Poster # 2543

AFNT-211: A FAS-41BB–enhanced TCR-T cell therapy with stem-like properties targeting KRAS G12V-expressing solid tumors

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Background

KRAS is the most common oncogenic driver mutation in solid tumors, promoting the initiation and progression of many uncurable cancers, including colorectal, pancreatic and lung cancer. While small molecule inhibitors to KRAS^{G12C} mutations have been approved, there are no targeted therapies available for patients with the highly prevalent KRAS^{G12V} mutations. TCR-T cell therapies have demonstrated remarkable responses in clinical trials, but their durability has been limited by the immunosuppressive tumor microenvironment (TME). AFNT-211 is an autologous T cell therapy engineered to express an HLA-A*11:01 KRAS^{G12V}-specific TCR, and further enhanced with CD8α/β coreceptor and a FAS-41BB switch receptor to drive T cell persistence and durable clinical responses. CD8α/β coreceptor enables a coordinated CD4$^+$/CD8$^+$ T cell response and FAS-41BB converts the FAS ligand (FASL) TME death signal into a costimulatory signal through 41BB activation[1,2].

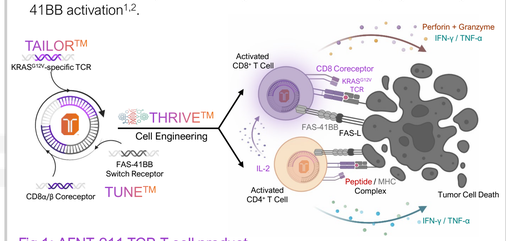


Fig 1: AFNT-211 TCR-T cell product

Methods

A potent and specific KRAS^{G12V} TCR was identified from healthy donors using the Tailor TCR discovery platform. AFNT-211 T cells are engineered with a 1) HLA-A*11:01 KRAS^{G12V}-specific TCR, 2) a CD8α/β coreceptor and 3) a FAS-41BB switch receptor.

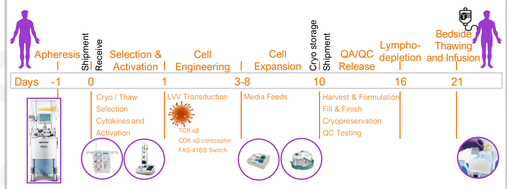
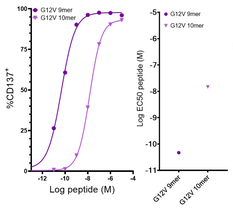


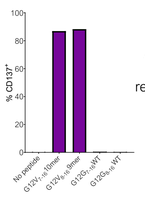
Fig 2: AFNT-211 10-day autologous manufacturing process (THRIVE™)

The Thrive biomanufacturing process was developed for optimal cell fitness and yield. The clinical manufacturing process consists of autologous CD4$^+$/CD8$^+$ T cells transduced with lentivirus and expanded using culture conditions that drive robust expansion while preserving stem-like properties. AFNT-211 drug product was assessed for efficacy *in vitro* against a panel of KRAS^{G12V}-expressing tumor cell lines. *In vitro* safety studies were performed to assess potential cross-reactivity, alloreactivity, and cytokine-independent growth. *In vivo* studies were performed using a human xenograft NSG mouse model.

Fig 3: AFNT-211 TCR Discovery

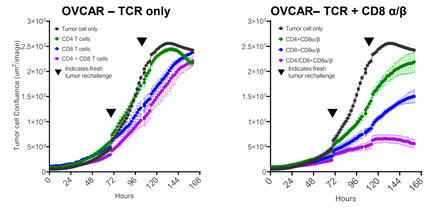


Identification of an HLA-A11:01 KRAS^{G12V} TCR with potent functional avidity against 9mer (pM EC50) and 10mer peptides



