244 AFNT-111, A SAFE AND EFFECTIVE TCR-ENGINEERED T CELL THERAPY TARGETING THE ONCOGENIC DRIVER KRAS G12V MUTATION

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Background Mutations in the *RAS* family of genes are responsible for approximately 30% of all human cancers. Mutated RAS proteins are truncal oncogenic driver antigens essential for cancer development and progression making them optimal targets for cancer therapies by limiting tumor escape. The AFNT-111 cell therapy consists of autologous $CD8^+$ and $CD4^+$ T cells expressing a TCR specific for the highly prevalent $KRAS_{G12V}$ mutation presented by HLA-A*11:01, one of the most common HLA alleles worldwide. AFNT-111 is also engineered to express the CD8α/β coreceptor, enabling a coordinated $CD4^+/CD8^+$ tumor response that aims to promote increased T cell activity and persistence while minimizing T cell exhaustion.

Methods Lentiviral vector was used to transduce primary human $CD4^+$ and $CD8^+$ T cells with the $KRAS_{G12V}$-specific TCR and CD8α/β coreceptor. Engineered T cells were assessed against $KRAS_{G12V}$ peptide and a panel of $KRAS_{G12V}$-expressing tumor cell lines for *in vitro* activation, proliferation, and cytotoxicity. *In vitro* safety studies were performed to evaluate self-peptide cross-reactivity and alloreactivity and *in vivo* efficacy studies were conducted using human $KRAS_{G12V}$ xenografts in NSG mice.

Results AFNT-111 demonstrated potent functional avidity for $KRAS_{G12V}$ peptide with no reactivity to wildtype KRAS. Several naturally expressing $KRAS_{G12V}$ human tumor cell lines, derived from lung, colorectal, and pancreatic cancer, triggered significant AFNT-111 T cell activation and proliferation, and potent cytotoxicity towards tumor cells. *In vitro* killing by AFNT-111 was consistently observed even after repeated tumor cell challenge. Robust *in vivo* anti-tumor efficacy was also observed in two established mouse xenograft tumor models. XScan studies using amino acid substitutions of the reference $KRAS_{G12V}$ peptide revealed a restrictive TCR recognition motif limiting risk of promiscuous off-target activation. Further, potentially cross-reactive self-peptides in the human proteome matching this motif were tested and no cross-reactivities with significant avidity were identified. A large lymphoblastoid cell line library covering >95% of the most common HLA alleles was assessed with no alloreactive responses detected. For clinical studies, a robust manufacturing process has been developed in which $CD4^+/CD8^+$ T cell ratios are controlled, and the final AFNT-111 drug product preserves stem-like properties.

Conclusions AFNT-111 preclinical data demonstrate a highly potent and specific TCR-engineered T cell product that is cytotoxic to $KRAS_{G12V}$-expressing tumor cells both *in vitro* and *in vivo*. Cross-reactivity and alloreactivity assessments established a strong safety profile of AFNT-111, supporting clinical translation. First-in-human clinical studies will focus on advanced or metastatic pancreatic, colorectal, and lung cancer indications.

Ethics Approval These studies were approved by Affini-T Therapeutics and Fred Hutchinson Cancer Research Center Ethics Boards, approval number EB17-010-303 and PROTO000050898, respectively.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0244