A Novel Strategy in HIV Eradication using CD8 CAR T cells

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INTRODUCTION

Latent HIV reservoir are a major burden for HIV cure as they are not recognised and killed by CD8 T cells. Guiding CD8 T cells to target these cells can be achieved by engineering chimeric antigen receptor (CAR) CD8 T cells using HIV broadly neutralising antibodies (bNAbs).

Accessing infected tissue resident CD4 memory T cells (T_{RM}) provides additional challenge in curing HIV. As most T_{RM} express CD69, incorporating CD69 ligand, Galectin-1 (Gal-1), into CD8 CAR T cells might help direct the CAR T cells into tissue.

Having a low-cost and high genetic capacity, *piggyBac* transposon system was used to deliver CAR genes via electroporation to generate CD8 CAR T cells.

HYPOTHESIS

1) CD8 CAR T cells will have specific activation and cytotoxicity against *in vitro* HIV model

2) Targeting HIV antigens combined with CD69 will be a novel strategy to eradicate latently infected CD4 T cells within tissues

AIMS

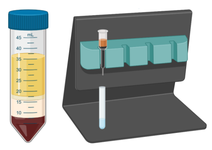
1) Generate and test **single** bNAb (PGT121, VRC07)-based and Galectin-1 (Gal-1) CD8 CAR T cells

2) Generate and test **Split Tandem** CD8 CAR (TanCAR) T cells expressing Gal-1 and two bNAbs utilising AND-gated strategy

METHOD

Day 1

Isolate CD8 T cells



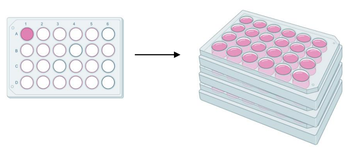
Transfection of CD8 T cells

piggyBac transposon delivered via electroporation:

$5x10^6$ cells

Day 1-21

CD8 CAR T cells Stimulation and Expansion



400 IU IL15 (every 2-3 days)
2:1 Irradiated PBMCs (day 1, 8, 15?)
0.2:1 Irradiated Cetuximab$^+$ K562 (day 1, 8, 15?)

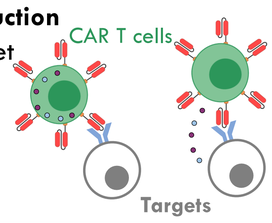
Assess CAR expression (GFP+) by flow cytometry

Day 22

CD8 CAR T cells Functional Assay

Cytokine induction
Effector: Target
1:1
IFN, TNF

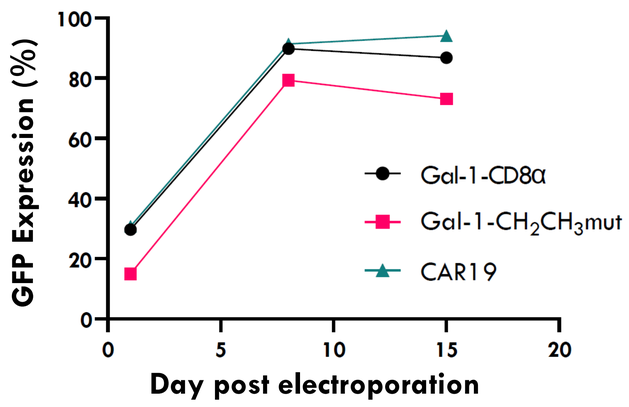
Killing
Effector: Target
2:1
5:1
10:1
20 :1



