NOVEL T-CELL IMMUNOTHERAPEUTICS ENABLE THE SELECTIVE GENERATION OF MORE POTENTLY CYTOTOXIC CD19 CHIMERIC ANTIGEN RECEPTOR T-CELLS (CAR-T CELLS) FROM CMV-SPECIFIC CYTOTOXIC T-CELLS

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Background: CAR-T therapy induces dramatic remissions of relapsed/refractory B-cell malignancies, but remission durability is frequently limited by reduced CAR-T persistence or poor function. We hypothesize CAR-T function and longevity would be increased by selectively deriving CAR-Ts from effector-memory virus-specific CD8+ cytotoxic T-lymphocytes (CTLs). To enable antigen-specific activation of T cells, we developed biologics termed synTacs (synapse for T-cell activation), which deliver TCR and costimulatory signals to selectively activate and expand CMV-specific CTLs. CMV-aCD28-synTacs consist of dimeric Fc domain scaffolds linking HLA-A2 molecules presenting a CMV-derived peptide (NLV/pp65) and an aCD28 binder (figure 1A) that induce selective in vitro and in vivo expansion of CMV-specific CTLs with potent antiviral activity (figure 1B, C). We used CMV-aCD28-synTacs to generate CAR-T cells from CMV-specific CTLs and compared their functionality to standard CAR-T cells generated by aCD3/aCD28 activation. We also evaluated an alternative strategy to generate CAR-T cells from CMV-specific T cells in vivo using synTac-based single-chain CMV-MHC-targeted lentiviral vectors.

Methods: We treated purified CD8+ T-cells from a CMV-responsive donor with CMV-aCD28-synTac or aCD3/aCD28 and then transduced them with lentivirus expressing an anti-CD19-CAR and a GFP reporter (figure 2A). CMV-specific CTLs and CAR-T cells were quantified by flow cytometry with tetramer staining or GFP expression, respectively.

Results: After 7 days, CTL treatment with CMV-aCD28-synTac rapidly induced robust activation and >50-fold selective expansion of CMV-specific CTLs (figure 1B). CMV-aCD28-synTac treatment activated CMV-specific T-cells, enabling their selective transduction with an anti-CD19-CAR lentivirus (figure 2C). These CMV-specific anti-CD19-CAR-T cells displayed more potent cytotoxic activity targeting donor B cells than those generated by traditional aCD3/aCD28 activation (figure 3B). We saw similar expansion and selective transduction after 2 weeks using the pp65-targeting lentivirus to generate pp65-responsive CD19 CAR-T cells (figures 2B and 3C, D).

Conclusions: CART cells derived from CMV-specific CTLs after CMV-aCD28-synTac activation exhibit enhanced cytotoxic activity against B cells as compared to standard CAR-Ts. Their co-expression of the CMV-specific T-cell receptor should also enable potent stimulation and activation by in vivo stimulation by endogenous CMV antigen or treatment with CMV-synTacs coupled to aCD28 or other costimulatory ligands or cytokines, enabling more potent and durable treatment of relapsed/refractory B-cell malignancies.

REFERENCE

Ethics Approval: This study was approved by the Albert Einstein College of Medicine’s Ethics Board; approval number 2017–8116.

