Molecular Therapy
Methods & Clinical Development
Original Article

Transcriptomic analysis reveals optimal cytokine combinations for SARS-CoV-2-specific T cell therapy products

Jessica Durkee-Shock,[1,2] Christopher A. Lazarski,[1] Mariah A. Jensen-Wachspress,[1] Anqing Zhang,[3] Aran Son,[2] Vaishnavi V. Kankate,[1] Naomi E. Field,[1] Kathleen Webber,[1] Haili Lang,[1] Susan R. Conway,[1] Patrick J. Hanley,[1,3,4] Catherine M. Bollard,[1,3,4] Michael D. Keller,[1,3,5,6] and Daniella M. Schwartz[2,6]

[1]Center for Cancer and Immunology Research, Children's National Hospital, Washington, DC, USA; [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; [3]GW Cancer Center, George Washington University, Washington, DC, USA; [4]Division of Blood and Marrow Transplantation, Children's National Hospital, Washington, DC, USA; [5]Division of Allergy and Immunology, Children's National Hospital, Washington, DC, USA

Adoptive T cell immunotherapy has been used to restore immunity against multiple viral targets in immunocompromised patients after bone-marrow transplantation and has been proposed as a strategy for preventing coronavirus 2019 (COVID-19) in this population. Ideally, expanded severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-virus-specific T cells (CSTs) should demonstrate marked cell expansion, T cell specificity, and CD8+ T cell skewing prior to adoptive transfer. However, current methodologies using IL-4 + IL-7 result in suboptimal specificity, especially in CD8$^+$ cells. Using a microexpansion platform, we screened various cytokine cocktails (IL-4 + IL-7, IL-15, IL-15 + IL-4, IL-15 + IL-6, and IL-15 + IL-7) for the most favorable culture conditions. IL-15 + IL-7 optimally balanced T cell expansion, polyfunctionality, and CD8+ T cell skewing of a final therapeutic T cell product. Additionally, the transcriptomes of CD4$^+$ and CD8$^+$ T cells cultured with IL-15 + IL-7 displayed the strongest induction of antiviral type I interferon (IFN) response genes. Subsequently, microexpansion results were successfully translated to a Good Manufacturing Practice (GMP)-applicable format where IL-15 + IL-7 outperformed IL-4 + IL-7 in specificity and expansion, especially in the desirable CD8$^+$ T cell compartment. These results demonstrate the functional implications of IL-15-, IL-4-, and IL-7-containing cocktails for therapeutic T cell expansion, which could have broad implication for cellular therapy, and pioneer the use of RNA sequencing (RNA-seq) to guide viral-specific T cell (VST) product manufacturing.

INTRODUCTION

During the coronavirus 2019 (COVID-19) pandemic, immunocompromised patients, including those with malignancy, inborn errors of immunity, or on immunosuppression therapy, have demonstrated increased morbidity and mortality in the setting of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[1–6] T cell immunity has a critical role in the resolution of natural infection as evidenced by the following: (1) CD4$^+$ and CD8$^+$ SARS-CoV-2-specific T cell responses are detected in COVID-19 convalescent donors, (2) patients with insufficient humoral responses are protected by T cells, and (3) T cells protect against reinfection in animal models.[7–11] Therefore, impaired T cell immunity may worsen outcomes in immunocompromised populations, and restoration of viral-specific T cell immunity through adoptive transfer is an attractive therapeutic modality.[12]

Hence, for individuals with impaired T cell immunity, we sought to produce SARS-CoV-2-specific T cells (CSTs) for therapeutic use from SARS-CoV-2 convalescent donors.[9] After rapid expansion in the presence of the growth and differentiation cytokines IL-4 + IL-7, we demonstrated statistically significant specificity, especially to the SARS-CoV2 antigens spike and membrane. However, CD4$^+$ T cell predominance and suboptimal proportions of antigen-specific T cells were evident in the final product. Previous cytokine cocktails used for culture of viral-specific T cell (VST) products have included IL-2, IL-15, IL-4, and IL-7 to promote T cell expansion and viral specificity.[13–16] However, the optimal conditions to maximize T cell yield and antiviral activity remain uncertain. Using a 96-well, microexpansion array platform to assess cytomegalovirus (CMV)-specific T cells, IL-15 + IL-6 and IL-4 + IL-7 were the optimal cocktails for CD3$^+$ T cell expansion and specificity, with IL-15 + IL-6 yielding more CD8$^+$ skewing.[17] Further, the impact of these cocktails on the expression of antiviral defense genes are unknown, and it is unclear if optimal VST culture conditions vary for differing antiviral targets.

Given the recent importance of CD8$^+$ T cells for effective SARS-CoV-2 immunity, we used a high-throughput microexpansion screen to interrogate multiple cytokine conditions, allowing us to increase the

Received 6 November 2021; accepted 25 April 2022;
https://doi.org/10.1016/j.omtm.2022.04.013.

[6]These authors contributed equally

Correspondence: Michael Keller, 111 Michigan Avenue NW, Washington, DC 20010, USA.
E-mail: mkeller@childrensnational.org

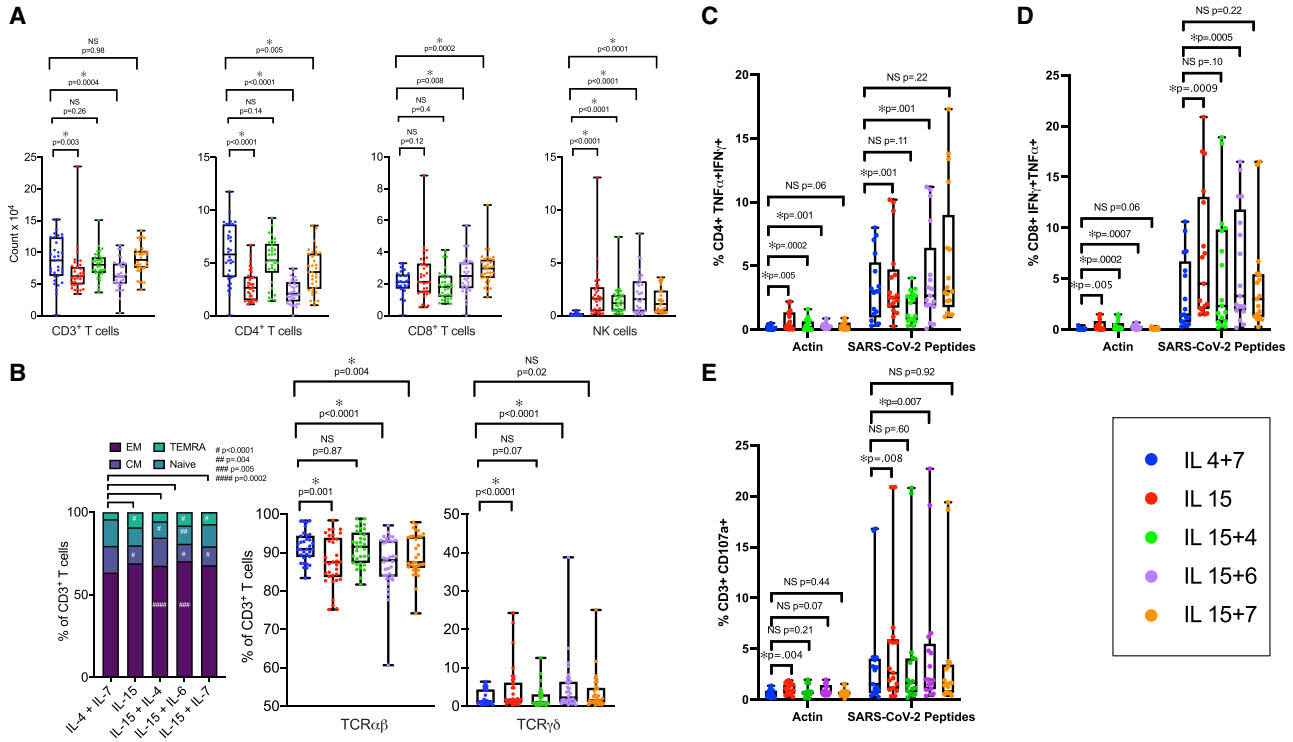

Figure 1. PBMCs from 9 donors were stimulated with SARS-CoV-2 pepmixes and then seeded at 200,000 cells per well in a 96-well plate

