CMVpp65 Vaccine Enhances the Antitumor Efficacy of Adoptively Transferred CD19-Redirected CMV-Specific T Cells

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Abstract

Purpose: T cells engineered with chimeric antigen receptors (CAR) recognizing CD19 can induce complete remission of B-cell malignancies in clinical trials; however, in some disease settings, CAR therapy confers only modest clinical benefit due to attenuated persistence of CAR T cells. The purpose of this study was to enhance persistence and augment the antitumor activity of adoptively transferred CD19/CAR T cells by restimulating CAR+ T cells through an endogenous cytomegalovirus (CMV)-specific T-cell receptor.

Experimental Design: CMV-specific T cells from CMV sero-positive healthy donors were selected after stimulation with pp65 protein and transduced with clinical-grade lentivirus expressing the CD19R-CD28ζ/4E8Fζ CAR. The resultant bispecific T cells, targeting CMV and CD19, were expanded via CD19 CAR-mediated signals using CD19-expressing cells.

Results: The bispecific T cells proliferated vigorously after engagement with either endogenous CMVpp65 T-cell receptors or engineered CD19 CARs, exhibiting specific cytolytic activity and IFNγ secretion. Upon adoptive transfer into immunodeficient mice bearing human lymphomas, the bispecific T cells exhibited proliferative response and enhanced antitumor activity following CMVpp65 peptide vaccine administration.

Conclusions: We have redirected CMV-specific T cells to recognize and lyse tumor cells via CD19CARs, while maintaining their ability to proliferate in response to CMV antigen stimulation. These results illustrate the clinical applications of CMV vaccine to augment the antitumor activity of adoptively transferred CD19CAR T cells in patients with B-cell malignancies. Clin Cancer Res; 21(13): 2993–3002. ©2015 AACR.

Introduction

Human studies of cancer and infectious diseases demonstrate that adoptive transfer of T cells of defined antigen specificity can establish or augment immunity to eradicate targeted malignant or infected cells. Adoptive transfer of in vitro expanded, chimeric antigen receptor (CAR)–redirected CD19-specific T cells can induce dramatic disease regression in patients with leukemia and lymphoma (1–4). However, the full potential of this emerging modality is hampered in some cancer settings by a significant rate of therapeutic failure arising from the attenuated engraftment and persistence of CAR-redirected T cells following adoptive transfer. In contrast, the adoptive transfer of native virus-specific T cells efficiently prevents progressive viral infections and exhibits longer-term persistence in patients (5–7).

The mechanisms for the differential persistence of adoptively transferred virus-specific T cells in hematopoietic cell transplantation (HCT) recipients versus tumor-reactive T cells in cancer patients is not fully understood, but possibly reflects both the environment into which the T cells are infused and qualitative attributes of the T cells that are isolated and expanded for adoptive transfer. In attempts to improve the efficacy of CAR T cells for tumor eradication, adoptive T cells with dual specificity have been created: isolated Epstein–Barr virus (EBV)–specific T cells modified to express GD2 or CD30 CARs recognizing tumors of neural crest origin (8–10), and isolated influenza A matrix protein 1 (MP1)–specific T cells modified to express CD19 CARs recognizing B-cell malignancies (11). These virus and CAR bispecific T cells demonstrate superior survival and antitumor activity compared with CAR T cells alone, possibly due to a more potent costimulation of virus-specific T cells after engagement of their native receptors. Recent studies demonstrate that adoptively transferred EBV × CMV × CD19CAR bi (tri)-specific T cells proliferate in patients as a result of CMV reactivation (12).

Cytomegalovirus (CMV) is a common virus for which 75% of adults in the United States test positive (13, 14) and it was the first virus targeted by adoptive transfer strategies. Pioneering immunotherapy trials (5–7) show that adoptive transfer of
virus-specific T cells is sufficient to reduce the incidence of CMV disease without toxicity (including GVHD). Phase I studies conducted at City of Hope demonstrate the safety and effectiveness of two different formulations of CMV vaccine for eliciting vaccine-driven expansion of pp65 specific T cells in healthy volunteers and transplant recipients (15).

