Poster # 342

KRAS G12V T cell receptor-engineered T cells expressing the durability FAS-41BB switch receptor exhibit a potent, persistent, coordinated CD4/CD8 anti-tumor response *in vitro* and *in vivo*

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Abstract

Adoptive cell therapy with genetically modified T cells has shown promising efficacy in solid tumors but has been limited by immunosuppressive mechanisms that interfere with sustained activity including FAS ligand-induced apoptosis of tumor-infiltrating, FAS receptor-positive lymphocytes [1]. As previously reported, T Cell Receptor (TCR)-engineered T cells expressing a FAS-41BB switch receptor, consisting of FAS extracellular and 41BB intracellular domains, demonstrated improved anti-tumor efficacy [2]. KRAS is a frequently mutated oncogene in cancers [3] and recent clinical evidence suggests that it is immunogenic and targetable via TCR-engineered T cells [4]. Targeting a mutated oncogenic driver such as KRAS G12V offers many advantages, including tumor dependence driving homogenous expression and decreasing risk of therapeutic escape. We are now reporting an optimized construct that achieves high functional co-expression of the KRAS TCR, CD8αβ, and FAS-41BB switch receptor in a single viral vector.

Methods

Human CD4/CD8 T cells isolated from healthy volunteers were lentivirally transduced with constructs encoding the KRAS TCR, CD8αβ chains, and FAS-41BB. Preclinical studies included peptide titrations with the index peptide and ones in which one residue was individually substituted to all possible amino acids (XScan), co-cultures with tumors or B-LCL, and *in vivo* subcutaneous xenografts.

Options To Optimize 5-Parameter Lentivirus

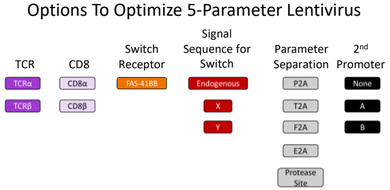


Fig 1: Expression

FACS analysis confirms expression of A11 G12V TCR, CD8αβ, and FAS-41BB in primary T cells.

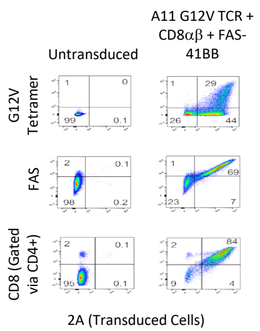


Fig 2: Cell Line Reactivity

CD137 expression on transduced CD8 T cells co-cultured with A11 KRAS G12V mutant cell lines demonstrates reactivity to endogenous KRAS mutant peptide presented by MHC class I.

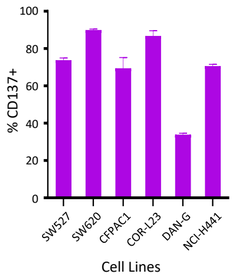


Fig 3: TCR-T Expansion

SW527 co-cultured with transduced mixed CD4/CD8 T cells, which were transferred to new well with fresh SW527 (↓). Dramatic expansion occurred in the absence of exhaustion despite repeat stimulations.

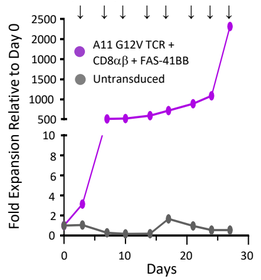


Fig 4: TCR-T Expansion

SW527 co-cultured with transduced CD4, CD8, or mixed CD4/CD8 T cells, which were transferred to new well with fresh SW527 (↑). Better expansion occurred with CD4 and CD8 T cell mixture.

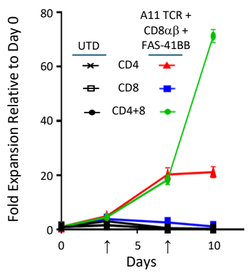


Fig 5: *In Vitro* Efficacy

Transduced CD4, CD8, or mixed CD4/CD8 T cells co-cultured and rechallenged (↑) with SW527. Better tumor control with CD4 and CD8 T cell mixture.

