HIV Eradication using CD8 CAR T cells

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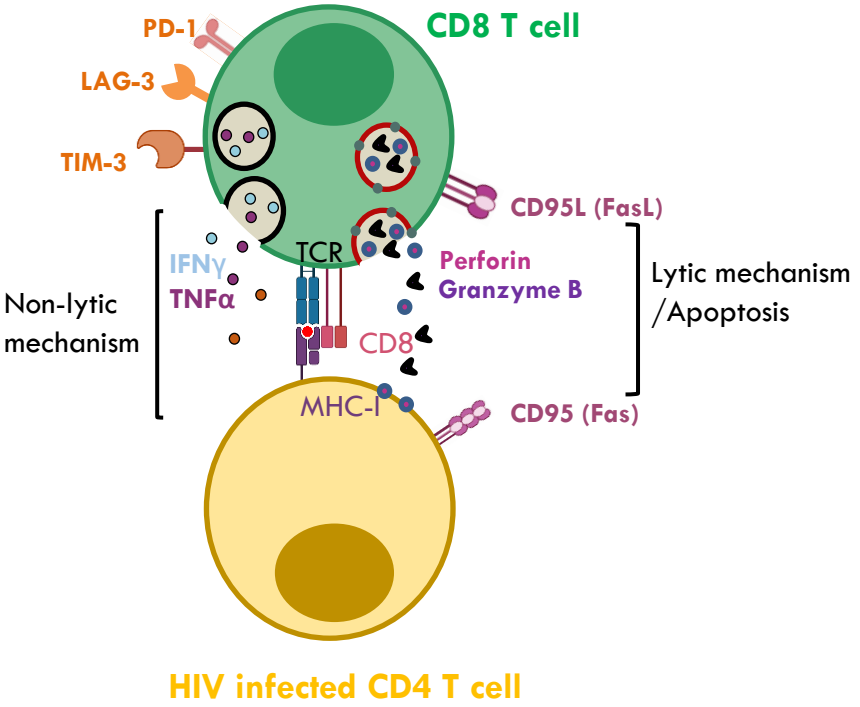
I have no financial interests or relationships to disclose

HIV reservoirs

- There remains no cure or vaccine for HIV
- 39 million people currently infected and 1.5 million new infections/year
- The only treatment is antiretroviral therapy (ART)
- It is lifelong, expensive and associated with many risk factors that decrease life expectancy
- A key challenge in curing HIV is that the virus establishes a latent reservoir
- If ART stops, HIV rebounds within 2-4 weeks

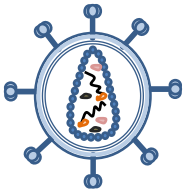
HIV evasion of CD8 T cell-mediated killing

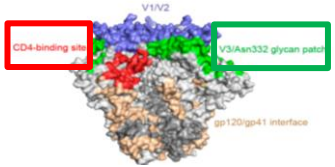
- All attempts to reduce the HIV reservoir have failed
- Latency reversing agents (LRA) were limited in reactivating silent HIV and/or the immune system did not kill reactivated cells
  - Nonfunctional exhausted cytotoxic CD8 T cells
  - Emergence of HIV mutants that evade the endogenous immune system
  - Down regulation of MHC-I expression on infected cells so minimal HIV is presented to CD8 T cells- reduced CD8 T cell cytotoxicity
- HIV localization in multiple tissues especially lymphoid follicles and CNS, where:
  - there is limited access to CD8 T cells
  - CD8 T cells are cytokine-producing rather than cytotoxic (Papadopoulos et al., Cell report 2025, Harper et al., JCI 2022, Mvaya et al., JCInsight 2022)



Our approaches to eliminate HIV reservoirs

Surface env gp120





Zhang *et al.*, 2016

To initiate infection, gp120 fuses with cell membrane via its

- CD4bs to CD4 receptor
- V3 binds to viral coreceptor CCR5

Broadly Neutralising Antibodies (bNAb)

- Secreted by 1-2% of PLH
- Recognise conserved regions of HIV gp120
- short half life!

