Generating Broadly Neutralizing Antibody-secreting CAR-T Cells Against HIV Infection

404

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BACKGROUND

Treatment with HIV-specific CAR-T cells and broadly neutralizing antibodies (bNAbs) are both important approaches to potentially provide the sustained anti-HIV activity required to achieve ART-free HIV remission. **We hypothesized that the anti-HIV activity of CAR-T cells could be further increased by enabling them to secrete bNAbs.** As a proof-of-concept, we engineered a lentiviral vector (LV) encoding the 3BNC117 bNAb which was co-transduced with a LV encoding an anti-HIV duoCAR construct targeting two distinct HIV gp120 epitopes with potent anti-HIV activity **(STM 2019; JCI Insight 2022)** to generate 3BNC-secreting duoCAR-T cells.

METHODS

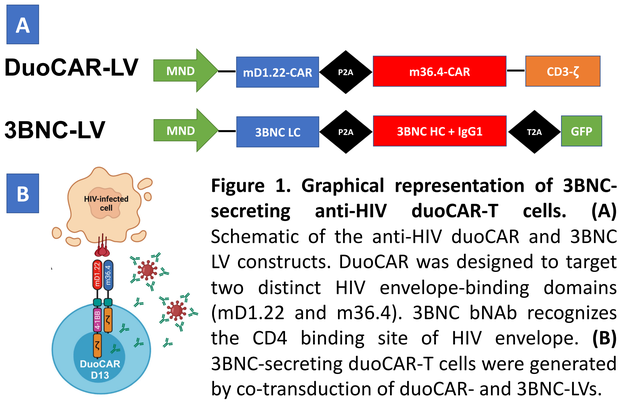

Figure 1. Graphical representation of 3BNC-secreting anti-HIV duoCAR-T cells. (A) Schematic of the anti-HIV duoCAR and 3BNC LV constructs. DuoCAR was designed to target two distinct HIV envelope-binding domains (mD1.22 and m36.4). 3BNC bNAb recognizes the CD4 binding site of HIV envelope. **(B)** 3BNC-secreting duoCAR-T cells were generated by co-transduction of duoCAR- and 3BNC-LVs.

RESULTS

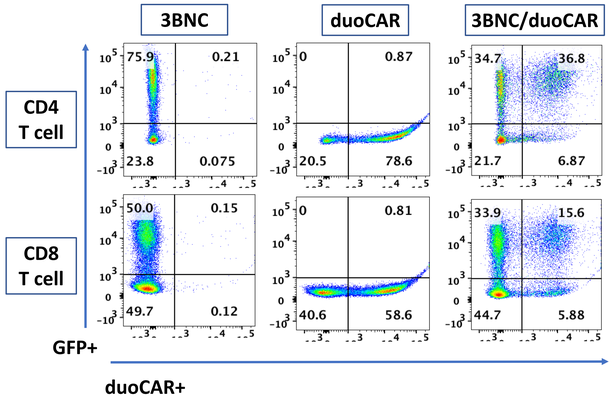

Figure 2. Generation of 3BNC-expressed duoCAR-T cells. CD3+ T cells purified from an HIV(-) donor were transduced with either 3BNC-LV alone, duoCAR-LV alone, or both. Transduction efficiency was measured by flow cytometry.

3BNC-secreting anti-HIV duoCAR-T cells display more potent antiviral activity as compared to single 3BNC or duoCAR LV-transduced cells.

