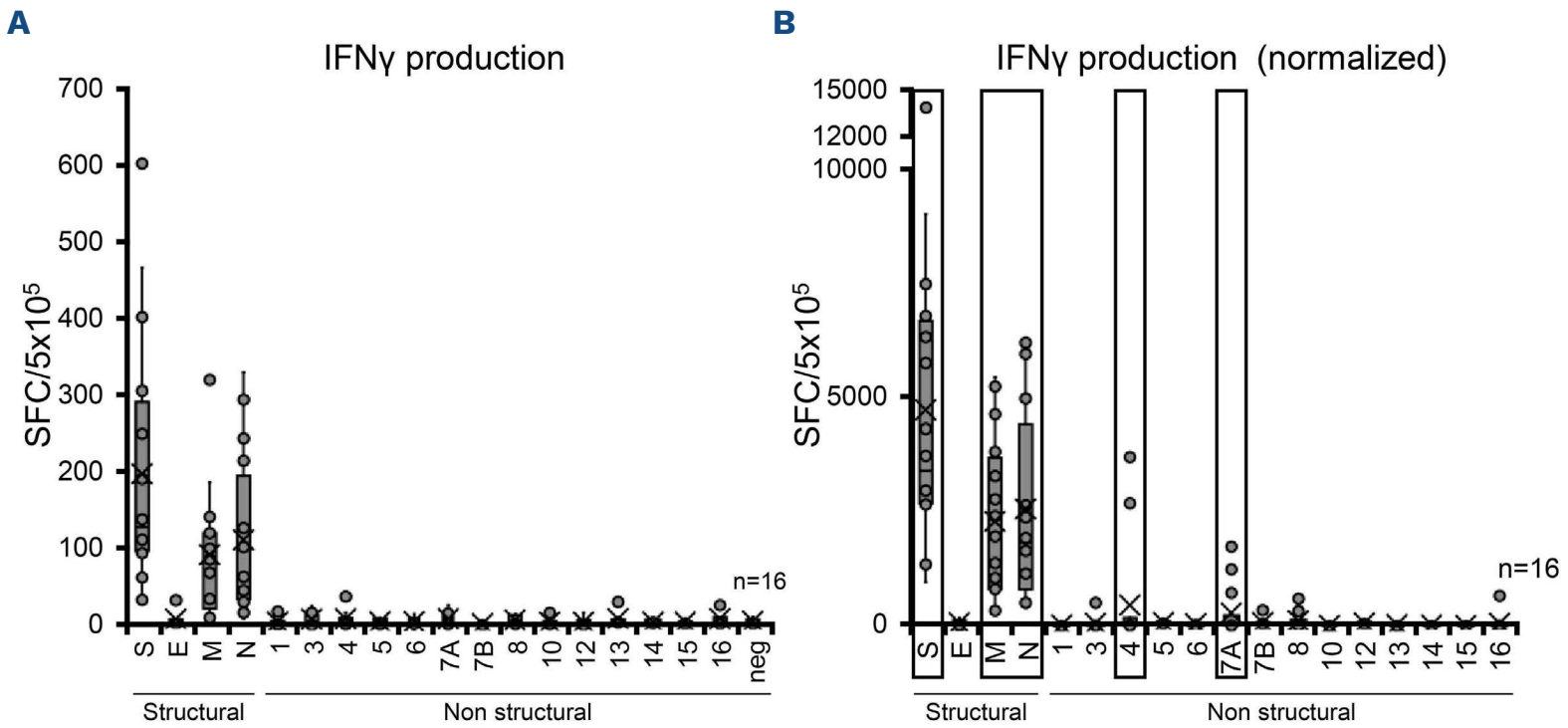
SARS-CoV-2-specific T cells are Th1-polarized, polyfunctional and kill virus-loaded targets but do not exhibit alloreactivity

To examine whether VST reactivity against S, N, M, AP7a and NSP4 was mediated by $CD4^+$, $CD8^+$, or both T-cell subsets, we performed intracellular cytokine staining, gating on $CD4^+$ and $CD8^+$ IFNγ-producing cells. T-cell activity was detected predominantly in the $CD4^+$ compartment, with a minor CD8 response (Figure 2A, representative donor [left] and summary data [right]). As the production of multiple pro-inflammatory cytokines and effector molecules correlates with enhanced cytolytic function and improved *in vivo* activity,[28,29] we additionally evaluated the production of the Th1 cytokines TNFα and granulocyte-macrophage colony-stimulating factor (GM-CSF) and other pro-inflammatory chemokines and effector molecules, including MIP-1α, MIP-1β, and granzyme B, in response to antigenic stimulation.

SARS-CoV-2 antigen-reactive T cells produced Th1-polarized/pro-inflammatory effector molecules including GM-CSF, TNFα, MIP-1α, MIP-1β, and granzyme B but not IL6 or IL10, as measured by Luminex and Granzyme-B ELIspot (Figure 2B, C). Furthermore, intracellular cytokine staining and multiparametric FluoroSPOT demonstrated that the majority (>60%) of all IFNγ-producing cells also produced TNFα (Figure 2D, representative donor [left] and summary data [right]) and/or granzyme B (Figure 2E, *Online Supplementary Figure S1*). Thus, our expanded SARS-CoV-2-specific T-cell lines were polyclonal, Th1-polarized, and polyfunctional. To investigate the cytolytic potential of these VST *in vitro*, we co-cultured SARS-CoV-2-specific T cells with ^{51}Cr-labeled, peptide-loaded autologous phytohemagglutinin-activated blasts. As shown in Figure 2F, SARS-CoV-2-loaded targets were specifically recognized and lysed by our expanded VST (80:1 effector:target ratio: 35.3±6.6%, n=16). Finally, there was no evidence of activity against non-infected autologous targets nor of alloreactivity (graft-*versus*-host potential) using allogeneic phytohemagglutinin-stimulated blasts as targets (Figure 2G), an important consideration if these cells are to be administered to individuals including transplant patients with COVID-19 who are at risk of disease progression.

