Cytomegalovirus Triplex vaccine in pediatric hematopoietic stem cell transplant patients at high risk for cytomegalovirus complications: evaluation of vaccine safety, immunogenicity and impact on viremia requiring antivirals

Cytomegalovirus (CMV) reactivation remains a leading clinical concern, contributing to substantial morbidity and mortality following allogeneic hematopoietic cell transplantation (HCT), in both adult and pediatric high-risk CMV-seropositive recipients.[1,2] Pre-emptive antiviral therapy (PET) greatly reduces the risk of CMV disease and mortality post-transplant, but it is associated with significant economic burden, toxicity and rates of failure.[3,4] In the case or pediatric patients, many PET antivirals have intolerable toxicity or have been insufficiently evaluated.[5,6] US Food and Drug Administration approved letermovir prophylaxis up day 200 post-transplant[7] is effective with minimal myelosuppression, and it is tolerated in pediatric patients.[8] Currently its use remains off-label in these patients, pending outcomes of an ongoing phase II trial (*clinicaltrials gov. Identifier: NCT03940586*). The main drawback of letermovir is the observed increase in clinically significant CMV infection and disease requiring antivirals, when prophylaxis is discontinued.[7,9] The decreased CMV reactivation and the consequent decreased CMV antigen exposure during letermovir prophylaxis may delay CMV-specific immune reconstitution leading to breakthrough CMV viremia requiring antivirals. Notably, polyfunctional T-cell responses specific for CMV pp65 and IE-1 proteins, which are involved in controlling CMV viremia are significantly decreased in HCT patients receiving letermovir prophylaxis, compared with HCT recipients receiving PET.[10] Additionally, there is no difference in mortality after 1 year between letermovir and PET-treated T-cell replete HCT recipients.[9] Infusion of CMV-specific T cells can promote restoration of durable, functional antiviral immunity. Despite the success of this strategy, ready availability of adoptive T-cell therapy remains difficult to achieve.[11] Furthermore, in December 2023 phase III trials of posoleucel,[12] an off-the-shelf, multi-virus specific T-cell therapy, including CMV were halted for futility. Consequently, immunotherapeutic strategies to suppress both early and late clinical reactivation, through enhancing and sustaining protective immune reconstitution against CMV remain an unmet need in the letermovir era.

Triplex is a modified vaccinia Ankara (MVA) vectored vaccine which expresses three immunodominant CMV proteins; pp65, IE1-exon4, and IE2-exon5. It has been designed to accelerate reconstitution of protective T-cell CMV-specific immunity. Triplex demonstrated tolerability and immunogenicity in healthy adults, autologous and allogeneic HCT recipients, in whom it reduced CMV viremia.[13] Based on Triplex favorable tolerability, immunogenicity and efficacy outcomes we investigated whether vaccinating HCT pediatric recipients, with Triplex safely promoted and expanded CMV-specific T cells protecting from clinical viremia requiring PET in the presence of letermovir.

We designed a non-randomized, open label, phase Ib pilot trial to evaluate safety, optimal dose, and immunogenicity of Triplex vaccine in CMV-seropositive pediatric allogeneic HCT recipients. The study was conducted in accordance with the Helsinki Declaration at City of Hope National Medical Center (COH), was approved by the local Institutional Review Board, and registered as *clinicaltrials gov. Identifier: NCT 03354728*.

Fifteen pediatric (age 1-21 years, as defined by the US Federal Food, Drug, and Cosmetic Act) CMV-seropositive allogeneic HCT transplant recipients were consented to be enrolled in the study, from August 2018 through January 2023. Several factors were associated with the trial slow accrual, including reassigning the study to a new principal investigator (PI), as the initial PI transferred to a different Institution; the significant COVID-19 pandemic disruption on cancer clinical trials and constrained financial resources. Clinical characteristics of the study population including Karnofsky performance score, conditioning regimen and stem cell source are described in Table 1. Exclusion criteria for administration of Triplex injection on day 28 post-HCT included: diagnosis of relapse, engraftment failure, acute graft-*versus*-host disease (GVHD) of grade 3 or 4 (according to the Keystone Consensus grading system), administration of steroids with a dosage above 1.0 mg/kg per day within 7 days, ongoing non-hematologic toxicity of grade 3 or higher (according to the Common Terminology Criteria for Adverse Events, version 4.03) and development of CMV viremia with quantitative polymerase chain reaction (qPCR) >500 copies/mL.

