Development of T cell-targeted Cocal-pseudotyped lentiviral particles for in-vivo CAR-T cell therapy

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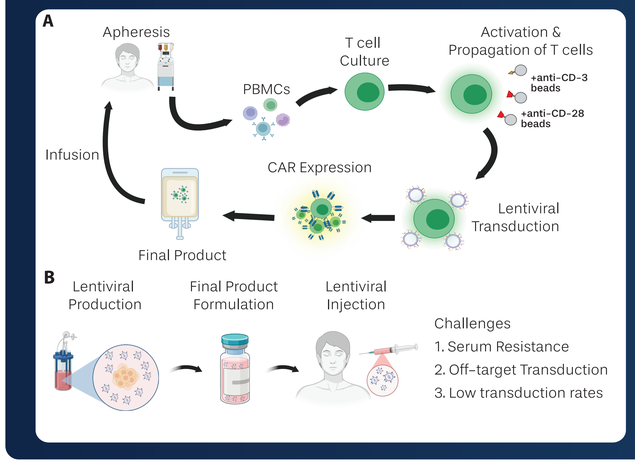
Background

- **CAR T-cell** therapy is a cancer treatment in which a patient's T cells are collected, genetically engineered to express a chimeric antigen receptor that recognizes a tumor antigen, expanded, and reinfused to find and treat the cancer.
- Despite showing remarkable success in **hematologic cancers**. their widespread use is hindered by high relapse rates, limited durability, and **complex manufacturing**[2][3]
- Most CAR-T therapies depend on **lentiviral transduction** to introduce the exogenous receptor into T cells[2]. However, due to the broad tropisms of commonly-used lentiviruses transduction of off-target cancerous cells can entirely abrogate CAR-T cell efficacy by inducing epitope shielding[4].
- Multiple clinical trial products are seeking to use **lentiviruses in-vivo**. However, problems have to be addressed

Traditional CAR Manufacturing (A) vs. Proposed In-Vivo (B)



Our Platform for study of in-vivo therapies

Multi-Domain Fusion (MDF) Ligand.
- Composed of CD80, CD3-scFv, CD58 connected by linkers

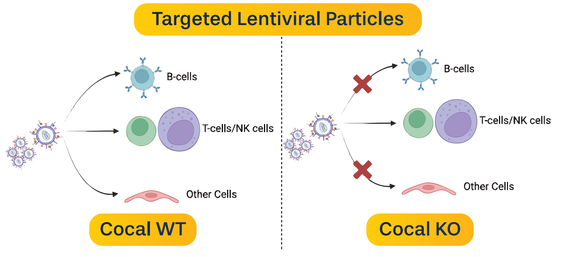
Cocal Envelope Glycoprotein
- Pseudotype of Cocal envelope protein is better suited for in-vivo applications due to its higher serum resistance compared to VSV-G pseudotyped lentiviruses[5].

Transduction Objective

Determine whether different Cocal fusogens, in combination with different costimulatory signals, reduce off-target transduction

Targeting Objective

Determine whether different Cocal fusogens, in combination with different costimulatory signals, produces distinct populations in PBMCs.

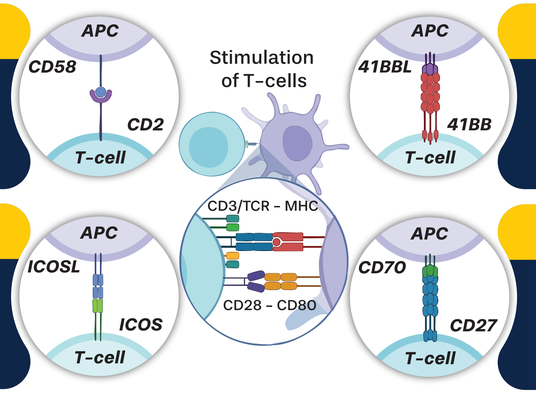


CD2 Costimulaton

In T cells, engagement of CD2 by its ligand CD58, broadly expressed on APCs such as dendritic cells, enhances proliferation, cytokine production, and effector function in **CD8+ T cells**[6].

ICOS Costimulation

ICOS was selected to promote differentiation toward the TH17 subset in **CD4+ T cells**, with this strategy shown to improve tumor control compared to CD28-mediated costimulation[7].



4-1BB Costimulation

4-1BB was included on the viral surface to preferentially expand **CD8+ cytolytic T cells**, based on reports of improved expansion and cytolytic potential over CD28 costimulation[8].

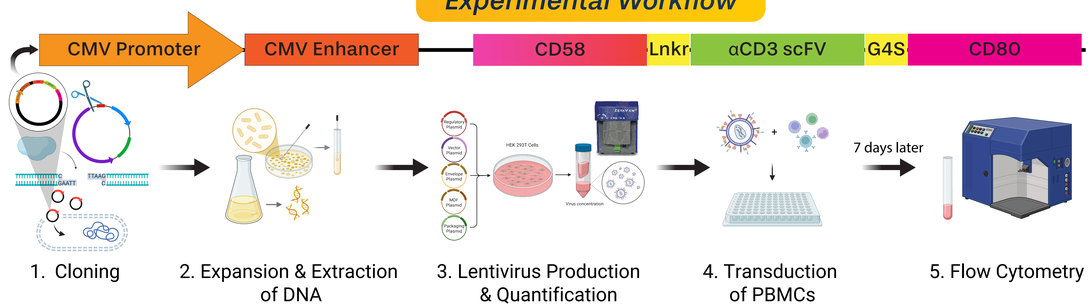
CD27 Costimulation

CD70 was included to lead antigen-dependent **CD8+ T-cell expansion** , and formation of functional cytotoxic memory. CD27 signaling programs more effector-biased, less durable memory[9][10].

