Current management strategies are suboptimal and associated with significant adverse effects. Patients undergoing alloSCT are profoundly immunocompromised and do not respond to vaccination with CMV antigens. Immunotherapy (IT) with CMV specific donor-derived cytotoxic T lymphocytes (CTL) is effective after alloSCT, but is expensive, labor intensive, and difficult to replicate. Non-toxic strategies are needed to improve outcomes. In earlier studies, we showed that *ex vivo* expanded activated T cells (ATC) armed with anti-CD3 × anti-Her2/neu bispecific antibody (Her2Bi) exhibit high levels of specific cytotoxicity directed at breast cancer cell lines. Using this strategy, we tested ATC armed with anti-CD3 (OKT3) × anti-CMV (Cytogam®) bispecific antibodies (CMVBi) to target and lyse CMV-infected fibroblasts. Normal donor ATC were generated and armed with CMVBi. Specific cytotoxicity was tested by ^{51}Cr release using CMV-infected or uninfected human fibroblasts. CMVBi alone, CMVBi-armed ATC (aATC), and ATC alone were tested for their ability to kill. ATC were armed with 0.001 to 500 ng of CMVBi/10^6ATC at effector:target (E:T) from 3.125:1 to 25:1. IFN$_\gamma$ EliSpot was used to assess IFN$_\gamma$ secreting cells in ATC and aATC after exposure to CMV-infected and uninfected fibroblasts. ATC armed with CMVBi as little as 0.001 ng/10^6 ATC was significantly more cytotoxic than unarmed ATC. There was higher cytotoxicity mediated by aATC as the multiplicity of infection (MOI) increased in target cells. At all E:T, CMVBi arming at a dose of 50 ng/10^6 ATC exhibited dramatically higher lysis of CMV-infected targets (MOI 1) than that exhibited by ATC alone. Cytotoxicity mediated by unarmed and armed ATC was background in uninfected targets. Immunofluorescent studies showed that aATC aggregated around GFP fluorescence-marked CMV-infected fibroblasts. Cytokine secretion analyzed by IFN$_\gamma$ EliSpot confirmed immune responses. The lack of MHC restriction in the mediation of cytotoxicity, polyclonal targeting of multiple CMV antigens and the simplicity of expanding donor T cells makes it easy to adapt this effective strategy against CMV infection post alloSCT.

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NAÏVE T CELLS FROM CORD BLOOD AND CMV-SERONEGATIVE DONORS RECOGNIZE ATYPICAL EPITOPES OF CMVpp65 AND CAN BE USED FOR ADOPTIVE IMMUNOTHERAPY

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Adoptive transfer of virus-specific T cells can effectively restore antiviral immunity after stem cell transplantation. However, protocols for eliciting virus-specific T cells from existing memory T cells of CMV-seropositive adult (CMVpos) donors have been ineffective when applied to naïve cord blood (CB) transplant (CBT) recipients due to absence of antigen-specific memory cells in these grafts. We previously demonstrated that CMVpp65-specific T cells could be generated from CB using a modified protocol and that 15/15 CB T cell lines recognized atypical epitopes of pp65. To test if naïve T cells from CMV-seronegative donors (CMVnegs) also recognize atypical epitopes, we again optimized our previous method. CD45RA+ naïve T cells were selected from peripheral blood and stimulated with pp65-pepmix-pulsed DC with IL7, IL12, and IL15. For subsequent stimulations T cells were stimulated with pp65-pepmix-pulsed EBV-LCL and IL15 or IL2. CMVpp65-specific T cells (CMV-CTL) expanded from 8 of 11 CMVnegs and were primarily CD8+ T cells (mean 71%). These T cells secreted markedly more IFN-γ in response to pp65 peptides (mean 224; range: 38-611 SFC/1x10^5 cells) than to irrelevant peptides (mean 12;Range 3-37) as measured in Elispot assays. These T cells lysed pp65-pulsed target cells (mean:48;Range:15-70%) but not negative controls (mean 22;Range 4-40%) in cytotoxicity assays. As with CB, CMVneg CMV-CTL were derived from naïve T cells and recognized only atypical pp65 epitopes. These findings raised the concern that the viral epitopes presented by endothelial cells infected with CMV in patients might not present the atypical peptides recognized by our CMV-CTL. We hypothesized that the epitopes presented to T cells by endothelial cells or other APCs after CMV infection are different to those presented in our in vitro system. We tested if different epitopes would dominate the naïve T cell response to CMV AD169-infected fibroblasts and CMV VR1814-infected DC. The responding naïve T cells were again specific for atypical epitopes showing that these epitopes were indeed presented by cells naturally infected with CMV. This reassured us that despite their unusual repertoire, T cells derived from CB or CMVnegs should control CMV infections. We are now evaluating their clinical efficacy in recipients of CBT and soon Hematopoietic Stem Cell Transplantation. These studies should determine if naïve T cells primed in vitro are able to persist and establish memory in vivo.

