PRECLINICAL STUDIES TOWARD A PHASE I/IIA TRIAL USING ANTI-HIV DUOCAR-T CELL THERAPY

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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). Paramount to translating such therapeutic candidates successfully into PWH will require anti-HIV CAR-T cells to migrate to lymphoid tissues in the body and eliminate reactivated HIV-infected cells. We hypothesized that clinical-grade anti-HIV duoCAR-T cells could traffic from the peripheral blood to the site of HIV infection in the spleen of humanized mice with HIV and potently suppress HIV infection.

METHODS

To test our hypothesis, we developed a GMP-complaint CAR-T cell manufacturing process using the CliniMACS Prodigy device to generate anti-HIV duoCAR-T cells at clinical scale. Clinical-grade anti-HIV duoCAR-T cells (2 x 10^6 total T cells) were intravenously injected into the tail-veins of PBMC-humanized NSG mice with intrasplenic HIV infection (hu-spl-PBMC-NSG). After 17-18 days of HIV infection, humanized mice were evaluated for signs of CAR-related toxicity and HIV infection quantified in the spleens of infected mice treated with and without duoCAR-T cell therapy.

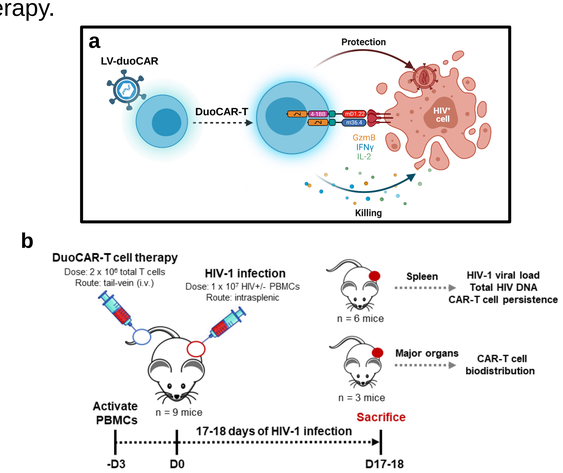

Fig 1. Experimental design of the preclinical *in vivo* study. a Illustration of anti-HIV duoCAR-T cell mediated killing of HIV-infected cells (created with BioRender.com). Primary T cells are converted to duoCAR-T cells via genetic modification using a lentiviral vector encoding the anti-HIV duoCAR (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). HIV infection, total HIV DNA, and CAR-T cell persistence were quantified after 17-18 days of HIV-1 infection. Major organs were collected at study endpoint followed by DNA extraction and analysis of CAR-T cell biodistribution by qPCR.

RESULT

A single intravenous injection of clinical-grade anti-HIV duoCAR-T cell therapy suppresses HIV infection *in vivo*

