Immunoagonist Converts CMV-Specific Cells Into More Potent HIV-Specific CAR-T Cells

277

Hang Su[1], Christopher Hiner[1], Moriah Rabin[1], Danica Lee[1], Stephanie Sabido[1], Scott J. Garforth[1], Jianhua Zheng[1], Kim Anthony-Gonda[2], Boro Dropulić[2], Steven C. Almo[1], Harris Goldstein[1]
[1]Albert Einstein College of Medicine, Bronx, NY, [2]Caring Cross, Gaithersburg, MD, United States

BACKGROUND

We developed novel biologics termed **synTacs** (**syn**apse for **T** cell **ac**tivation) to selectively activate and expand virus-specific CD8+ T cells *in vitro* and *in vivo* ***(JCI 2021)***. SynTacs consist of dimeric Fc-domain scaffolds linking CD28-specific agonists to HLA-A2 MHC molecules covalently-tethered to a virus-derived peptide enabling delivery of TCR-specific and costimulatory signals to antigen-specific T cells **(Fig. 1A). We hypothesized that we could produce CAR-T cells with more potent and sustained activity than those produced by standard αCD3/αCD28 antibody activation, through selective synTac-induced activation and expansion of virus-specific effector-memory CD8+ T cells that enabled selective transduction by the CAR lentivector.** As a proof-of-concept, we used a duoCAR construct **(Fig. 1B)** we developed targeting two distinct HIV gp120 epitopes (HIV-CAR) that potently eliminate HIV-infected cells ***(STM 2019),*** to determine if selectively engineering HIV-CAR into CMV-specific memory CD8+ T cells that recognize a CMV pp65 epitope (NLVPMVATV) generated more potent HIV-CAR-T cells than those generated by standard αCD3/αCD28 polyclonal T cell activation.

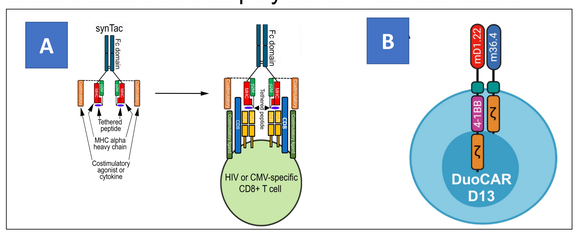


Figure 1. Illustration of synTac and duoCAR-T constructs. (A) SynTacs consist of dimeric Fc-domain scaffolds linking CD28- or 4-1BB–specific ligands to HLA-A2 MHC molecules covalently tethered to HIV- or CMV-derived peptides, recapitulating antigen presenting cells to activate antigen-specific T cells. **(B)** DuoCAR was designed to target two distinct HIV envelope-binding domains (mD1.22 and m36.4), with 4-1BB costimulatory domain and CD3 ζ T cell signaling motif.

METHODS

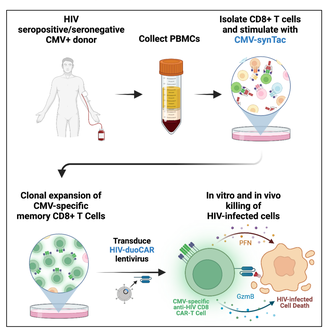


Figure 2. Experimental strategy. CD8+ T cells purified from an HIV(-/+) and CMV(+) donor were activated either by standard αCD3/α28 antibody or CMV-synTac, and then transduced with the HIV-duoCAR lentivector. HIV-CAR-T cells were either cocultured or co-injected into NSG mice with HIV-infected autologous PBMCs to measure *in vitro* and *in vivo* viral suppression.

CMV-synTac selectively expands and converts CMV-specific CD8+ T into more potent HIV-CAR-T cells than standard αCD3/αCD28-generated HIV-CAR-T cells.

