Attacking Latent HIV with convertibleCAR-T Cells, a Modular Killing Platform

336

Eytan Herzig[1], Kaman Chan Kim[2], Thomas A. Packard[1], Noam Vardi[1], Roland Schwarzer[1], Andrea Gramatica[1], Steven G. Deeks[3], Steven R. Williams[2], Kyle Landgraf[2], Nigel Killeen[2], David W. Martin[2], Leor Weinberger[1], Warner C. Greene[1]
[1]Gladstone Institutes/UCSF, San Francisco , Ca, USA, [2]Xyphos Biosciences, Inc. South San Francisco, Ca, USA, [3] University of California San Francisco, San Francisco, Ca USA

BACKGROUND

Reducing the size of the latent HIV reservoir and controlling subsequent viral rebound by immune engineering could lead to a sustained viral remission in HIV-infected individuals in the absence of ART. CTLs could reduce the size of the reservoir by recognizing and killing reactivated reservoir cells. However, cellular exhaustion and the presence of CTL-resistant viruses may undermine their effectiveness. We have tested a new approach to reservoir reduction where convertibleCAR-T cells (cCAR-Ts) programmed with multiple HIV-specific broadly neutralizing antibodies (bNAbs) are deployed.

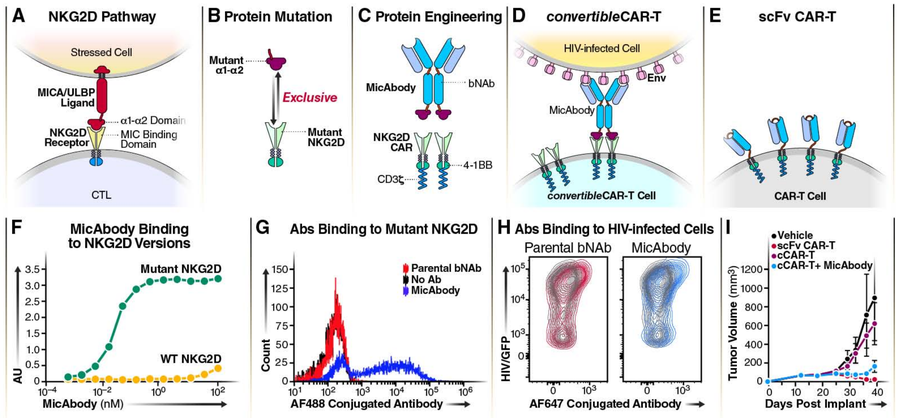
METHODS

cCAR-Ts utilize a mutated, inert form of the NKG2D receptor. Orthogonal MIC ligands that bind to inert NKG2D but not wild-type NKG2D are fused to antibodies to generate bispecific MicAbodies for directing cCAR-T targeting and activation. cCAR-Ts can therefore be readily redirected by altering the antibody component of the MicAbody and furthermore, MicAbodies can be multiplexed. 4 bNAbs were engineered as MicAbodies and tested for their ability to kill tonsil, spleen, or blood cells infected with GFP-tagged R5 or X4-tropic or transmitted/founder viruses. Specificity of infected cell killing was monitored by loss of GFP+ vs GFP- cells. Reactivated CD4 T cells from HIV-infected individuals on ART were assayed for loss of cell-associated viral RNA in the presence cCAR-Ts either armed or not armed with bNAbs. The platform was checked in vivo, in NSG mice model of cancer, by measuring size reduction of cancer tumors.

RESULTS

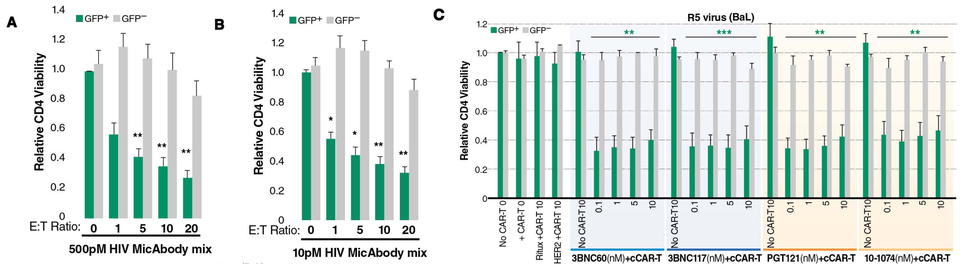
In the presence of bNAb-MicAbodies, CD8 cCAR-Ts effectively killed HIV-infected, but not uninfected, cells from tonsil, spleen and blood. Killing was strictly dependent on the presence of bNAb-MicAbodies targeting HIV Env. cCAR-T cells also reduced by more than half the inducible reservoir present in blood of HIV-infected individuals on ART. Administration of cCAR-Ts cells in a mice cancer model, demonstrated highly effective in vivo killing.