Cells were split on day 7 and then proceeded directly to intracellular flow cytometry on day 10. Expansion, phenotype, and anti-viral T cell specificity were evaluated in 5 cytokine conditions: IL-4 + IL-7, IL-15, IL-15 + IL-4, IL-15 + IL-6, and IL-15 + IL7. (A) Absolute counts of $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, and $CD3^-CD56^+$ NK cells on day 10. An approximation of fold expansion is provided in Figure S4. (B) Proportions of naive, central memory, effector memory, terminal effector, T cell receptor (TCR)αβ, and TCRγδ $CD3^+$ T cells on day 10. (C) $CD4^+$ T cell specificity as measured by absolute count of $CD4^+IFN\gamma TNF\text{-}\alpha^+$ cells on day 10. (D) $CD8^+$ T cell specificity as measured by absolute count of $CD8^+IFN\gamma TNF\text{-}\alpha^+$ cells on day 10. (E) $CD3^+$ T cell specificity as measured by absolute count of $CD3^+CD107a^+$ cells on day 10. *Statistically significant, $p < 0.0125$ corrected for multiple comparisons. Error bars represent 95% confidence intervals.

$CD8^+$ proportion of CSTs while preserving $CD4^+$ T cell specificity and optimizing cell expansion.[18–20] Additionally, we used mRNA sequencing of the T cell products to identify the cytokine cocktail that most strongly induced antiviral interferon-stimulated genes (ISGs). Finally, we demonstrated that these optimized conditions could be successfully upscaled to a Good Manufacturing Practice (GMP)-applicable platform in preparation for clinical administration of an adoptive T cell product targeting SARS-CoV-2 in immunocompromised hosts.

RESULTS

Donors

Peripheral blood mononuclear cell (PBMC) samples from SARS-CoV-2 convalescent donors were obtained. Donors were selected based on history of T cell response to SARS-CoV-2 structural proteins.[9] Donor details are listed in Table S1.

IL-15-containing conditions enhance CD8+ T cell polyfunctionality and central-memory expansion

To determine which conditions produced the largest number of CSTs for adoptive transfer, we expanded PBMCs from 9 convalescent donors in replicate for 10 days in five culture conditions: IL-4 (400 IU/mL) + IL-7 (10 ng/mL), IL-7 (10 ng/mL) + IL-15 (5 ng/mL), IL-4 (400 IU/mL) + IL-15 (5 ng/mL), IL-6 (100 ng/mL) + IL-15 (5 ng/mL), and IL-15 alone (5 ng/mL). IL-4 + IL-7 was used as the reference comparator based on our current FDA-approved CST clinical trials (ClinicalTrials.gov: NCT01945814, NCT02510417) and as previously published.[9,17] Final cell counts obtained were utilized as a surrogate for cell expansion. Total $CD3^+$ T cell expansion was comparable after culture with IL-4 + IL-7, IL-15 + IL-4, and IL-15 + IL-7 but substantially lower when using IL-15 alone or IL-15 + IL-6. Absolute numbers of $CD4^+$ T cells were highest after culture with IL-4 + IL-7 and lowest after culture with IL-15 + IL-6. In contrast, IL-4-containing cultures were less effective at increasing absolute numbers of $CD8^+$ T cells. All cytokine cocktails containing IL-15 produced significantly ($p < 0.0125$ adjusted for multiple comparisons) more natural killer (NK) cells, which was maximized with IL-15 alone. But when IL-6 or IL-7 was added to the cytokine cocktail, a 24%–40% increase in CD8+ T cells was observed compared with the non-IL-15-containing culture (Figure 1A) (for median values and interquartile ranges, please refer to Table S2). Taken together, these results suggested that IL-4 and IL-7 promote $CD4^+$ expansion

but that the presence of IL-4 constrains $CD8^+$ expansion. In contrast, IL-15 promotes $CD8^+$ and NK expansion.

Since protective VSTs are in the effector-memory compartment, we also measured the effect of various culture conditions on the expansion of memory T cell populations. Proportions of effector memory T cells (EMs) were comparable among the tested cytokine cocktails. Importantly, central memory T cells may enhance persistence after adoptive T cell therapy compared with EMs or terminal effector cells (TEMRAs).[21–24] The IL-15 + IL-4 cocktail produced the most central memory T cells (median 17.3%), with relatively high proportions under the influence of IL-4 + IL-7 (median 14.7%) or IL-15 + IL-7 (11.6%) (Figure 1B). TEMRAs, which may be especially important for effective antiviral immunity, were higher in IL-15-containing cultures (median range 5.4%–6.0%) compared with non-IL-15-containing culture (median 3.5%).[25] Expansion with IL-4 + IL-7 resulted in higher $CD45RO^-CCR7^+$ T cells (which may include both naive and stem cell memory) compared with cultures containing IL-15 (Figure 1B).

In prior studies, polyfunctional antiviral specificity has been considered a key measure for predicting therapeutic efficacy of adoptively transferred VSTs.[26,27] Among the five tested culture conditions, IL15 + IL-7 was comparable to IL-4 + IL-7 at expanding polyfunctional virus-specific tumor necrosis factor alpha $(TNF\text{-}\alpha)^+IFN\gamma^+$ $CD4^+$ T cells, while IL-15 + IL-4 was the least effective (Figures 1C and S1). In the $CD8^+$ T cell compartment, IL-15-containing conditions increased the expansion of virus-specific $TNF\text{-}\alpha^+IFN\gamma^+$ $CD8^+$ T cells relative to IL-4 + IL-7. This increase was significant in cells cultured with IL-15 alone and with IL-15 + IL-6, and there was a trend toward expansion of $TNF\text{-}\alpha^+IFN\gamma^+$ $CD8^+$ virus-specific T cells in IL-15 + IL-4- and IL-15 + IL-7-containing cultures (Figures 1D and S1). We also assessed antigen-specific surface expression of CD107a as a marker of immune cell activation and cytotoxic degranulation.[28–31] No appreciable differences in CST CD107a expression were observed regardless of which cytokine cocktail was used (Figure 1E). Taken together, these microexpansion results suggest that IL-15 + IL-7 might be superior to IL-4 + IL-7 for CST manufacture since IL-7 and IL-15 support the expansion of $CD4^+$ and $CD8^+$ virus-specific T cells, respectively. In contrast, our so-called "standard" approach using IL-4 and IL-7 results in a CST product that predominantly favors CD4+ T cell expansion. In summary, IL-15 + IL-7 improved $CD8^+$ T cell expansion and specificity while preserving $CD4^+$ T cell expansion and specificity as well as total T cell expansion (which were compromised in the other IL-15-containing cytokine conditions) and thus was chosen for further GMP-compatible experiments.

IL-15 + IL-7 conditions enhance IFN-response gene expression compared with IL-4 conditions

Based on our observation that IL-15-containing conditions were more effective than IL-4 + IL-7 at inducing $CD4^+$ and $CD8^+$ antigen-specific VSTs, we hypothesized that IL-15 might be a major inducer of antiviral pathways. TNF-α and IFNγ production are both important predictors of antiviral activity in T cells, although they are largely driven by type 1 IFN responses.[32] Type 1 IFNs induce antiviral ISGs through the ISGF complex comprising STAT1/STAT2/IRF9. To determine the effect of the different culture conditions on antiviral ISGs, we measured the transcriptomes of $CD4^+$ and $CD8^+$ T cells derived from 4 donors expanded without cytokines or with the five CST-promoting culture conditions.

