Investigating Chimeric Antigen Receptor (CAR) T Cell Nonspecific Killing via CRISPR KO Screen

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BACKGROUND

Chimeric Antigen Receptor (CAR) T Cell Therapy

- Re-engineering of patient immune T cells to contain surface markers (CAR) that recognize and eradicate target cancer cells
- Products against B cell malignancies target CD19 antigen and contain CD137 (41BB) or CD28 costimulatory domains
- Post-treatment relapse remains a prominent issue that is not fully understood

Preliminary Findings

- CD137-costimulated CAR T kills non-target cells lacking CD19 target, unlike CD28-costimulated CAR T
- Understanding this alternative killing mechanism can enhance patient treatment plans and stratification, improving clinical outcomes

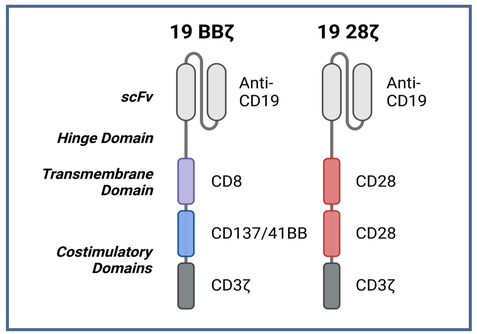

Figure 1. Anti-CD19 CAR construct designs.

What is the mechanism by which CD137-costimulated CAR T cells eliminate cancer cells lacking regular target molecules?

METHODS

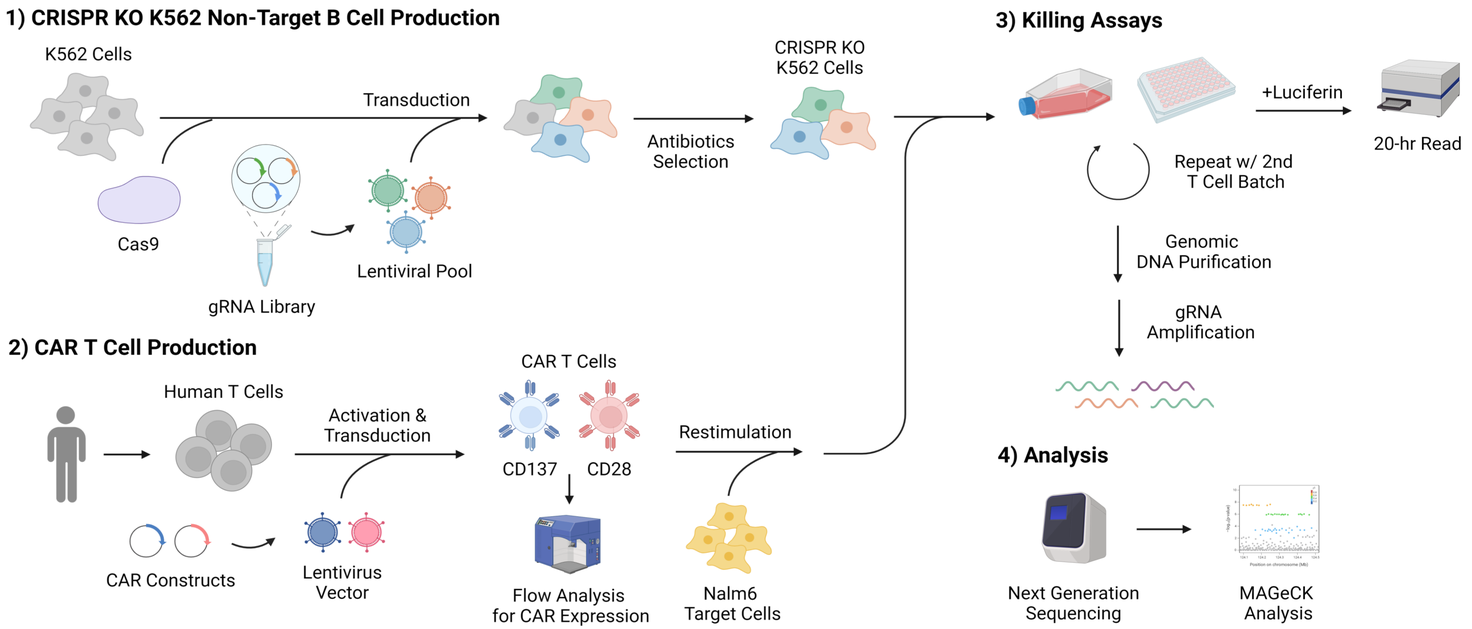

Figure 2. Flowchart for genome-wide CRISPR KO screen.