On the basis of the clinical observation that enhanced antiviral efficacy can be achieved using a vaccine recognized by an endogenous T-cell receptor (TCR), we have transduced native CMV-specific T cells with a CD19CAR lentivirus to determine whether CD19CAR-directed CMV-specific T cells can respond to a CMV vaccine with rapid expansion and enhanced antiviral activity.

Materials and Methods

Antibodies and flow cytometry

Fluorochrome-conjugated isotype controls, anti-CD3, anti-CD4, anti-CD8, anti-CD28, anti-CD45, anti-CD27, anti-CD4, anti-CD127, anti-IFNγ, and streptavidin were obtained from BD Biosciences. Biotinylated cetuximab was generated from cetuximab purchased from the City of Hope pharmacy. The IFNγ Secretion Assay—Cell Enrichment and Detection Kit and CMVpp65 protein were purchased from Miltenyi Biotec. Phycoerythrin (PE)-conjugated CMV pp65 (NLVPMVATV)-HLA-A2*0201 ITAg MHC tetramer, PE-conjugated multi-allele negative tetramer was obtained from Beckman Coulter. Carboxyfluorescein diacetate succinimidyl ester (CFSE) was purchased from Invitrogen. All monoclonal antibodies, tetrads, and CFSE were used according to the manufacturer’s instructions. Flow cytometry data acquisition was performed on a MACSQuant (Miltenyi Biotec) or FACScalibur (BD Biosciences), and the percentage of stimulated CMV-specific T cells after CMVpp65 protein stimulation was calculated using FCS Express V3 (De Novo Software).

Cell lines

EBV-transformed lymphoblastoid cell lines (LCL) were made from peripheral blood mononuclear cells (PBMC) as previously described (16). To generate LCL, OKT3, allogeneic LCLs were resuspended in nucleofection solution using the Amaxa Nucleofector kit T, OKT3-2A-Hygromycin_pEK plasmid was added to 5 × 10^5 cells, the cells were electroporated using the Amaxa Nucleofector I, and the resulting cells were grown in RPMI-1640 with 10% FCS containing 0.4 mg/ml hygromycin. To generate firefly luciferase+ GFP+ LCLs (BfluGFP+LCLs), LCLs were transduced with lentiviral vector encoding eGFP-fluc. Initial transduction efficiency was 48.5%, so the GFP+ cells were sorted by FACS for >98% purity. To generate CD19 NIHI3T3 cells, parental NIHI3T3 cells (ATCC) were transduced with a retrovirus encoding CD80, CD54, and CD58 (17). The established cell line was further engineered to express CD19GFP by lentiviral transduction. GFP+ cells were purified by FACS sorting and expanded for the use of stimulation of bispecific T cells. To generate pp65 stimulator cells, U251T cells derived from human glioblastoma cells from an HLA A2 donor (ATCC) were transduced with a lentiviral vector encoding full-length pp65 fused to green fluorescent protein (GFP). pp65U251T cells were purified by GFP expression using flow cytometry. Banks of all cell lines were authenticated for the desired antigen/marker expression by flow cytometry prior to cryopreservation, and thawed cells were cultured for less than 6 months prior to use in assays.

Peptides

The pp65 peptide NLVPMVATV (HLA-A 0201 CMVpp65) at >90% purity was synthesized using automated solid phase peptide synthesis in the TVR (Beckman Research Institute of City of Hope). MP1 GIGFVFTL peptide (HLA-A 0201 influenza) was synthesized at the City of Hope DNA/RNA Peptide Synthesis Facility (Duarte, CA). pepMix HCMVA (pp65; pp65pepmix) was purchased from JPT peptide Technologies (GmbH). All peptides were used according to the manufacturer’s instructions.