In $CD4^+$ T cells targeting SARS-CoV-2 spike and membrane proteins, CST expansion regulated 450 differentially expressed genes (DEGs) in >1 condition (Figure 2A). CST expansion induced genes important for viral defense across multiple culture conditions, indicating that expansion upregulates desired antiviral responses in $CD4^+$ T cells. Conversely, IL-4-containing culture condition induced a gene cassette that was enriched for negative regulation of host defense, suggesting that IL-4 might reduce the protective capacity of $CD4^+$ T cells. As in $CD4^+$ T cells, CST expansion regulated three cassettes of genes in $CD8^+$ T cells: a group of expansion-repressed genes, a set of common expansion-induced genes, and a cassette of genes induced primarily in IL-4-containing cultures (Figure 2B). The IL-4-induced gene cassette was enriched for negative regulation of host defense (*ARG2, TNFRSF11A*), indicating that IL-4 might also inhibit the immune function of $CD8^+$ T cells.

To specifically investigate the antiviral transcriptomic signature of the different CSTs, we investigated a cassette of ISGs critical for antiviral host defense across human diseases and murine models.[33] Using gene set enrichment analysis (GSEA), we compared enrichments of this cassette among the various culture conditions, finding that IL-15 + IL-7 was the most effective at inducing antiviral ISGs in both $CD4^+$ and $CD8^+$ T cells (Figures 2C and 2D, respectively).[34,35] Accordingly, STAT1 target genes were also strongly enriched in $CD4^+$ T cells cultured with IL-15 + IL-7, whereas STAT3 and STAT6 target genes were not enriched (Figure S2). These findings strongly suggest that IL-4 represses genes important for host defense, while IL-15 + IL-7 are most effective at inducing antiviral response genes in $CD4^+$ and $CD8^+$ CSTs.

Hence, IL-15 + IL-7 was selected as the GMP-compatible manufacturing platform for enhanced expansion and specificity of CSTs, as well as an increased $CD8^+$ T cell component and maximization of the antiviral transcriptomic signature.

IL-7 + IL-15 optimized expansion and specificity in a GMP-applicable format

To facilitate translation to the clinic, PBMCs from 6 additional SARS-CoV-2 convalescent donors and 1 repeat donor were selected to expand a GMP-compliant product using G-Rex 10 bioreactors, as were used for our other VST products.[36,37] Six out of 7 donor-derived CSTs demonstrated numerically increased expansion in G-Rex 10 bioreactors in the setting of IL-15 + IL-7 compared with IL-4 + IL-7, with a mean fold expansion of 3.2 versus 1.9 ($p = 0.08$). (Figure 3A).

More than 90% of lymphocytes generated with IL-4 + IL-7 and IL-15 + IL-7 were $CD3^+$ T cells, with minimal presence of residual

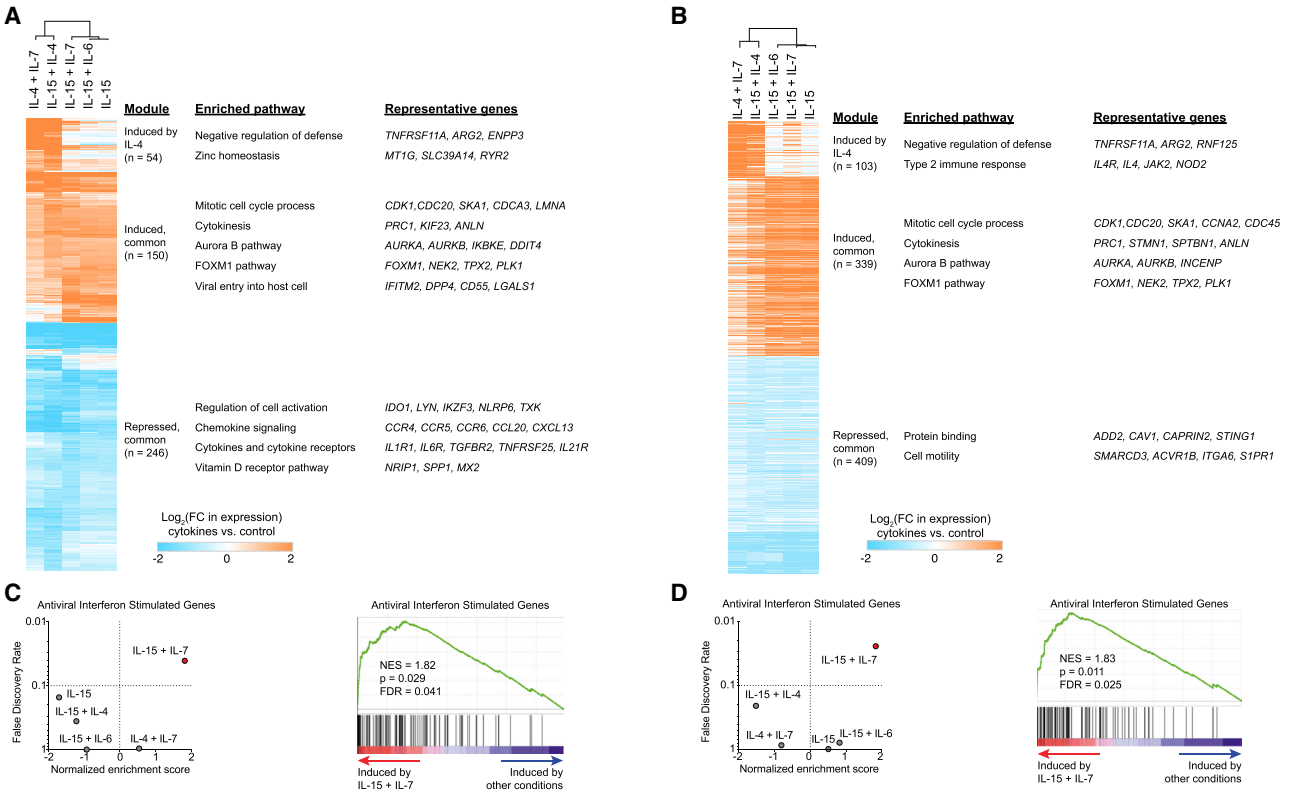

Figure 2. Transcriptomic analysis of CSTs shows that IL-15 + IL-7 increases stimulation of important antiviral interferon response genes

(A) Heatmap displays 450 differentially expressed genes (DEGs) in $CD4^+$ T cells cultured in at least one cytokine-containing condition versus $CD4^+$ T cells cultured without cytokines (fold change >2 or <-2, false discovery rate [FDR] <0.05, ANOVA, Partek). Colors denote average $\log_2$(fold change in gene expression) for cells cultured in cytokine-containing conditions versus cells cultured without cytokines. Selected enriched pathways and representative genes (metascape) are shown for each module of DEGs. (B) Heatmap displays 851 DEGs in $CD8^+$ T cells cultured in at least one cytokine-containing condition versus $CD4^+$ T cells cultured without cytokines (fold change >2 or <-2, FDR<0.05, ANOVA, Partek). Colors denote average $\log_2$ fold change in gene expression for cells cultured in cytokine-containing conditions versus cells cultured without cytokines. Selected enriched pathways and representative genes (metascape) are shown for each module of DEGs. (C) Scatterplot displays normalized enrichment score (NES) and FDR for an antiviral interferon-stimulated gene cassette (Immunological Genome Project, GEO: GSE75306) in $CD4^+$ T cells. Dashed line denotes an FDR of 10%. NESs are shown for each condition versus all other conditions. Conditions where antiviral gene cassette is significantly induced are marked in red; conditions where antiviral gene cassette is significantly repressed are marked in blue. Representative GSEA plot for $CD4^+$ T cells cultured in IL-15 + IL-7 versus other conditions. (D) Scatterplot displays NES and FDR for an antiviral interferon-stimulated gene cassette (Immunological Genome Project, GEO: GSE75306) in $CD8^+$ T cells. Dashed line denotes an FDR of 10%. NESs are shown for each condition versus all other conditions. Conditions where antiviral gene cassette is significantly induced are marked in red; conditions where antiviral gene cassette is significantly repressed are marked in blue. Representative GSEA plot for $CD8^+$ T cells cultured in IL-15 + IL-7 versus other conditions.