RESULTS

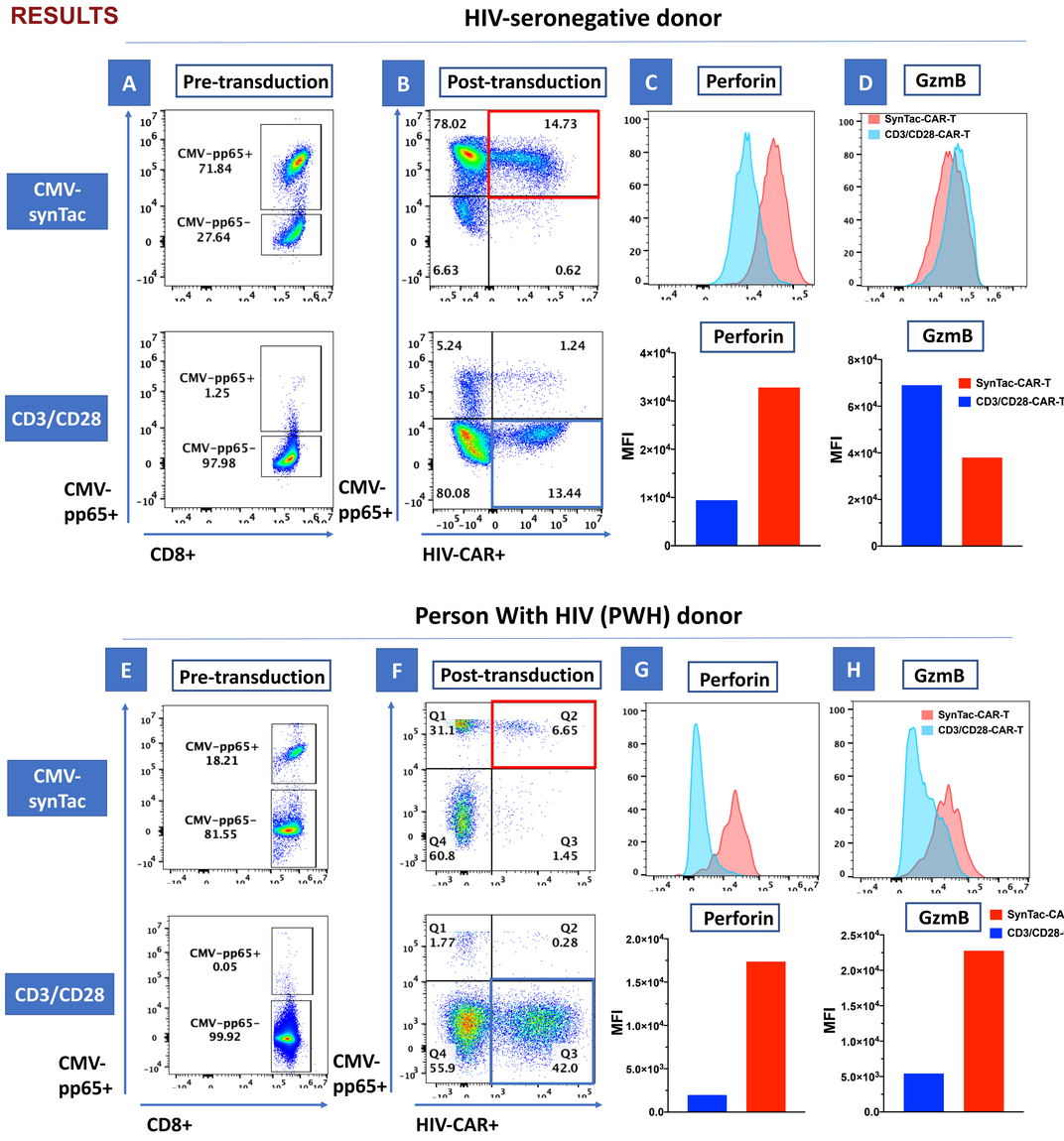


Figure 3. Intracellular perforin and granzyme B expression by CMV-synTac- or αCD3/αCD28-generated HIV-CAR-T cells. Purified CD8+ T cells from either an HIV-seronegative **(upper panel)** or a PWH **(lower panel)** CMV-positive donor were stimulated with CMV-synTac or αCD3/αCD28. **(A and E)** CMV-specific CD8+ T cells, **(B and F)** HIV-CAR-expressed CD8+ T cells, and **(C and G)** intracellular perforin and **(D and H)** granzyme B expression were measured by flow cytometry.

