NK-Cell Immunotherapy Administered at the Time of HIV Rebound Is Associated With Viral Control

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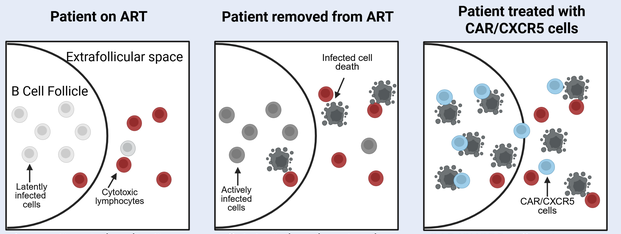
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BACKGROUND

Although ART therapy has greatly improved patients' survival rate and quality of life, these drugs require lifelong adherence and are not a cure for HIV. Efficacious HIV therapeutics are critical for public health and for the operational readiness of U.S. service members. Reservoirs of HIV persist in B cell follicles of lymphatic tissues even during ART treatment, driven by a paucity of HIV-specific cytotoxic cells in these locations, as they largely do not express the chemokine receptor CXCR5.

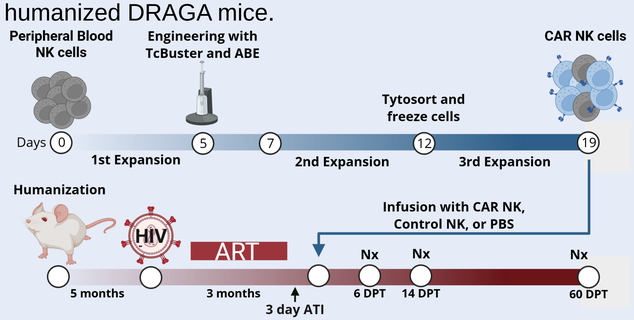
Our group pioneered the development of HIV-specific CAR/CXCR5 T cell therapies, which kill HIV-Env-expressing cells and migrate to B cell follicles.



CAR T therapies are typically autologous, as treatment with allogeneic T cells can cause graft-versus-host disease. However, developing autologous CAR T cells from immunocompromised people living with HIV can pose engineering challenges. As an alternative, we explored CAR/CXCR5 NK cells as they can be used as allogeneic treatments and have endogenous roles in immunosurveillance of HIV.

METHODS

We engineered HIV-specific CAR/CXCR5 NK cells armored with IL-15 and PD-1 KO. We assessed the efficacy and safety of these therapies in HIV-infected humanized DRAGA mice.

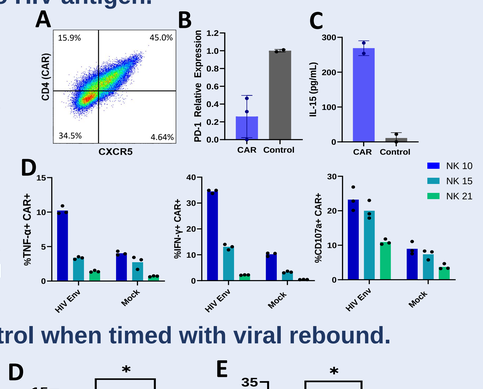


We evaluated the efficacy of novel NK cell immunotherapies in a humanized mouse model of HIV. The data suggest that treatment timing in relation to viral rebound is important for therapeutic efficacy.

RESULTS

CAR NK cells produce cytotoxic cytokines in response to HIV antigen.

Figure 1: *in vitro* analysis of NK therapies. A) Expression of CAR (MBL) and CXCR5 molecules was confirmed in the cell product using flow cytometry. Cells were pre-gated sequentially on lymphocytes, singlets, live cells, CD56+, and CD3-. B) PD-1 knockout was detected at the RNA level by RT-PCR, CAR NK (blue) relative to control NK cells (black). C) IL-15 expression in CAR NK (blue) and control NK (black) cells via IL-15 ELISA of cell culture supernatant. D) Pro-inflammatory cytokine (IFN-γ and TNF-α) and degranulation marker (CD107a) production was measured after CAR NK cells were cocultured with either HIV-Env or mock PBMCs. Measured via ICS assay. Three deidentified human NK cell donors were evaluated, labeled NK 10 (dark blue), NK 15 (teal), and NK 21(turquoise).



CAR and control NK cells were associated with viral control when timed with viral rebound.