RESULTS

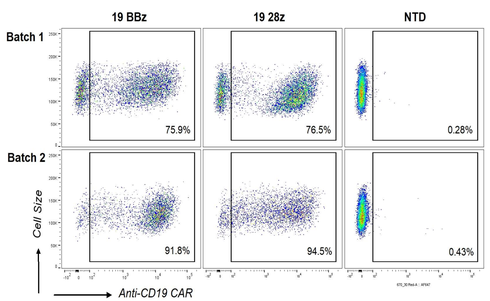

Figure 3. CAR transduction efficiencies for two T cell batches using flow cytometry.

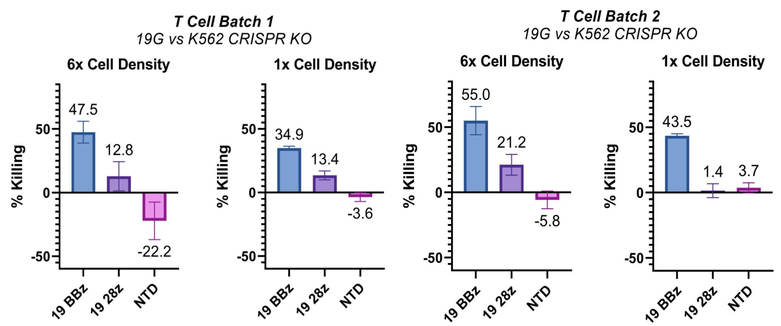

Figure 4. Luciferase-based cytotoxicity assays 20-hr read. Assays were performed with 1:3 effector: target ratio at 1x and 6x cell densities. 2 CAR T cell batches were added 2 days apart to the same K562 cells.

Note: These result track the process of a single sequencing run. This process was repeated multiple times to compile data for final analysis.

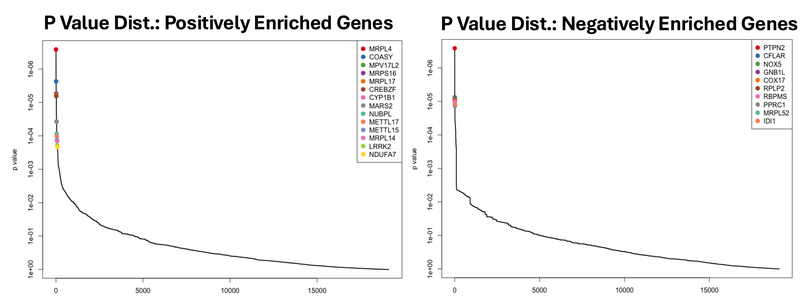

Figure 5. MAGeCK analysis of NGS. P value distributions reveal positively and negatively enriched gRNAs in CD137 CAR T-exposed K562 cells when compared to CD28 CAR T-exposed cells. Top gene hits were analyzed for common pathways.

CONCLUSIONS/FUTURE DIRECTIONS

- Luciferase-based cytotoxicity assays confirms significantly greater nonspecific killing performed by CD137-costimulated CAR T cells on non-target K562 cells.
- MAGeCK analysis of CRISPR KO screen revealed gRNAs that persisted when comparing CD137-exposed K562 cells to CD28-exposed control, with several top hits being mitochondrial related, including MRPL4, COASY, and MPV17L2.
- Overrepresentation of these genes indicate their significance in K562 cell survival and thus the novel killing mechanism performed by CD137-costimulated CAR Ts.

Further experiments:

- Single gene KO experiments on top gene hits to confirm their contribution
- Examine potential pathways involved using caspase inhibitors, ferroptosis inhibitors, anti-IFNγ, and more

REFERENCES

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This research was supported by a College Alumni Research Grant and Frances Velay Fellowship.