Single chain variable fragments of bnAbs (scFv)

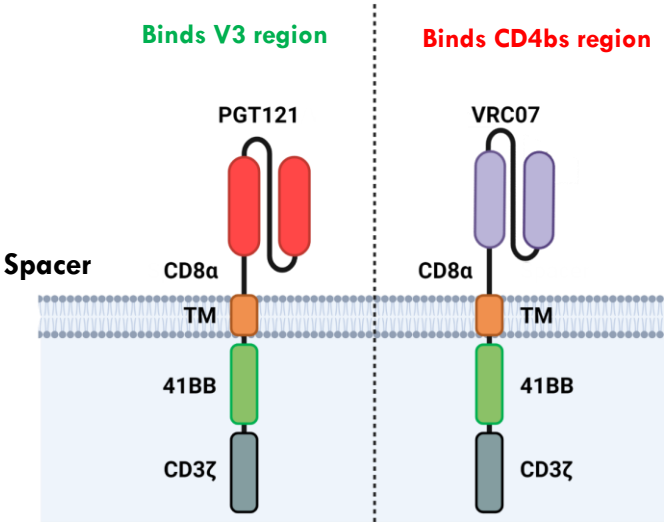
Construct of CAR receptor

CAR
Antigen-binding domain – target gp120
Spacer – provide flexibility
Transmembrane – anchor CAR to membrane
Activation/ persistence (41BB)
Intracellular signal – activation of T cells (CD3ζ)

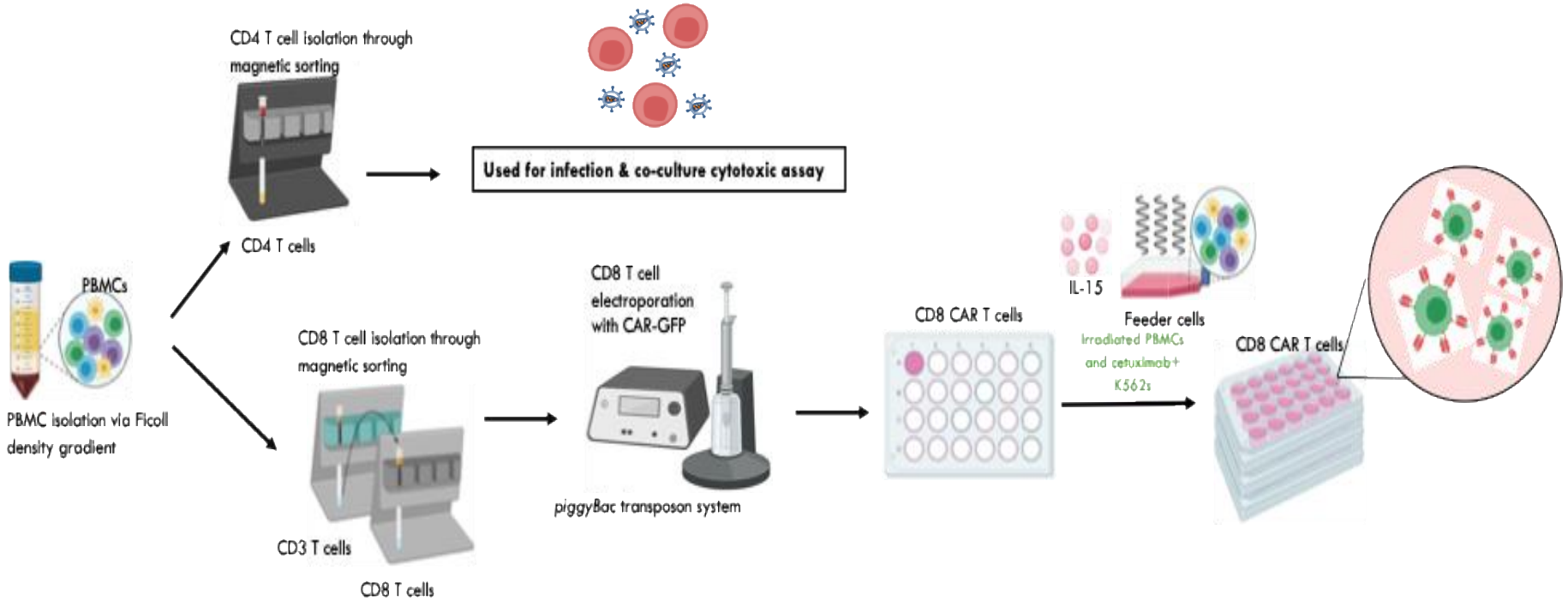
Aim

Generate gp120-specific CD8 CAR T cells to induce killing of infected CD4 T cells thus bypassing MHC Class I downregulation and HIV mutations