Variant coverage

Our VST were generated using pepmixes spanning S, N, M, AP7a and NSP4, which were synthesized based on the parental strain (NC_045512.2). To address whether our cells were able to target emerging clinically important viral variants we examined the cross-reactive potential of the cells against alpha (B.1.1.7), beta (B.1.351), gamma (P.1), epsilon (B.1.429), kappa (B.1.617.1), delta (B.1.617.2) and omicron (B.1.1.529) strains. In these assessments, we specifically focused on Spike, which is the most mutated antigen across the different variants with 0.3-1.0% sequence variation. Given the polyclonality and TCR diversity of our product we predicted that our cells would be able to react to each of the variants and indeed, when we exposed our VST to variant-derived S sequences we saw activity at a level that was not significantly different from that induced against the stimulating (parental) sequence (P>0.05) (Figure 3A). We next assessed specific cross-reactivity of the T-cell response at the epitope level. To do this we identified a cohort of mutated sequences present



Continued on following page.

Figure 1. Immunogenicity of SARS-CoV-2-derived antigens, target antigen selection, specificity and polyclonality of *ex vivo*-expanded SARS-CoV-2-specific T cells. (A, B) Reactivity against 18 SARS-CoV-2-derived antigens tested in peripheral blood mononuclear cells (A) and *ex vivo*-expanded SARS-CoV-2-VST (B) of 16 convalescent donors as measured by IFNγ ELIspot using all 18 antigens as a stimulus. Data are shown in box plots as spot-forming cells (SFC); mean and median values are indicated. (C) Schematic of the ALVR109 manufacturing process using the five selected immunodominant antigens. (D-G) Characterization of *ex vivo*-expanded SARS-CoV-2-VST. (D) Fold expansion. (E) Specificity as measured by IFNγ ELIspot for 16 lines generated using all five antigens as a stimulus. Data are shown as SFC ± standard error of mean (SEM) and each color represents an individual antigenic specificity. (F) Phenotype and memory/activation profile. Data are shown in box plots; mean and median values are indicated. (G) T-cell receptor vβ repertoire of *ex vivo*-expanded SARS-CoV-2-VST; representative donor (left) and summary data are shown as mean ± SEM (right). SFC: spot-forming cell; IFNγ: interferon gamma; S: spike; E: envelope; M: membrane; N: nucleocapsid; PBMC: peripheral blood mononuclear cells; IL: interleukin; AP: accessory protein; TEM: effector memory T cells; TCM: central memory T cells; TEMRA: terminally differentiated effector memory T cells; TCR: T-cell receptor; VST: virus-specific T cells; SARS-CoV-2; severe acute respiratory syndrome coronavirus-2.