monocytes or B cells (Figure 3B). Proportions of $CD4^+$ and $CD8^+$ T cells were comparable between the 2 cytokine conditions. Reflecting microexpansion results, cultures containing IL-15 demonstrated increased $CD3^-CD56^+$ NK cells (for median values and interquartile ranges, please refer to Table S3). CSTs cultured with IL-15 + IL-7 had proportionally higher EMs (IL-4 + IL-7 median 10.1%; IL-15 + IL-7 median 48.1%) and TEMRAs (IL-4 + IL-7 median 4.7%; IL-15 + IL-7 median 6.2%), while central memory populations were similar (IL-4 + IL-7 median 15.9%; IL-15 + IL-7 median 15.9%) (Figure 3C).

Finally, VSTs cultured with IL-15 + IL-7 showed increased $CD8^+$ and $CD4^+$ T cell specificity to both membrane and spike peptide pools, without increasing nonspecific T cell activation (Figures 3D and 3E). Additionally, IL-15 + IL-7 cultures improved CST cytotoxicity, as represented by CD107a expression (Figure 3F). Specificity testing via $IFN\gamma^+$ enzyme-linked immunospot (ELISpot) after culture in IL-15 + IL-7 similarly showed a trend toward higher specificity as compared with IL-4 + IL-7 (Figure 3G). Cytotoxicity was assessed using a calcein-based flow-cytometry assay, and minimal cytotoxicity was demonstrated by CSTs cultured with IL-15 + IL-7 (data not shown).

DISCUSSION

Here, we have shown that antiviral specificity and the antiviral IFN transcriptome of CSTs can be dramatically improved by optimizing the cytokines used for proliferation and differentiation during cell culture. Multiple reports have demonstrated the importance of $CD8^+$ T cells in natural immunity to SARS-CoV-2.[9] In those studies,

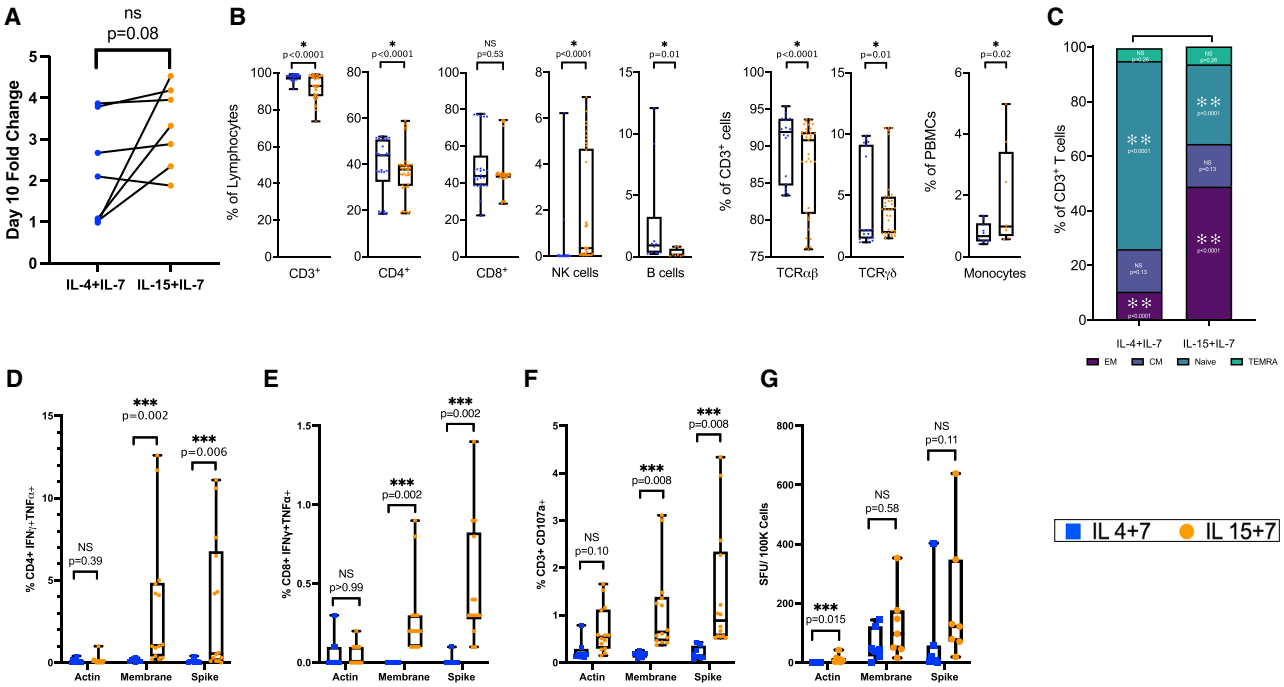

Figure 3. Microexpansion of donor PBMCs identifies optimal cytokine conditions for production of CD4$^+$ and CD8$^+$ CSTs

(A) Fold expansion of live cells following 10 day expansion. (B) Proportions of total T cells (CD3$^+$), CD4$^+$ T cells, CD8$^+$ T cells, TCR$\alpha\beta$ T cells, TCR$\gamma\delta$ T cells, CD56$^+$CD3$^-$ NK cells, CD19$^+$ B cells, and CD14$^+$ monocytes after expansion. (C) Proportion of CD3$^+$ total T cells identified as naive (CCR7$^+$, CD45RO$^-$), central memory (CCR7$^+$, CD45RO$^+$), effector memory (CCR7$^-$, CD45RO$^+$), and terminal effector (CCR7$^-$, CD45RO$^-$) cells after expansion. (D) CD4$^+$ T cell specificity with differing cytokine conditions (IL-4 + IL-7 versus IL-15 + IL-7) as measured by proportion of IFNγ^+TNF-α^+-secreting cells. (E) CD8$^+$ T cell specificity with differing cytokine conditions (IL-4 + IL-7 versus IL-15 + IL-7) as measured by proportion of IFNγ^+TNF-α^+-secreting cells. (F) CD3$^+$ T cell specificity with differing cytokine conditions (IL-4 + IL-7 versus IL-15 + IL-7) as measured by proportion of CD107a$^+$-expressing cells. (G) Antiviral specificity of SARS-CoV-2-specific T cells (day 10) expanded with IL-4 + IL-7 or IL-7 + IL-15 to spike and membrane peptide libraries as measured by spot forming units (SFU) per 100,000 cells on IFNγ ELIspot assay. *Statistically significant, $p < .05$. **Statistically significant, $p < .0125$, corrected for multiple comparisons for (B). ***Statistically significant, $p < 0.017$, corrected for multiple comparisons for (C). Error bars represent 95% confidence intervals.