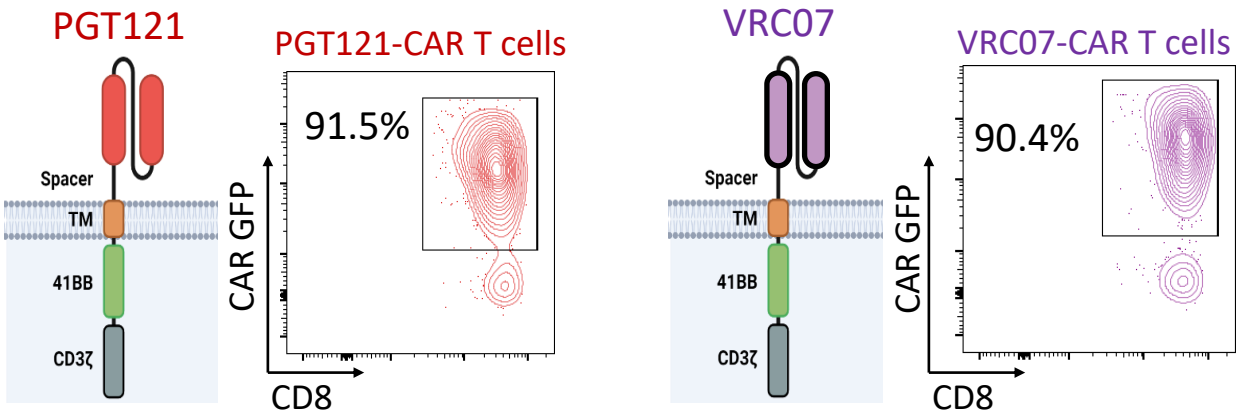
Chimeric Antigen Receptors against HIV gp120

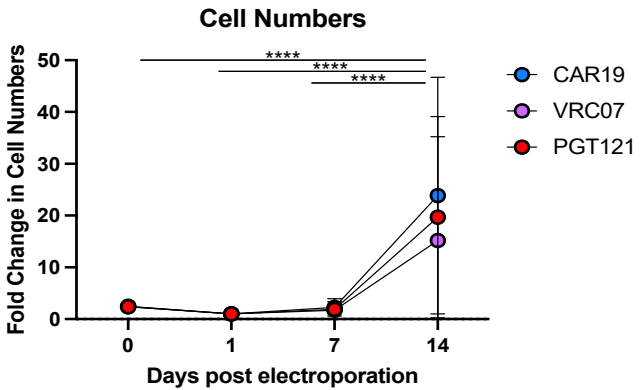


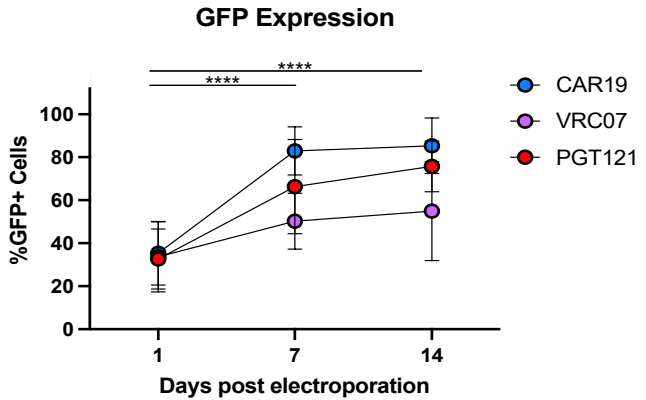
Generation/Expansion of CD8 CAR T cells & infection of CD4 T cells