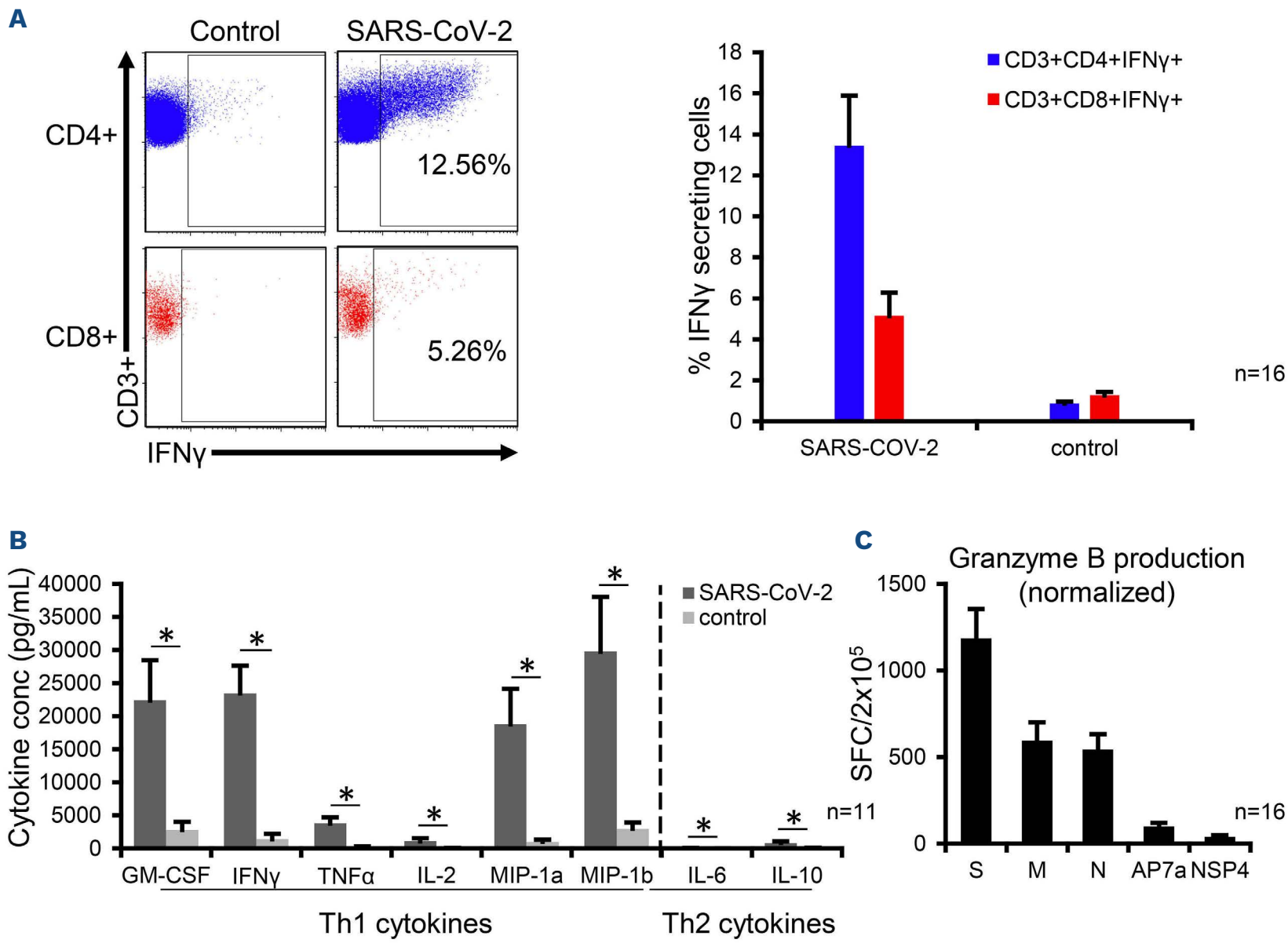
in the viral variant strains (Figure 3B, *Online Supplementary Figure S2*) and generated a panel of individual peptides incorporating these mutated sequences and their wildtype counterparts. Additionally, we generated peptides spanning immunogenic epitopes in parts of Spike that were conserved across all sequences, which we called unique immunogenic epitopes and which served as positive controls in our assays (*Online Supplementary Figure S2*). Figure 3C shows results from a representative donor who had a strong response to Spike antigen and two unique immunogenic epitopes (black bars). When we investigated reactivity to variant peptides we saw that some mutations had no impact on immunogenicity (shown in green - 69/70 del, P681H, N501Y) while others abrogated peptide recognition (shown in yellow - P681R, D614G). Results from 16 donors tested are summarized in Figure 3D. Of note, each donor retained activity against unique immunogenic epitopes and to multiple mutated Spike peptides. Ultimately, these VST also targeted four additional viral antigens, thereby minimizing the potential risk of immune escape from our therapy.[30]

Feasibility of administering "off-the-shelf", virus-specific T cells to patients with COVID-19

We prepared a bank of 15 VST lines for clinical use (see *Online Supplementary Table S2* for VST characteristics). Four hospitalized patients with COVID-19 who met protocol eligibility criteria were referred for participation in this clinical trial. Low-resolution HLA-typing was conducted on the patients with results available within 48 hours in all cases. We were able to identify and infuse a suitably HLA-matched VST line for all four referrals (100%) within 8 to 72 hours after referral. The infused VST were matched at 2/8 to 5/8 of the recipients' HLA alleles (*Online Supplementary Table S3*). Patients infused had a baseline World Health Organization ordinal score of 3 to 4 (Table 1) and had symptoms for 5-14 days prior to receipt of VST. These patients were all at high risk of disease progression due to the presence of risk factors including cancer, prior hematopoietic stem cell transplant, age, hypertension and/or diabetes. They were concomitantly receiving other standard-of-care therapies including corticosteroids, remdesivir and convalescent plasma but were ineligible for monoclonal antibody therapy as they were hospitalized. None had been vaccinated. As summarized in *Online Supplementary Table S4* there were no immediate post-infusion toxicities and none of the patients developed graft-*versus*-host disease. One patient (#4) developed grade III cytokine release syndrome 13 days after infusion, which was transient in nature and most likely related to COVID-19 progression rather than to VST. Patients #1, #2 and #4 achieved complete resolution of infection while patient #3 had transient disease improvement, followed by COVID-19 progression and death approximately 5 weeks after VST. As shown in Figure 4 we observed a significant increase in the frequency of SARS-CoV-2-reactive T cells after infusion in all four patients, accompanied by detection of infused VST (as assessed by TCR deep sequencing analysis) for up to 6 months following VST treatment. By comparing TCR clonotypes detected against a publicly available COVID-TCR database (immunoSEQ T-MAP/COVID) we were able to confirm SARS-CoV-2-antigen specificity of line-derived clones in all patients (*Online Supplementary Table S5*).

Discussion

In this study, we characterized the cellular T-cell immune response to 18 structural and non-structural proteins encoded by SARS-CoV-2 and established a hierarchy of immunodominance based on the profile of T-cell activity detected in 16 healthy convalescent individuals. Of these proteins, three structural (S, M, and N) and two non-structural (NSP4 and AP7a) were advanced to clinical VST manufacturing. Our intent was to produce VST that were polyclonal (mix of $CD4^+$ and $CD8^+$ T cells), that were diverse with respect to TCR repertoire and that recognized multiple epitopes within antigens expressed at different stages of the life cycle of the virus, thereby minimizing the risk of immune escape. Indeed, the *ex vivo*-expanded cells induced using this cohort of antigens were Th1-polarized, produced multiple effector molecules, killed antigen-loaded targets and were able to recognize the



Continued on following page.