Construction of MicAbody/convertibleCAR-T Platform



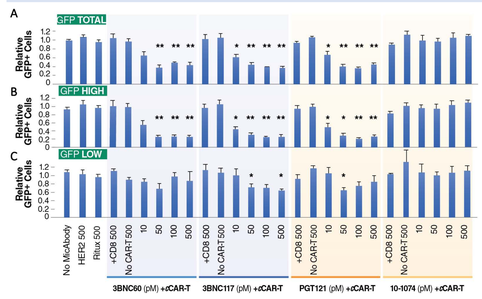
(A) The MIC/ULBP-ligand family are natural ligands for NKG2D receptors present on NK cells and CTLs. NKG2D binds to the α1- α2 part of the ligands. (B) Protein engineering of the α1- α2 ligand domain and NKG2D receptor to create a cognate ligand-receptor pair that no longer recognizes the natural ligand or receptor. (C) Protein engineering of bispecific antibody based on bNAb and mutated α1- α2 on the antibody (MicAbody), and a mutated NKG2D CAR fused to 4-1BB and CD3ζ as the signaling domains. (D) Construction of cCAR-T cell based on the mutated NKG2D. The *convertible*CAR system allows specific binding of MicAbody to the mutated NKG2D-based CAR expressed on the T cell. (E) Conventional scFv-based CAR-T cell. (F) ELISA binding assay of MicAbody to WT NKG2D receptor or to the mutated form. AU, arbitrary absorbance units. (G) Antibodies conjugated to Alexa Fluor (AF) fluorophore were assessed for selective binding to cCAR-T cell with mutated NKG2D. Blue, MicAbody; red, parental bNAb; black, no antibodies. (H) MicAbody binds to HIV/GFP+ cells similarly to the parental bNAb. Red, parental bNAb; blue, MicAbody; gray, isotype control. (I) *in vivo* killing by the cCAR-T platform. Comparison of the effectiveness of cCAR-T platform with the scFv conventional CAR-T platform in controlling Raji lymphoma cell growth in NSG mice. n = 3 for each cohort.

Specific Killing of HIV-infected Primary CD4 T Cells by convertibleCAR-T Combined with HIV Env-Specific MicAbodies



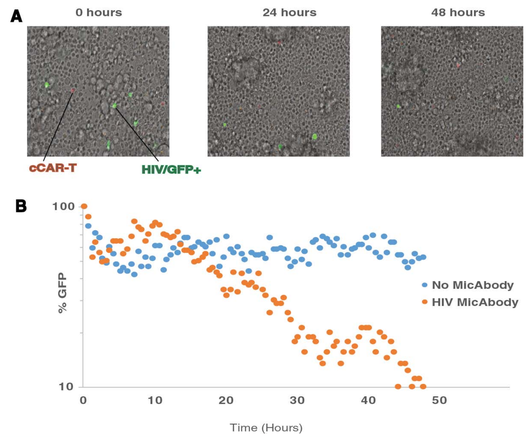
To determine the optimal effector to target cell ratio, one million tonsil-derived cells (10^4 HIV/GFP-infected target cells) were incubated with a range of cCAR-T effector cells from zero (0:1) to $2X10^5$ (20:1) cCAR-T cells: target cells for 48 h with the a mix of four HIV Env-specific MicAbodies (Mix). In the absence of cCAR-T cells (0:1), the donor-matched untransduced CD8 T cells were present. Live GFP+/CD3+/CD8- cells were counted to assess reduction in target cells (GFP+) and live GFP-/CD3+/CD8- cells were counted to assess off-target killing (GFP-).(A and B) HIV-MicAbody mix was tested with high (500 pM) (A) or low (10 pM) (B) concentration of each individual MicAbody. Data derived from three independent experiments; mean + SEM
(C) R5 tropic HIV-1 (BaL) was used to infect tonsil cells followed by testing of individual HIV-specific MicAbody for specific killing by cCAR-T cells. One million tonsil-derived cells were incubated with 10^5 cCAR-T cells for 48 h, in the presence of different concentrations (0.1–10 nM) of HIV Env-specific MicAbodies. B cell-specific MicAbody (Ritux) and anti-HER2-specific MicAbody (HER2) were used as negative control MicAbodies.