Six patients did not meet inclusion and did not receive the planned study intervention (*Online Supplementary Figure 1S*). Triplex injections were administered to nine eligible recipients on days 28 and 56 post-transplant, consistent

with timing of vaccination in our previous patient studies.[13] Two Triplex vaccine dose levels (DL1=10^8 PFU and DL2=5x 10^8 PFU) were evaluated in two age groups (11-21 and 1-10 years old). DL2 is the Triplex dose administered to healthy adults and HCT patients in our published trials.[13] Triplex-related adverse event (AE) assessment, including GVHD, non-relapse mortality, relapse, CMV reactivation requiring PET and CMV disease were prospectively monitored, recorded according to institutional standard of care, and summarized in Table 1 and *Online Supplementary Table 1S*. The impact of Triplex on cellular immunity was investigated and focused on magnitude, function, and quality of CMV-specific CD4 and CD8 T-cell expansion. Longitudinal immune monitoring was performed measuring 4-1BB (CD137) surrogate marker of T-cell activation by surface staining of peripheral blood mononuclear cells (PBMC), following 24 hours of stimulation with either pp65, IE1 or IE2 peptide libraries and medium as control. The CD137 assay was combined with the monitoring of CD28, CD45RA memory phenotype markers,[14] to achieve a comprehensive immunological assessment of the Triplex vaccine effect on quantity and quality of CMV-specific T-cell functional expansion. PBMC from blood draws obtained before Triplex vaccination at day 28 and afterwards on days 42, 56, 70, 84, 100, 140, 180, and 270 were analyzed by fluorescence-activated cytometry in the research laboratory setting (FC; Gallios™, Beckman Coulter with Kaluza analysis software, Brea, CA).[2] Concentrations of pp65, IE1- or IE2-specific $CD3^+CD4^+CD137^+$ and $CD3^+CD8^+CD137^+$ T cells were longitudinally measured using multiparameter (6 colors) flowcytometry. When either CMV-specific $CD3^+CD8^+CD137^+$ T-cell or $CD3^+CD4^+CD137^+$ T-cell populations were ≥0.2%, a further analysis for CD28 and CD45RA memory membrane markers could be performed. $CD45RA^+CD28^+$ cells were classified as naïve; $CD45RA^-$ $CD28^+$ cells, as central memory (TCM); and $CD28^-$ cells, as effector T cells. Within the effector T-cell group, two subpopulations were identified: $CD45RA^-CD28^-$ cells (TEM) and $CD45RA^+CD28^-$ effector "revertant" T cells, re-expressing the RA isoform of the CD45 surface marker (TEMRA).

From August 2018 to April 2023, nine eligible patients each received two injections of Triplex with no safety concerns and no dose limiting toxicity. Triplex was well tolerated at both DL and no AE grade ≥2 possibly or probable related to the vaccine was recorded (Table 1). Enrollment for DL1 vaccinees included three transplant 11-21-year-old recipients and three 1-10-year-old recipients. In the DL2, three transplant 11-21-year-old recipients were vaccinated. All nine participants completed the two injection Triplex vaccination regimen. All except one patient (COH002) received letermovir prophylaxis. CMV reactivation was treated with PET on day 44 for COH002 and on day 216 for COH011 following >1.0 mg/kg steroid treatment for chronic GVHD. COH014 withdrew from study on day 140 post-HCT, to return to their home country; no history of CMV reactivation was observed through 1-year post-HCT. COH013 experienced relapse on day 158 post-HCT and further research blood draws were suspended.

We found high levels of functional CMV-specific T cells fol-

Table 1. Characteristics and outcomes of the Triplex-vaccinated pediatric hematopoietic stem cell transplant recipients.