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EFFECT OF A CO-STIMULATORY ENDODOMAIN ON THE PERFORMANCE OF T CELLS EXPRESSING A CHIMERIC ANTIGEN RECEPTOR DIRECTED AT CD19 IN PATIENTS WITH RELAPSED/REFRACTORY B-CELL MALIGNANCIES

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First generation chimeric antigen receptors (CAR) usually combine the antigen binding domain of a monoclonal antibody with the ζ signaling domain of the T cell receptor/CD3 complex. When expressed in T cells, those CAR provide potent antigen-specific, non MHC-restricted effector function against tumor cells in vitro, but clinical trials have shown limited expansion and persistence of these lymphocytes in vivo. This limitation is likely attributable to the failure of these CAR to fully activate T cells following target antigen engagement on tumor cells, since tumors lack expression of costimulatory molecules required for sustained T cell activation. Incorporation of costimulatory endodomains within the CAR, such as CD28, increases proliferation and activity of the modified T cells in vitro and may enhance their benefit in vivo but, as yet, the relevance and value of these modifications in human subjects remains speculative.

We now present results from a phase I trial of T cells redirected to CD19 given to patients with refractory/relapsed B-cell malignancies. Patients were simultaneously infused with 2 autologous T cell products expressing CAR with identical CD19-specific exodomains. In one product, the endodomain contained only the ζ sequence (CAR.19ζ) while, in the second product, we added a costimulatory CD28 domain (CAR.19-28ζ). We could thus directly measure and compare the expansion, persistence and effectiveness of each cell population in every patient. Six subjects have been treated at 3 cell dose levels (Table). Persistence of CAR+ T cells was assessed in blood by Q-PCR assays specific for each population.

Table 1. Characteristics of the infused CD19-redirected CAR-bearing T cell products (N=6)

	CD4+	CD8+	Naïve (CD45RA+)	Memory (CD45RO+ CD62L+)	Specific lysis (20:1 E:T ratio)
CAR.19ζ T cells	44 ± 23	49 ± 22	6 ± 5	50 ± 24	53 ± 10
CAR.18-28ζ T cells	47 ± 26	48 ± 22	6 ± 6	47 ± 66	65 ± 19

All numbers are shown as mean percentage ± standard deviation. T cell products were generated by activation of autologous peripheral blood mononuclear cells with immobilized OKT3, gene modification with retroviral vectors encoding either CAR.19ζ or CAR.19-28ζ and ex vivo expansion for a median of 14 days (range 6-18) in the presence of IL-2. Three dose levels have been administered: 2×10^7, 1×10^8 and 2×10^8 cells of each product/m^2.

All infusions were well tolerated. CAR.19-28ζ cells were detected at a low level after infusion, but progressively increased 7- to 63-fold, peaking at 1-2 wk, before declining to background levels over the