Directing a high avidity KRAS G12D-specific TCR engineered with a CD8α/β co-receptor and chimeric cytokine receptor using non-viral knock-in enhances anti-tumor responses

Poster # 355

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Abstract

T cells engineered with T cell receptors (TCRs) recognizing epitopes derived from intracellular oncogenic drivers like mutant KRAS, the most frequently altered driver oncogene in human cancers, have the potential to induce durable responses in patients with solid tumors. AFNT-212 is an engineered T cell therapy that uses non-viral targeted knock-in (KI) at the TCRα constant chain (*TRAC*) locus to express a multi-cistronic cassette that includes 1) a high-affinity TCR specific for the *KRAS G12D* mutation, 2) a CD8α/β coreceptor, and 3) a chimeric cytokine receptor. AFNT-212 cells demonstrated cytotoxicity against endogenously expressing HLA-A*11:01+/KRAS G12D+ cell lines *in vitro*, and mediated robust anti-tumor activity *in vivo*. Engineered cells also demonstrated a favorable safety profile for the *KRAS G12D* specific TCR and gene editing reagents. Our work supports the planned clinical development of AFNT-212 as a novel non-viral KI TCR-engineered T cell therapy for *KRAS*-mutant solid tumors.

AFNT-212: CRISPR-based non-viral KRAS G12D TCR T cell therapy

Autologous CD4+ and CD8+ T cells were engineered using MG29-1, a novel type V CRISPR-Cas system[1,2], to knock-out the endogenous *TRAC* locus and simultaneously knock-in (KI)[3] non-virally delivered transgenes within the *TRAC* locus. AFNT-212 cells express:

- A high avidity HLA-A*11:01-restricted TCR specific for the KRAS G12D mutant peptide
- The CD8 α/β co-receptor that can drive a coordinated CD8/CD4 T cell response by allowing for CD4 stimulation that promotes CD8+ T cell functional persistence
- An Interleukin Receptor, ILR, a fusion protein that promotes anti-tumor activity through increased T cell proliferation and survival

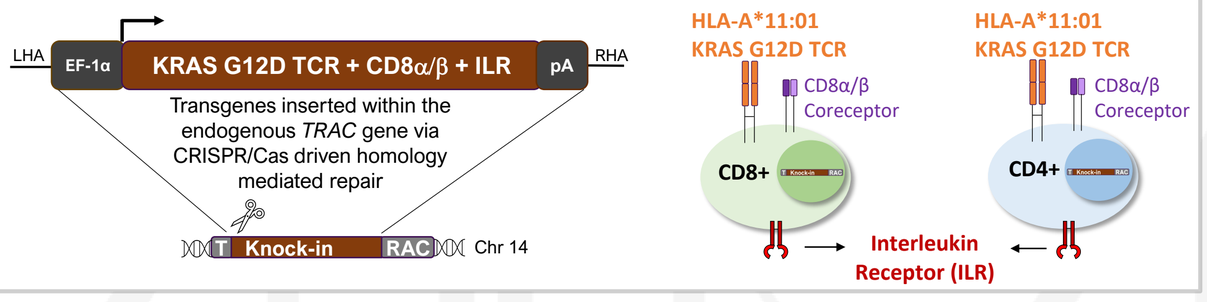


Fig. 1: AFNT-212 T cells bind KRAS G12D peptide with high functional avidity and show robust cytotoxicity *in vitro*

AFNT-212 TCR T cells are activated specifically by the KRAS G12D peptide even at sub-nanomolar concentrations (left) and showed robust cytotoxicity against HuCCT1 tumor cells (expressing endogenous KRAS G12D and HLA-A*11-01).