Figure 2. *Ex vivo*-expanded SARS-CoV-2-VST are Th1-polarized, polyfunctional and specifically kill virus-loaded targets. (A) SARS-CoV-2-directed IFNγ production detected within the $CD4^+$ and $CD8^+$ compartments by ICS; representative donor (left) and summary data shown as mean ± standard error of mean (SEM) (right). (B, C) Th1-polarized effector molecule production by SARS-CoV-2-VST as measured by Luminex (B) and Granzyme B ELIspot (C). ELIspot data have been normalized to background levels and are shown as SFC ± SEM. (D, E) Simultaneous production of multiple effector molecules by SARS-CoV-2-VST as measured by intracellular cytokine staining (D): representative donor [left] and summary data [right]) and multi-parametric FluoroSpot analysis (E). (F, G) Specific lysis of virus-expressing targets by SARS-CoV-2-VST (F) and lack of cytolytic activity against autologous or allogeneic targets (G). *Statistically significant differences (P<0.05). SARS-CoV-2; severe acute respiratory syndrome coronavirus-2; IFNγ: interferon gamma; GM-CSF; granulocyte-macrophage colony-stimulating factor; TNFα: tumor necrosis factor alpha; MIP: macrophage inflammatory protein; IL: interleukin; SFC: spot-forming cells; S: spike; M: membrane; N: nucleocapsid; GrB: granzyme B; PBMC: peripheral blood mononuclear cells; PHA: phytohemagglutinin; E:T: effector to target ratio; VST: virus-specific T cell.

parental SARS-CoV-2 strain as well as an array of variant strains including delta and omicron. We have also demonstrated the feasibility of translating these VST to high-risk COVID-19 patients, with clinical experience both at our center and by other groups who have utilized these banked VST under emergency investigational new drug applications.[31] In our cohort of four patients, we observed the expansion of SARS-CoV-2-reactive T cells after infusion and the persistence of our cells for up to 6 months.

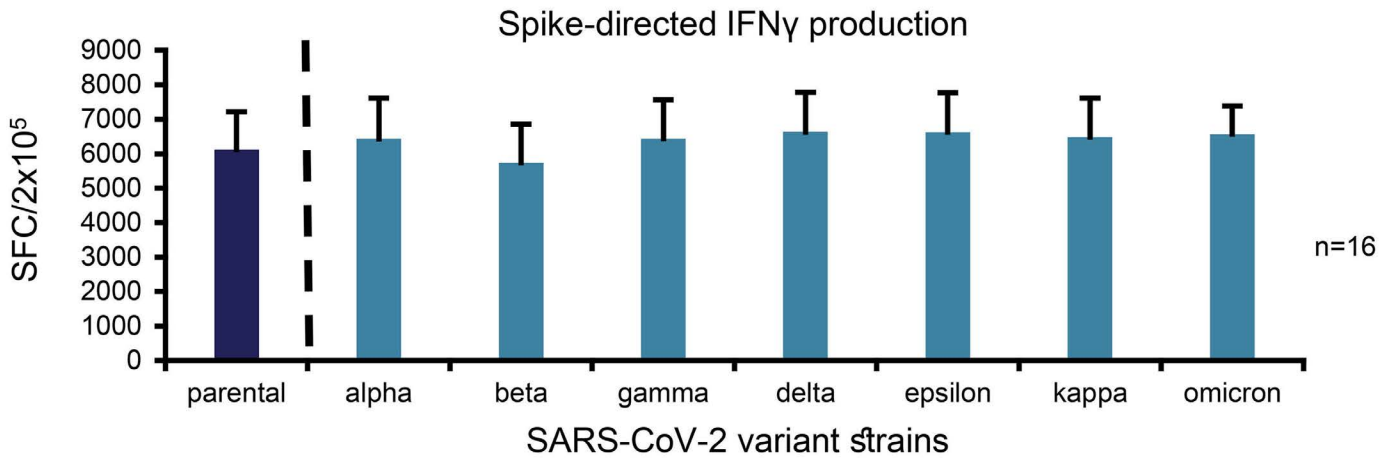
There is emerging evidence that deficiencies in T-cell immunity render SARS-CoV-2-infected individuals at increased risk of disease progression and COVID-19-related death.[12,13,17] This signature initially emerged in the pre-vaccine era, with hospitalized patients presenting with severe lymphopenia that was most pronounced in critically ill patients in the Intensive Care Unit and in whom residual T cells exhibited an exhausted/terminally differentiated phenotype.[1-7,32-36] Even in the post-vaccine era, patients with underlying immune compromise including those receiving cancer treatment, immunosuppressive agents such as high-dose corticosteroids and TNF blockers,[11,37] as well as recipients of solid-organ and stem cell transplants, mount poor immune responses to the vaccine.[8-11] Thus, despite the availability of agents that effectively prevent serious infections in the immunocompetent host, there remains a need for effective and safe therapeutic agents to treat vulnerable individuals.