patients with CD8$^+$ T cell lymphopenia had worse disease, while robust CD8$^+$ T cell responses were associated with better outcomes.[18,19] Our work demonstrates that expansion of a CST product results in different CD4$^+$ and CD8$^+$ T cell populations when using IL-4 + IL-7 versus IL-15 + IL-7. The latter combination improved expansion, shifted the memory phenotype, improved specificity in the CD8$^+$ T cell compartment, and increased antigen-specific CD107a expression. Together, these results suggest a more effective CD8$^+$ T cell response after culture with IL-15 + IL-7, which should enhance clinical potency of this novel therapeutic *in vivo*. Of note, while cytotoxicity of CSTs cultured with IL-7 + IL-15 was not demonstrated, cytotoxicity assays reflect only a single effector function of VSTs. Furthermore, peptide-pulsed phytohemagglutinin (PHA) blasts were used as the target cells because a validated pulmonary model for target cells is not currently available. Since SARS-CoV-2 does not have a prominent viremia, even in immunocompromised hosts, cytotoxicity of hematologic cells, or lack thereof, is unlikely to be clinically relevant. As expected, expansion of CD56$^+$CD3$^-$ NK cells increased in the setting of IL-15 + IL-7 given the role of IL-15 in the proliferation and maturation of both CD8$^+$ T cells and NK cells.[38] While NK cells are typically rare in a T cell therapeutic product, NK cells may be clinically beneficial here due to their importance in natural SARS-CoV-2 immunity.[39] NK therapy is also being considered independently for SARS-CoV-2 therapy; thus, a joint NK- and T cell-specific product may further reconstitute viral-specific immunity in patients with high-risk immunocompromised states (ClinicalTrials.gov: NCT04280224, NCT04365101).[40]

The type I IFN/antiviral transcriptome of VSTs for adoptive immunotherapy has not been previously assessed, although RNA sequencing (RNA-seq) has been used extensively to understand the efficacy and differential responses to chimeric antigen receptor (CAR)-T cells and other anti-tumor T cells.[41–44] Here, we successfully demonstrated that IL-15 + IL-7 maximally enhanced expression of desirable ISG cassettes for cell replication and viral defense, as well as STAT1 target genes. In comparison, cocktails containing IL-4 induced genes that repressed host defense as well as undesirable, atopic type II immune-response genes, especially in CD8$^+$ T cells. This reflects prior studies reporting deleterious effects of type II cytokines on CD8$^+$ T cell responses.[45] A strong type I IFN response may be especially important for successful SARS-CoV-2 immunity given the observation that patients with defective type I IFN response

have markedly worse clinical outcomes.[46,47] These findings could have broader implications for expansion of VSTs specific for other viral targets, as alteration of type I IFN responses would not be apparent on routine phenotyping assays. Furthermore, these genes could be included in a high-throughput, rapid-screening transcript panel to be used during VST optimization.

In the broader context of antiviral cellular therapy, we have also confirmed that cytokine cocktails screened in microexpansion can be successfully translated to a GMP-applicable format in preparation for a clinical product.[17] Extensive screening of cytokine conditions is limited at a GMP scale due to time, cost, and the need for large numbers of PBMCs from an individual donor for paired comparisons. Of note, higher specificity was demonstrated in microexpansion compared with G-Rex, although trends of improved expansion and specificity in cytokine conditions remained reproducible in both formats. The etiology of enhanced specificity in microexpansion remains unexplained but may be due to enhanced cellular interactions, cellular density, and/or co-stimulation in U-bottomed 96-well plates compared with larger, flat-bottomed G-Rex bioreactors.

Although this study systematically investigated the efficacy of multiple conditions on CST expansion, the focus on CSTs raises the possibility that these findings may not be generalizable to other viral targets. For instance, IL-15 + IL-6 was previously found to be optimal for CMV-specific T cell expansion.[17] Given the possible role of IL-6 in severe COVID-19, IL-6 was felt to be undesirable for CST product development, but this may not be as concerning for other viral targets.[48] We anticipate that this platform and VST product analysis, both using RNA-seq and high-throughput cytokine screening, could be used for optimizing VST production for many other viral targets of interest. This study was also performed on a relatively small number of donors; other donors could have different responses that might affect the final product. For instance, some donors, based on their human leukocyte antigen (HLA) types, may have a more T helper 2 (TH2)-skewed response in the presence of IL-4 compared with other donors, based on underlying conditions. Further investigations are needed to identify screening procedures to select the most appropriate third-party donors for clinical T cell adoptive transfer. Finally, despite improvements in cell expansion by changing manufacturing from IL-4 + IL-7 to IL-7 + IL-15 in a GMP-applicable format, mean expansion had only a 3-fold change from day 0 to 10, thereby requiring substantial, but not unattainable, blood-draw volumes from eligible donors for third-party banking.

Given the maladaptive and hyperinflammatory role of T cells in mid and late SARS-CoV-2-related disease, optimal timing of clinical CST administration will be critical.[49–51] There may be a window at the onset of SARS-CoV-2 infection, prior to immune-mediated inflammatory complications, where augmentation of T cell immunity would be beneficial in patients with impaired T cell immunity and an elevated risk of severe COVID-19; however, this window is likely to be brief. If the CSTs are administered too late in disease course, they could augment, instead of ameliorate, COVID-19. Therefore, prophylactic use of CSTs may be more advantageous to prevent severe disease in immunocompromised patients. We also hypothesize that CSTs could be beneficial in immunocompromised patients with long-term viral shedding. Furthermore, the possible role of IL-6 in the pathogenesis of COVID-19 discouraged our use of IL-6 for manufacturing of CSTs despite improved $CD8^+$ T cell specificity over Il-4+IL-7.[52] Chalangari et al. recently published a single case of a patient status post cardiac transplant with ongoing hospitalization, viral shedding, and COVID-19 after 3 weeks of illness despite standard therapies. The patient had substantial clinical and viral improvement, with undetectable SARS-CoV-2 viral load 11 days after administration, and discharge of the patient occurring 12 days after CST administration.[53] Furthermore, 4 patients were treated with ALVR109, a CST product, with 1 serious event of cytokine release syndrome, which was transient and likely due to COVID-19 progression. All 4 patients had initial clinical response, 1 of which had later recurrence and death. The other 3 patients clinically improved with resolution of viral shedding (ID week oral abstract, 2021: https://www.biospace.com/article/releases/data-presented-at-idweek-2021-demonstrate-that-alvr109-allovir-s-investigational-sars-cov-2-specific-t-cell-therapy-is-reactive-against-a-broad-range-of-variants-including-delta/).

In conclusion, IL-15 + IL-7 optimized the expansion, phenotype, and specific polyfunctional antiviral signature of CSTs, both on intracellular cytokine staining flow cytometry and RNA transcriptome analysis when compared with a variety of other cytokine culture conditions. Importantly, results were reproducible in a GMP-compliant format. This study provides evidence for the utility of screening culture conditions in microexpansion format and provides an important step in the manufacturing of CSTs for adoptive therapeutic use in individuals with impaired T cell immunity.

METHODS

Donors

PBMCs from local and regional convalescent volunteers with previously demonstrated T cell responses to SARS-CoV-2 were collected under informed consent approved by the Institutional Review Board of Children's National Hospital in accordance with the Declaration of Helsinki (CNMC IRB: Pro00004033).

Microexpansion experiments

Stimulation and expansion

PBMCs from 9 donors were thawed and incubated with media containing overlapping peptide libraries containing SARS-CoV-2 structural proteins for spike, membrane, envelope, and nucleocapsid (A&A Peptide, San Diego, CA, USA), as previously reported.[9] Pulsed PBMCs were plated in 96-well plates at 200,000 cells/well in media with one of six cytokine conditions, specifically IL-4 (400 IU/mL) + IL-7 (10 ng/mL), IL-7 (10 ng/mL) + IL-15 (5 ng/mL), IL-4 (400 IU/mL) + IL-15 (5 ng/mL), IL-6 (100 ng/mL) + IL-15 (5 ng/mL), IL-15 alone (5 ng/mL), or no cytokines (R&D Systems, Minneapolis, MN, USA). Cytokine concentrations were chosen based on foundational

work by Lazarski et al.[17] Cells were split on day 7 of culture and supplemented with additional media and cytokines.