Patient characteristics	
Median age in years (IQR)	13 (8-18)
Female sex	5 (55.6)
Race	
Caucasian	7 (77.8)
Asian	2 (22.2)
Primary diagnosis	
Acute lymphoblastic leukemia	5 (55.6)
Acute myeloid leukemia	2 (22.2)
Severe aplastic anemia	2 (22.2)
Karnofsky performance score (at conditioning)	
100	6 (66.7)
90	3 (33.3)
Conditioning regimen	
Reduced intensity	2 (22.2)
Myeloablative	7 (77.8)
Stem cell source	
Bone marrow	6 (66.7)
Peripheral blood	3 (33.3)
Donor type	
Matched related	2 (22.2)
Haploidentical	3 (33.3)
Matched unrelated	4 (44.4)
Donor CMV serostatus	
Positive	6 (66.7)
Negative	3 (33.3)
Primary outcomes	
CMV viremia or disease requiring antiviral treatment[1]	1 (11.1)
Non-relapse mortality[1]	0
Severe (grade 3-4)[2] acute GVHD[3]	0
Grade 3-4 AE[3,4]	0
Secondary outcomes	
Late CMV viremia or disease requiring antiviral treatment[5]	1 (11.1)
Delayed engraftment	0
Chronic GVHD	1 (11.1)
Relapse	1 (11.1)
All-cause mortality	0
Cellular immunity (see Figures 1, 2)	

CMV: cytomegalovirus; IQR: interquartile range; AE: adverse event; GVHD: graft-*versus*-host disease. [1]Through day 100 after transplant; [2]according to the Keystone Consensus grading system; [3]within 2 weeks from each vaccination; [4]on the basis of Common Terminology Criteria for Adverse Events (CTCAE), version 4.03 at least probably or definitely related to the Triplex vaccine; [5]>100 days after transplant; as per standard of care, pre-emptive antiviral therapy (PET) was started in high-risk hematopoietic stem cell transplant; patients receiving letermovir prophylaxis when 2 consecutive CMV quantitative polymerase chain reaction (qPCR) were >500 copies/mL or when one qPCR was >500 copies/mL, if letermovir prophylaxis was not used. Low-risk patients started PET with CMV qPCR ≥1,500 copies/mL or with rising qPCR regardless of prophylaxis (1 copy/mL ≈0.93 IU/mL). *Values are numbers of patients (percentages) unless otherwise indicated.

lowing Triplex vaccination, which had a similar longitudinal profiles and often surpassed those observed in healthy adults and HCT recipients from previous Triplex vaccination studies[13] (Figure 1; *Online Supplementary Figure S2*). High levels of functional CMV-specific T-cell response were measured in patients treated at both Triplex DL, in both age groups, in transplant recipients with either CMV-seropositive or CMV-seronegative donors undergoing matched related, unrelated or haploidentical HCT, using bone marrow or peripheral stem cell sources. The elevated response may have contributed to restoring protective antiviral cellular immunity after transplant in the Triplex-vaccinated pediatric recipients, who did not receive PET to control CMV reactivation. However, a randomized study is required to confirm such a hypothesis. A possible explanation of the vigorous response to Triplex in pediatric recipients may be linked to the effective activity of the thymus, which is highly functional in children, protecting against serious infections and leading to good vaccine responses.[15] High-dose post-transplant cyclophosphamide used in haploidentical HCT pediatric recipients to prevent acute GVHD, did not induce CMV-specific T-cell depletion, in agreement with previous studies in adult recipients.[16] As often reported in the adult transplant population, use of high dosage corticosteroids for GVHD reduced CMV-specific T-cell responses[3,14] and in COH011 led to late PET treatment. As visualized by the gray bands in Figure 2A, functionally activated pp65, IE1, IE2-specific T cells increased post-transplant during immune-reconstitution. Linear mixed models were used to evaluate the longitudinal changes on log10-transformed concentration of CMV-specific T cells. The analysis showed a significant increase in pp65-specific T cells/μL (left plot) after day 28 first Triplex injection through day 270 post-HCT (P=0.013). As previously found in healthy adults and in HCT recipients vaccinated with Triplex,[13] longitudinal pp65-specific T-cell expansions were the most consistent and the highest in magnitude among the current recipient population, while enhanced levels of both IE1- and IE2-specfic T cells were observed in some pediatric patients (Figure 1). The memory phenotype analysis (Figure 2B) focused on pp65-specific T-cell responses, and showed that functionally activated pp65-specific $CD137^{+}CD8^{+}$ T cells had a predominant TCM profile early post-transplant, and reduced naïve T-cell subsets.[13] In the vaccinated recipients, pp65-specific CD8 T cells showed a memory phenotype pattern mainly consisting of antigen-experienced TCM, immediately after transplant. By linear mixed models, the TCM subset significantly decreased following Triplex first injection (P=0.043) acquiring enhanced effector (TEMRA + TEM) functions (P=0.026), through day 270 post-HCT. Strong predominance of long-lived and functional effector T cells has been reported during primary CMV infection, since these cells are critical for viremia control.[17]

