CRISPR-Cas9 Knockout of Src Homology-2 Domain-Containing Phosphatases Enhances Anti-Tumor Activity of CAR T cells

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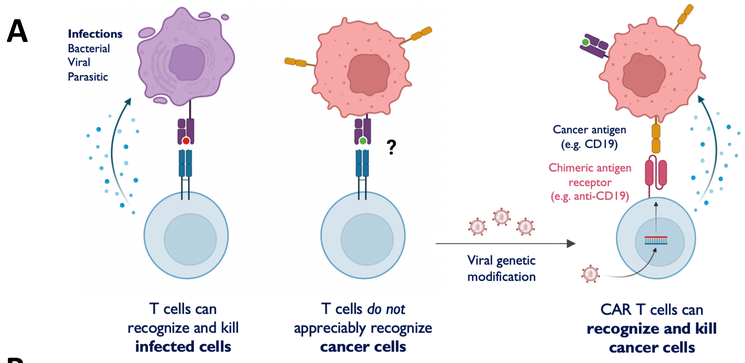
Introduction: Next-generation CAR T Cell Therapy

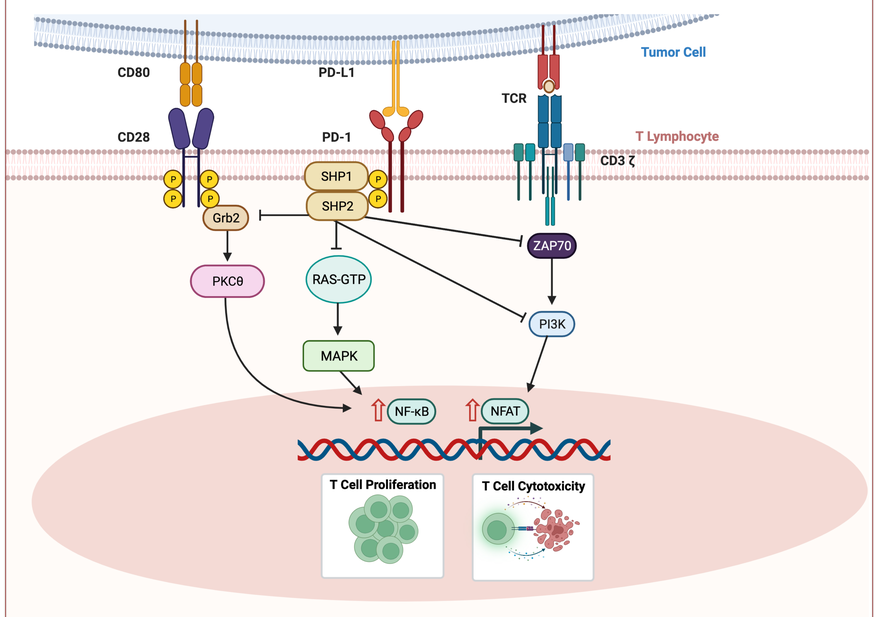


Problem: CAR19 T cell therapies have shown remarkable clinical outcomes in CD19+ B-cell malignancies. However, many patients fail treatment and relapse due to mechanisms of resistance.[2]

Background: Suboptimal CART performance is driven by high expression of immune checkpoints such as PD-1.[3] Mediators of PD-1, such as SHP1 and SHP2, play a large role in suppressing the T cell response.

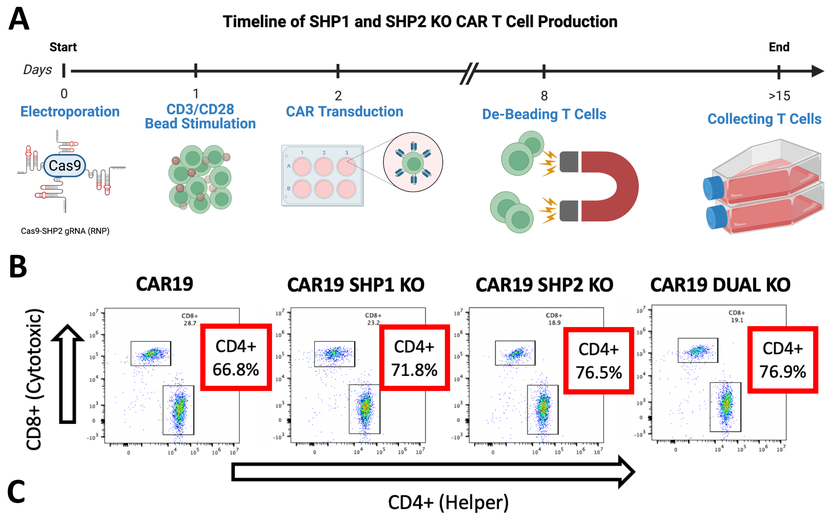
Hypothesis: Expression of SHP1 and SHP2 in CAR19 can be deleted via CRISPR-Cas9 to enhance anti-tumor potential.[4]

