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

Hubert Lam*, Xingyue He, Cheryl Black, Michele Hoffmann, Joshua Francis, James Parsons, Christian Roy, Jinsheng Liang, Hongjing Qu, Martin Campbell, Tomasz Sebastiani, Jessica Webb, Aude Chapuis, Thomas Schmitt, Philip Greenberg, Damien Hallet, Markus Vallaster, Piotr Pierog, Gary Shapiro, Loic Vincent. Affini-T Therapeutics, Watertown, MA, USA; Fred Hutchinson Cancer Research Center, Seattle, WA, USA

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 KRASG12V 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 KRASG12V-specific TCR and CD8α/β coreceptor. Engineered T cells were assessed against KRASG12V peptide and a panel of KRASG12V-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 KRASG12V peptide with no reactivity to wildtype KRAS. Several naturally expressing KRASG12V 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 KRASG12V 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 KRASG12V-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.