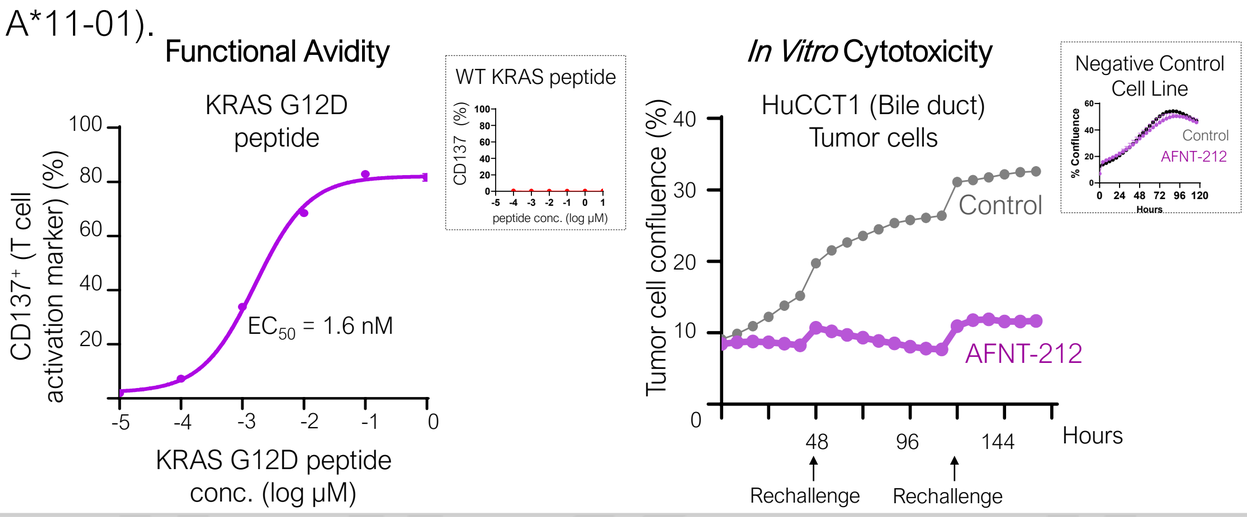


Fig. 2: AFNT-212 T cells shows robust tumor cell control *in vivo*

AFNT-212 cells were intravenously administered in HuCCT1 or CL40 tumor bearing NSG mice. Treatment with AFNT-212 cells resulted in durable robust responses in both murine xenograft models including the CL40 model injected with a low dose of TCR T cells to stress test AFNT-212 T cells.

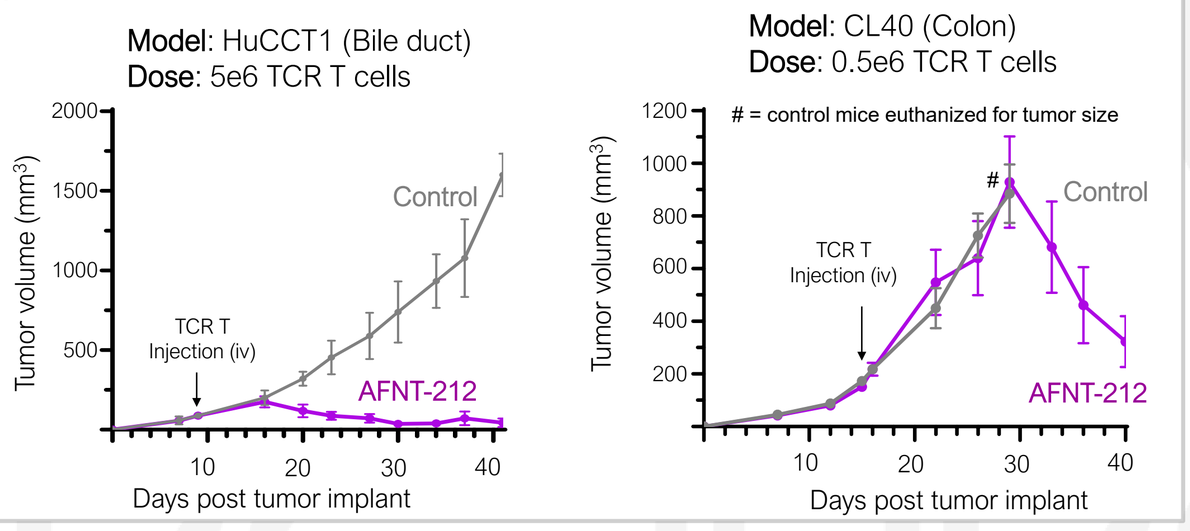
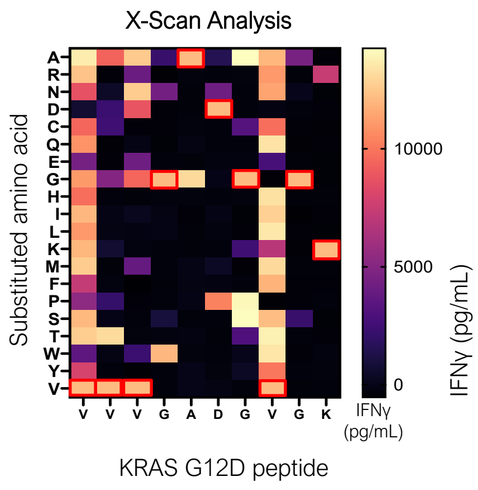


Fig. 3: AFNT-212 T cells exhibit low risk of cross-reactivity

Potential cross-reactive peptides from the human genome were identified using an X-scan assay. None of the candidate potentially cross-reactive peptides activated TCR T cells even at a supraphysiologic concentration demonstrating high specificity of the KRAS G12D TCR.



