T_{SCM}/T_{CM}-Enriched Anti-HIV DUOCAR-T Cells Exert Potent HIV Control

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BACKGROUND

Anti-HIV chimeric antigen receptor (CAR) T cell therapies are candidates to functionally cure HIV infection in people with HIV (PWH). Translating such therapeutic candidates successfully into PWH will require anti-HIV CAR-T cells to persist long term and eliminate reactivated HIV-infected cells. Interestingly, recent clinical studies have shown a positive correlation between the early-memory phenotype of pre-infusion anti-CD19 CAR-T cell therapies and their long-term *in vivo* persistence and therapeutic efficacy. Here, we hypothesized that early-memory enriched anti-HIV duoCAR-T cells generated using a GMP-compliant CAR-T cell manufacturing process would exert potent HIV control in humanized mice with productive HIV-1 infection.

METHODS

To test this hypothesis, we developed an 8-day CAR-T cell manufacturing process and profiled the T cell differentiation state of pre-infusion anti-HIV duoCAR-T cell products using multiparametric flow cytometry and CyTOF analyses. The therapeutic efficacy of early-memory enriched anti-HIV duoCAR-T cells was evaluated in a humanized NSG mouse model of intrasplenic HIV-1 infection (hu-spl-PBMC-NSG).

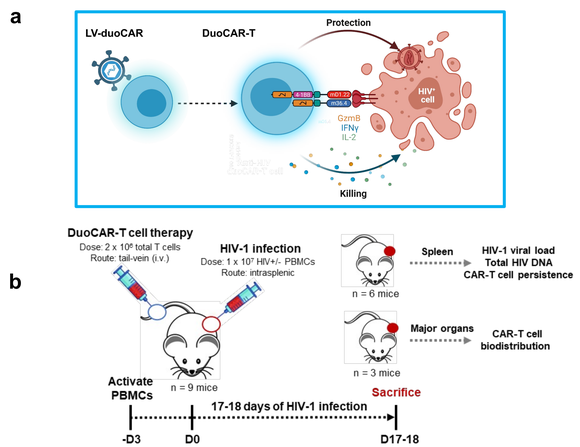

Fig 1. Experimental design to test anti-HIV DuoCAR-T cell activity. a) Illustration of anti-HIV duoCAR-T cell mediated killing of HIV-infected cells (created with BioRender.com). Primary T cells are converted to duoCAR-T cells via genetic modification using a lentiviral vector encoding the anti-HIV duoCAR (LV-duoCAR). **b** A single intravenous injection of duoCAR-T cells were administered via the tail-vein to PBMC-humanized NSG mice with intrasplenic HIV infection (hu-spl-PBMC-NSG).

RESULTS

Functional and Phenotypic Characterization of Clinic-Ready CAR-T Products

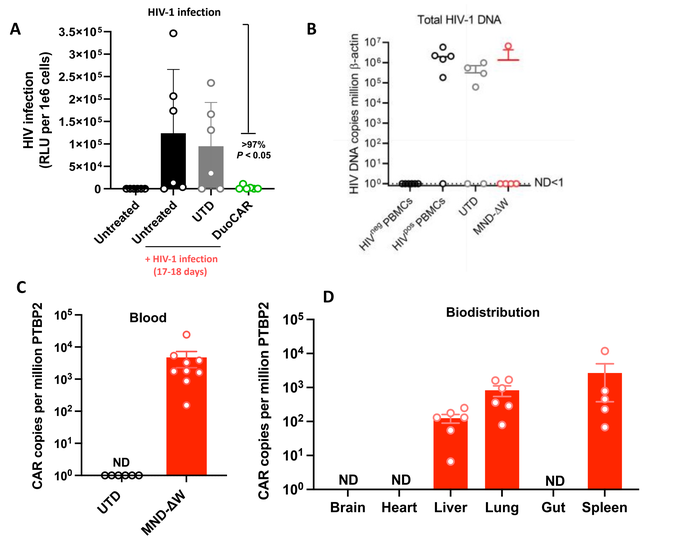

Figure 1. In vivo localization and anti-HIV efficacy of i.v.-administered anti-HIV duoCAR T cell therapy in humanized HIV-infected mice. A) HIV viral load as measured by luciferase signal (RLU) in spleens of humanized mice (huNSG) 17-18 days post infection. **B**) Quantification of cell-associated HIV-1 DNA in the spleens of huNSG, expressed as HIV-1 Gag DNA copies per million-actin copis, ND not detected. **C and D)** Persistence and biodistribution of i.v. anti-HIV duoCAR T cells in the blood and major organs of huNSG 17-18days post-infection. Data are expressed as CAR DNA copies per million PTBP2 copies. Data shown are mean +/-SD of samples tested (5-9 mice). *P<0.05