Lentivirus vector construction

The lentivirus CAR construct was modified from the previously described CD19-specific scFvFc:ζ chimeric immunoreceptor (18), to create a third-generation vector. The CD19CAR containing a CD28ζ costimulatory domain carries mutations at two sites (L235E; N297Q) within the CH2 region on the IgG4-Fc spacers to ensure enhanced potency and persistence after adoptive transfer (Supplementary Fig. S1). The lentiviral vector also expressed a truncated human epidermal growth factor receptor (hEGFRt), which includes a cetuximab (Erbitux) binding domain but excludes the EGF-ligand binding and cytoplasmic signaling domains. A T2A ribosome skip sequence links the codon-optimized CD19RC:CD28ζ sequence to the huEGFRt sequence, resulting in coordinate expression of both CD19RC:CD28ζ and EGFRt from a single transcript (CD19RCRD28EGFRt: ref. 19). The CD19RCRD28EGFRt DNA sequence (optimized by GeneArt) was then cloned into a self-inactivating (SIN) lentiviral vector pHIV7 (gift from Jiing-Kuan Yee, Beckman Research Institute of City of Hope, Duarte, CA) in which the CMV promoter was replaced by the EF-1α promoter.

Enrichment of CMV-specific T cells after CMVpp65 protein stimulation

PBMCs were isolated by density gradient centrifugation over Ficoll-Paque (Pharmacia Biotech) from peripheral blood of consented healthy, HLA-A2 CMV-immune donors under a City of Hope Internal Review Board–approved protocol. PBMCs were frozen for later use. After overnight rest in RPMI medium containing 5% Human AB serum (Gemini Bio Products) without
cytokine, the PBMCs were stimulated with current good manufacturing practice (cGMP) grade CMVpp65 protein (Miltenyi Biotec) at 10 μL/10^5 cells for 16 hours in RPMI-1640 (Irvine Scientific) supplemented with 2 mmol/L L-glutamine (Irvine Scientific), 25 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; Irvine Scientific), 100 U/mL penicillin, 0.1 mg/mL streptomycin (Irvine Scientific) in the presence of 5 μL/mL IL2 and 10% human AB serum. CMV-specific T cells were selected using the IFNγ capture (Miltenyi Biotec) technique according to the manufacturer’s instructions.

**Derivation and expansion of bispecific T cells**

The selected CMV-specific T cells were transduced on day 2 after IFNγ capture with lentiviral vector expressing CD19CAR-D28EGFRt at MOI 3. Seven to 10 days after lentivirus-transduction, the bispecific T cells were expanded by stimulation through CAR-mediated activation signals using 8,000 cGy-irradiated CD19-expressing NIH 3T3 cells at a 10:1 ratio (T cells: CD19 NIH3T3) once a week as described previously (17) in the presence of IL2 50 μU/mL and IL15 1 ng/mL. After two rounds of expansion, the growth and functionality of the bispecific T cells was evaluated in vitro and in vivo.

**Intracellular cytokine staining**

Bispecific T cells (10^5) were activated overnight with 10^5 LCL OKT3, LCL, or KG1a cells in 96-well tissue culture plates, and with 10^5 U251T and engineered pp65-expressing U251T cells (pp63U251T) in 24-well tissue culture plates in the presence of Brefeldin A (BD Biosciences). The cell mixture was then stained using anti-CD8, cdxetuximab, and streptavidin to analyze surface coexpression of CD8 and CAR, respectively. Cells were then fixed and permeabilized using the BD Cytofix/Cytoperm kit (BD Biosciences). After fixation, the T cells were stained with an anti-IFNγ antibody.

**CFSE proliferation assays**

Bispecific T cells were labeled with 0.5 μmol/L CFSE and cocultured with stimulator cells LCL OKT3, LCLs, and pp65 U251T for 6 days. Cocultures with U251T and KG1a cells were used as negative controls. Proliferation of CD3- and CAR-positive populations was determined using multicolor flow cytometry.

**Cytokine production assays**

T cells (10^5) were cocultured overnight in 96-well tissue culture plates with 10^5 LCL OKT3, LCL, or KG1a cells and in 24-well tissue culture plates with 10^5 U251T and engineered pp65-expressing U251T cells. Supernatants were then analyzed by cytometric bead array using the Bio-Plex Human Cytokine 17-Plex Panel (Bio-Rad Laboratories) according to the manufacturer’s instructions.

**Cytotoxicity assays**

Four-hour chromium-release assays (CRA) were performed as previously described (20) using effector cells that had been harvested directly after two rounds of CD19 Ag stimulations.