CD8 CAR T cells expanded and expressed high levels of GFP



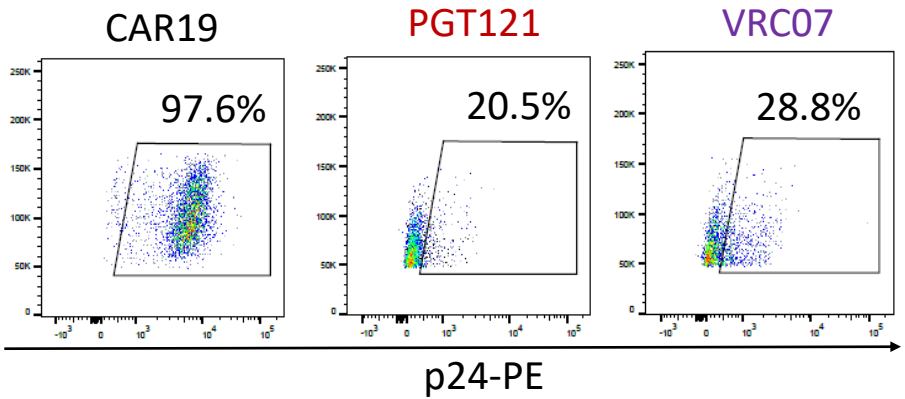


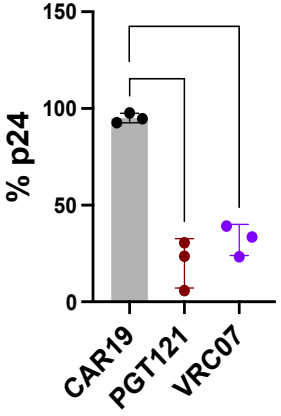


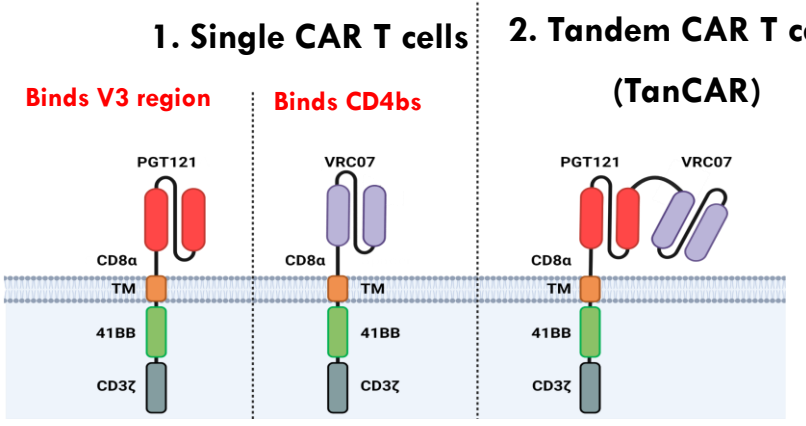
CD8 CAR T cells induced efficient killing in coculture with HIV+ SupT1s

Infect target cells with HIV for 72h

Coculture target cells with CAR T cells for 6 hours

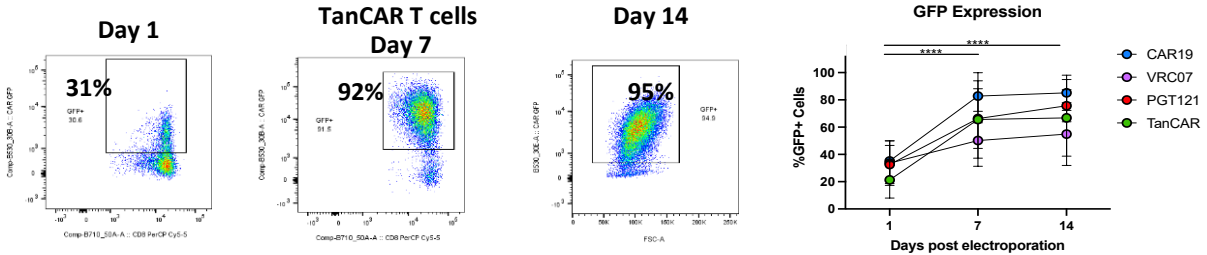




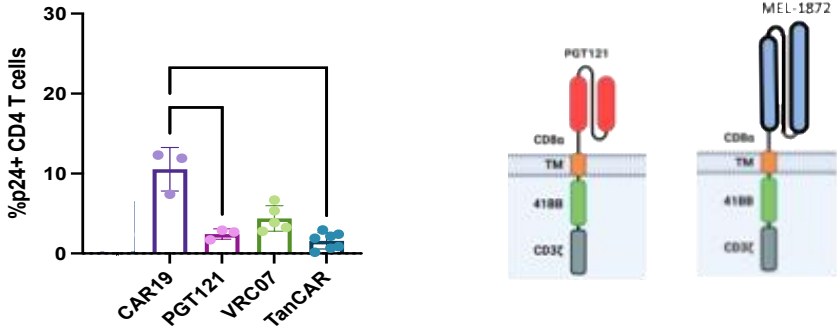


Construction of a tandem CAR T cell to overcome resistance to a single CAR

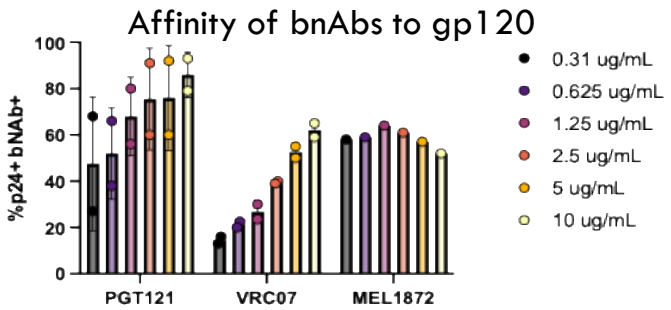
TanCAR T cells expanded and expressed GFP