RESULTS

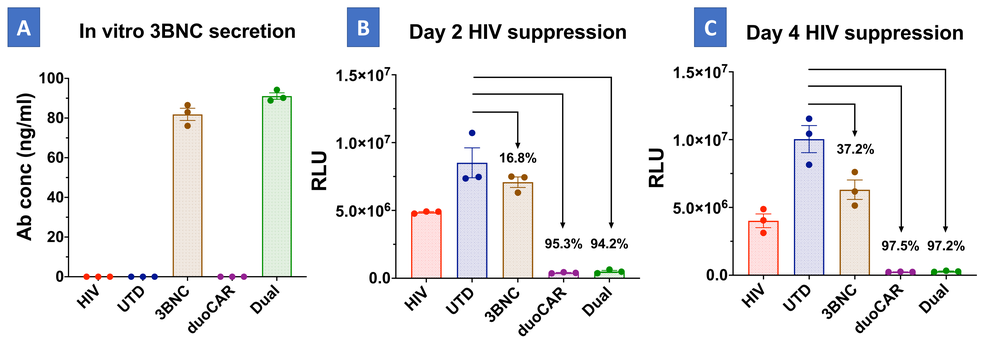

Figure 3. 3BNC/duoCAR-LV-transduced T cells secreted functional 3BNC and showed potent in vitro suppression of HIV infection in autologous PBMCs. CD3+ T cells purified from an HIV(-) donor were transduced with either 3BNC-LV alone, duoCAR-LV alone, or both, and co-cultured with autologous PBMCs infected with HIV-1 that contains a Renilla luciferase reporter gene (HIV_{Luc}), at an E:T ratio of 1:1. Untransduced T cells (UTD) were used as control. **(A)** Cell culture media was collected after 2 days to measure 3BNC concentration by ELISA. **(B)** 2 days and **(C)** 4 days after co-culture, cell lysate luciferase activity was measured, as an indicator of HIV infection. Percentage suppression of HIV infection by indicated effector cells are listed. Experiments were done in triplicate.

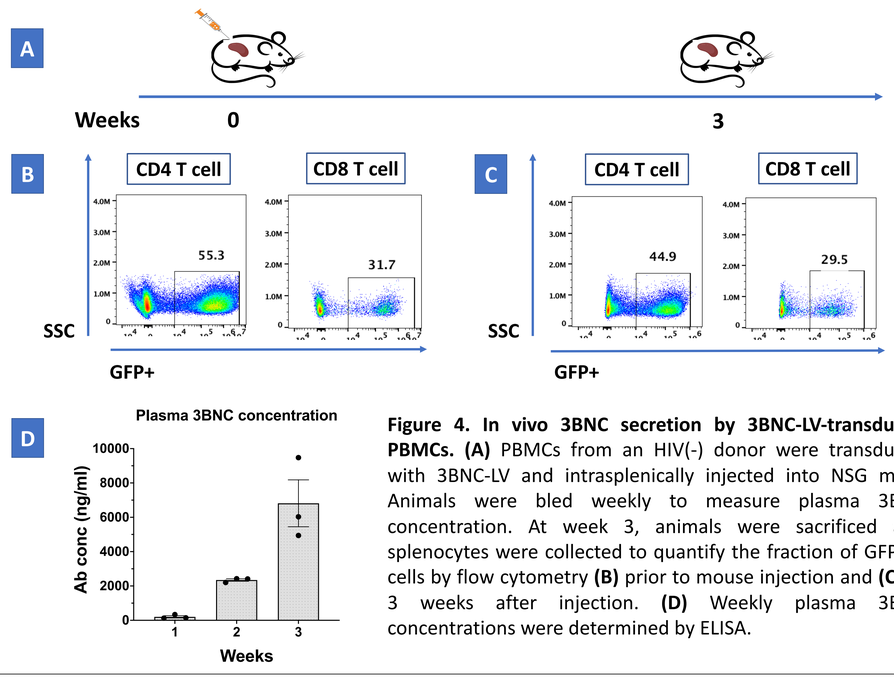

Figure 4. In vivo 3BNC secretion by 3BNC-LV-transduced PBMCs. (A) PBMCs from an HIV(-) donor were transduced with 3BNC-LV and intrasplenically injected into NSG mice. Animals were bled weekly to measure plasma 3BNC concentration. At week 3, animals were sacrificed and splenocytes were collected to quantify the fraction of GFP+ T cells by flow cytometry **(B)** prior to mouse injection and **(C)** at 3 weeks after injection. **(D)** Weekly plasma 3BNC concentrations were determined by ELISA.