Arming cCAR-T Cells with HIV MicAbodies Promotes Effective Killing of Target Cells Infected with a Transmitted/Founder Virus Proportionally to GFP/Env Expression



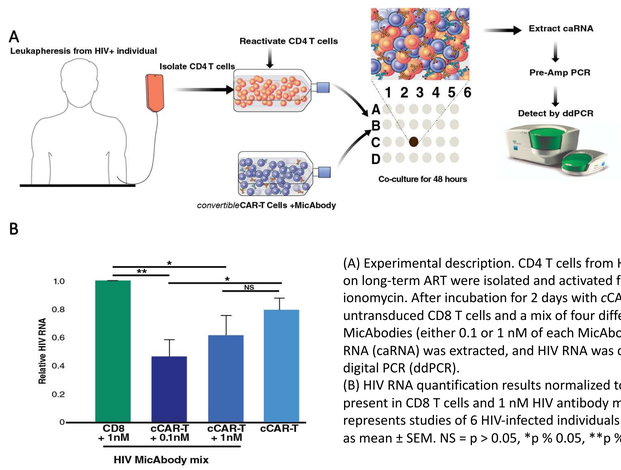
One million tonsil-derived cells, including 10^4 CD4 T cells infected with the F4 T/F virus containing a GFP reporter (F4-GFP), were incubated with 10^5 cCAR-T cells in the presence of different concentrations (10–500 pM) of four different HIV-specific MicAbodies. After a 48 h incubation, survival was assessed for the whole GFP-expressing population (A) and for cells gated on high (B) and low (C) GFP expression. Negative controls for the HIV Env-specific MicAbodies included B cell-specific MicAbody (Ritux) or anti-HER2-specific MicAbody (HER2). Additional controls for cCAR-T included no cCAR-T or CD8 cells supplemented with the highest MicAbody concentration tested (500 pM). Results are cumulated from four independent experiments. Data are represented as mean + SEM. *p < 0.05, **p < 0.01.

Time-Lapse Microscopy at Single-Cell Resolution Shows Delay in Killing



(A) Representative time course of primary cells' survival after infection with GFP-tagged F4-HIV and exposure to cCAR-T cells armed with a mix of the four HIV Env-specific MicAbodies (500 pM each). Snapshots of 36 fields of view were taken every 30 min for 48 h for bright field (cell borders), GFP (HIV-positive cells), and RFP (*c*CAR-T cells) in X20 magnification on a confocal spinning disc microscope. Scale bar, 50 mm.
(B) Quantitative analysis of HIV-positive cells killing assay over 48 h with *c*CAR-T and with or without MicAbodies. GFP quantification was made by ImageJ after reduction of background.

Ex Vivo Killing of Reactivated CD4 T Cells from HIV-1-Infected Individuals on ART by cCAR-T and MicAbodies



(A) Experimental description. CD4 T cells from HIV-infected individuals on long-term ART were isolated and activated for 72 h with PMA and ionomycin. After incubation for 2 days with *c*CAR-T cells or matched untransduced CD8 T cells and a mix of four different HIV-specific MicAbodies (either 0.1 or 1 nM of each MicAbody), cell-associated RNA (caRNA) was extracted, and HIV RNA was quantitated by droplet digital PCR (ddPCR).
(B) HIV RNA quantification results normalized to HIV RNA levels present in CD8 T cells and 1 nM HIV antibody mix. This experiment represents studies of 6 HIV-infected individuals. Data are represented as mean ± SEM. NS = p > 0.05, *p % 0.05, **p % 0.01.

CONCLUSIONS

An attractive feature of cCAR-Ts is that it is a modular platform that not only allows for multiplexing of MicAbodies, but also targeted delivery of kill switches if needed or cytokines for cCAR-T rejuvenation. This platform could be an important tool for reducing and controlling the size of the latent HIV reservoir.