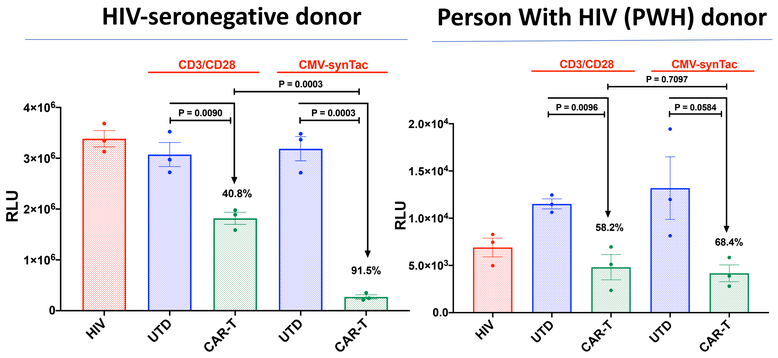


Figure 4. CMV-synTac-generated HIV-CAR-T cells showed more potent in vitro suppression of HIV infection in autologous PBMCs than standard αCD3/αCD28-generated HIV-CAR-T cells. PBMCs from either an HIV-seronegative **(left)** or PWH **(right)** donor were infected with HIV-1 that contains a Renilla luciferase reporter gene. Autologous infected PBMCs were cultured alone (red), cocultured with synTac- or αCD3/αCD28-generated HIV-CAR-T cells (green), or respective untransduced (UTD) cells (blue) at an E:T ratio of 1:1. After 48 hours, cells were collected and measured for luciferase activity. The viral suppression efficiency by HIV-CAR-T cells compared to respective UTD cells was listed. All the conditions were set up in triplets.

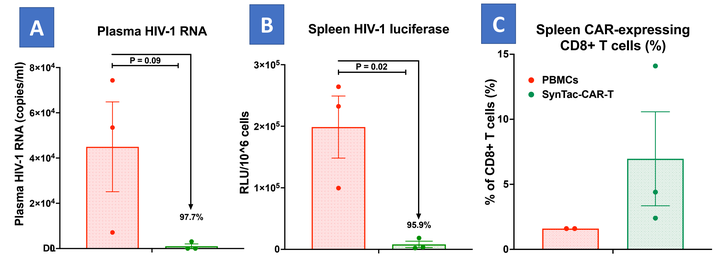


Figure 5. CMV-synTac-generated HIV-CAR-T cells suppress in vivo HIV infection in humanized mice. PBMCs from an HIV-seronegative donor were infected with HIV-1 luciferase virus, and then either injected alone (n=3, red), or co-injected with autologous CMV-synTac-generated HIV-CAR-T cells at an E:T ratio of 1:1 (n=3, green), into humanized mice intrasplenically. After 12 days, animals were sacrificed and **(A)** plasma HIV-1 RNA, **(B)** spleen HIV-1 luciferase signal, and **(C)** spleen CAR-expressing CD8+ T cells were determined. The viral suppression efficiency in HIV-CAR-T cells-treated compared to untreated animals were listed.

CONCLUSIONS

- CMV-synTac selectively activates and expands CMV-specific CD8+ T cells, enabling their selective transduction with an HIV-CAR lentivector and conversion into HIV-CAR-T cells.
- CMV-synTac-generated HIV-CAR-T cells exhibited more potent suppressive HIV activity than standard αCD3/αCD28-generated HIV-CAR-T cells.
- Our preliminary data support further development of a new strategy to selectively generate more potent HIV-CAR-T cells.

REFERENCES

- Anthony-Gonda K, et al. Multispecific anti-HIV duoCAR-T cells display broad in vitro antiviral activity and potent in vivo elimination of HIV-infected cells in a humanized mouse model. *Sci Transl Med*. 2019;11(504):eaav5685.
- Li M, et al. T cell receptor–targeted immunotherapeutics drive selective in vivo HIV- and CMV-specific T cell expansion in humanized mice. *J Clin Invest*. 2021;131(23):11051.