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Poster
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A Non-Virally Engineered T Cell therapy Targeting The Hotspot Mutation R175H in TP53 with Signals 1, 2, and 3 (TCR, Co-stimulation, and Cytokine) Drives A Coordinated Antitumor CD4/8 T Cell Response

Santosh Narayan[1], Ken Gareau[1], Josh Ferrell[1], Monique Peyreau[1], Jinsheng Liang[1], Luhua Shen[1], Tanya Tetrault[1], Ankit Gupta[1], Nicholas Rouillard[1], Tyler Warner[1], Joshua Francis[1], Xingyue He[1], Damien Hallet[1], Patrick J. Browne[2], Rebecca Lamothe[2], Meghan D. Storlie[2], Gregory J. Cost[2], Thomas M. Schmitt[3], Philip D. Greenberg[3], Smita S. Chandran[4], Michael Gormally[4], Christopher A. Klebanoff[4], Gary Shapiro[1], Kim Nguyen[1], Loïc Vincent[1]

[1]Affini-T Therapeutics, Inc., Watertown, MA,[2]Metagenomi, Inc., Emeryville, CA,[3]Program in Immunology and Translational Science and Therapeutics Division, Fred Hutchinson Cancer Research Center, Seattle, WA,[4]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

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Abstract

Background
Adoptive T cell therapy (ACT) has demonstrated antitumor activity in solid tumor patients but requires further optimization. T cell receptor (TCR)-engineered T cells recognize peptides derived from intracellular and surface proteins presented in the context of MHC class I. TP53 is the most frequently mutated gene across human cancers, with a recurrent arginine to histidine hotspot alteration in codon 175 that generates a peptide presented in the context of HLA-A*02:01. AFNT-313 is engineered to overcome the challenges faced by T-cell transfer and delivers all three signals (TCR, co-stimulation, cytokine) required for optimal T cell stimulation by including a: 1) TCR specific for TP53 R175H mutation and CD8αβ coreceptor, 2) FAS-41BB switch receptor, and 3)chimeric cytokine receptor.
Methods
Human CD4+ and CD8+ T cells isolated from healthy volunteers were genetically engineered by a novel CRISPR-Cas nuclease and gRNA targeting the TRAC locus that resulted in knock-out of the endogenous TCR and simultaneous integration of the non-viral plasmid-based transgene cassette. Engineered T cells were assessed against TP53 R175H peptide presented by HLA-A*02:01 and a panel of TP53R175H-expressing tumor cell lines for *in vitro* activation, proliferation, and cytotoxicity. *In vitro* tolerability studies were performed along with *in vivo* activity studies in human tumor-derived xenografts.
Results
AFNT-313 showed specific recognition of the TP53 R175H peptide, demonstrated cytotoxicity against endogenously expressing HLA-A*02:01/TP53 R175H+ cell lines in tumor cell re-challenge assays *in vitro*, and induced robust anti-tumor activity *in vivo*. Inclusion of the chimeric cytokine receptor resulted in improved knock-in efficiency and with a potent anti-tumor response putatively stemming from improved T cell expansion and resistance to exhaustion. The FAS-41BB switch receptor, engineered to deliver co-stimulation allowing for more enhanced functionality, inhibited FASL-induced apoptosis. The knock-in strategy drove robust expression of all six encoded parameters regardless of location within the 7 kilobase polycistronic construct. AFNT-313 demonstrated a favorable tolerability profile indicated by no off-target liabilities identified upon co-incubation with all possible peptides in the human proteome matching the TCR recognition motif. In the presence of FASL-induced co-stimulation via FAS-41BB, AFNT-313 activity continued to be gated on TCR signaling and demonstrated no cytokine-independent survival.
Conclusions
We report a first-in-class TCR gene therapy approach selectively targeting mutant TP53 R175H-harboring tumors with a coordinated CD4/CD8 T cell response demonstrating a promising activity and tolerability profile. Our work supports the planned clinical development of AFNT-313 as a novel non-viral knock-in TCR-engineered T cell therapy for treating TP53-mutant tumors.

Fig 1: AFNT-313 TP53 R175H Non-Viral Knock-In TCR-T

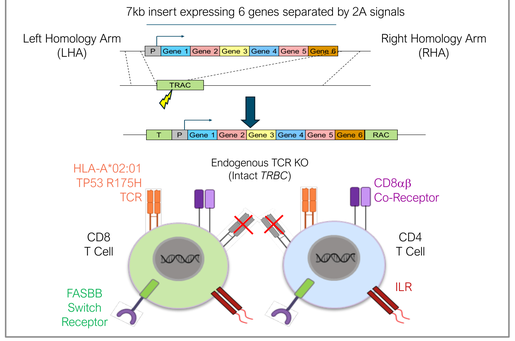
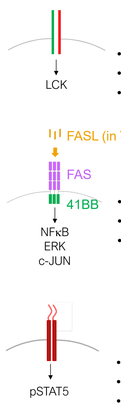


Fig. 2: Armoring in Product



CD8αβ
- Augment signal 1 (TCR) in CD4+ T cells
- Coordinated immune response
- Stimulation & prevents exhaustion

FAS-41BB
- Signal 2 (co-stim) upon FASL binding
- Memory development
- Proliferation, metabolism, & survival

ILR
- Constitutive signal 3 (cytokine)
- Proliferation, survival, & trafficking
- No cytokine independent survival

Fig. 3: Epitope-Specific Activation

Cytokine secretion only by genetically-engineered primary CD4/8 TCR-T cells (AFNT-313) co-cultured with endogenously expressing HLA-A*02:01 and mutant TP53 R175H cell lines demonstrates specific reactivity to endogenous TP53 mutant peptide presented by restricted MHC class I.