**Xenograft models**

All mouse experiments were approved by the City of Hope Institutional Animal Care and Use Committee. Six- to 10-week-old NOD/ScidIl2Rγnull (NSG) mice were injected intravenously (i.v.) on day 0 with 2.5 × 10^6 fflucGFPLCLs cells. Three days after tumor inoculation, recipient mice were injected i.v. with 2 × 10^6 bispecific T cells that had undergone two rounds of CD19 stimulation. To generate antigen-presenting T cells (T-APC) for vaccine, REM-expanded T cells from the autologous donor were pulsed (2 hours at 37°C in CM) with 10 μg/mL of either HLA-A2–restricted pp65 peptide (NPVPMVATV), 1 μg/mL pp65 pep-mix depending on whether bispecific T-cell products are pp65 tetramer dominant (GIGFVFTL, donor 2) or not (pp65 pepmix donor 1) or 10 μg/mL HLA-A2–restricted control peptide specific for MIP1 (GIGFVFTL). Following one wash with phosphate-buffed saline (PBS), 5 × 10^6 T-APC that had been irradiated with 3,700 cGy were injected i.v into the T cell–treated mice. Tumor burden was monitored with Xenogen imaging twice a week. Human T-cell engraftment in peripheral blood, bone marrow, and spleen was determined by flow cytometry.

**Results**

**Enrichment of CMV-specific T cells from PBMC of healthy donors after stimulation with cGMP grade CMVpp65 protein**

To demonstrate the consistency of this clinically feasible process, the selection was repeated five times using PBMC from three different donors. IFNγ-positive T cells were consistently enriched from a baseline mean of 3.8% (range, 1.8–5.6) to a postcapsule mean of 71.8% (range, 61–81) and contained polyclonal CD8+ (34%) and CD4+ T cells (37%) after selection (Supplementary Table S1 and Fig. 1A). Moreover, the selected CMV-specific T cells included both CD4 and CD8 subsets and represented the entire spectrum of CMV-specificity, showing responsiveness to CMVpp65 pep-mix stimulation with broad recognition.

**Genetic modification of enriched CMV-specific T cells to express CD19 CAR and in vitro expansion of the bispecific T cells**

In the clinically adaptable procedure, IFNγ-captured CMV-specific T cells were transduced 2 days after the selection, without OKT3 activation, using the second-generation CD19RCD28EGFRt lentiviral construct containing the IgG4 Fc hinge region mutations (E235Q; E237Q). To demonstrate the consistency of this clinically feasible process, the selection was repeated five times using PBMC from three different donors. IFNγ-positive T cells were consistently enriched from a baseline mean of 3.8% (range, 1.8–5.6) to a postcapsule mean of 71.8% (range, 61–81) and contained polyclonal CD8+ (34%) and CD4+ T cells (37%) after selection (Supplementary Table S1 and Fig. 1A). Moreover, the selected CMV-specific T cells included both CD4 and CD8 subsets and represented the entire spectrum of CMV-specificity, showing responsiveness to CMVpp65 pep-mix stimulation with broad recognition.

**Bispecific T cells exhibited specified effector function after stimulation through predefined viral TCR and CD19CAR**

Recapitulating our previous studies (23), the ex vivo–expanded CMV-specific T cells possessed an effector phenotype and no longer expressed the central memory markers of the originally selected cells, such as CD62L, CD28, and IL7Ra (Fig. 1A and Supplementary Fig. S2). However, levels of CD27 remained high, suggesting a greater proliferative potential that has been associated with greater clinical efficacy (24). To investigate bispecific T-cell effector function via signaling by both the endogenous CMV-specific TCR and the introduced CD19CAR, we evaluated response to engineered pp65-expressing U251T cells from HLA-A2 donors, and also allogeneic CD19+ LCLs, based on
After selection, the cells were stained with antibodies specific to IFNγ, CD4, and CD8. The frequency of each population is presented after exclusion of dead cells labeled with CFSE and cocultured with different stimulators, and response to CD19 or pp65 antigen stimulation, T cells were enriched prior to lentiviral transduction. CAR expression in response to CD19 or pp65 antigen stimulation, 24% to 53% of the T cells in the population were CAR positive (LCL cells) or the CMV-specific TCR (pp65U251T cells, Fig. 4). Building on these findings, we next performed in vivo experiments to examine the effects of CMV peptide vaccine on the expansion and antilymphoma efficacy of adoptively transferred bispecific T cells.

