bNAb-Secreting Hybrid CAR-T Cells Show HIV-Specific Activation *In Vitro* and Tissue Viral Suppression

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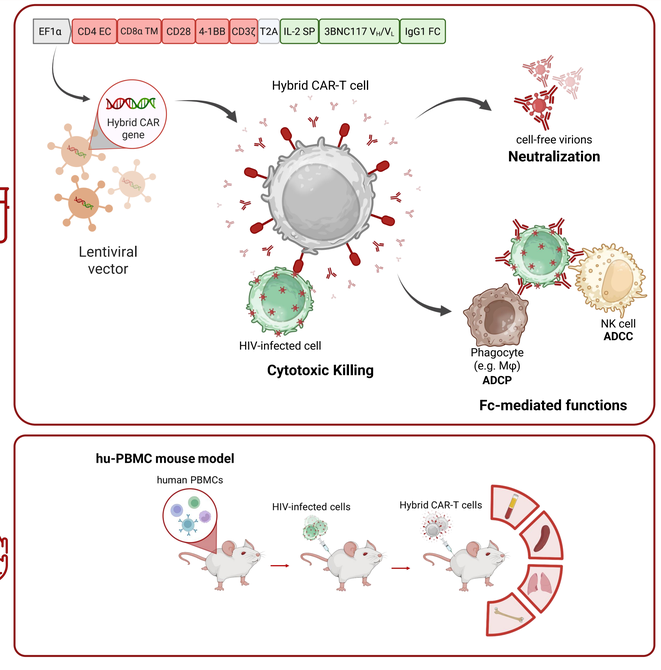
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BACKGROUND

- **ART** is highly effective at suppressing HIV replication but **is not curative** and **viral rebound** occurs rapidly upon **ART interruption**
- Achieving a **functional cure** will likely require combination strategies
- **Chimeric Antigen Receptor (CAR) T cells** and **broadly neutralizing antibodies (bNAbs)** constitute key therapeutic strategies with potential for durable viral control
- We introduce the **Hybrid CAR platform**; a dual-function therapeutic that combines the **cytotoxic killing** of HIV-infected cells and **bNAb secretion**, bridging together cellular and humoral mechanisms.

METHODS



- We generated a bicistronic lentiviral vector that encodes for a CD4-based 2nd generation CAR and for a 3BNC117 V_H/V_L fused to IgG1-Fc.
- Hybrid CAR-T cells were assessed *in vitro* for cytotoxicity, activation, cytokine secretion and antibody production.
- *In vivo* evaluation was performed using a PBMC-humanized mouse model.

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bNAb-secreting CAR-T cells preserve intrinsic CAR-T functionality, while bNAb secretion mediates Fc-dependent immune effector functions *in vitro* and supports multi-tissue HIV control *in vivo*.

RESULTS

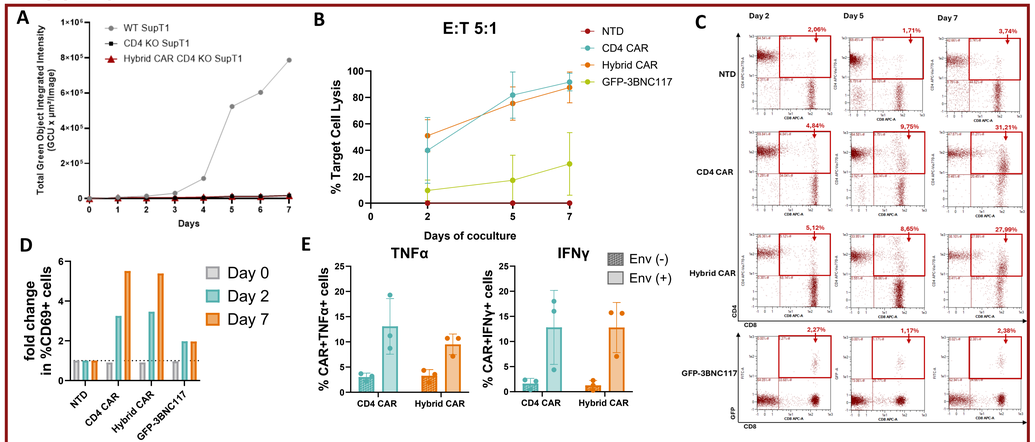


Figure 1. Functional characterization of Hybrid CAR-T cells. (A) Hybrid CAR+ CD4$^{KNOCK-OUT}$ SupT1 cells were exposed to HIV and infection was assessed for 7 days. (B) Cytotoxicity of Hybrid CAR-T cells cocultured with autologous HIV-infected CD4+ T cells at 5:1 effector-to-target (E:T) ratio, for 7 days; each dot represents the mean % target lysis from four donors (n=4). (C) Antigen-specific expansion of Hybrid CAR-T cells in cocultures with autologous HIV-infected CD4+ T cells. (D) Flow cytometric analysis of (Hybrid) CAR- and GFP-3BNC117-transduced T cells after co-culture with autologous HIV-infected CD4+ T cells. CD69 fold-change was assessed at Day 0, 2, and 7 of coculture. Representative data from one donor. (E) Percentage of cytokine-producing CAR-T cells in presence of Env+ targets; each dot represents one donor (n=3).