In developing our SARS-CoV-2-targeted T-cell therapy we sought to mirror the cellular immune landscape present in convalescent (never hospitalized) individuals whose endogenous T cells were apparently protective.[18-20,38] Hence, we initiated our studies by interrogating the circulating memory T-cell response in these recovered individuals to identify which antigens were most frequently recognized and induced the highest frequency of IFNγ-producing T cells, with the objective of advancing the top candidates for VST manufacturing and clinical testing. To prepare a clinical product that would effectively target any viral strain and prevent the emergence of immune escape variants, we generated VST that recognized multiple immunogenic structural and non-structural proteins. In addition, to preserve the breadth of antigen/epitope specificities present in the circulating memory T-cell pool of our convalescent donors, we stimulated donor PBMC with overlapping peptide libraries (15mers overlapping by 11 amino acids that contain all possible HLA class I epitopes and many class II) spanning our target antigens. Thus, the resultant VST were polyclonal, and recognized multiple epitopes within multiple antigens. This is in contrast to traditional peptide-based platforms, which typically rely on stimulation with selected epitopes, resulting in VST that can be used only in a subset of individuals bearing the relevant restricting HLA allele(s).[39] We selectively enriched for polyclonal SARS-CoV-2-VST by culture in medium supplemented with the pro-inflammatory/survival cytokines IL4 and IL7, which we have previously shown to selectively promote the expansion and survival of both $CD4^+$ and $CD8^+$ VST recognizing multiple epitopes.[40] This combination should favor the subsequent sustained expansion of transferred cells *in vivo*. Notably, this breadth of activity – at both the antigen and epitope level – conferred our VST the ability to react with all clinically important viral variants that have emerged to date, including the delta and omicron strains.

We administered our VST to four patients who were hospitalized with COVID-19 and at high risk of disease progression. VST treatment was not accompanied by clinically relevant alloreactivity as we saw no graft-*versus*-host disease. One patient did develop transient grade III cytokine release syndrome 13 days after infusion; this was considered likely to be secondary to COVID-19. All recipients had a significant increase in the frequency of VST after infusion, accompanied by detection of the transferred cells, which were confirmed to be COVID-specific, for as long as 6 months. Furthermore, three of the four infused patients achieved complete resolution of infection. The potential of these VST to address viral variants was also clinically confirmed in a heart transplant recipient with recalcitrant COVID-19 due to SARS-CoV-2 delta strain who failed to respond to remdesivir, corticosteroids, and tocilizumab, but proved clinically and virologically responsive to ALVR109 cells administered as an emergency investigational new drug.[31]

Our group has a long history of preparing and clinically utilizing partially HLA-matched VST targeting viruses, including cytomegalovirus, Epstein-Barr virus, adenovirus,

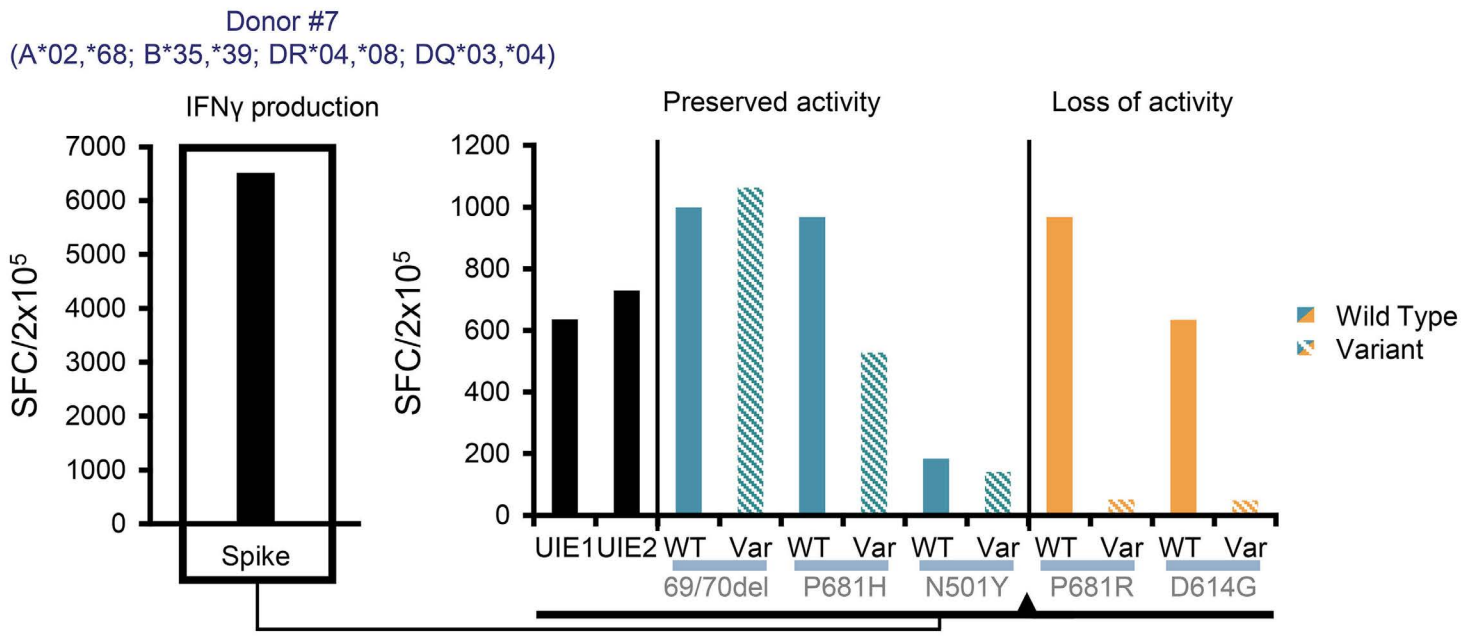
A



B

	D614G	69/70del	P681H	K417N	K417T	E484K	N501Y	P681R	L452R	E484Q	UIEs
Parental	–	–	–	–	–	–	–	–	–	–	√
Alpha	√	√	√	–	–	–	√	–	–	–	√
Beta	√	–	–	√	–	√	√	–	–	–	√
Gamma	√	–	–	–	√	√	√	–	–	–	√
Delta	√	–	–	–	–	–	–	√	√	–	√
Epsilon	√	–	–	–	–	–	–	–	√	–	√
Kappa	√	–	–	–	–	–	–	√	√	√	√
Omicron	√	√	√	√	–	√	√	√	–	–	√

