VACCINE & IMMUNOTHERAPY CENTER
THE WISTAR INSTITUTE

BEAT-HIV DELANEY COLLABORATORY

Combination CAR T Cell Therapy Restricts HIV Escape and Durably Suppresses HIV Replication In Vivo

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BACKGROUND

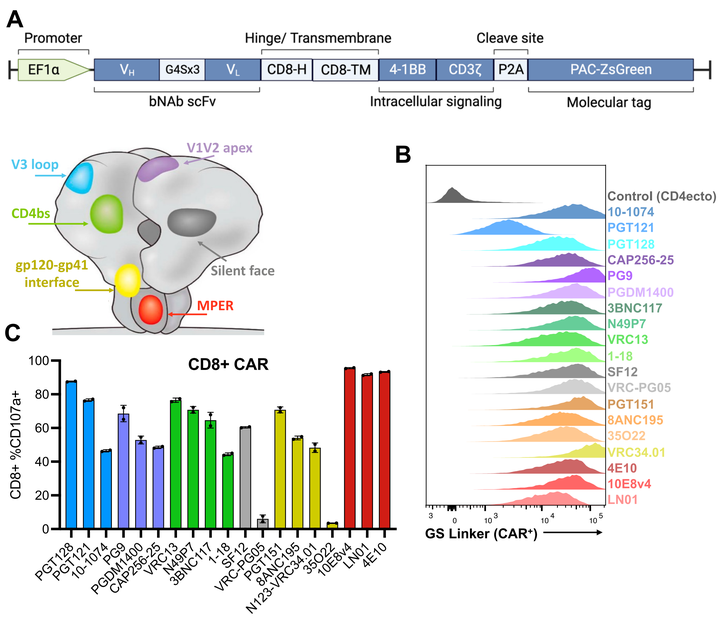
In most cases, the cellular immune response is unable to durably control HIV replication. Chimeric antigen receptor (CAR) T cell therapy may provide an avenue to engineer a more potent T cell response.

We hypothesize:

- CAR T cells with enhanced antigen sensitivity may be required to effectively clear HIV infected cells *in vivo*.
- Antigen sensitivity is influenced by epitope accessibility and CAR avidity.
- Combinations of CAR specificities will be required to effectively restrict HIV escape in order to suppress viremia in *vivo*.

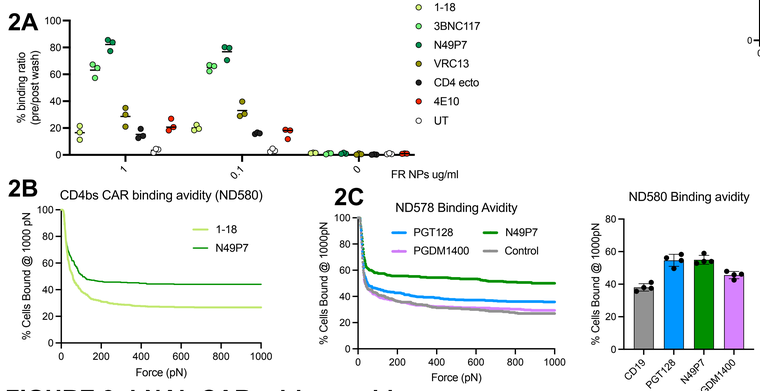
METHODS

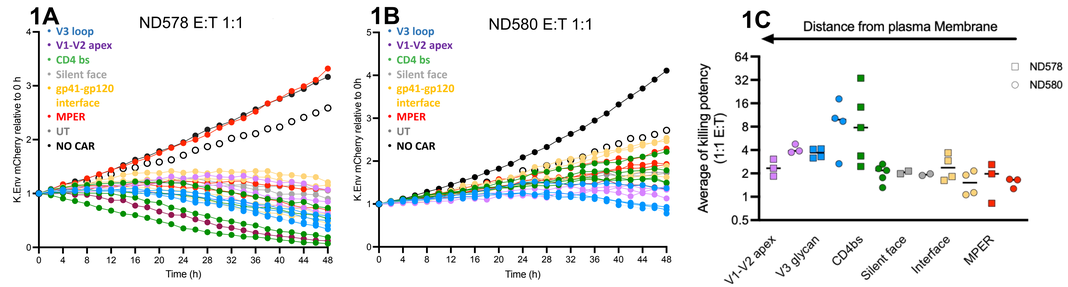
CAR plasmid constructs **(A)** encoding the scFv of 19 broadly neutralizing antibodies were generated to manufacture CAR T cells confirmed to express the bNAb scFv **(B)** and demonstrating responsiveness to targets cells by degranulation **(C).**



RESULTS

FIGURE 1: Killing potency associated with distal epitopes. Killing potency, as measured by K.Env killing kinetics, was heterogeneous among bNAb CAR constructs in the two donors tested, but clustered between epitope specificities **(1A, 1B)**. Despite different ranks among donors, Env epitopes most distal from the plasma membrane like V1/V2 apex, V3 loop and CD4bs, were associated with greater potency **(C)**.