Antilymphoma activity of adoptively transferred bispecific T cells was augmented in vivo by vaccination with CMVpp65 peptide antigen

Our preliminary studies have demonstrated that engineered CD19CAR T cells can target and lyse CD19-positive lymphoma in vivo. However, the antitumor efficacy is suboptimal and tumor reduction represents a transient event followed by eventual tumor progression (data not shown) unless high doses of CAR T cells were infused (21). In this study, we wanted to tease out the differences between the targeted and control vaccines. Therefore, we chose a suboptimal T-cell dose (2 × 10⁶ T cells), which is 5 times lower than the curative dose we used previously (10 × 10⁶; ref. 21). We attempted to augment antitumor efficacy using a CMV peptide vaccine boost (Fig. 5A). As expected, as few as 2 × 10⁶ bispecific T cells were able to induce a specific tumor reduction as compared with untreated and CMV-monospecific T cell–treated mice. We observed augmented antitumor activity after vaccination with pp65-peptide-pulsed T-APC of two
different formulations [pp65pepmix (Fig. 5B) and HLA A2-restricted CMVpp65 peptide (Fig. 5C)], but not in mice that were vaccinated using T-APC loaded with the irrelevant peptide MP1 (HLA A2–restricted). Interestingly, mice that received bispecific T-cell treatment had to be euthanized around the same time as control mice even though the tumor signals were dramatically lower (Fig. 5). Our further studies indicated that there were highly elevated levels of human-specific IFNγ and IL6 in the mouse serum (Supplementary Fig. S3) and it is probable that the mice died of cytokine release syndrome (CRS; 25) rather than tumor. More interestingly, augmented antitumor efficacy induced by pp65 vaccine was supported in a relapsed tumor model (Supplementary Fig. S3) and it is probable that the mice died of exposure to CD19 antigen in vivo. This was supported by the finding that there were lower levels of engraftment of CMV-specific T cells as compared with bispecific T cells in tumor-bearing mice, even though the same pp65 peptide vaccine was used to stimulate both types of T cells (Fig. 6A). These data suggested that bispecific T cells were able to proliferate and expand in vivo in response to stimulation of the TCR as well as the CD19 CAR.

Figure 2.
Bispecific T cells exhibit specific effector function after engagement with CD19+ and CMVpp65+ tumors. A, 7 days after the second CD19 Ag stimulation, T cells were stained with HLA A2–restricted pp65 tetramer, cetuximab–biotin, anti-CD8, and antibodies specific to central memory T-cell surface markers. Percentage of positive cells is indicated after dead cell exclusion with DAPI, gating based on pp65 tetramer and cetuximab double-positive, and isotype-matched stained samples. B, 4-hour 51Cr release assays were performed using the bispecific T cells and indicated 51Cr-labeled target cells at different effector:target (E:T) ratios. OKT3-expressing LCLs were used as positive controls, KG1A and U251T as negative controls. CD19+ LCL and engineered pp65U251T cells were used as targets for CD19 and CMV-specific T cells, respectively. Data from a representative donor are presented. C, bispecific T cells (10⁵) were activated overnight with 10⁵ LCL, OKT3, LCL, or KG1A in 96-well tissue culture plates and 10⁵ U251T and engineered pp65-expressing U251T cells (pp65U251T) in 24-well tissue culture plates. Supernatants were collected after overnight coincubation of bispecific T cells and stimulators. Cytokine levels with indicated stimulators (means ± SEM of triplicate wells) were determined using cytometric bead array.
Adoptively transferred bispecific T cells are efficiently ablated by cetuximab-mediated antibody-dependent cell-mediated cytotoxicity in vivo

The impressive clinical efficacy of CAR T-cell therapy and the frequently associated on/off-target toxicities, such as CRS, have highlighted the need for T-cell ablation strategies (1, 3, 4, 26). 