C



D

Donor ID	HLA class I		HLA class II			Spike mutations									
	A	B	DR	DQ	UIEs	D614G	69/70del	P681H	K417N	K417T	E484K	N501Y	P681R	L452R	E484Q
#1	02,26	44,50	04,07	02,03											
#2	11,30	15,40	08,14	03,04											
#3	02,25	18,40	11,15	03,06											
#4	24,24	07,15	12,14	02,03											
#5	24,24	40,45	10,16	03,05											
#6	02,03	15,38	07,15	02,06											
#7	02,68	35,39	04,08	03,04											
#8	02,68	44,51	04,07	02,03											
#9	02,11	18,44	04,04	03,03											
#10	11,29	35,44	07,14	02,05											
#11	24,24	07,13	07,12	02,03											
#12	02,11	15,40	08 09	03,04											
#13	24,68	15,35	11,15	03, 05											
#14	24,68	07,07	15,15	06,06											
#15	02,03	15,44	04,12	03,03											
#16	02,36	35,58	11,13	03,06											

Loss of activity Preserved activity

Continued on following page.

Figure 3. *Ex vivo*-expanded SARS-COV-2-VST provide coverage against clinically important viral variants. (A) SARS-CoV-2-VST generated against the parental strain maintain Spike-directed reactivity against all variant strains as measured by IFNγ ELIspot. Data are shown as spot-forming cells (SFC) ± standard error of mean (SEM). (B) Selected mutations of the Spike protein and their prevalence among the different viral variants. (C) Example of a representative donor with a strong response to Spike antigen and the two unique immunogenic epitopes and varying levels of reactivity against variant peptides, as shown by IFNγ ELIspot. Data are reported as SFC ± SEM. (D) Summary results of all 16 donors tested. Each donor retains activity against unique immunogenic epitopes and to multiple mutated Spike peptides. SFC: spike-forming cells; IFNγ: interferon gamma; SARS-CoV-2; severe acute respiratory syndrome coronavirus-2; UIE: unique immunogenic epitope; WT: wildtype; Var: variant; ID: identity; VST: virus-specific T cell.

Table 1. Clinical summary of the enrolled patients.

Pt ID	Sex	Age, years	Risk factors	VST dose	N of matched HLA alleles	Other treatments	WHO score at baseline	Clinical outcomes
1	F	67	Age Hodgkin lymphoma Diabetes	$1x10^7$	5/8	Steroids, remdesivir, convalescent plasma	4	Recovered, discharged D7
2	F	70	Age Hypertension	$1x10^7$	4/8	Steroids, remdesivir, convalescent plasma	4	Recovered, discharged D6
3	F	50	CNS lymphoma Post auto-SCT	$1x10^7$	2/8	Steroids	3	Died, initial recovery followed by progression on D26 due to worsening COVID-19
4	M	52	CML Post allo-SCT	$2x10^7$	2/8	Steroids, remdesivir	3	Recovered, transferred to outside facility D9

Pt ID: patients' identification; VST: virus-specific T cells; HLA: human leukocyte antigen; WHO; World Health Organization; F: female; M: male; D: day; CNS; central nervous system; auto: autologous; allo; allogeneic; SCT: stem cell transplantation; CML: chronic myeloid leukemia; COVID-19: coronavirus disease-2019.