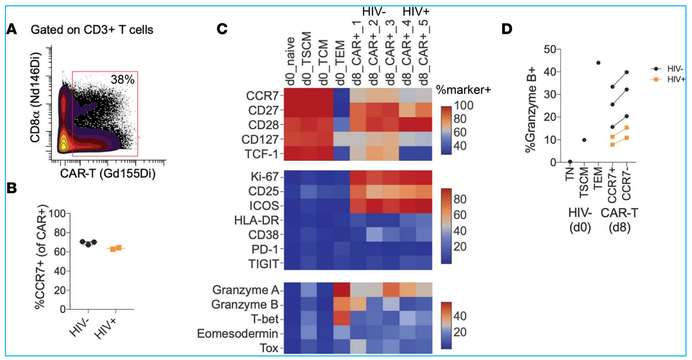

Figure 2. CyTOF analysis of preinfusion anti-HIV duoCAR T cell products reveals a TCM/TSCM phenotype primed for lymphoid tissue homing and effector function. A) Gating strategy to identify anti-HIV duoCAR T cells from our 8-day manufacturing procedures using mass cytometry staining. **B**) Frequency of summed TSCM _ TCM phenotype (CCR7+) duoCAR T cells at D8 among HIV seronegative (HIV-, CAR+_1, CAR+_2, CAR+_3; n=3) and seropositive donors (HIV+, CAR+_4, CAR+_5; n=2). **C**) Heatmap showing phenotype of duoCAR T cells from HIV- and HIV+ donors post-manufacturing compared to untransduced (D0) T cell subsets from an HIV- donor. **D**) Expression of Granzyme B in D0 T cell subsets compared to CCR7+ and CCD7- subsets from a final CAR+ prodict. TSCM, stem cell momory; TCM, central memory, TEM, effector memory.

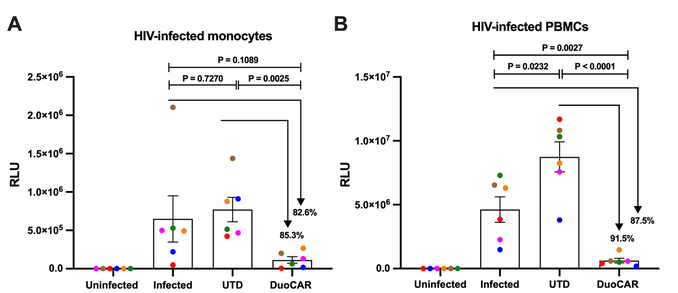

Figure 3. Anti-HIV duoCAR T cells recognize and potently kill HIV-infected monocytes. A) CD14+ cells from PBMC were cultured for 3 days in complete media and GM-CSF, then infected with HIV_{BaL}-LucR HIV for 2 days, B) or **B)** parallel infected unfractioned PBMC (infected for 2 days) were either: untreated/uninfected, infected alone, treated with donor matched non-LV transduced T cells (UTD), or treated with MND-ΔW duoCAR T cells at an E:T ratio of 1:1 for an additional 3 days. HIV-infection in each culture was quantified by measuring luciferase signal (RLU, y-axis). Each donor is denoted by a different color circle, and data shown are mean +/-SEM from triplicate wells. Percent suppression is shown above the DuoCAR bars.

RESULTS

MND-ΔW duoCAR T cells derived from PWH potently kill HIV infected cells

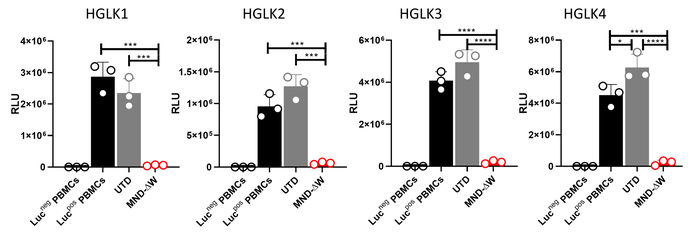

Figure 4. Modification of T cells from PWH on suppressive ART with LV-encoding DuoCARs generates cells capable of killing autologously infected PBMC. PBMC were superinfected with Luc+ HIV (BaL Enc, clade B), and cultured for 3 days immediately after infection, or infection was allowed to continue for 3 days prior to the addition of CAR-T to the culture, and subsequently cultured 3 additional days (not shown). Four donors are shown. HIV-1 infection was quantified by luciferase activity (RLU, y-axis) from uninfected PBMC (Luc^{neg} PBMC, open circles), untreated infected PBMC (Luc^{pos} PBMC, black bars), cultures treated with untransduced control T cells (gray bars) or duoCAR-T cells (MND-DW, red circles). Data shown are means and SD of triplicate wells, ***P<0.001.

CONCLUSIONS

1. Ani-HIV DuoCAR-T cells express an early-memory phenotype along with markers of T cell activation and effector function.
2. HIV DuoCAR-T are active in humanized mouse models i.v., can control infection in monocytes/macrophages, and are readily generated from PWH on suppressive ART.
3. Effector T cells population of this phenotype may be associated with a durable therapeutic response in PWH.
4. These studies support translation of anti-HIV duoCAR-T cell therapy in our open phase I/IIa clinical trial (NCT04648046, Steven Deeks, MD, PI).

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