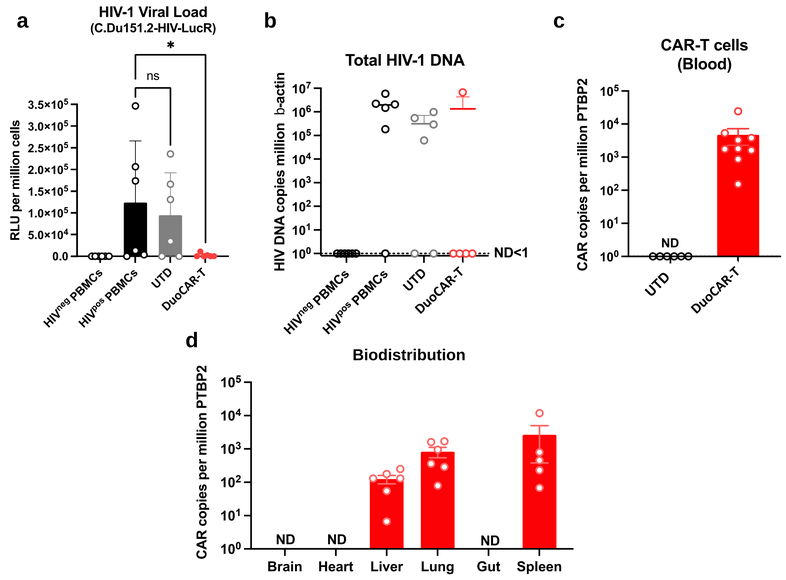

Fig 2. Intravenously administered anti-HIV duoCAR-T cells potently suppresses HIV in humanized mice with HIV. a Quantification of HIV-1 viral load via *Renilla* luciferase (LucR) activity in the spleens of humanized mice after 17-18 days of HIV-1 infection. **b** Quantification of cell-associated total HIV-1 DNA in the spleens of humanized mice after 17-18 days of HIV-1 infection. Results are expressed as HIV-1 Gag DNA copies per one million β-actin copies. ND = not detected. One of the mice in the duoCAR-T cell treated group had insufficient cells for total HIV-1 DNA analysis and therefore only five samples were evaluated in this group. **c, d** Persistence and biodistribution profile of intravenously administered anti-HIV duoCAR-T cells in the (**c**) blood and (**d**) major organs of humanized mice after 17-18 days of HIV-1 infection. Data is expressed as CAR DNA copies per 1 million polypyrimidine tract binding protein 2 (PTBP2) copies. Error bars represent mean +/- SD of samples tested (n = 5-9 mice). Statistical analysis was performed by one-way ANOVA followed by Tukey's or Dunnett's multiple comparisons posttest. Significance is considered $P < 0.05$ (* $P < 0.05$).

RESULT

Table 1. Successful manufacture of clinical-grade anti-HIV duoCAR-T cell products from PWH on suppressive ART

Release test	Specification	Donor PID: 2286	Donor PID: 2529
Sterility	**No growth at 14 days**	**No growth**	**No growth**
Mycoplasma	Negative	Negative	Negative
Endotoxin	< 5.0 EU/kg/hr	<2.31 EU/kg/hr	<2.61 EU/kg/hr
Viability (7-AAD)	≥ 70%	87.20%	86.41%
T cell purity	≥ 70%	98.20%	98.73%
% $CD4^+$ T cells	Report	73.88%	81.43%
% $CD8^+$ T cells	Report	25.53%	18.49%
% CAR^+ $CD3^+$	≥ 10%	51.32%	59.83%
% CAR^+ $CD4^+$	Report	38.77%	52.15%
% CAR^+ $CD8^+$	Report	12.79%	11.64%
% DuoCAR$^+$ $CD8^+$	Report	33.55%	33.02%
Potency	Report	6.42 x 10^8 CAR^+ T cells	7.96 x 10^8 CAR^+ T cells
VCN	< 5 copies per transduced cell	2.27	3.29
Transgene Mobilization	Not detected or decline	Not detected	Not detected
RCL VSV-G DNA	Not detected or decline	Not detected	Not detected
RCL culture assay	Not detected	Not detected	Not detected
Total HIV-1 DNA	Not detected or decline	Not detected < 1	Not detected < 1

RESULT

First-in-human study to evaluate anti-HIV duoCAR-T in PWH

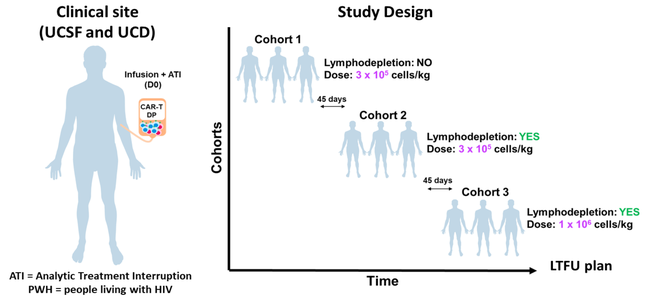

Fig 3. Clinical trial design for the anti-HIV duoCAR-T cell study. Clinical trial NCT04648046 "*CAR-T cells for HIV infection*" is a first-in-human phase I/IIa study to evaluate the safety and efficacy of anti-HIV duoCAR-T cell therapy in PWH. Cohorts of 3 participants will be enrolled at a specified dose level (-/+ cyclophosphamide) in a 3+3 trial design for a total of 9 participants with the possibility of an expansion cohort. Participants will stop antiretroviral drugs on the day of CAR-T cell infusion. During ATI, participants will be monitored for HIV rebound, changes in $CD4^+$ T cell count, CAR-T cell persistence, and inflammatory cytokines (e.g., IL-6, IFN-gamma). Upon study conclusion, participants will be followed for up to 15 years as part of the long-term follow-up (LTFU) plan.

CONCLUSIONS

1. Intravenously administered anti-HIV duoCAR-T cells traffic from the peripheral blood to the spleen of mice with HIV and exert potent anti-HIV effects against cells with HIV.
2. High quality anti-HIV duoCAR-T cell products can be successfully manufactured at clinical-scale from HIV seropositive individuals using the Prodigy cell manufacturing device.
3. This work supports the safety and efficacy of anti-HIV duoCAR-T cell therapy in our presently open phase I/IIa clinical trial (NCT04648046).

ACKNOWLEDGEMENTS

BELIEVE MARTIN DELANEY COLLABORATORY
NIH National Institutes of Health
CIRM CALIFORNIA'S STEM CELL AGENCY
UCSF University of California San Francisco
CFAR Einstein-Rockefeller-CUNY Center for AIDS Research
EINSTEIN Albert Einstein College of Medicine
UCDAVIS HEALTH

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