Poster # 9

AFNT-212: A TRAC-knocked-in KRAS G12D-specific TCR-T cell product enhanced with CD8αβ and a chimeric cytokine receptor for treatment of solid cancers

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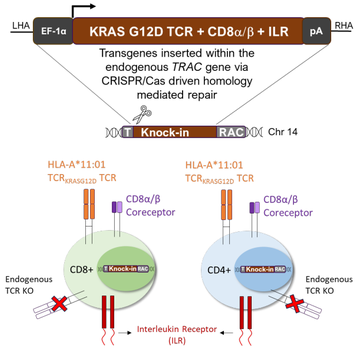
Abstract

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 and durable 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 with enhanced activities for KRAS-mutant solid tumors.

Fig.1: AFNT-212: CRISPR-based non-viral KRAS G12D TCR T cell therapy



AFNT-212 is a cellular therapy consisting of autologous CD4+ and CD8+ TCR-T cells engineered to recognize the HLA-A*11:01-restricted oncogenic driver KRAS G12D mutation. AFNT-212 T cells express:

- A high avidity HLA-A*11:01-restricted TCR specific for the KRAS G12D mutant peptide
- The wildtype CD8 (CD8α/β) coreceptor intended to trigger a coordinated CD4+ and CD8+ T cell immune response against cancer
- An Interleukin Receptor, ILR, that may promote anti-tumor activity through increased T cell proliferation, survival and trafficking

T cells are engineered using MG29-1, a CRISPR-Cas12a system, to knock-in (KI) the transgenes within the TRAC locus where the transgenes are delivered non-virally as a plasmid. The transgene KI simultaneously disrupts the endogenous TRAC locus abrogating the endogenous TCRα chain expression.

Fig. 2: 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 as assessed using CD137 expression (left). Wildtype KRAS peptide did not activate AFNT-212 TCR-T cells (left inset). AFNT-212 TCR-T cells showed robust cytotoxicity against HLA-A*11:01 restricted KRAS G12D expressing CL40 colon tumor cells even following rechallenges with fresh tumor cells (↑) (right). Control cell line expressing wildtype KRAS did not induce cytotoxicity (right inset).

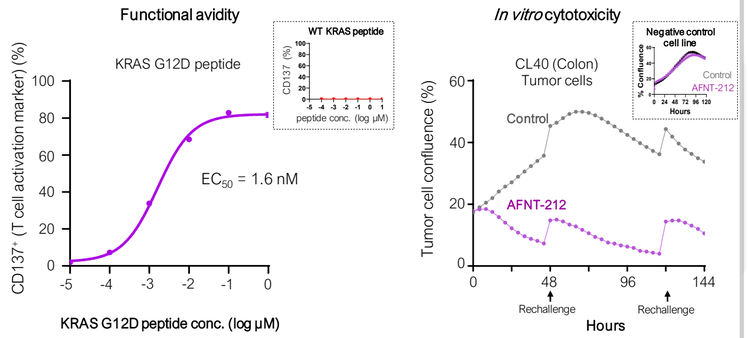


Fig. 3: AFNT-212 T cells exhibit low risk of cross-reactivity

Potential cross-reactive peptides from the human genome were identified using an X-scan assay. None of the potentially cross-reactive peptides activated TCR-T cells even at a supraphysiologic concentration demonstrating high specificity of the KRAS G12D TCR (top). No alloreactivity was observed for AFNT-212 TCR-T cells when tested against a panel of B-Lymphoblastoid Cell Lines (B-LCLs) expressing the most frequent HLA subtypes reported in >99% of the US population (bottom).

Validation of putative cross-reactive peptides

EC90	Potential cross-reactive peptides identified by x-scan	Validated cross-reactive peptides
197 nM	211	0

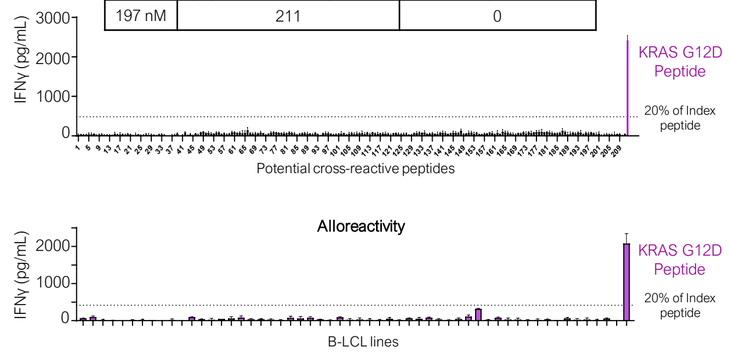


Fig. 4: AFNT-212 T cells shows robust tumor cell control *in vivo*

AFNT-212 cells were intravenously administered in CL40 tumor bearing NSG mice. Treatment with AFNT-212 cells resulted in dose dependent robust responses (top) and maintained complete durable responses in 5/5 treated mice until end of study (bottom).