Taking advantage of the properties of the EGFRt receptor transgene, we demonstrated the necessity for T-cell ablation strategies (1, 3, 4, 26). W

Adoptively transferred CMV-specific T cells can control latent CMV infection during HCT, are efficiently expanded in response to vaccine, and are able to control viral reactivation in immunocompromised patients (5–7). CAR-engineered adoptive T-cell therapy is a developing area in cancer immunotherapy, with the potential for augmentation of antitumor efficacy by manipulating the processes prior to (proximal) and after (distal) T-cell infusion. One strategy to improve the persistence of in vitro–expanded effector cells is to isolate defined T-cell subtypes with an intrinsic capacity to persist during in vitro manipulation. Our studies have shown that central-memory–derived viral- or tumor-specific T cells exhibit superior persistence upon adoptive transfer compared with effector-memory–derived T cells (23, 27). We now seek approaches to magnify the potency of tumor-specific T cells after adoptive transfer.

Initially the major obstacle to the clinical application of CMV-specific T cells was the lengthy process required for their generation and selection (28, 29). In this study, our use of IFNγ capture of CMV-specific T cells consistently and efficiently enriched CMV-specific T cells while preserving the broad spectrum of CMV repertoires. Moreover, these cells remained amenable to gene modification after a brief CMVpp65 stimulation, avoiding the need for CD3/CD28 bead activation prior to transduction, which can cause activation-induced cell death (AICD) of CMV-specific T cells (30). Engineering the bulk IFNγ-captured T cells with the CD19CAR lentivirus followed by stimulation with CD19 antigen resulted in 50% to 70% of the CAR+ T cells responding to pp65 stimulation, representing the subset of functional bispecific T cells. The bispecific T cells exhibited specific cytolytic activity and secreted IFNγ, as well as proliferating vigorously after engagement of endogenous CMVpp65 T-cell receptors or engineered CD19 CARs. Upon transfer into tumor-bearing mice, the bispecific T cells mediated CRS, which has been found to correlate with antitumor efficacy in the clinic (2, 31). We have demonstrated the IFNγ-capture platform for derivation of bispecific T cells to be clinically feasible, and were able to generate therapeutic doses of...
functional bispecific T cells within 3 to 4 weeks, ensuring timely production for clinical application.

Efficient \textit{in vivo} activation of virus-specific T cells through the TCR demands that viral antigens are processed and presented in a human leukocyte antigen (HLA)–dependent manner. In mouse models, we generated APC by loading autologous T cells with either pp65 peptide or a full-length pp65pepmix. The effects of vaccination were indistinguishable whether using pp65 peptide or a full-length pp65pepmix. Both approaches elicited bispecific T-cell responses and induced enhanced antitumor activity compared with irrelevant MP1 challenge. We anticipate that the tetramer-negative population has disproportionately expanded \textit{in vivo} compared with tetramer-positive cells, because this subset includes cells expressing mouse xeno-reactive native T-cell receptors. It is also possible that another contribution to the decline in the proportion of pp65\(^+/\)CAR\(^+\) cells from the input population could be a result of these double-positive cells undergoing AICD after killing tumor cells, due to their effector T-cell characteristics (Fig. 2A). AICD could be thought of as a deleterious effect of the vaccine on pp65\(^+/\)CAR\(^+\), but could actually be a measure of effectiveness as demonstrated by decreased tumor burden (Fig. 6B). Ongoing studies on the functional responses to CMV vaccine of the different T-cell subsets of the infused product will further reveal the mechanisms of the enhanced antitumor activity.