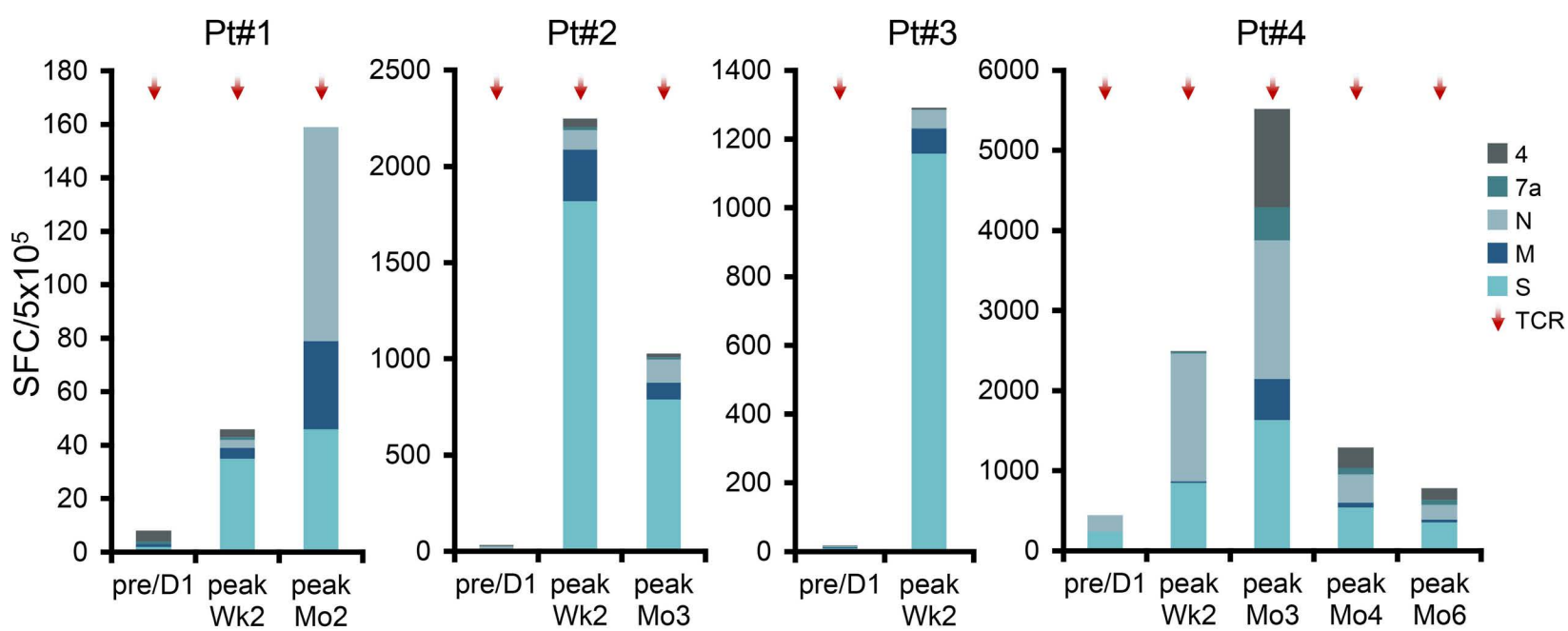


Figure 4. Detection of SARS-CoV-2-reactive T cells before and after infusion in the four infused patients. Specific cells are measured by ELIspot using the five targeted antigens as a stimulus. Results are reported as spot-forming cells ± standard error of mean and each color represents an individual antigenic specificity. In addition, the red arrows indicate each time point at which T-cell receptor deep sequencing confirmed the presence of infused virus-specific T cells in the patients. SFC: spot-forming cells; D1: day 1; Wk: week; Mo: month; N: nucleocapsid; M: membrane; S: spike; TCR: T-cell receptor.

BK virus and human herpes virus-6, for the treatment of refractory viral infections in allogeneic hematopoietic stem cell transplant recipients.[25-27,41,42] However, this study establishes the feasibility of preparing and delivering off-the-shelf, SARS-CoV-2-directed VST to patients with COVID-19. In addition it is the first in which VST are used to address a public health issue afflicting other vulnerable groups, including the elderly, the very young and those with underlying conditions.[34,36,43] These VST can be rapidly and efficiently produced in scalable quantities, with excellent long-term stability, so they are suited for clinical use in high-risk individuals in immediate need of therapeutic intervention.

Disclosures

SV, MK and YV are consultants to AlloVir. BJG owns QBRegu-

latory Consulting which has consulting agreements with Tessa Therapeutics, Marker Therapeutics, LOKON, and ViraCyte. HEH is a co-founder with equity in Allovir and Marker Therapeutics, has served on advisory boards for Tessa Therapeutics, Kiadis, Novartis, Gilead Biosciences, Fresh Wind Biotechnologies and GSK and has received research support from Kuur Therapeutics and Tessa Therapeutics. CMR and MKB have stock and other ownership interests with Coya, Bluebird Bio, Tessa Therapeutics, Marker Therapeutics, AlloVir, Walking Fish, Allogene Therapeutics, Memgen, Kuur Therapeutics, Bellicum Pharmaceuticals, TScan Therapeutics, Abintus Bio; have consulting or advisory roles with Abintus Bio, Adaptimmune, Brooklyn Immunotherapeutic, Onk Therapeutics, Tessa Therapeutics, Memgen, Torque, Walking Fish Therapeutics, TScan Therapeutics, Marker Therapeutics, Turnstone Bio; and have received research funding from Kuur Therapeutics. AML is a co-founder and equity holder of AlloVir and Marker Therapeutics and a consultant to AlloVir. PL is a member of the advisory board for Karyopharm. LH, AGW.

Contributions

SV, MK, AGW, AW, YV and SL performed research; SV, MK, AGW and TNE analyzed data; KM was involved in research coordination; BJG was in charge of regulatory issues; NL, HEH, CMR and MKB supervised the study; LH, GC, KAG and PDL were involved in patients' care; SV, LH, AML and PDL wrote the manuscript.

Acknowledgments

The authors thank Walter Mejia for assistance with figure formatting.

Funding

This work was supported by a sponsored research grant from AlloVir, Inc.

Data-sharing statement

Datasets are maintained in an electronic database at the Center for Cell and Gene Therapy; data are available from the corresponding author on reasonable request.