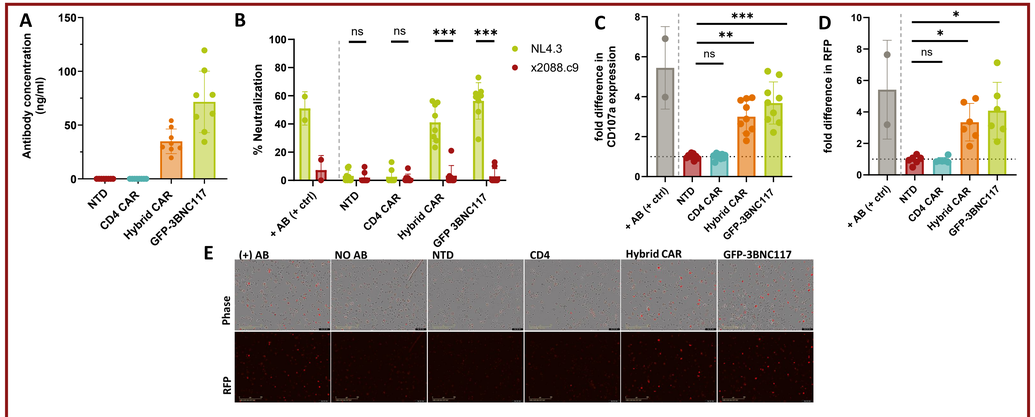


Figure 2. Hybrid CAR-T cells secrete 3BNC117-IgG1Fc. Secreted antibodies elicit Fc-mediated effector functions. (A) Mean antibody concentration of 3BNC117scFv-IgG1Fc in the supernatants of non-transduced and Hybrid CAR-T cells, as measured by ELISA. (B) Cell culture supernatants can neutralize HIV NL4.3 but not the 3BNC117-resistant strain HIV x088.c9; unpaired non-parametric t-test; n=9. (C) Antibody dependent NK cell degranulation assay: Primary human NK cells were analyzed for surface expression of the degranulation marker CD107a; One-way ANOVA, Kruskal-Wallis test; n=9. (D) Amount of red fluorescence emitted at the peak of the ADCP assay; One-way ANOVA, Kruskal-Wallis test; n=6. (E) Representative brightfield and red fluorescent channel images at the peak of the ADCP assay (S3 Live Cell Imaging System).

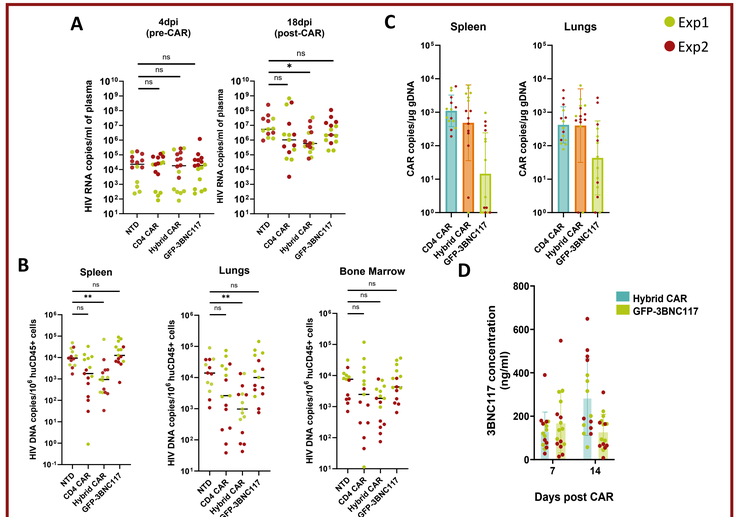


Figure 3. *In vivo* cytotoxic activity and sustained bNAb secretion by Hybrid CAR-T cells in humanized mice. (A) Plasma HIV viral loads expressed as copies per ml of plasma; One-way ANOVA, Kruskal-Wallis test. (B) HIV-DNA levels in murine tissue compartments expressed as DNA copies per 10^6 huCD45+ cells; One-way ANOVA, Kruskal-Wallis test. (C) CAR-T cell distribution in the spleen and the lungs. CAR gene copies were detected by dPCR. (D) Plasma levels of 3BNC117scFv-IgG1Fc, 7 and 14 days post CAR administration as measured by ELISA.

CONCLUSIONS

- Hybrid CAR-T cells retain intrinsic CD4-CAR cytotoxic function, showing robust cytotoxic killing, activation and cytokine production in response to their specific targets.
- Hybrid CAR expression does not facilitate HIV entry.
- Hybrid CAR-T cells secrete functional 3BNC117scFv-IgG1Fc that neutralize HIV and mediate Fc-dependent effector functions.
- In a PBMC-humanized mouse model, Hybrid CAR-T cells support systemic viral control, with reduced plasma viremia and decreased HIV-DNA levels in multiple tissue compartments.
- CAR gene copies were detected in spleen and lung, confirming *in vivo* trafficking.
- Hybrid CAR-T cells mediate antibody secretion *in vivo.*
- Together, these findings validate the Hybrid CAR platform as a dual-mechanism strategy that integrates cellular cytotoxicity and humoral Fc-mediated immunity within a single therapeutic product.

PLAIN LANGUAGE SUMMARY
We developed an HIV cure strategy that can both kill HIV-infected cells and produce antibodies against the virus. In mouse studies, this dual approach helped reduce HIV levels in blood and tissues.