355 DIRECTING A HIGH AVIDITY KRAS G12D-SPECIFIC TCR ENGINEERED WITH A CD8αβ CO-RECEPTOR AND CHIMERIC CYTOKINE RECEPTOR USING NON-VIRAL KNOCK-IN ENHANCES ANTI-TUMOR RESPONSES

[1]Allison P Drain, [1]Nicholas Rouillard, [1]Nathaniel Swanson, [1]Santosh Narayan, [1]Tyler Warner, [1]Nicole Danek, [1]Ken Gareau, [1]Cheryl Black, [1]James Parsons, [1]Anthony Thomas, [1]Jinsheng Liang, [1]Luhua Shen, [1]Tanya Tetrault, [1]Vince Nguyen, [1]Iqraa Priyata, [1]Sarah Vidyasagar, [1]Joshua Francis, [1]Xingyue He, [2]Patrick J Browne, [3]Rebecca C Lamothe, [3]Meghan D Storlie, [3]Gregory J Cost, [4]Thomas M Schmitt, [4,5]Philip D Greenberg, [6]Smita S Chandran, [6]Christopher A Klebanoff, [1]Ankit Gupta*, [1]Damien Hallet, [1]Gary Shapiro, [1]Kim Nguyen, [1]Loic Vincent. *[1]Affini-T Therapeutics, Watertown, MA, USA; [2]Metagenomi Inc, Los Angeles, CA, USA; [3]Metagenomi Inc, Emeryville, CA, USA; [4]Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [5]University of Washington School of Medicine, Seattle, WA, USA; [6]Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Background Adoptive T cell therapy has demonstrated clinical activity in a subset of patients with solid tumors; however, consistent responses will require 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. Immunologic targeting of recurrently mutated oncogenic drivers, such as KRAS, overcomes many of the major obstacles of this modality because the resulting epitope is: 1) tumor-specific, 2) essential for cancer cell fitness, and 3) derived from a stably expressed non-self protein. AFNT-212 is a next-generation engineered T cell therapy that uses non-viral targeted knock-in (KI) at the TCRα constant (*TRAC*) locus to express a multi-cistronic cassette that includes 1) a high-affinity TCR specific for $KRAS_{G12D}$ mutation, 2) a CD8αβ coreceptor, and 3) a chimeric cytokine receptor.

Methods Human $CD4^+$ and $CD8^+$ T cells were genetically engineered by a novel CRISPR-Cas nuclease and gRNAs targeting *TRAC* and the TCRβ constant (*TRBC*) genes allowing for knock-out of the endogenous TCR loci and simultaneous integration of the non-viral plasmid-based transgene cassette. Engineered T cells were assessed for specificity and potency, including activation, proliferation, and cytotoxicity, against KRAS G12D peptide presented by HLA-A*11:01 and a panel of KRAS G12D-expressing tumor cell lines. *In vitro* safety studies were performed along with *in vivo* efficacy studies in multiple human xenograft models.

Results Engineered primary T cells showed specific recognition of KRAS G12D peptide, demonstrated cytotoxicity against endogenously expressing HLA-A*11:01$^+$/KRAS G12D$^+$ cell lines in tumor cell re-challenge assays *in vitro*, and mediated robust anti-tumor activity *in vivo*. Inclusion of the chimeric cytokine receptor allowed for a more potent anti-tumor response stemming from improved T cell expansion and resistance to exhaustion. No off-target liabilities were identified upon co-incubation of AFNT-212 with all possible peptides in the human proteome matching the xScan-defined epitope recognition motif for the TCR, demonstrating specificity. Gene editing safety evaluation did not reveal any off-target activity for the CRISPR-Cas nucleases and engineered T cells did not show cytokine-independent proliferation, collectively supporting a favorable pre-clinical safety profile for AFNT-212.

Conclusions We report a novel TCR gene therapy approach targeting mutant KRAS G12D-expressing tumors with a coordinated CD4/CD8 T cell response that has a promising efficacy and safety profile. 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.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0355