Flow cytometry

On day 10, expanded VSTs were re-stimulated with SARS-CoV-2 structural protein pepmixes, staphylococcal enterotoxin B (SEB), or actin with CD28/CD49d (BD Biosciences, San Jose, CA, USA) and anti-CD107a-Pe-Cy7 antibody. After 1 h of stimulation, brefeldin A (Golgiplug; BD Biosciences, San Jose, CA) and monensin (GolgiStop; BD Biosciences) were added. Cells were then incubated for an additional 4 h. Cell viability was assessed using Live-Dead Aqua. VSTs were surface stained with fluorophore-conjugated antibodies against CD3-BV785, CD4-BV605, CD8-BV421, TCRαβ-PerCP/Cy5.5, TCRγδ-APC-Fire750, CCR7-FITC, CD45-RO-PEDazzle, HLA-DR-Alexaflour700, and CD56-BV650 (Miltenyi Biotec; BioLegend). Cells were fixed and permeabilized with Cytofix/Cytoperm solution (BD Biosciences) and subsequently stained with IFN-γ-APC and TNF-α-PE (Miltenyi Biotec). All samples were acquired on a CytoFLEX Cytometer (Beckman Coulter, Brea, CA, USA). The gating strategy for analysis is presented in Figure S3.

RNA-seq

On day 10, expanded VSTs from 4 donors were re-stimulated with SARS-CoV-2 pepmixes encompassing spike, nucleocapsid, membrane, and envelope with CD28/CD49d (BD Biosciences) and incubated for 4 h. Cell viability was assessed using Live-Dead-near-infrared (IR). VSTs were surface stained with fluorophore-conjugated antibodies against CD4-BV605, CD8-BV421, CD19-FITC, CD16-FITC, and CD14-FITC (Biolegend) and then sorted for $CD4^+$ and $CD8^+$ cells. Cells were not stained with $CD3^+$ antibodies to prevent non-specific cell activation. Cells were then frozen and preserved in Trizol (Invitrogen, Waltham, MA, USA). Total RNA was extracted from 10,000–50,000 cells, and mRNA sequencing libraries were generated as previously described.[54] Novaseq6000 was used for 50-cycle single-end read sequencings, which were processed with bcl2fastq 2.17.1 to generate FastQ files. Reads were mapped to the human transcriptome hg19 using STAR 2.7.0; gene expression values (fragments per kilobase exon per million mapped reads [fpkm]) were calculated with RSEM 1.3.0 and normalized as previously described.[54] GSEA was performed as described.[54] Enrichment-score curves and member ranks were generated by the GSEA software (Broad Institute). RNA-seq datasets were used in conjunction with the following user-generated gene sets: (1) antiviral ISGs (GEO: GSE75306, 109 genes), (2) IFNα-induced Stat1target genes (GEO: GSE40666; 83 genes), (3) IL-6-induced Stat3 target genes (GEO: GSE65621; 76 genes), and (4) IL-4 induced Stat6 target genes (GEO: GSE22801, 167 genes)

G-Rex 10 expansion

Briefly, PBMCs from convalescent donors were incubated with media containing overlapping peptide libraries for spike and membrane structural proteins (JPT, Berlin, Germany). Stimulated cells were then aliquoted and seeded into G-Rex 10 Bioreactors (Wilson Wolf, New Brighton, MN, USA) at 10–20 million cells each.[9] Thirty mL media with one of 2 cytokine conditions chosen from the Microexpansion experiments, specifically IL-4 (400 IU/mL) + IL-7 (10 ng/mL) or IL-7 (10 ng/mL) + IL-15 (5 ng/mL). Cells were supplemented with additional media and cytokines on day 7. Cells were harvested and counted on day 10 and then aliquoted at 200–500 cells/sample for intracellular flow cytometry as described above.

IFNγ ELISpot assay

Antigen specificity of T cells was measured by IFNγ ELISpot (Millipore, Burlington, MA, USA). Day 10 VSTs were plated at 100,000 cells/well with no peptide, actin (control), each of the individual SARS-CoV-2 pepmixes (200 ng per peptide per well), or SEB. Plates were then sent for IFNγ spot counting (Zellnet Consulting, Fort Lee, NJ, USA).

Statistical analysis

Data were analyzed using a Wilcoxon matched-pairs signed rank test. Bonferroni correction was applied for multiple comparisons within an analysis, and corrected p values are provided in figure legends as appropriate. For RNA-seq, ANOVA (Partek Genomics Suite) was used to develop a list of DEGs (fold change ≥ 2 or ≤ -2, and false discovery rate <0.05). K-means clustering was performed to develop modules of DEGs, with the number of clusters determined using the elbow method. We identified pathways enriched in each module using metascape (metascape.org) and performed GSEA for each condition compared with pooled expression from all other conditions.[34] Antiviral ISGs were obtained from public datasets.[33]

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtm.2022.04.013.

ACKNOWLEDGMENTS

The authors thank the staff members of the Center for Cancer and Immunology Research and the National Institute of Allergy and Infectious Diseases (NIAID) Laboratory of Allergic Disease and our referring collaborators for enabling this work. The authors also are very grateful to all their patients and their families for participating in this work. This work was supported by grants from the Board of Visitors of the Children's National Health System, the Katzen Foundation, the Connor Family Foundation, and the intramural research programs of the NIH/NIAID.

AUTHOR CONTRIBUTIONS

J.D.S., C.A.L., P.J.H., C.M.B, M.D.K., and D.M.S. conceived and designed the experiments; J.D.-S., C.A.L., M.A.J.-W., A.S., N.E.F., M.D.K., S.R.C., V.V.K., K.W., H.L., and D.M.S. conducted the research; J.D.-S., C.A.L., M.D.K., and D.M.S. analyzed data. J.D.-S., C.A.L., M.A.J.-W., M.D.K., and D.M.S. wrote the manuscript; and all authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

P.J.H. is a cofounder and is on the board of directors of Mana Therapeutics. P.J.H. is on a scientific advisory board for Cellevolve. M.D.K.

is on a scientific advisory panel for Gilead Sciences. The remaining authors declare no competing financial interests.