PG121 and TanCAR T cells induced efficient killing of primary activated CD4 T cells

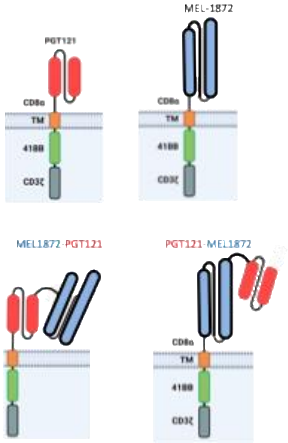


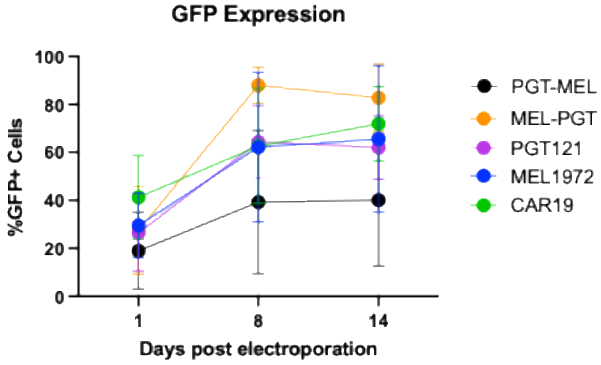
- Low affinity binding of VRC07 to CD4 binding site
- Substitued VRC07 with MEL-1872 (Heydarchi, et a., Cell reports Medicine 2022)
- MEL-1872 has an extraordinary breadth, potency, superior binding affinity to gp120 bs on CD4 due to its ultralong CDRH3 regions compared to previous bNAbs
- It does not cause any auto-reactivity or polyreactivity

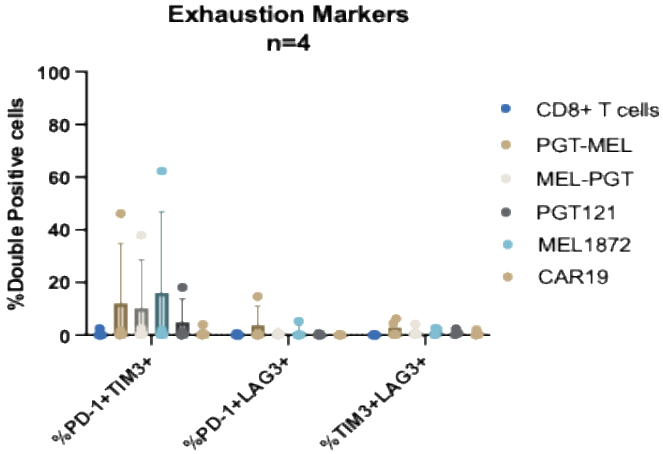


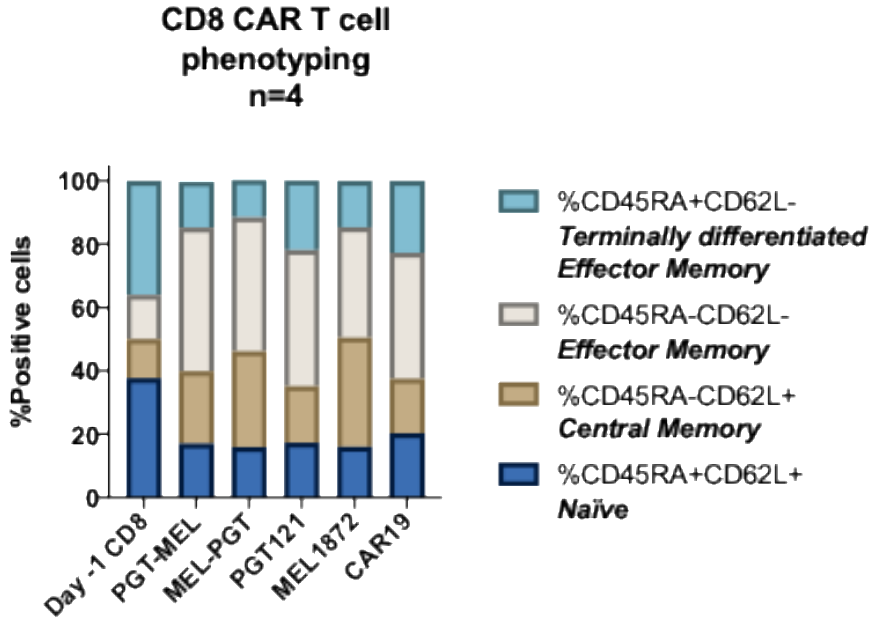
- Infected primary CD4 T cells were incubated with bNAbs (0.31-10µg/mL) against different HIV gp120 epitopes
- They were then stained with secondary Abs to detect bNAbs, followed by intracellular p24
- Double positive cells were quantified to determine the level of infected cells that bound bNAbs