Validation of putative cross-reactive peptides

EC90	Potential cross-reactive Peptides Identified by X-Scan	Validated Cross-reactive Peptides
197 nM	211	0

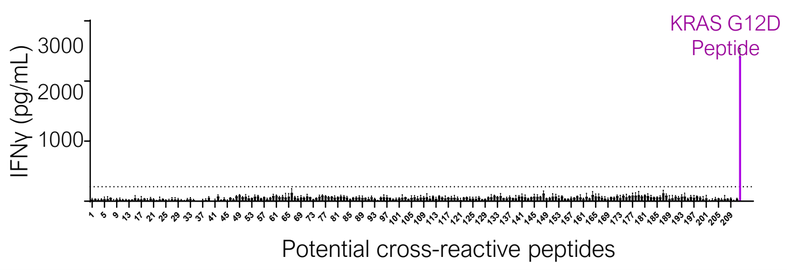


Fig. 4: Gene editing (GE) reagents show high specificity

Potential off-target sites for GE reagents[1,2] (MG29-1 and *TRAC* gRNA) identified using *in silico* prediction (left) and oligo-capture method (right) were assayed for insertions and deletions in GE T cells using a targeted sequencing assay. None of the potential off-targets showed significant activity.

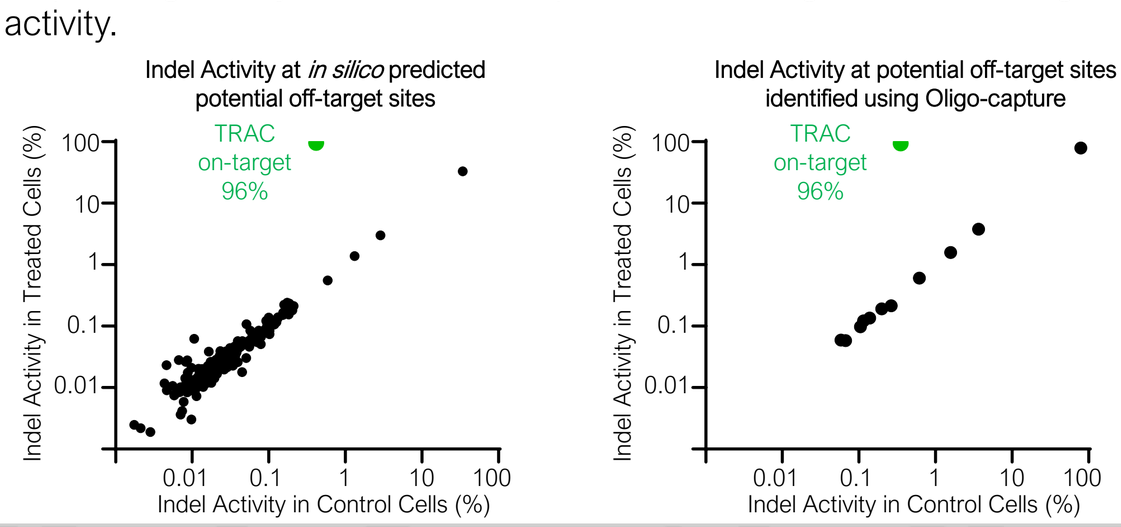


Fig. 5: Non-viral KI achieves high efficiency of transgene integration

Non-viral KI can achieve >40% transgene integration efficiency in T cells from healthy donors. The process performed similarly at a research scale and a 10X scale up version. Engineered T cells demonstrated robust expansion and readily achieved sufficient cell yields for clinical application.