REFERENCES

1. Liang, W., Guan, W., Chen, R., Wang, W., Li, J., Xu, K., Li, C., Ai, Q., Lu, W., Liang, H., et al. (2020). Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol. *21*, 335–337. https://doi.org/10.1016/S1470-2045(20)30096-6.
2. Meyts, I., Bucciol, G., Quinti, I., Neven, B., Fischer, A., Seoane, E., Lopez-Granados, E., Gianelli, C., Robles-Marhuenda, A., Jeandel, P.Y., et al. (2021). Coronavirus Disease 2019 in patients with inborn errors of immunity: an international study. J. Allergy Clin. Immunol. *147*, 520–531. https://doi.org/10.1016/j.jaci.2020.09.010.
3. Vijenthira, A., Gong, I.Y., Fox, T.A., Booth, S., Cook, G., Fattizzo, B., Martin-Moro, F., Razanamahery, J., Riches, J.C., Zwicker, J., et al. (2020). Outcomes of patients with hematologic malignancies and COVID-19: a systematic review and meta-analysis of 3377 patients. Blood *136*, 2881–2892. https://doi.org/10.1182/blood.2020008824.
4. Azzi, Y., Bartash, R., Scalea, J., Loarte-Campos, P., and Akalin, E. (2021). COVID-19 and solid organ transplantation: a review article. Transplantation *105*, 37–55. https://doi.org/10.1097/TP.0000000000003523.
5. Shields, A.M., Burns, S.O., Savic, S., and Richter, A.G.; UK PIN COVID-19 Consortium (2021). COVID-19 in patients with primary and secondary immunodeficiency: the United Kingdom experience. J. Allergy Clin. Immunol. *147*, 870–875.e1. https://doi.org/10.1016/j.jaci.2020.12.620.
6. Dai, M., Liu, D., Liu, M., Zhou, F., Li, G., Chen, Z., Zhang, Z., You, H., Wu, M., Zheng, Q., et al. (2020). Patients with cancer appear more vulnerable to SARS-CoV-2: a multicenter study during the COVID-19 outbreak. Cancer Discov. *10*, 783–791. https://doi.org/10.1158/2159-8290.CD-20-0422.
7. Weiskopf, D., Schmitz, K.S., Raadsen, M.P., Grifoni, A., Okba, N.M.A., Endeman, H., van den Akker, J.P.C., Molenkamp, R., Koopmans, M.P.G., van Gorp, E.C.M., et al. (2020). Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci. Immunol. *5*, eabd2071. https://doi.org/10.1126/sciimmunol.abd2071.
8. Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S., et al. (2020). Targets of T Cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell *181*, 1489–1501.e15. https://doi.org/10.1016/j.cell.2020.05.015.
9. Keller, M.D., Harris, K.M., Jensen-Wachspress, M.A., Kankate, V.V., Lang, H., Lazarski, C.A., Durkee-Shock, J., Lee, P.H., Chaudhry, K., Webber, K., et al. (2020). SARS-CoV-2-specific T cells are rapidly expanded for therapeutic use and target conserved regions of the membrane protein. Blood *136*, 2905–2917. https://doi.org/10.1182/blood.2020008488.
10. Braun, J., Loyal, L., Frentsch, M., Wendisch, D., Georg, P., Kurth, F., Hippenstiel, S., Dingeldey, M., Kruse, B., Fauchere, F., et al. (2020). SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature *587*, 270–274. https://doi.org/10.1038/s41586-020-2598-9.
11. Noh, J.Y., Jeong, H.W., Kim, J.H., and Shin, E.C. (2021). T cell-oriented strategies for controlling the COVID-19 pandemic. Nat. Rev. Immunol. *21*, 687–688. https://doi.org/10.1038/s41577-021-00625-9.
12. Keller, M.D., and Bollard, C.M. (2020). Virus-specific T-cell therapies for patients with primary immune deficiency. Blood *135*, 620–628. https://doi.org/10.1182/blood.2019000924.
13. Gerdemann, U., Katari, U.L., Papadopoulou, A., Keirnan, J.M., Craddock, J.A., Liu, H., Martinez, C.A., Kennedy-Nasser, A., Leung, K.S., Gottschalk, S.M., et al. (2013). 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. *21*, 2113–2121. https://doi.org/10.1038/mt.2013.151.
14. Gerdemann, U., Vera, J.F., Rooney, C.M., and Leen, A.M. (2011). Generation of multivirus-specific T cells to prevent/treat viral infections after allogeneic hematopoietic stem cell transplant. J. Vis. Exp. 2736. https://doi.org/10.3791/2736.
15. Kaeuferle, T., Krauss, R., Blaeschke, F., Willier, S., and Feuchtinger, T. (2019). Strategies of adoptive T -cell transfer to treat refractory viral infections post allogeneic stem cell transplantation. J. Hematol. Oncol. *12*, 13. https://doi.org/10.1186/s13045-019-0701-1.
16. Verhagen, J., van der Meijden, E.D., Lang, V., Kremer, A.E., Volkl, S., Mackensen, A., Aigner, M., and Kremer, A.N. (2021). Human CD4(+) T cells specific for dominant epitopes of SARS-CoV-2 Spike and Nucleocapsid proteins with therapeutic potential. Clin. Exp. Immunol. *205*, 363–378. https://doi.org/10.1111/cei.13627.
17. Lazarski, C.A., Datar, A.A., Reynolds, E.K., Keller, M.D., Bollard, C.M., and Hanley, P.J. (2021). Identification of new cytokine combinations for antigen-specific T-cell therapy products via a high-throughput multi-parameter assay. Cytotherapy *23*, 65–76. https://doi.org/10.1016/j.jcyt.2020.08.006.
18. De Biasi, S., Meschiari, M., Gibellini, L., Bellinazzi, C., Borella, R., Fidanza, L., Gozzi, L., Iannone, A., Lo Tartaro, D., Mattioli, M., et al. (2020). Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat. Commun. *11*, 3434. https://doi.org/10.1038/s41467-020-17292-4.
19. Chen, Z., and John Wherry, E. (2020). T cell responses in patients with COVID-19. Nat. Rev. Immunol. *20*, 529–536. https://doi.org/10.1038/s41577-020-0402-6.
20. McMahan, K., Yu, J., Mercado, N.B., Loos, C., Tostanoski, L.H., Chandrashekar, A., Liu, J., Peter, L., Atyeo, C., Zhu, A., et al. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature *590*, 630–634. https://doi.org/10.1038/s41586-020-03041-6.
21. Berger, C., Jensen, M.C., Lansdorp, P.M., Gough, M., Elliott, C., and Riddell, S.R. (2008). Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. J. Clin. Invest. *118*, 294–305. https://doi.org/10.1172/JCI32103.
22. Kueberuwa, G., Gornall, H., Alcantar-Orozco, E.M., Bouvier, D., Kapacee, Z.A., Hawkins, R.E., and Gilham, D.E. (2017). CCR7(+) selected gene-modified T cells maintain a central memory phenotype and display enhanced persistence in peripheral blood in vivo. J. Immunother. Cancer *5*, 14. https://doi.org/10.1186/s40425-017-0216-7.
23. Hurton, L.V., Singh, H., Najjar, A.M., Switzer, K.C., Mi, T., Maiti, S., Olivares, S., Rabinovich, B., Huls, H., Forget, M.A., et al. (2016). Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells. Proc. Natl. Acad. Sci. U S A *113*, E7788–E7797. https://doi.org/10.1073/pnas.1610544113.
24. Golubovskaya, V., and Wu, L. (2016). Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. Cancers *8*, 36. https://doi.org/10.3390/cancers8030036.
25. Weiskopf, D., Bangs, D.J., Sidney, J., Kolla, R.V., De Silva, A.D., de Silva, A.M., Crotty, S., Peters, B., and Sette, A. (2015). Dengue virus infection elicits highly polarized CX3CR1+ cytotoxic CD4+ T cells associated with protective immunity. Proc. Natl. Acad. Sci. U S A *112*, E4256–E4263. https://doi.org/10.1073/pnas.1505956112.
26. Leen, A.M., Myers, G.D., Sili, U., Huls, M.H., Weiss, H., Leung, K.S., Carrum, G., Krance, R.A., Chang, C.C., Molldrem, J.J., et al. (2006). Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. Nat. Med. *12*, 1160–1166. https://doi.org/10.1038/nm1475.
27. Blyth, E., Clancy, L., Simms, R., Ma, C.K.K., Burgess, J., Deo, S., Byth, K., Dubosq, M.C., Shaw, P.J., Micklethwaite, K.P., and Gottlieb, D.J. (2013). Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. Blood *121*, 3745–3758. https://doi.org/10.1182/blood-2012-08-448977.
28. Marshall, N.B., and Swain, S.L. (2011). Cytotoxic CD4 T cells in antiviral immunity. J. Biomed. Biotechnol. *2011*, 954602. https://doi.org/10.1155/2011/954602.
29. Betts, M.R., Brenchley, J.M., Price, D.A., De Rosa, S.C., Douek, D.C., Roederer, M., and Koup, R.A. (2003). Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. J. Immunol. Methods *281*, 65–78. https://doi.org/10.1016/s0022-1759(03)00265-5.
30. Betts, M.R., Price, D.A., Brenchley, J.M., Lore, K., Guenaga, F.J., Smed-Sorensen, A., Ambrozak, D.R., Migueles, S.A., Connors, M., Roederer, M., et al. (2004). The functional profile of primary human antiviral CD8+ T cell effector activity is dictated by cognate peptide concentration. J. Immunol. *172*, 6407–6417. https://doi.org/10.4049/jimmunol.172.10.6407.
31. Grossman, W.J., Verbsky, J.W., Barchet, W., Colonna, M., Atkinson, J.P., and Ley, T.J. (2004). Human T regulatory cells can use the perforin pathway to cause

autologous target cell death. Immunity *21*, 589–601. https://doi.org/10.1016/j.immuni.2004.09.002.