References

1. Arunachalam PS, Wimmers F, Mok CKP, et al. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. Science. 2020;369(6508):1210-1220.
2. De Biasi S, Meschiari M, Gibellini L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun. 2020;1(1):3434.
3. Diao B, Wang C, Tan Y, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). Front Immunol. 2020;11:827.
4. Kusnadi A, Ramirez-Suastegui C, Fajardo V, et al. Severely ill COVID-19 patients display impaired exhaustion features in SARS-CoV-2-reactive CD8(+) T cells. Sci Immunol. 2021;6(55):eabe4782.
5. Liao M, Liu Y, Yuan J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med. 2020;26(6):842-844.
6. Mathew D, Giles JR, Baxter AE, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science. 2020;369(6508):eabc8511.
7. Song JW, Zhang C, Fan X, et al. Immunological and inflammatory profiles in mild and severe cases of COVID-19. Nat Commun. 2020;11(1):3410.
8. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. Blood. 2021;137(23):3165-3173.
9. Mair MJ, Berger JM, Berghoff AS, et al. Humoral immune response in hematooncological patients and health care workers who received SARS-CoV-2 vaccinations. JAMA Oncol. 2022;8(1):106-113.
10. Ribas A, Dhodapkar MV, Campbell KM, et al. How to provide the needed protection from COVID-19 to patients with hematologic malignancies. Blood Cancer Discov. 2021;2(6):562-567.
11. Romano E, Pascolo S, Ott P. Implications of mRNA-based SARS-CoV-2 vaccination for cancer patients. J Immunother Cancer. 2021;9(6):e002932.
12. Ameratunga R, Woon ST, Steele R, Lehnert K, Leung E, Brooks AES. Severe COVID-19 is a T cell immune dysregulatory disorder triggered by SARS-CoV-2. Expert Rev Clin Immunol. 2022;18(6):557-565.
13. Fung M, Babik JM. COVID-19 in immunocompromised hosts: what we know so far. Clin Infect Dis. 2021;72(2):340-350.
14. Avanzato VA, Matson MJ, Seifert SN, et al. Case study: prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. Cell. 2020;183(7):1901-1912.
15. Corey L, Beyrer C, Cohen MS, Michael NL, Bedford T, Rolland M. SARS-CoV-2 variants in patients with immunosuppression. N Engl J Med. 2021;385(6):562-566.
16. Shah V, Ko Ko T, Zuckerman M, et al. Poor outcome and prolonged persistence of SARS-CoV-2 RNA in COVID-19 patients with haematological malignancies; King's College Hospital experience. Br J Haematol. 2020;190(5):e279-e282.
17. Garcia-Vidal C, Puerta-Alcalde P, Mateu A, et al. Prolonged viral replication in patients with hematologic malignancies hospitalized with COVID-19. Haematologica. 2022;107(7):1731-1735.
18. Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. Immunity. 2020;52(6):971-977.
19. Peng Y, Mentzer AJ, Liu G, et al. Broad and strong memory CD4(+) and CD8(+) T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol. 2020;21(11):1336-1345.
20. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell. 2020;183(1):158-168.
21. Basar R, Uprety N, Ensley E, et al. Generation of glucocorticoid-resistant SARS-CoV-2 T cells for adoptive cell therapy. Cell Rep. 2021;36(3):109432.
22. Bonifacius A, Tischer-Zimmermann S, Santamorena MM, et al. Rapid manufacturing of highly cytotoxic clinical-grade SARS-

CoV-2-specific T cell products covering SARS-CoV-2 and its variants for adoptive T cell therapy. Front Bioeng Biotechnol. 2022;10:867042.
23. Sivapalan R, Liu J, Chakraborty K, et al. Virus induced lymphocytes (VIL) as a novel viral antigen-specific T cell therapy for COVID-19 and potential future pandemics. Sci Rep. 2021;11(1):15295.
24. Papayanni PG, Chasiotis D, Koukoulias K, et al. Vaccinated and convalescent donor-derived severe acute respiratory syndrome coronavirus 2-specific T cells as adoptive immunotherapy for high-risk coronavirus disease 2019 patients. Clin Infect Dis. 2021;73(11):2073-2082.
25. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood. 2013;121(26):5113-5123.
26. Tzannou I, Papadopoulou A, Naik S, et al. 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. J Clin Oncol. 2017;35(31):3547-3557.
27. Tzannou I, Watanabe A, Naik S, et al. "Mini" bank of only 8 donors supplies CMV-directed T cells to diverse recipients. Blood Adv. 2019;3(17):2571-2580.
28. Harari A, Dutoit V, Cellerai C, Bart PA, Du Pasquier RA, Pantaleo G. Functional signatures of protective antiviral T-cell immunity in human virus infections. Immunol Rev. 2006;211:236-254.
29. Rossi J, Paczkowski P, Shen YW, et al. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. Blood. 2018;132(8):804-814.
30. Dolton G, Rius C, Hasan MS, et al. Emergence of immune escape at dominant SARS-CoV-2 killer T cell epitope. Cell. 2022;185(16):2936-2951.
31. Martits-Chalangari K, Spak CW, Askar M, et al. ALVR109, an off-the-shelf partially HLA matched SARS-CoV-2-specific T cell therapy, to treat refractory severe COVID-19 pneumonia in a heart transplant patient: case report. Am J Transplant. 2022;22(4):1261-1265.
32. Bange EM, Han NA, Wileyto P, et al. CD8(+) T cells contribute to survival in patients with COVID-19 and hematologic cancer. Nat Med. 2021;27(7):1280-1289.
33. Meckiff BJ, Ramirez-Suastegui C, Fajardo V, et al. Imbalance of regulatory and cytotoxic SARS-CoV-2-reactive CD4(+) T cells in COVID-19. Cell. 2020;183(5):1340-1353.
34. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell. 2020;183(4):996-1012.
35. Wilk AJ, Rustagi A, Zhao NQ, et al. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. Nat Med. 2020;26(7):1070-1076.
36. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-1062.
37. Vormehr M, Lehar S, Kranz LM, et al. Dexamethasone premedication suppresses vaccine-induced immune responses against cancer. Oncoimmunology. 2020;9(1):1758004.
38. Tan AT, Linster M, Tan CW, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell Rep. 2021;34(6):108728.
39. Hont AB, Powell AB, Sohai DK, et al. The generation and application of antigen-specific T cell therapies for cancer and viral-associated disease. Mol Ther. 2022;30(6):2130-2152.
40. Gerdemann U, Keirnan JM, Katari UL, et al. Rapidly generated multivirus-specific cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections. Mol Ther. 2012;20(8):1622-1632.
41. Gerdemann U, Katari UL, Papadopoulou A, et al. Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. Mol Ther. 2013;21(11):2113-2121.
42. Papadopoulou A, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med. 2014;6(242):242ra83.
43. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell. 2021;184(4):861-880.