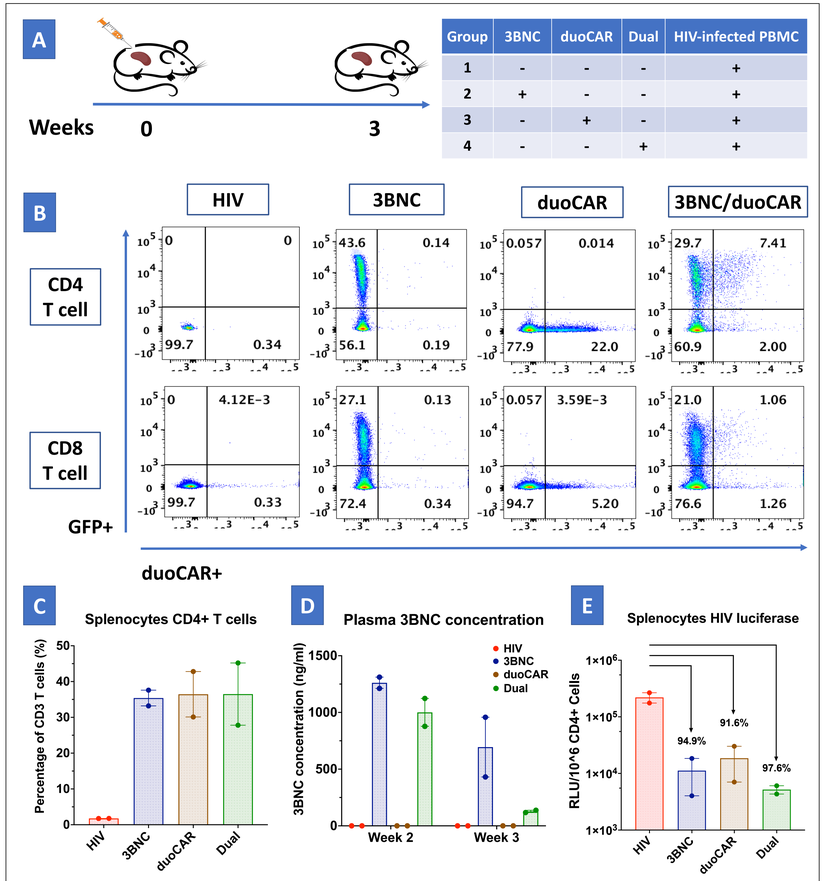

Figure 5. 3BNC-secreting duoCAR-T cells showed superior in vivo suppression of HIV infection as compared to single-LV-transduced T cells. (A) CD3+ T cells purified from an HIV(-) donor were transduced with either 3BNC-LV alone, duoCAR-LV alone, or both, and intrasplenically co-injected with autologous HIV_{Luc}-infected PBMCs into NSG mice, at an E:T ratio of 2:1. Animals were sacrificed at week 3 post-injection for subsequent analysis. **(B)** GFP+ and duoCAR+ and **(C)** CD4+ splenocytes were quantified by flow cytometry. **(D)** Plasma 3BNC concentrations at week 2 and 3 post-injection were measured by ELISA. **(E)** Spleen HIV infection was measured by quantitating luciferase activity and percentage suppression of HIV infection by indicated effector cells are listed for each group.

CONCLUSIONS

- **We successfully engineered anti-HIV CAR-T cells that secret the broadly neutralizing antibody 3BNC against HIV in vitro and in vivo.**
- **3BNC-secreting anti-HIV CAR-T cells display more potent in vivo antiviral activity as compared to single 3BNC or duoCAR LV-transduced cells.**
- **This "2 in 1" approach provides a new strategy to generate more potent immunotherapeutics to contribute to a functional cure of HIV infection.**