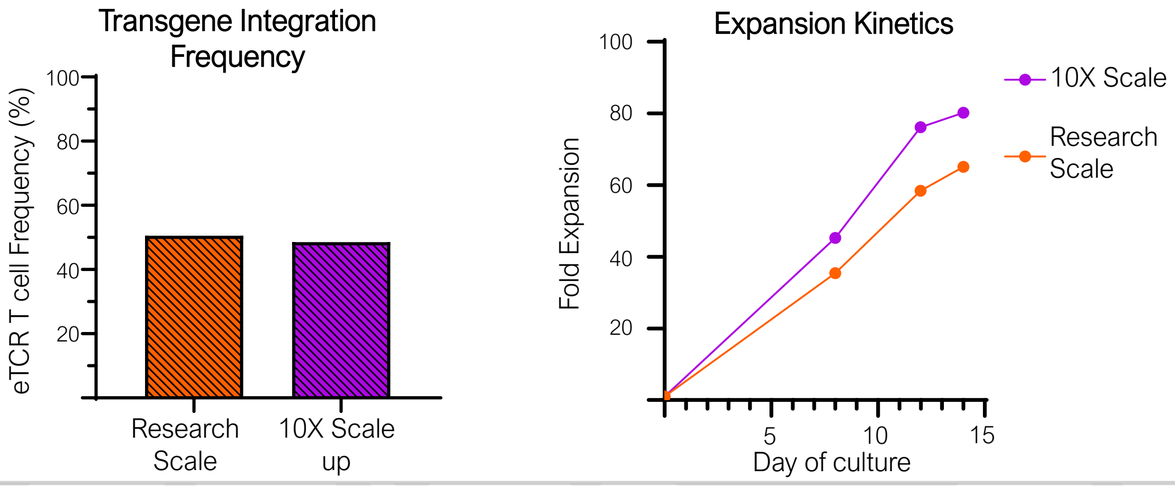
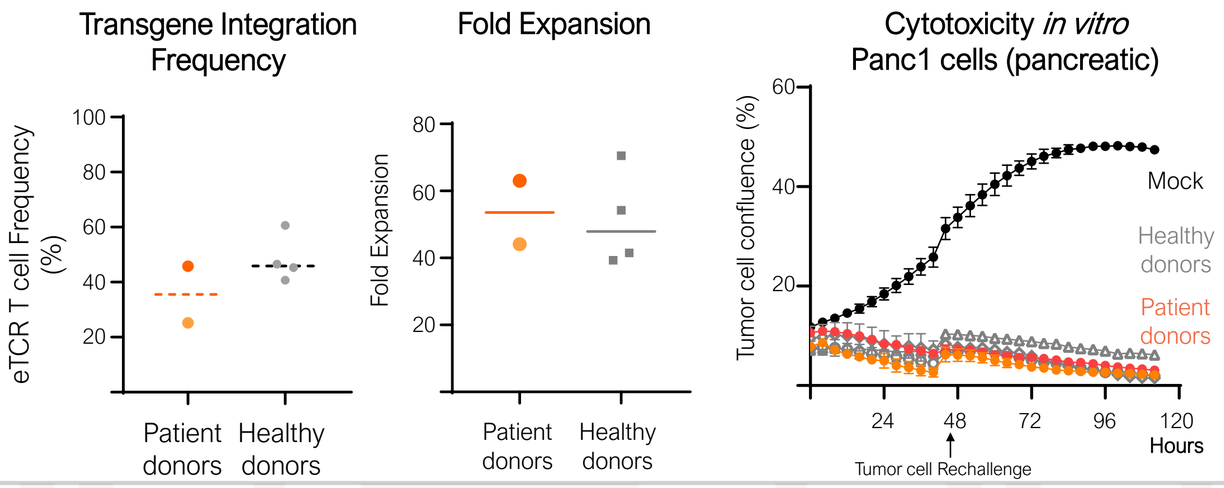


Fig. 6: KI-engineered T cells from patient donors show robust expansion and cytotoxicity

T cells from patient donors and healthy donors were engineered using non-viral KI process. Patient TCR T cells showed similar KI efficiency, expansion and cytotoxicity as healthy donor TCR T cells.



Summary

- AFNT-212 engineered TCR T cells show high functional avidity and *in vitro* cytotoxicity against a panel of *KRAS G12D* positive tumor cell lines.
- Engineered TCR T cells control tumor cell growth *in vivo* even with as few as 500,000 cells suggesting the transferred TCR T cells exhibit capacity to proliferative.
- AFNT-212 has low risk of mediating off-target/off-tumor toxicity from cross-reactivities
- Gene editing reagents used for manufacturing show low risk of genotoxicity
- Non-viral KI can achieve high transgene integration efficiency and cell growth to yield sufficient numbers of engineered TCR T cells for clinical application
- AFNT-212 program is poised to enter clinical testing in 2024

References

1. Goltsman et al., Novel Type V-A CRISPR effectors are active nucleases with expanded targeting capabilities, CRISPR J., 2020.
2. Lamothe et al., Novel CRISPR-associated gene-editing systems discovered in metagenomic samples enable efficient & specific genome engineering. CRISPR J. 2023
3. Schober et al., Orthotopic replacement of T-cell receptor α- and β-chains with preservation of near-physiological T-cell function, Nature Biomed. Engg., 2019.

See SITC Abstract #1223 for CRISPR/Cas Mediated Non-Viral Knock-In