SHP Cascade Inhibits T Cell Proliferation and Cytotoxicity

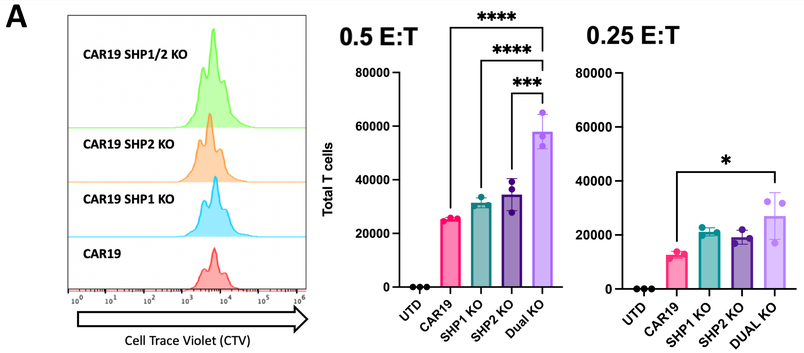


- SHP phosphatase cascade inhibits systemic proliferation, antigen-specific cytotoxicity, and functional metabolism by decreasing activation of NF-κB and NFAT among other protein factors.[4]
- SH2 domains bind to immune receptor tyrosine-based inhibitory motifs (ITIM) and immune receptor tyrosine-based switch motifs (ITSM) on the cytosolic surface of PD-1 receptors.

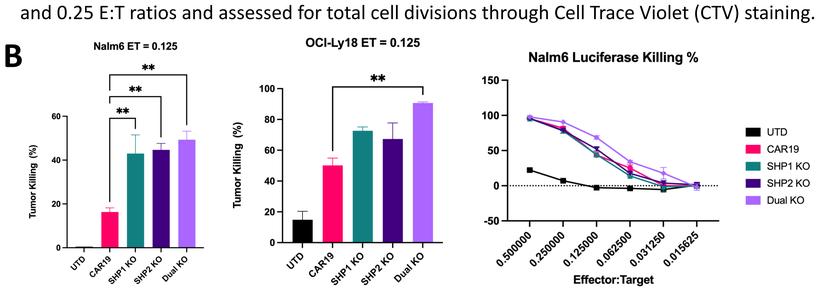
Methods: CART Manufacturing Timeline and Expansion Summary



Results: CAR19 SHP KO Enhances Anti-Tumor Response

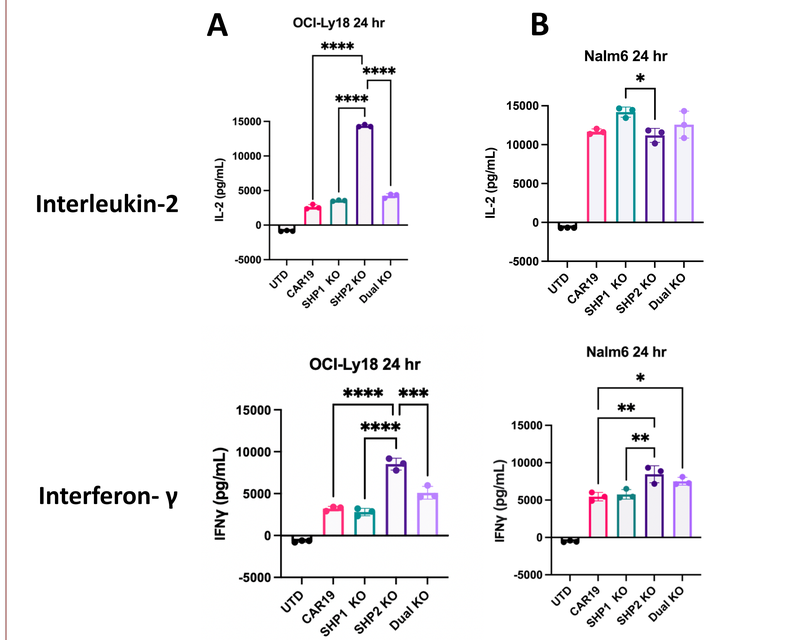


Cell Trace Violet Proliferation Assay: CAR19 effectors and irradiated OCI-Ly18 were plated at 0.5 and 0.25 E:T ratios and assessed for total cell divisions through Cell Trace Violet (CTV) staining.



Luciferase Killing Assay: CAR19 effectors, Nalm6, and OCI-Ly18 cancer cells were plated at various E:T ratios and assessed for cytoxicity through luciferase uptake at 24, 48, and 72-hour timepoints.

Results: ELISA and Cytokine Secretion



- SHP2 KO led to substantial spikes in secretion of IL-2 and IFN**γ** in Oci-Ly18.
- SHP1 and SHP2 KO secrete similar levels of IL-2 and TNFα in Oci-Ly18 and Nalm6.

Enzyme-Linked Immunosorbent Assay: CAR19 effectors, OCI-Ly18, and Nalm6 cancer cells were plated at 0.25 E:T ratio and protein-rich supernatant was collected at 24 and 48 hrs.

Conclusions and Future Directions

Conclusions:

- CAR19 SHP KO T cells have higher absolute counts, luciferase killing %, and cytokine secretion compared to standard CAR19.
- Expansion of CAR19 SHP KO selectively favors CD4+ differentiation, revealing a potential exhaustion-resistant mechanism.
- SHP2 has low expression in resting T-cells and may be activation-dependent.

Future Directions:

- Initiate murine *in-vivo* studies to understand proliferation and localization of CAR T cells.
- Explore the role of phosphatases in human T cells through screens and NGS experiments.

References and Acknowledgements

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