

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 CD8 α / 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 CD8 α / 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.

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