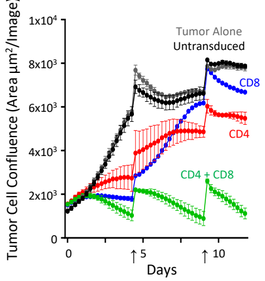


Fig 6: XScan

Transduced CD8 T cells stimulated with potential off-target peptides predicted by XScan. Even at high peptide concentration only the RAB7B peptide shows any evidence of reactivity.

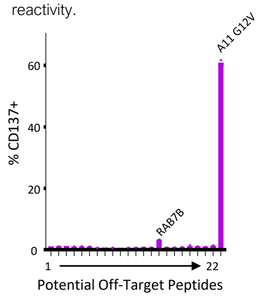


Fig 7: Off-Target

XScan predicted a single potential off-target in the genome, RAB7B, but peptide failed to stimulate transduced CD8 T cells at physiologic concentrations demonstrating lack of autoreactivity.

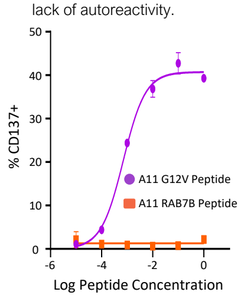


Fig 8: Cytokine-Independent Persistence

Transduced CD4/CD8 T cells expanded with cytokine support for 2 weeks, followed by cytokine removal. Lack of persistence in the absence of exogenous cytokines supports potential safety profile.

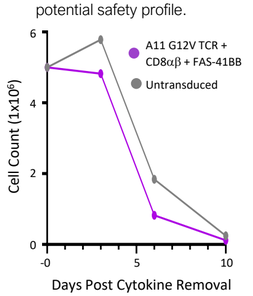


Fig 9: *In Vivo* Efficacy

Single intravenous administration of $1x10^7$ CD4/CD8 TCR-T 10 days after subcutaneous inoculation of SW527 leads to 4 out 5 mice achieving a complete response.

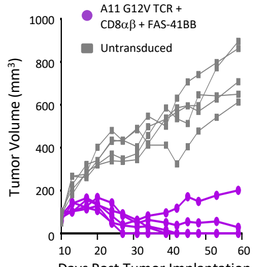
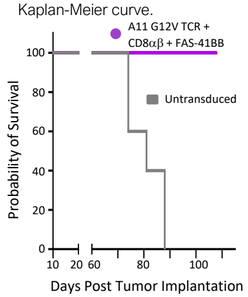


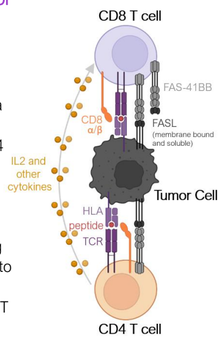
Fig 10: *In Vivo* Survival

Single intravenous administration of $1x10^7$ CD4/CD8 TCR-T 10 days after subcutaneous inoculation of SW527 leads to long term survival as demonstrated by Kaplan-Meier curve.



Coordinated CD4/CD8 Response and FAS-41BB Switch Receptor

- T cells transduced with high affinity TCR specifically recognize KRAS G12V presented by HLA-A*11:01
- CD8 α/β co-receptor drives a coordinated CD8/CD4 T cell response by allowing for CD4 stimulation that promotes CD8+ T cell functional persistence
- FAS-41BB switch receptor increases fitness while acting as a FAS dominant negative to protect from tumor, endothelium, and stimulated T cell-derived FASL-induced apoptosis



Summary

- 5-parameter lentivirus able to express A11 KRAS G12V TCR + CD8ab + FAS-41BB switch receptor
- Inclusion of CD8αβ allows for stimulation of CD4 T cells and coordinated immune response resulting in a superior product
- Transduced T cells respond to multiple cell lines expressing endogenous KRAS G12V, allowing for *in vivo* efficacy
- No cytokine-independent persistence and off-target specificities not detected
- Preclinical studies support the clinical development of this first-in-class product

References

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