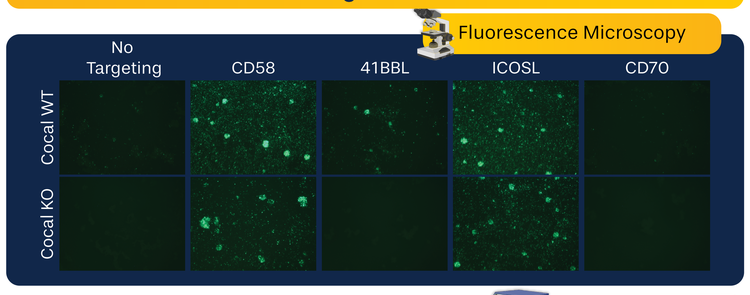
Hypothesis

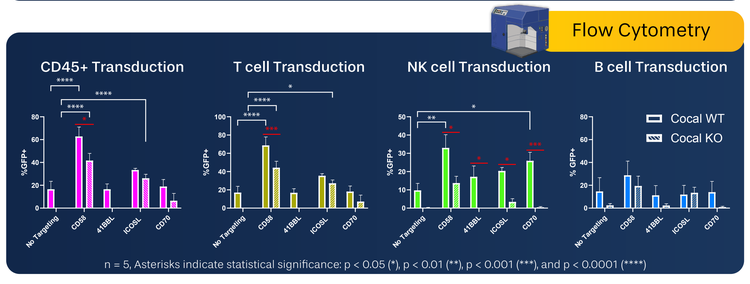
Transduction using mutated, targeted Cocal-pseudotyped lentiviral vectors significantly reduce off-target transduction, while allowing for more control over the population of transduced cells

Experimental Workflow



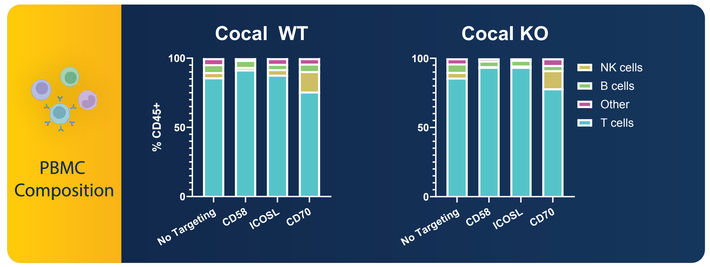
Knocked-out cocal pseudotyped lentiviral particles decrease off-target transduction rates

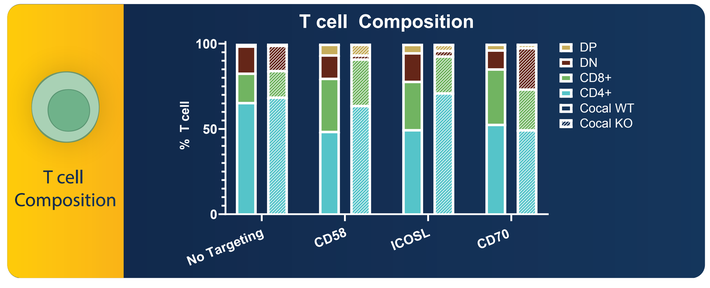




n = 5, Asterisks indicate statistical significance: p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), and p < 0.0001 (****)

Different costimulation signals result in different cell population compositions





Transduction – Observations

- **CD45+ & T cell:** CD58 and ICOSL result in significant transduction. CD58 T cell transduction is affected by Cocal KO.
- **NK Cells:** Cocal KO results in a reduction of transduction in all conditions. CD70 and CD58 increase transduction rate in NK cells.
- **B Cells:** High variability in B cell transduction results in no statistical significance between groups.
- 41BBL is not statistically significant in any of the categories observed.

Targeting – Observations

PBMC Composition
- CD58 and ICOSL results in T-cell outgrowth.
- CD70 results in a NK cell outgrowth
- ICOSL (KO) results in greater T cell outgrowth

T Cell Composition
- ICOSL (KO) increases CD4+ populations.
- CD70 increases DN populations, with a greater effect in KO condition

Conclusions

- **Transduction:** Using Cocal KO envelopes decreases off-target transduction in NK cells. Further testing required for B cells.
- **Targeting:** Using various costimulation signals on lentiviral particle surface allows us to influence PBMC and T cell population compositions

Future Direction

- **Functional Testing:** Cocal KO CD20 CAR Testing with PBMCs, then in small-animal models.
- **Cytokine Profiling:** assess whether different costimulations result in different cytokine release profiles

Acknowledgements

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