1223 NON-VIRAL TARGETED KNOCK-IN OF A KRAS G12D SPECIFIC TCR, CD8αβ, AND CHIMERIC CYTOKINE RECEPTOR IN THE *TRAC* LOCUS OUTPERFORMS LENTIVIRAL-BASED ENGINEERING OF T CELLS

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Background The AFNT-212 cell therapy consists of autologous CD8+ and CD4+ T cells expressing 1) a T Cell Receptor (TCR) specific for the prevalent oncogenic driver KRAS G12D mutation presented by HLA-A*11:01, 2) a chimeric cytokine receptor, and 3) the CD8α/β coreceptor enabling a coordinated CD4+/CD8+ anti-tumor response to promote T cell activity while minimizing exhaustion. While viral vectors, including lentivirus (LVV), have been a standard modality to deliver transgenes for cell therapies, they are limited by cargo size and manufacturing complexity. In comparison, gene-editing mediated targeted knock-in (KI) of a non-virally delivered transgene cassette overcomes limitations of LVV-mediated delivery and improves function and safety profile of the engineered cells.

Methods Human CD4+ and CD8+ T cells from healthy volunteers or patients were engineered by a novel CRISPR-Cas nuclease and gRNAs targeting the T-cell receptor α constant (*TRAC*) and T-cell receptor β constant (*TRBC*) genes to knock-out the endogenous TCR and simultaneously integrate a non-viral plasmid-based transgene cassette. Human CD4+ and CD8+ T cells were engineered in parallel with lentivirus encoding the same transgene cassette and CRISPR-Cas targeting *TRAC* and *TRBC* genes to knock-out endogenous TCR. Engineered T cells were assessed via KRAS G12D peptide stimulation and co-culture with KRAS G12D-expressing tumor cells for *in vitro* activation and cytotoxicity. *In vitro* safety studies were performed and *in vivo* efficacy studies were conducted using human KRAS G12D xenografts in NSG mice.

Results Non-viral KI generated lower vector copy number per cell than LVV but drove higher transgene expression, suggesting the EF1α promoter within the KI construct outperforms the MSCV promoter of the LVV. T cells engineered with either non-viral KI or LVV demonstrated specific and sensitive recognition of the target KRAS G12D peptide. However, KI-engineered cells demonstrated improved cytotoxicity against endogenously-expressing HLA-A*11–01 and KRAS G12D cell lines in tumor cell rechallenge assays *in vitro*. KI-engineered cells also showed superior anti-tumor activity in established subcutaneous tumor bearing mice. Off-target assessment was similar for the KI and LVV products, as identified by co-incubation with all possible peptides in the human proteome matching the TCR recognition motif. Optimized KI process generated large number of transgenic cells with naïve and memory phenotype potentially suitable for clinical applications.

Conclusions Non-viral targeted KI engineered AFNT-212 cells drive a robust coordinated CD4/CD8 T cell response against KRAS G12D-harboring tumors and outperform LVV-engineered cells. Our work supports the planned clinical development of this novel TCR-engineered T cell therapy for treating KRAS-mutant solid tumors.

Ethics Approval These studies were approved by Affini-T Therapeutics and Explora Biolabs' Institutional Animal Care and Use Committee, approval number EB17–010-303.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1223