32. McNab, F., Mayer-Barber, K., Sher, A., Wack, A., and O'Garra, A. (2015). Type I interferons in infectious disease. Nat. Rev. Immunol. *15*, 87–103. https://doi.org/10.1038/nri3787.

33. Mostafavi, S., Yoshida, H., Moodley, D., LeBoite, H., Rothamel, K., Raj, T., Ye, C.J., Chevrier, N., Zhang, S.Y., Feng, T., et al. (2016). Parsing the interferon transcriptional network and its disease associations. Cell *164*, 564–578. https://doi.org/10.1016/j.cell.2015.12.032.

34. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., and Mesirov, J.P. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U S A *102*, 15545–15550. https://doi.org/10.1073/pnas.0506580102.

35. Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstrale, M., Laurila, E., et al. (2003). PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat. Genet. *34*, 267–273. https://doi.org/10.1038/ng1180.

36. Harris, K.M., Horn, S.E., Grant, M.L., Lang, H., Sani, G., Jensen-Wachspress, M.A., Kankate, V.V., Datar, A., Lazarski, C.A., Bollard, C.M., and Keller, M.D. (2020). T-cell therapeutics targeting human parainfluenza virus 3 are broadly epitope specific and are cross reactive with human parainfluenza virus 1. Front. Immunol. *11*, 575977. https://doi.org/10.3389/fimmu.2020.575977.

37. McLaughlin, L.P., Lang, H., Williams, E., Wright, K.E., Powell, A., Cruz, C.R., Colberg-Poley, A.M., Barese, C., Hanley, P.J., Bollard, C.M., and Keller, M.D. (2016). Human parainfluenza virus-3 can be targeted by rapidly ex vivo expanded T lymphocytes. Cytotherapy *18*, 1515–1524. https://doi.org/10.1016/j.jcyt.2016.08.010.

38. Carson, W.E., Giri, J.G., Lindemann, M.J., Linett, M.L., Ahdieh, M., Paxton, R., Anderson, D., Eisenmann, J., Grabstein, K., and Caligiuri, M.A. (1994). Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J. Exp. Med. *180*, 1395–1403. https://doi.org/10.1084/jem.180.4.1395.

39. van Eeden, C., Khan, L., Osman, M.S., and Cohen Tervaert, J.W. (2020). Natural killer cell dysfunction and its role in COVID-19. Int. J. Mol. Sci. *21*, 6351. https://doi.org/10.3390/ijms21176351.

40. Market, M., Angka, L., Martel, A.B., Bastin, D., Olanubi, O., Tennakoon, G., Boucher, D.M., Ng, J., Ardolino, M., and Auer, R.C. (2020). Flattening the COVID-19 curve with natural killer cell based immunotherapies. Front. Immunol. *11*, 1512. https://doi.org/10.3389/fimmu.2020.01512.

41. Chen, G.M., Chen, C., Das, R.K., Gao, P., Chen, C.H., Bandyopadhyay, S., Ding, Y.Y., Uzun, Y., Yu, W., Zhu, Q., et al. (2021). Integrative bulk and single-cell profiling of premanufacture T-cell populations reveals factors mediating long-term persistence of CAR T-cell therapy. Cancer Discov. *11*, 2186–2199. https://doi.org/10.1158/2159-8290.CD-20-1677.

42. Fraietta, J.A., Lacey, S.F., Orlando, E.J., Pruteanu-Malinici, I., Gohil, M., Lundh, S., Boesteanu, A.C., Wang, Y., O'Connor, R.S., Hwang, W.T., et al. (2021). Author Correction: determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat. Med. *27*, 561. https://doi.org/10.1038/s41591-021-01248-2.

43. Caushi, J.X., Zhang, J., Ji, Z., Vaghasia, A., Zhang, B., Hsiue, E.H.C., Mog, B.J., Hou, W., Justesen, S., Blosser, R., et al. (2021). Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. Nature *596*, 126–132. https://doi.org/10.1038/s41586-021-03752-4.

44. Krishna, S., Lowery, F.J., Copeland, A.R., Bahadiroglu, E., Mukherjee, R., Jia, L., Anibal, J.T., Sachs, A., Adebola, S.O., Gurusamy, D., et al. (2020). Stem-like CD8 T cells mediate response of adoptive cell immunotherapy against human cancer. Science *370*, 1328–1334. https://doi.org/10.1126/science.abb9847.

45. Fox, A., Harland, K.L., Kedzierska, K., and Kelso, A. (2018). Exposure of human CD8(+) T cells to type-2 cytokines impairs division and differentiation and induces limited polarization. Front. Immunol. *9*, 1141. https://doi.org/10.3389/fimmu.2018.01141.

46. Bastard, P., Rosen, L.B., Zhang, Q., Michailidis, E., Hoffmann, H.H., Zhang, Y., Dorgham, K., Philippot, Q., Rosain, J., Beziat, V., et al. (2020). Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science *370*, eabd4585. https://doi.org/10.1126/science.abd4585.

47. Zhang, Q., Bastard, P., Liu, Z., Le Pen, J., Moncada-Velez, M., Chen, J., Ogishi, M., Sabli, I.K.D., Hodeib, S., Korol, C., et al. (2020). Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science *370*, eabd4570. https://doi.org/10.1126/science.abd4570.

48. Jones, S.A., and Hunter, C.A. (2021). Is IL-6 a key cytokine target for therapy in COVID-19? Nat. Rev. Immunol. *21*, 337–339. https://doi.org/10.1038/s41577-021-00553-8.

49. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet *395*, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

50. Mehta, P., McAuley, D.F., Brown, M., Sanchez, E., Tattersall, R.S., Manson, J.J., and Hlh Across Speciality Collaboration, U.K. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet *395*, 1033–1034. https://doi.org/10.1016/S0140-6736(20)30628-0.

51. Wang, C., Kang, K., Gao, Y., Ye, M., Lan, X., Li, X., Zhao, M., and Yu, K. (2020). Cytokine levels in the body fluids of a patient with COVID-19 and acute respiratory distress syndrome: a case report. Ann. Intern. Med. *173*, 499–501. https://doi.org/10.7326/L20-0354.

52. Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Qi, Y., Sun, R., Tian, Z., Xu, X., and Wei, H. (2020). Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. Natl. Sci. Rev. *7*, 998–1002. https://doi.org/10.1093/nsr/nwaa041.

53. Martits-Chalangari, K., Spak, C.W., Askar, M., Killian, A., Fisher, T.L., Atillasoy, E., Marshall, W.L., McNeel, D., Miller, M.D., Mathai, S.K., and Gottlieb, R.L. (2021). 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. Transpl. *22*, 1261–1265. https://doi.org/10.1111/ajt.16927.

54. Schwartz, D.M., Farley, T.K., Richoz, N., Yao, C., Shih, H.Y., Petermann, F., Zhang, Y., Sun, H.W., Hayes, E., Mikami, Y., et al. (2019). Retinoic acid receptor alpha represses a Th9 transcriptional and epigenomic program to reduce allergic pathology. Immunity *50*, 106–120.e10. https://doi.org/10.1016/j.immuni.2018.12.014.