Preclinical studies with engineered CAR T cells in different xenotransplant tumor models have demonstrated variable potency with some showing tumor eradication in the short window tested and some reporting eventual tumor relapse (17, 22, 32, 33). Several variables of these artificial systems, such as the aggressiveness of the tumor cell line, tumor burden at the time of CAR T-cell infusion, dose of CAR T cells may account for perceived differences in CAR potency, making it difficult to compare between xenograft models. Optimal growth signals are required for efficient and sustained expansion of transfused effector T cells \textit{in vivo}. These signals encompass T-helper cell interactions, native TCR/CD3 complex signaling, and the activation of costimulatory signals. Although the CAR is designed to mimic the TCR and transmit activation signaling, the lack of \textit{in vivo} persistence of some CAR T cells has been attributed to incomplete stimulation after engagement of the CAR (8, 10). This study suggests that the interaction of CAR T cells with tumor cells is inadequate to completely eradicate the transplanted tumor. This could be a result of insufficient growth signal transmission through the CAR for T-cell expansion and activation, or insufficient cytolytic activation of T cells to kill tumor targets. T-cell activation through viral TCRs has several advantages over self-antigen TCR in promoting robust T-cell expansion. Signaling through a viral TCR is generally far more robust than through a self-antigen–specific TCR, because the viral-specific TCR affinity to antigen has not been dampened by the effects of tolerance and negative selection (34). A recent study is emblematic of the contrast in T-cell activation caused by stimulation through a self-antigen such as p53 and the immune response to antigens expressed from a viral vector (35). Because the viral TCR is expressed from the same cell as the CAR, the robust T-cell activation caused by an antiviral TCR could lead to enhanced antitumor activity as a consequence of the expansion of CMV-specific CAR T cells.
Efficiently controlling proliferation to avoid cytokine storm and off-target toxicity is an important hurdle for the success of T-cell immunotherapy. The EGFRt incorporated in the CD19CAR lentiviral vector will serve not only as a marker for detection and selection of CAR T cells, but may also act as suicide gene to ablate the CAR+ T cells using cetuximab. These results illustrate the clinical applications of CMV vaccine to augment the antitumor activity of adoptively transferred CD19CAR T cells in several scenarios: (i) to salvage patients not achieving complete remission or relapsing after CAR T-cell therapy, (ii) vaccine boost when CD19 CAR T cells are failing to persist regardless of tumor responses at that time, (iii) planned vaccination on days 28 and 58 post-CD19 CAR T cells, which has been shown an effective immune-stimulation in our CMV peptide vaccine. There is also potential benefit of using the bispecific T cells preemptively postallogeneic HCT, both to eliminate minimal residual disease (MRD) and control CMV, potentially preventing reactivation of virus or undergoing expansion in response to latent CMV reactivation. City of Hope is currently accruing patients on clinical trials of two CMV vaccines (NCT01588015 and NCT01941056; ref. 15), enabling us to advance this combined therapy to the clinic rapidly, because we already hold INDs for both the CD19CAR T-cell vector and the CMV vaccines. Moreover, this CMV vaccine strategy has the potential to profoundly affect the general field of adoptive T-cell therapy,

Figure 5.
Antitumor activity of adoptively transferred bispecific T cells is enhanced by CMVpp65 vaccination. A, NSG mice were injected i.v. on day 0 with $2.5 \times 10^6$ GFPfluorescent LCL cells. Three days after tumor inoculation, recipient mice were injected i.v. with $2 \times 10^6$ bispecific cells that underwent two rounds of CD19 stimulation. Vaccine was given by i.v. injection of peptide pulsed autologous T cells. Fourteen to 17 days after T-cell infusion, $5 \times 10^6$ pp65peptide (A) or pp65 peptide (C; or MP1) loaded autologous T cells were irradiated and injected (i.v.) into T cell-engrafted mice as vaccine. pp65 vaccine was also supplemented to the mice that were treated with $2 \times 10^6$ CMV-specific T cells from the same donor, and untreated mice were used as another type of control. Tumor growth was evaluated by Xenogen imaging. N = 5 for each group in the experiments. The Mann–Whitney test was used for statistical analysis.
because by transducing a variety of tumor-directed CARs into our CMV-specific T cells, we have the potential to tailor this strategy to a wide range of malignancies and tumor targets.

Disclosure of Potential Conflicts of Interest
M.C. Jensen reports receiving commercial and other research grants from, has ownership interest (including patents) in, and is a consultant/advisory board member for Juno Therapeutics, Inc. No potential conflicts of interest were disclosed by the other authors.

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