This is the first-in-pediatric HCT recipient study of Triplex. The early post-HCT administration of a biologic vaccine has few precedents in pediatric clinical trial history. Though clinical research is of considerable importance in developing safe medications, pediatric trials pose many ethical and clinical challenges. Our pilot trial outcomes are of interest in the transplant context, as they demonstrated

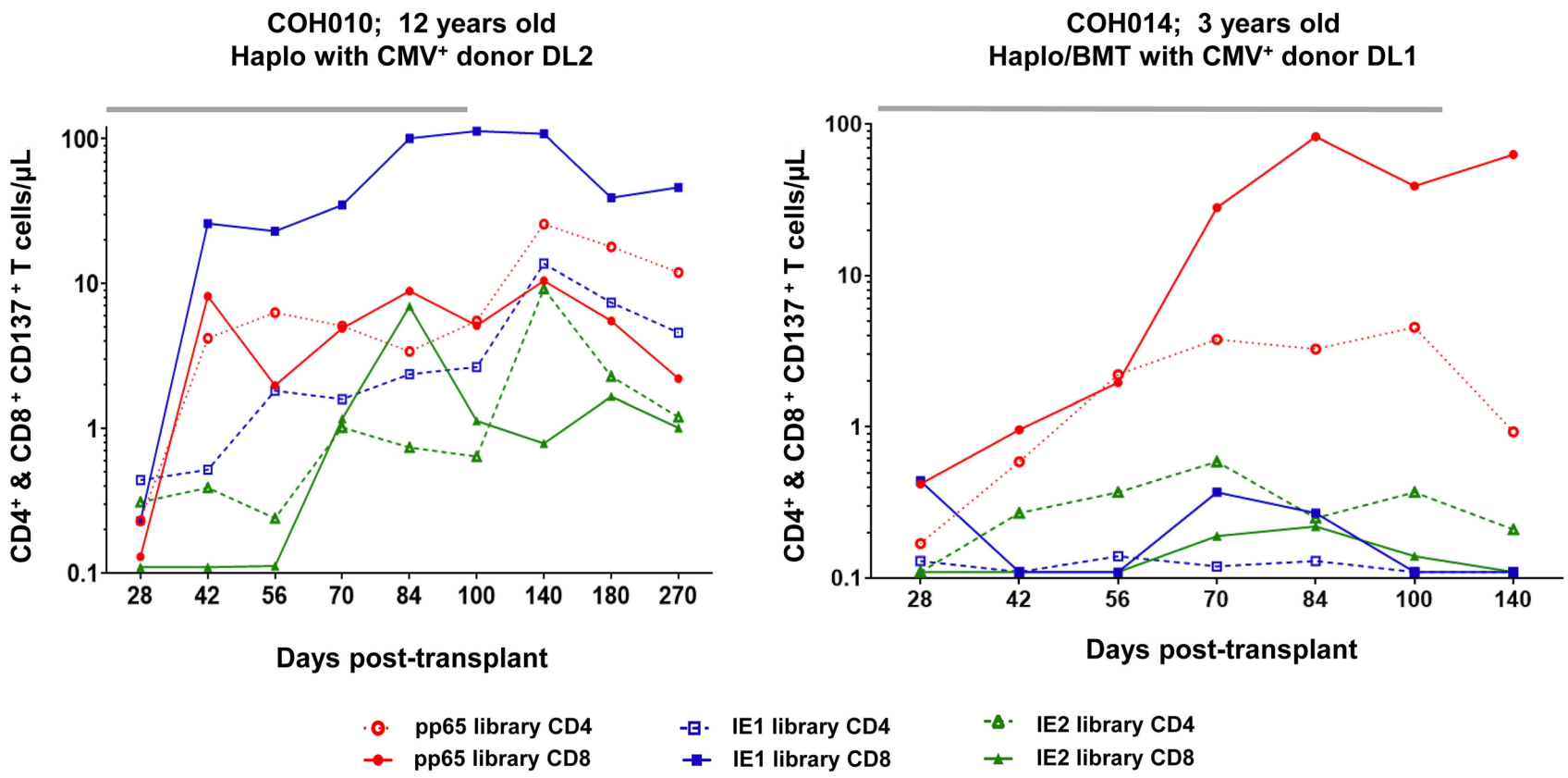

Figure 1. Cytomegalovirus-specific T-cell responses. Longitudinal immune profiles of COH010 and COH014 pediatric haploidentical (Haplo) recipients in response to Triplex vaccination. Cytomegalovirus (CMV)-specific T cells/μL are shown by distinct colors (pp65-, IE1-, and IE-2) and line types (CD4 and CD8), as indicated in legend. Letermovir duration is shown by the black line on plot top (Haplo). BMT: bone marrow transplant; DL: dose level.