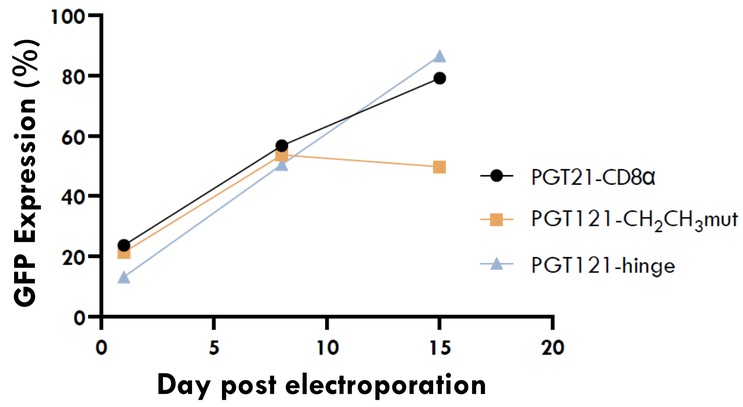
RESULTS

GFP expression on CD8 CAR T cells

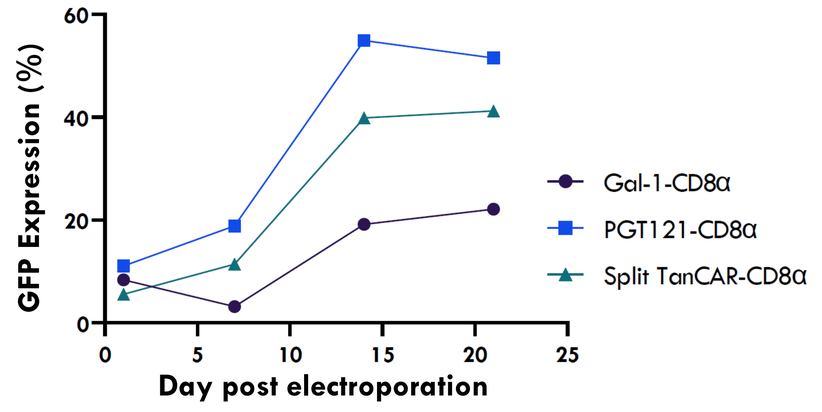
Single Gal-1 CAR T cells



Single bNAb-based (PGT121) CAR T cells

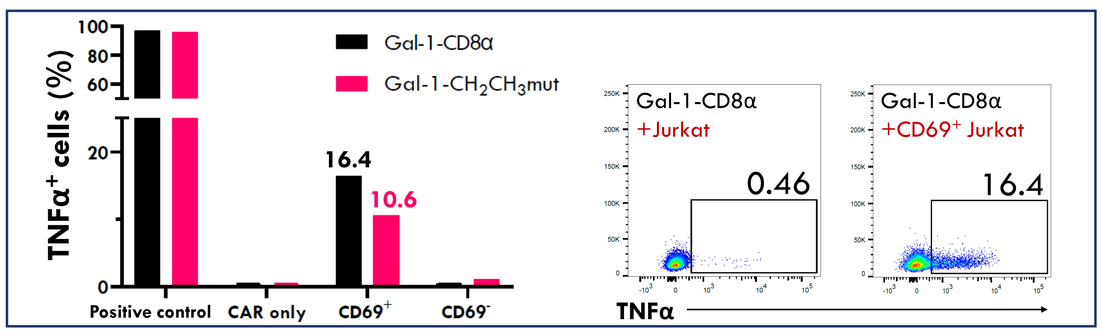


Split TanCAR T cells

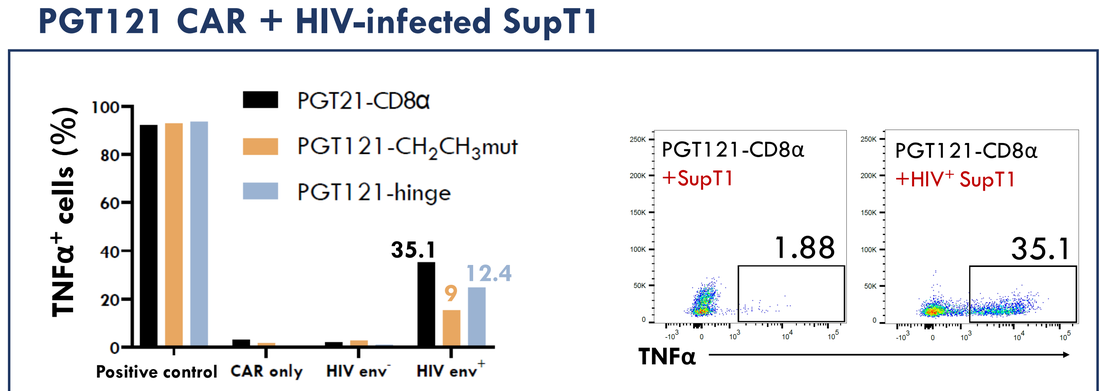


Specific target cells activate CD8 CAR T cells to express anti-viral cytokines

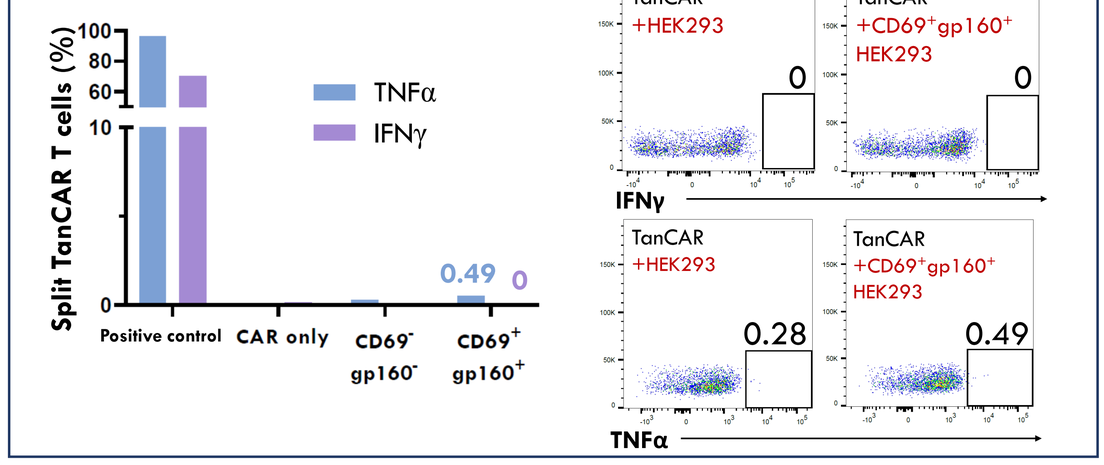
Gal-1 CAR + CD69$^+$ Jurkat



PGT121 CAR + HIV-infected SupT1



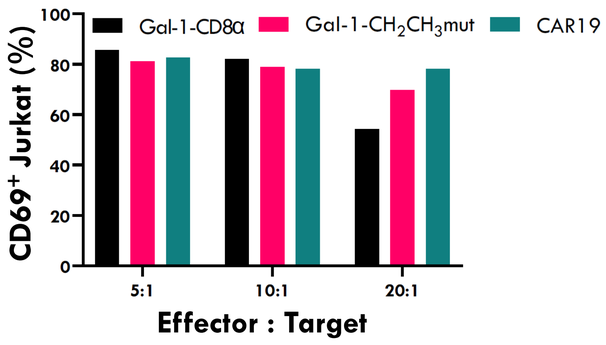
Split TanCAR + CD69$^+$ gp160$^+$ HEK293



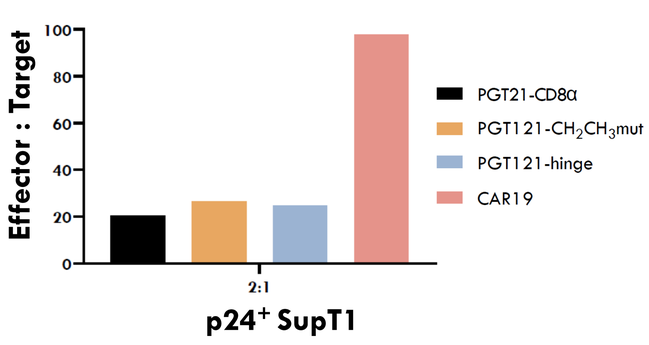
Flow cytometry data shows TNFα and IFNγ (not shown for single CARs) expressing-cells after co-culture of single or split TanCAR CD8 T cells with their target cells for 4 hours.

CD8 CAR T cells exhibit cytotoxicity against targets

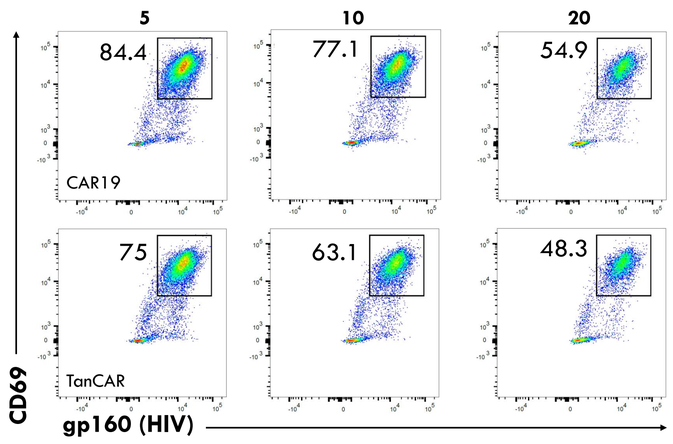