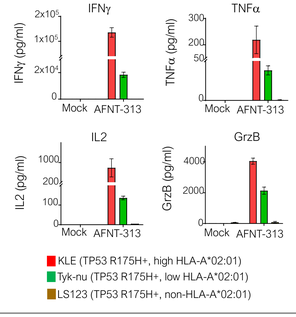


Fig. 4: Cytotoxicity & Selectivity *In Vitro*

Genetically-engineered primary CD4/CD8 TCR-T cells (AFNT-313) control TYK-nu tumor cells, an endogenously expressing HLA-A*02:01 TP53 R175H line, even after rechallenges ( ). No control of antigen-negative SW527 (HLA-A*02:01 TP53 R273H, P309S) even at higher effector to target ratio (10:1, inset).

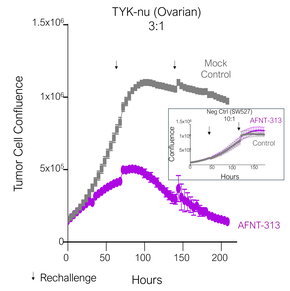


Fig. 5: Low Risk of Cross-Reactivity

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

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

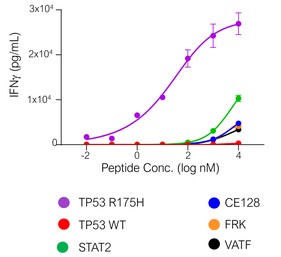


Fig. 6: No Cytokine Independent Growth

Cryo-preserved AFNT-313 (produced for primary CD4/CD8 TCR-T cells derived from 2 donors) were thawed in cytokine-containing or cytokine-free medium. Lack of persistence in the absence of exogenous cytokines and in the presence of FASL (inset) supports potential tolerability profile of AFNT-313.

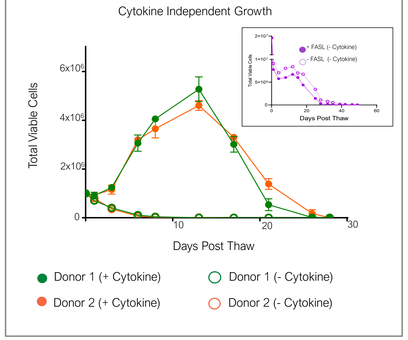


Fig. 7: High Transgene Expression

Transgene expression similar between primary CD4/CD8 TCR-T cells genetically-engineered with knock-in expressing 4-, 5-, or 6-genes demonstrating minimal impact of length or position within the construct.

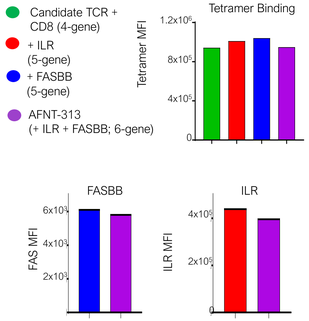


Fig.8: ILR Increases Knock-In Over Time

ILR expression causes increase in genetically-engineered TCR-T from 6 to 12 days post knock-in allowing for ~50% efficiency with 7kb construct expressing 6 genes. Higher knock-in percentage may increase maximum manufacturable dose level while decreasing cost-of-goods.

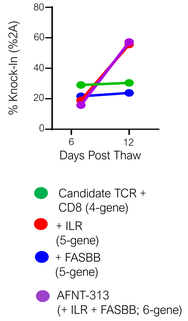


Fig.9: Rescue From FAS Ligand-Induced Death

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.

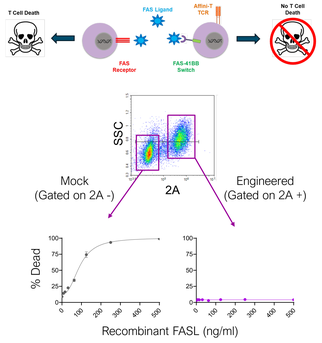
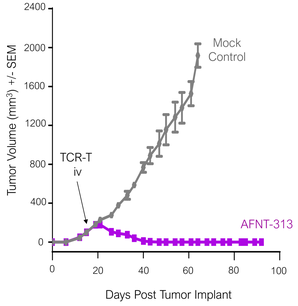


Fig. 10: Complete Responses *In Vivo*

Single intravenous administration of 10x10[6] primary CD4/CD8 TCR-T cells (AFNT-313) 20 days after subcutaneous inoculation of TYK-nu tumor cells led to 100% complete response with no loss in body weight (not shown).



Summary

- AFNT-313 includes all 3 signals for full stimulation of both CD8+ & CD4+ T cells
- Robust & specific cytotoxicity demonstrated both *in vitro* and *in vivo*
- Promising preclinical tolerability with no off-targets & no cytokine-independent growth
- High percentage of engineered T cells and high expression of all 6 transgenes
- Program currently in preclinical IND-enabling studies

Partners

Memorial Sloan Kettering Cancer Center

Fred Hutch Cancer Center

Metagenomi

Presenter & Corresponding Author:
Santosh.Narayan@Affinittx.com

https://affinittx.com/