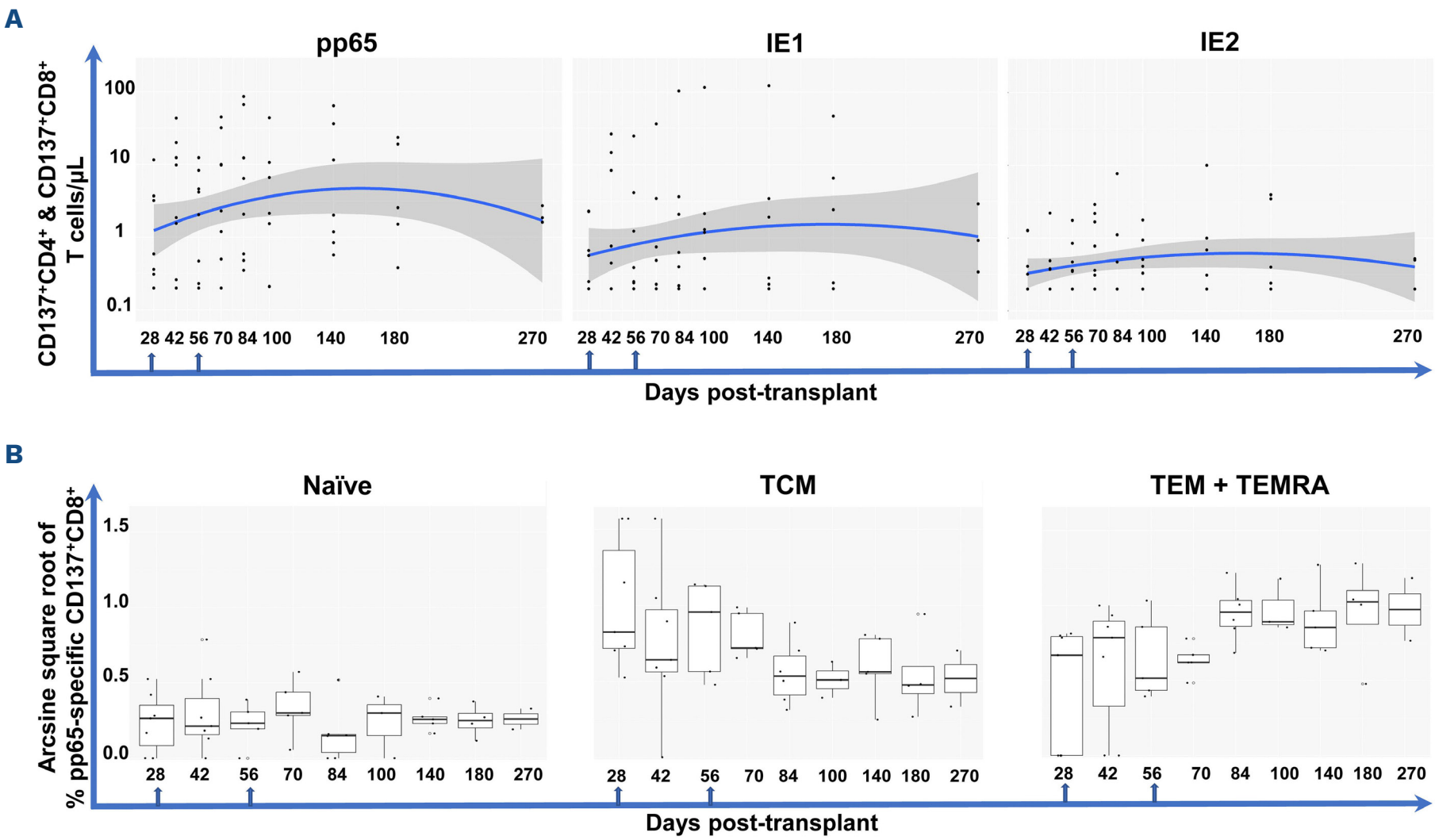

Figure 2. Levels and memory phenotypes of functional cytomegalovirus-specific T cells in the Triplex-vaccinated pediatric recipients. (A) Longitudinal levels of combined $CD137^+CD3^+$ $CD4^+$ and $CD137^+CD3^+$ $CD8^+$ T cells specific for pp65, IE1 and IE2 antigens (as specified at the top of each plot). The band shown was computed using the loess scatterplot smoother providing the marginal geometric mean concentrations through time. A 95% confidence band is shown in gray, and individual measurement trajectories are shown for each participant up to 7 days before cytomegalovirus (CMV) reactivation, requiring antivirals (COH002 and COH011). Linear mixed models were used to evaluate the changes on log10-transformed concentration of CMV-specific T cells from day 28 through day 270. Both linear and quadratic terms of study days were included in the models on CMV-specific T cells. An autoregressive covariance matrix was used to account for the correlation in participant's T-cell responses over time. Logarithmic spacing is used to aid visualization. Arrows indicate Triplex injections on days 28 and 56 post-transplant. (B) Box plots showing percentages on the arcsine scale of pp65-specific, $CD137^+CD8^+$ naïve, T central (TCM) and effector (TEM + TEMRA) memory phenotype subset (as indicated at the top of each plot). The box spans the interquartile range, the central bar shows the median, and the whiskers extend to 1.5-times the interquartile range. Percentages of memory subsets from day 28 though day 270 were evaluated using linear mixed models and study day was included as a categorical predictor.

feasibility and safety of vaccinating immunosuppressed pediatric patients with Triplex. The encouraging preliminary results suggest that in HCT pediatric patients receiving letermovir prophylaxis, Triplex safely expands large numbers of long lasting functional CMV-specific T cells early post-transplant, which may prevent subsequent CMV reactivation requiring PET. The current key outcomes laid the groundwork for the planning of a multicenter, randomized placebo-controlled phase II trial. Though Triplex vaccination can overcome the letermovir-induced immune impairment,[10] our long-term goal is to eliminate this daily oral medication for young patients who already have polypharmacy burdens. Novel successful strategies of vaccinating the immunocompetent HCT donor and possibly also the recipient with Triplex may suffice to prevent CMV reactivation by promoting early post-HCT sustained, protective CMV-immune reconstitution.