Gal-1 CAR + CD69$^+$ Jurkat

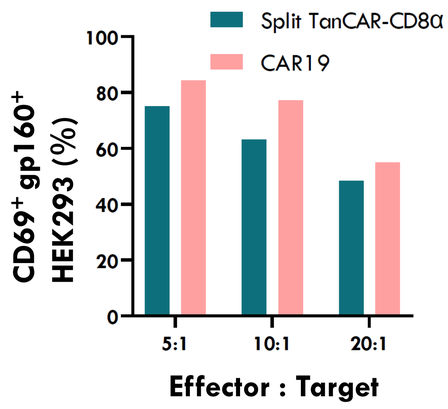


PGT121 CAR + HIV-infected SupT1



Split TanCAR + CD69$^+$ gp160$^+$ HEK293





Flow cytometry data shows number of live target cells after co-culture with different effector to target ratio of single or split TanCAR CD8 T cells with their target cells for 6 hours compared to non-specific CAR19.

CONCLUSIONS AND FUTURE DIRECTIONS

- Single CARs utilising the CD8α spacer were the best in effector functions. Therefore, split TanCAR was generated using CD8α.
- Split TanCAR did not exert any effector function.

Future directions: Investigate steric hindrance on split TanCAR T cells by generating other CAR iterations (Split or Tandem CAR)

Significance: Development of dual target immunotherapy may provide a novel approach in accessing HIV tissue reservoir and overcoming antibody resistance.