Phenotype of CAR T cells

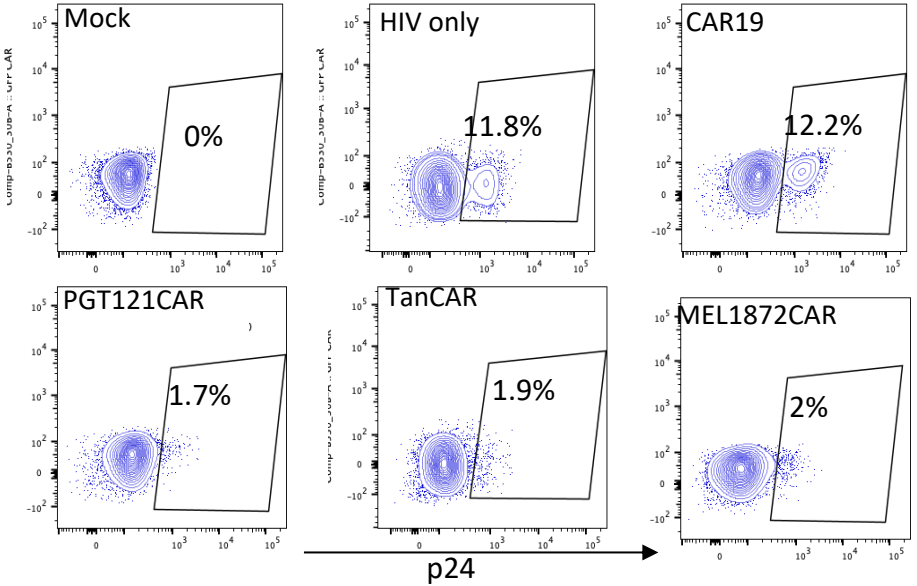


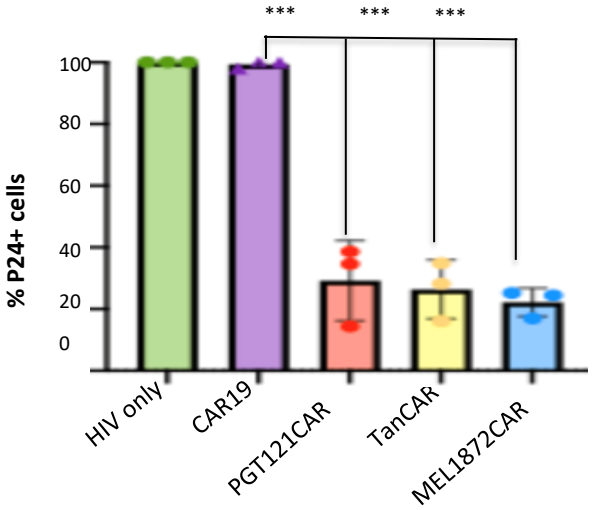






Efficient killing of in-vitro infected HIV CD4 T cells with our CAR designs





Future Directions

- Neutralisation assay to assess whether both CARs on TanCARs are functional
- Assess our CAR constructs ex vivo on cells derived from PLH upon their reactivation with an LRA
- Based on ex vivo data, we will progress the most potent CAR construct for testing in a humanised mice model (WEHI)

Significance

- **Tandem CAR T cells:** by Incorporating multiple bNAbs into CD8 CAR T cells, we can overcome mutation, antibody resistance to a single CAR, and HIV downregulation of MHC-I to have a potent CAR T cell

Acknowledgements

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