Poster #10

Non-Viral Engineered T Cell Therapy Specific for the Hotspot Mutation p53 R175H that Integrates Signal 1 (TCR), Signal 2 (Co-Stimulation) and Signal 3 (Cytokine) and Co-Opts FASL-Dependent Apoptosis to Achieve a Sustained Coordinated Antitumor CD4/8 T Cell Response

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Abstract

Adoptive T cell therapy (ACT) has demonstrated antitumor efficacy in patients with solid cancers but requires further optimization to become a reproducibly effective treatment. T cell receptor (TCR)-engineered T cells recognize peptides derived from intracellular and surface proteins presented in the context of MHC class I. Targeting mutated oncogenic drivers addresses many of the major obstacles of this modality, in that the antigenic epitope is: 1) tumor-specific, 2) essential for tumor survival, and 3) derived from a stably expressed protein. However, the immune-suppressive tumor microenvironment makes further optimization of engineered T cells necessary to bring long-term clinical benefit to patients. For an optimal anti-tumor response, T cells require three signals: TCR, co-stimulation, and cytokine signaling. The tumor suppressor *TP53* is the most frequently mutated gene across human cancers, with a highly recurrent arginine to histidine hotspot alteration in codon 175 leading to novel tumor-dependent functions. Here we report the use of a novel CRISPR-Cas nuclease system to knock-in a six-gene multi-cistronic cassette into the *TRAC* locus with high efficiency. We employed several strategies to maximize the potency and durability of a TCR-T cell product targeting the p53 R175H oncogenic driver, including: 1) A high-affinity TCR (α and β chains) specific for the p53 R175H mutation presented by HLA-A*02:01 permits the recognition of tumor cells expressing even low levels of the epitope (**Signal 1**), 2) Inclusion of the CD8αβ co-receptor drives stimulation of CD4+ T cells with the MHC class I restricted TCR, allowing for a physiologic coordinated immune response required for maximal efficacy, 3) A FAS-41BB switch receptor acts as a dominant negative to the FASL-inducing apoptotic signal in the tumor microenvironment and drives stimulation and persistence of the T cell product via 41BB co-stimulatory signaling (**Signal 2**), 4) A chimeric cytokine receptor (constitutive Interleukin Receptor) promotes expansion and survival while avoiding immunologic exhaustion (**Signal 3**). Together, these strategies deliver the three signals required for maximal T cell function: antigen-driven activation, co-stimulation, and growth/survival-promoting cytokine signaling. The non-viral *TRAC*-knocked-in T cells demonstrate robust and specific cytotoxicity against endogenously expressing HLA-A*02:01 and p53 R175H cell lines *in vitro* and effective anti-tumor activity *in vivo* while maintaining a favorable preclinical safety profile. These data support the planned clinical development of a novel non-viral *TRAC*-knocked-in T cell therapy for the treatment of p53 R175H-mutant solid tumors.

Fig 1: TP53 R175H CRISPR-Based Non-Viral TCR-T

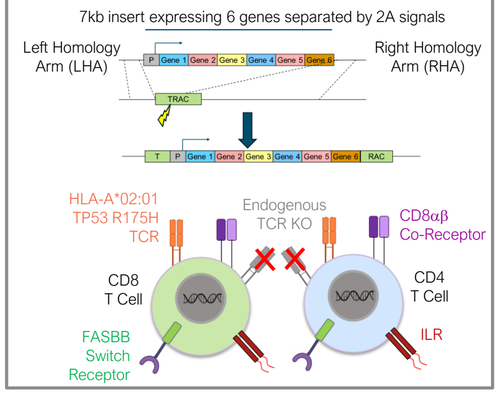


Fig. 2: Armoring in Product

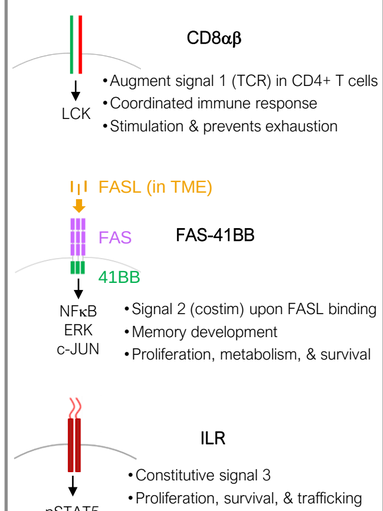


Fig. 3: Epitope-Specific Activation

IFNγ secretion only by genetically-engineered primary CD4/8 TCR-T cells co-cultured with HLA-A*02:01 TP53 R175H mutant cell line demonstrates specific reactivity to endogenous TP53 mutant peptide presented by restricted MHC class I.

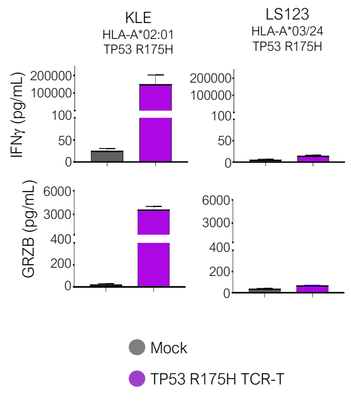


Fig. 4: Cytotoxicity & Selectivity *In Vitro*

Genetically-engineered primary CD4/CD8 TCR-T cells control TYK-nu tumor cells, an endogenously expressing HLA-A*02:01 TP53 R175H line, even after rechallenges (↑). No control of antigen-negative HCT116 (HLA-A*02:01 TP53 WT) even at higher effector to target ratio (inset).