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Disclosures

CLR reports receiving consulting fees and research funding from Helocyte Inc. DJD reports receiving fees for royalties, research funding, and fees for serving on the advisory board of Helocyte Inc. DJD is co-inventor on a patent application covering the design and construction of the synthetic modified vaccinia Ankara platform (PCT/US2021/016247); is co-inventor on a patent application covering the development of a COVID-19 vaccine (PCT/US2021/032821); is an employee of City of Hope National Medical Center (Duarte, CA, USA), which developed and funded the vaccine trial; receives consulting fees, patent royalties, research funding, and fees for serving on the advisory board of Helocyte Inc.; in addition, DJD has two patents 8,580,276 and 9,675,689 that are licensed to Helocyte; is a co-inventor of the Patent Cooperation Treaty (PCT) application that covers the development of a COVID-19 vaccine (PCT/US2021/032821) licensed to GeoVax Labs Inc.; receives consulting fees and research support from GeoVax Labs Inc. DJD and CLR are co-inventors on a patent covering the "Vaccination of hematopoietic stem cell donors with cytomegalovirus Triplex composition" (PCT/US2023/032052).

Contributions

AP, WS, NK, and JC treated and clinically assessed the patients for adverse events and acute graft-*versus*-host disease. DJD and WS designed the study. YP, QZ, and TK performed specimen processing, reagent preparation, and immunological assays. CLR and YP analyzed the immune monitoring data. DY performed the statistical analyses. CLR and DJD wrote the initial manuscript; and all authors approved the final version.

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Data-sharing statement

The clinical trial protocol and the anonymized data presented in this study can be made available upon reasonable request.

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