Expanding the repertoire of V3 glycan supersite-specific CAR T cell products with distinct binding dependencies

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Background: Antiretroviral therapy (ART) is highly successful in suppressing HIV replication but is not curative and requires lifelong treatment. HIV-specific chimeric antigen receptor (CAR) T cells could provide ART-free remission with a single infusion. Our prior work has defined the potency hierarchy of broadly neutralizing antibody (bNAb)-based CAR T cells, identifying the V3 glycan supersite (V3S-CAR) as particularly potent. Aside from their superior avidity and functional potency, we observe that distinct V3S-CARs exhibit variability in their escape pathways, providing the potential to multiplex bnAb-CARs from our most potent epitope class to inform the design of bispecific CAR T cell products.

Methods : We generated an expanded panel of 15 second-generation, 4-1BBz V3S-CAR constructs that include additional V3S-targeting and non-neutralizing antibodies to broaden the suite of CAR T cells potently binding the V3 region of the HIV Envelope (Env) protein. We identified prominent escape mutations associated with our most potent V3S-CARs *in vivo* and validated their unique binding dependencies by engineering 293T cell lines and infectious molecular clones expressing Envs harboring these mutations. Escape mutant Envs were tested for recognition by the panel of 15 V3S-CARs through intracellular cytokine staining, real-time killing assays, and *in vitro* suppression assays as functional readouts.

Results : *In vitro and in vivo* escape mapping demonstrated that the majority of Type-I (N332 glycan-dependent) V3S-CARs were heavily dependent on the presence of the N332 glycan. However, PGT128 and PGT121 V3S-CARs required secondary mutations to mediate significant escape from CAR T cell pressure. A subset of non-neutralizing and Type-II (N332 glycan-independent) V3S-CARs exhibited unique escape patterns. Through *in vitro* suppression assays with replication-competent viruses harboring combinations of these mutations, we demonstrated that, due to their unique binding dependencies, these non-Type-I V3S-CAR T cells can efficiently target Env variants that express canonical V3S-CAR-associated escape mutations, which otherwise render current V3S-CARs ineffective. This suggests that combinations of potent Type-I and non-canonical V3S-CARs in bi-specific CAR products could further restrict HIV escape and improve viral control.

Conclusions : Our results further corroborate the potency of CAR T cells targeting the V3 glycan supersite, while highlighting the importance of expanding the repertoire of V3S-CARs with distinct binding footprints and escape dependencies in order to design multi-specific CAR T cell products capable of restricting HIV escape and improving viral control.