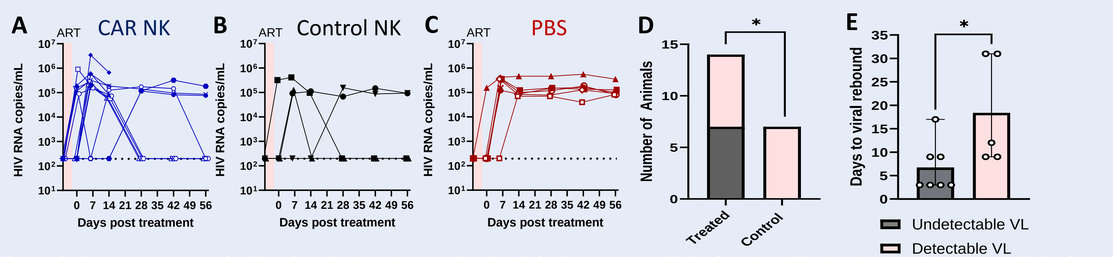


Figure 2: Post-treatment viral loads. Viral loads over time in A) CAR NK-treated DRAGA mice (blue), B) control NK-treated DRAGA (black), and C) PBS-treated DRAGA (red). At 56 DPT, 5/8 CAR, 2/4 Mock, and 0/6 PBS-treated animals had undetectable viral loads. D) Number of animals in either the treated (CAR and control NK) or untreated (PBS) groups at 56 DPT, with the percentage of controllers (gray) or non-controllers (pink) at 56 DPT (P=0.0377, Fisher's exact test of proportion within treated versus PBS groups). E) Controllers (gray) or non-controllers (pink) at 56 DPT by number of days to viral rebound post-ART interruption (P=0.0278, Mann-Whitney test). Mean values with SD are shown.

NK cell concentration peaked at 6 days post-treatment, with CAR NK cells persisting up to 28 days.

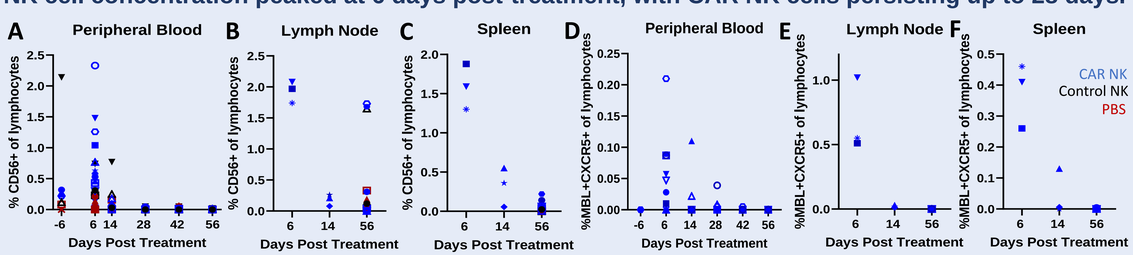
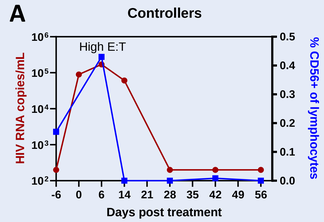
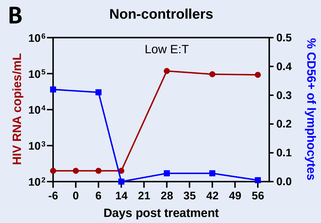


Figure 3: NK levels post-treatment. NK (CD56+) cells in CAR (blue), control (black), or PBS (red) treated groups from A) Peripheral Blood, B) Lymph node (at necropsies), and C) Spleen (at necropsies). Mice were euthanized by CO2 inhalation followed by cervical dislocation. CAR+CXCR5+ NK cells over time in D) Peripheral Blood, E) Lymph node (at necropsies), and F) Spleen (at necropsies). Flow was pre-gated on Lymphocytes, Singlets, Live Cells, human CD45+, mouse CD45-, CD3- and then CD56+ MBL+/CXCR5+.

Peak NK cell concentration at the time of viral recrudescence is correlated with viral control.



In controllers, peak NK cell concentration coincided with peak viral loads post-ATI, resulting in relatively high in vivo E:T ratios at viral peak.



In non-controllers, NK cell concentration diminished before viral rebound, resulting in relatively low E:T at viral peak.

Figure 4: E:T ratios of NK cells to HIV viral loads. Median viral loads (dark red) and CD56+ NK cell levels (teal) post-treatment in A) animals that controlled HIV infection or B) animals that did not control HIV infection.

CONCLUSIONS

- Infusion with CAR and control NK cells is safe and led to undetectable viral loads in 58.3% of treated animals.
- Timing of NK cell treatment with viral recrudescence is associated with therapeutic efficacy.
- Future studies could explore multiple rounds of NK cell infusions to achieve superior efficacy.
- This study demonstrates the promise of NK therapies for viral suppression of HIV in the absence of ART.

ACKNOWLEDGEMENTS

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PLAIN LANGUAGE SUMMARY

We tested NK-cell therapies to see if they can safely treat HIV in an animal model. These therapies were most effective when given at the time when levels of HIV were increasing after stopping ART drugs.