Abstract 295 Figure 1 SynTac construct design and pp65 (NLV)-synTac treatment-stimulated in vitro expansion of functional pp65-specific CD8+ T cells from HLA-A*0201 CMV-seropositive donors. (A) synTacs consist of split sc-pMHC-Fc fusion proteins, with the F2M and the MHC HLA-A*0201 alpha chain linked through engineered interchain disulfide bonds and the costimulatory domains linked to the carboxy end of the F2M. synTacs recapitulate the signaling that takes place at the immunological synapse by presenting MHC-peptide to the TCR and providing a secondary costimulatory signal. (B) Representative flow plot demonstrating specific expansion of pp65 (NLV)-αCD28-specific CD8+ T cells by CMV-pp65 (NLV)-αCD28 synTac stimulation of purified CD8+ T cells from a CMV-responsive HLA-A*0201 donor (HGLK0055), as compared to lack of expansion in purified CD8+ T cells stimulated with an irrelevant HIV-SL9-αCD28 synTac. (C) Results from four independent experiments as described in (B) showing %pp65 (NLV)-specific purified CD8+ T cells from a CMV-responsive donor (HGLK0055) 7 days after treatment with either pp65 (NLV)-αCD28-synTac or irrelevant HIV (SL9)-αCD28-synTac with mean ± s.d. shown and statistical significance assessed by ordinary one-way ANOVA followed by Tukey’s multiple comparisons test.
Abstract 295 Figure 2  Lentiviral vector constructs and in vitro specific transduction of CMV-specific CD8+ T cells with standard anti-CD19 CAR lentiviral vector or specific transduction of Jurkat cells with NLV-scMHC GFP lentiviral particles (LVPs). (A) Structural representation of anti-CD19 CAR lentiviral vector. The CD19 scFv was cloned into a lentiviral vector driven by a spleen forming focus virus (SFFV) promoter, and also expresses a dGFP for identification via flow cytometry. (B) Lentiviral vector map containing the NLV-scMHC targeting envelope (pEnv-NLV-scMHC), the mutagenized fusogenic glycoprotein (pENV-VSVGmut), a packaging plasmid (psPAX) and the transfer plasmid expressing the anti-CD19 or GFP and driven by an MND promoter, and structural representations of the mutagenized fusogenic glycoprotein and the NLV-scMHC LVP. (C) CD8+ T cells were isolated from CMV-exposed donor (HGLK0055) PBMCs, treated with pp65 (NLV)-aCD28 synTac (0.01nM), and cultured in complete IMDM with IL-2 (100 U/mL) for 7 days, or left untreated and then activated for 48 hours after 5 days in culture with CD3 (100ng/mL) and aCD28 (2ug/mL). 7 days after synTac treatment, CD8+ T cells were transduced with CD19 CAR lentivirus and analyzed 3 days later by flow cytometry, displaying selective transduction of pp65 (NLV)-tetramer+ cells in the condition treated with pp65 (NLV)-aCD28 synTac. (D) Targeted delivery of NLV-scMHC GFP LVPs in a CMV-specific Jurkat cell line in vitro. Jurkat cells expressing the CMV (NLV)-specific TCR (RA14 TCR) were transduced with 3.3e5 TU of NLV-scMHC LVP expressing GFP or VSVGmut LVP, which lacks a targeting envelope. 3 days post transduction, flow cytometric analysis showed selective transduction of cells expressing the RA14 TCR (pp65 tetramer+), as well as no significant transduction when cells were transduced with VSVGmut GFP LVP.
Abstract 295 Figure 3  Functional activity of CMV-specific anti-CD19 CAR-T cells and in vitro delivery of NLV-scMHC LVPs both expands and induces transduction of primary donor-derived CMV-specific CD8+ T-cells. (A) Representative histograms of CMV-specific anti-CD19 CAR-T cell functional activity. CD8+ T cells were isolated from CMV-exposed donor (HGLK0055) PBMCs, treated with pp65 (NLV)-αCD28 synTac (0.01nM), and cultured in complete IMDM with IL-2 (100 U/mL) for 7 days, or left untreated and then activated for 48 hours after 5 days in culture with αCD3 (100ng/mL) and αCD28 (2ug/mL). 7 days after synTac treatment, CD8+ T cells activated with either pp65 (NLV)-αCD28 synTac or αCD3/αCD28 were transduced with CD19 CAR lentivirus with pp65 (NLV) tetramer expression transduction efficiency analyzed 3 days later by flow cytometry as in figure 2C. CMV-specific-anti-CD19 CAR T cells and traditional αCD3/αCD28-activated CAR T-cells were co-cultured on a 96-well plate in triplicate with PKH-stained purified primary CD19+ cells at the effector to target ratios shown. After 24 hours, CD19 cell death was analyzed via flow cytometry using 7-AAD live/dead dye. CMV-specific anti-CD19 CAR-T cells were more potently cytotoxic against primary B-cells than traditional αCD3/αCD28 CAR T-cells. (B) Results from four independent experiments as described in (A) showing %PKH+ CD19 cells dead 24 hours after co-culture with either CMV-specific-anti-CD19 CAR T cells or traditional αCD3/αCD28 CAR T-cells at the effector to target ratios shown. Mean ± s.d. shown and statistical significance were assessed by ordinary one-way ANOVA followed by Tukey’s multiple comparisons test. (C) Expansion of pp65 (NLV)+ CD8+ T-cells 14 days after transduction with 1–6e7 NLV-scMHC LVPs. Statistical significance assessed through one-way ANOVA of 4 samples. (D) PBMCs were cultured in the presence of IL-2 (50U/mL) and transduced with NLV-scMHC LVPs expressing GFP or left untransduced. GFP expression in CMV-specific CD8+ T-cells was assessed 14 days post transduction. Representative flow cytometric plots show GFP expression in donor-derived (HGLK0055) CMV-specific T-cells gated based on NLV tetramer binding.

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