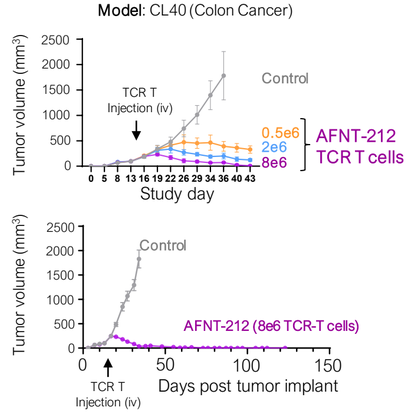


Fig. 5: Gene editing (GE) reagents show high specificity

Target locus amplification (TLA) analysis showed transgene integration within the desired *TRAC* locus demonstrating specificity of GE reagents[1,2] (MG29-1 and *TRAC* gRNA) and the HDR-based knock-in process (left).
Further, potential off-target sites for GE reagents identified using *in silico* prediction 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.

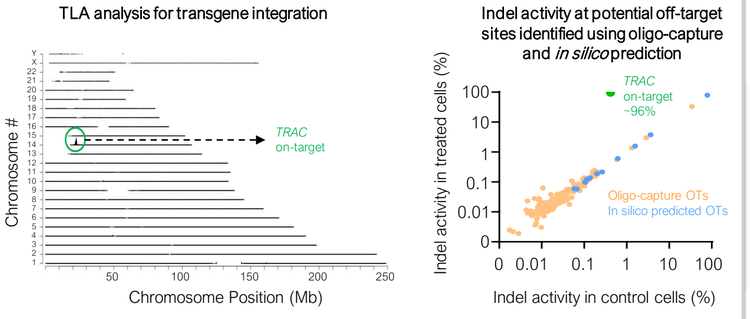
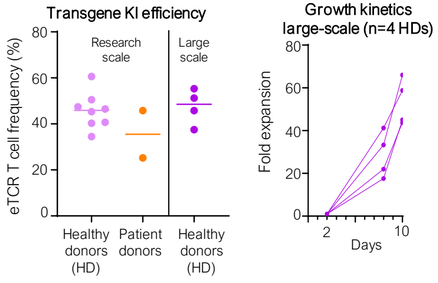
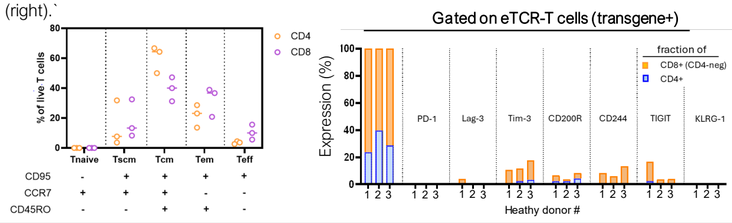


Fig. 6: THRIVE™ platform enables robust and scalable manufacturing of TCR T cells

Non-viral THRIVE platform achieves high transgene knock-in efficiency in T cells from healthy and patient starting material in both research scale and large- scale process. Engineered AFNT-212 T cells demonstrated robust proliferative capacity and readily achieved relevant cell yields for clinical application.



Non-viral THRIVE platform preserves early differentiation status with high proportion of central memory T-cells in both CD4+ and CD8+ subsets (left). The manufactured AFNT-212 product displays minimal expression of canonical exhaustion biomarkers, such as PD-1, LAG-3 and TIM-3 (right).`



Summary

- AFNT-212 engineered TCR-T cells show high functional avidity and *in vitro* cytotoxicity against KRAS G12D positive tumor cell lines including CL40 (colon), PANC-1 and HPAF-II (pancreas), SK-LU-1 (lung), HuCCT1 (cholangiocarcinoma) etc.
- Engineered TCR-T cells show robust and durable anti-tumor activity *in vivo*. AFNT-212 showed tumor control even with as few as 500,000 cells/mice suggesting the transferred TCR-T cells exhibit capacity to proliferate.
- AFNT-212 has low risk of off-target/off-tumor toxicity.
- Gene editing reagents used for manufacturing show low risk of genotoxicity.
- THRIVE™, non-viral KI platform can achieve high transgene integration efficiency and cell growth to yield relevant numbers of engineered TCR-T cells for clinical application.
- AFNT-212 program is poised to enter clinical testing in 2024

References

1. 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.
2. Goltsman *et al.*, Novel Type V-A CRISPR effectors are active nucleases with expanded targeting capabilities, CRISPR J., 2020.
3. Lamothe *et al.*, Novel CRISPR-associated gene-editing systems discovered in metagenomic samples enable efficient & specific genome engineering. *CRISPR J.* 2023 (In press)
4. Oda, S. K. et al. A Fas-4-1BB fusion protein converts a death to a pro-survival signal and enhances T cell therapy. J Exp Med 217 (2020)

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