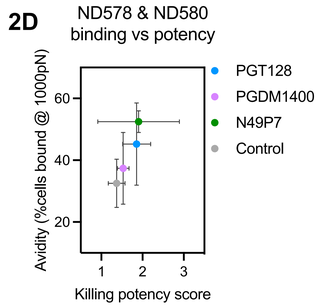
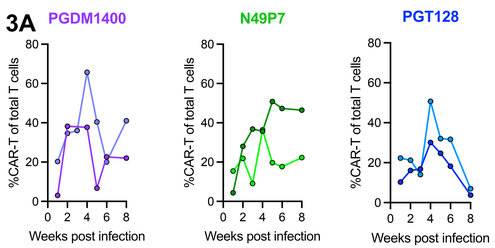


FIGURE 2: Binding avidity influences killing potency Binding avidity as measured by bNAb CARs binding to plate-bound Env nanoparticles **(2A)**, or binding to K.Env target cells **(2B-D)**, was variable among CARs within the same specificity. Importantly, CARs with higher binding avidity are associated with greater potency both within a specific epitope **(2B)** or among different epitopes **(2D)**.

FIGURE 3: bNAb CARs drive rapid escape bNAb CARs adoptively transferred into HIV-infected humanized mice demonstrate robust antigen-driven expansion *in vivo* **(3A).** HIV *env* deep-sequenced from bNAb CAR-treated humanized mice demonstrates putative escape mutations at known contact residues **(3B)**.



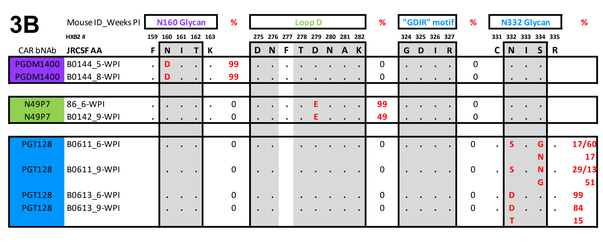
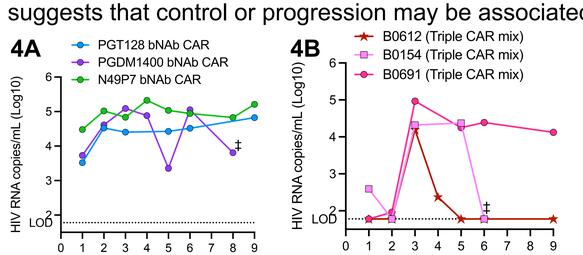
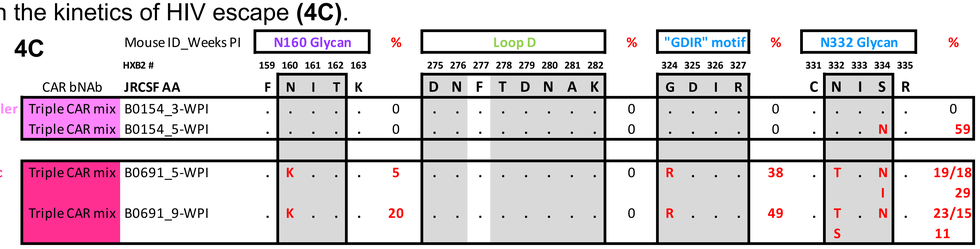


FIGURE 4: Combination CAR therapy restricts escape and suppresses viremia CAR T cell therapy with mono-specificity CAR T cells did not result in suppression of HIV viremia **(4A)**, whereas a combination of bNAb CARs specific for 3 distinct epitopes demonstrated full HIV suppression in 2/3 treated mice **(4B)**. *Env* sequencing of Triple-CAR treated mice with disparate suppression outcomes suggests that control or progression may be associated with the kinetics of HIV escape **(4C)**.





CONCLUSIONS

- Env epitopes distal to the plasma membrane are associated with CAR T cells demonstrating enhanced killing potency, suggesting epitope accessibility is an important constraint.
- CAR binding avidity may further dictate CAR potency, especially for CARs within the same epitope class.
- Restricting HIV escape through targeting multiple Env epitopes that are associated with orthogonal escape pathways may be a viable strategy to robustly suppress HIV replication *in vivo*.