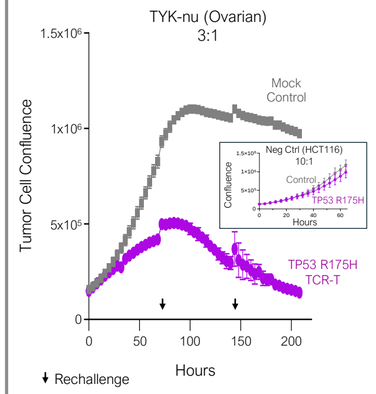


Fig. 5: Low Risk of Cross-Reactivity

X-Scan predicted 294 potential off-targets in the genome but testing showed only 4 surpassed 10% activity of index peptide. However, all >1000-fold less sensitive suggesting low probability of autoreactivity.

X-Scan		
EC90	Potential Reactive Peptides	Validated Peptides
1uM	294	0

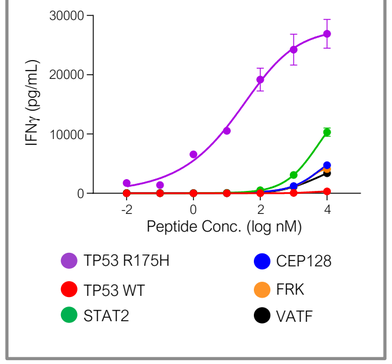


Fig. 6: No Cytokine Independent Survival

Genetically-engineered primary CD4/CD8 TCR-T cells were expanded with cytokine support for 2 weeks, followed by cytokine removal. Lack of persistence in the absence of exogenous cytokines supports potential safety profile of 6-gene construct.

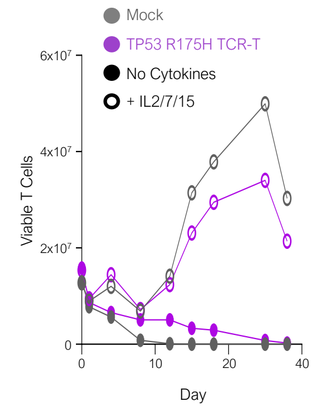


Fig. 7: High Transgene Expression

Transgene expression similar between primary CD4/CD8 TCR-T cells genetically-engineered with knock-in expressing either 5- or 6-genes demonstrating minimal impact of length or position within the construct.

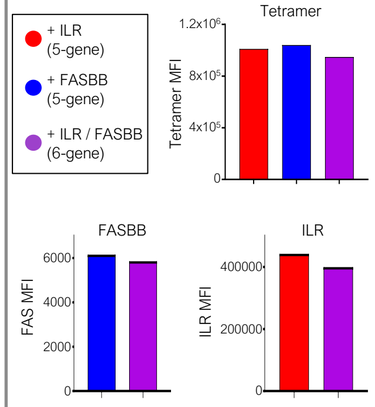


Fig.8: ILR Increases Knock-In Over Time

ILR expression leads to an increase in genetically-engineered TCR-T from 6 to 12 days post knock-in allowing for >50% efficiency with the 7kb construct expressing 6 genes. A higher knock-in percentage may further increase maximum manufacturable dose level while decreasing cost-of-goods.

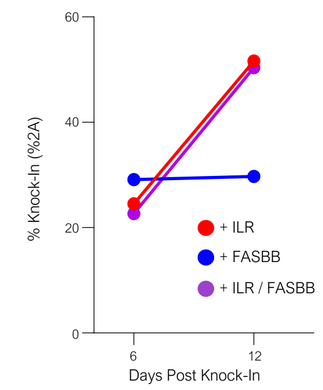


Fig.9: Rescue From FAS Ligand Death

FASBB switch receptor acts as a FASR dominant negative in engineered primary CD4/CD8 TCR-T allowing for resistance to apoptosis-inducing FASL. This may protect tumor-infiltrating TCR-T cells from death.

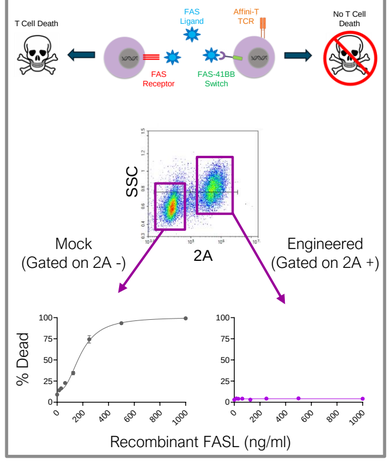
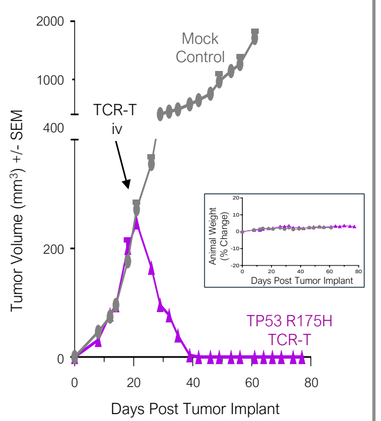


Fig. 10: Complete Responses *In Vivo*

Single intravenous administration of $5x10^6$ primary CD4/CD8 TCR-T 20 days after subcutaneous inoculation of TYK-nu leads to 5 of 5 mice achieving a complete response in the absence of body weight loss (inset).



Summary

- Delivery of all 3 signals for full stimulation of both CD8+ & CD4+ T cells
- Robust & specific cytotoxicity demonstrated both *in vitro* and *in vivo*
- Promising preclinical safety with minimal off-target recognition & no cytokine-independent survival
- Methodology yields high percentage of engineered T cells expressing transgenes
- Program poised to enter preclinical IND-enabling studies in 2024

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