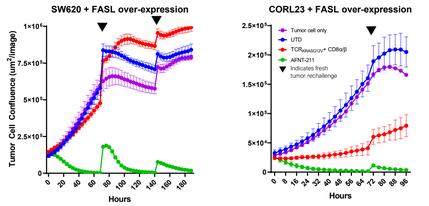
AFNT-211 does not recognize WT KRAS

Fig 4: CD8α/β Coreceptor Enhanced Activity



TCR-T cells expressing CD8α/β coreceptor exhibit more robust cancer cell killing than TCR alone (1:1 E:T)

Fig 5: FAS-41BB Switch Receptor



Expression of FAS-41BB switch receptor enhances the cytotoxic activity of TCR-T cells by over-coming cell death caused by FASL (5:1 E:T)

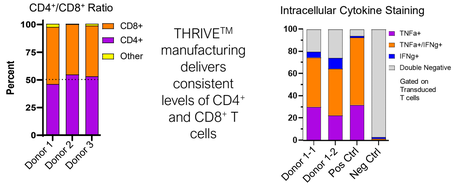
Table 1: Robust AFNT-211 Manufacturing Process Developed

Scale	Fold Expansion	Total Viable Cells (e9)	Total Viable TCR+ T cells	Drug Substance Viability (%)
At-scale (n=2)	170 – 350	60.4 ± 7.6	30 e9	93 ± 3.0
Mid-scale (n=3)	246 – 418	83.5 ± 15.3	42 e9	93 ± 1.2

Process range: 30-60 x 10^9 TCR$^+$ T cells. Option to generate higher dose levels with process scale out

THRIVE™ manufacturing enables high clinical doses

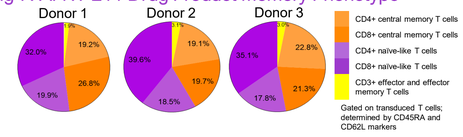
Fig 6: AFNT-211 Drug Product Purity and Potency



THRIVE™ manufacturing delivers consistent levels of CD4$^+$ and CD8$^+$ T cells

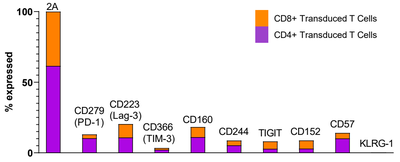
INFγ and TNFα ICS indicates high levels of polyfunctional AFNT-211 T cells

Fig 7: AFNT-211 Drug Product Memory Phenotype



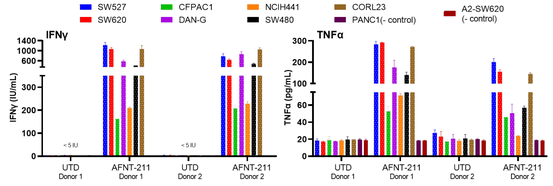
AFNT-211 drug product consists of large numbers of central memory and naïve-like T cell phenotypes

Fig 8: AFNT-211 Drug Product Extended Characterization



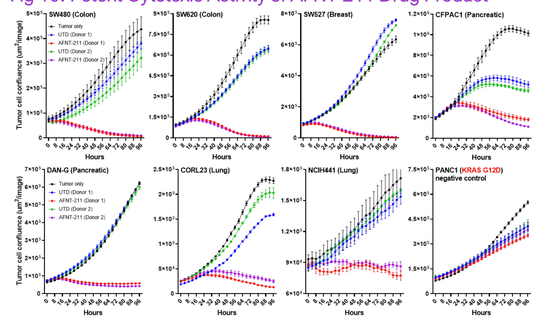
AFNT-211 drug product expresses low levels of exhaustion and senescence markers. Note: exhaustion marker expression is similar to input T cells of manufacturing process

Fig 9: AFNT-211 Response to Diverse KRAS^{G12V} Tumor Cells



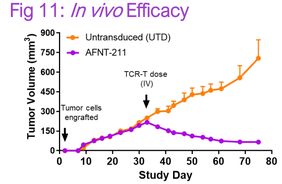
Polyfunctional cytokine secretion of AFNT-211 in response to all KRAS^{G12V}+ tumor cell lines tested

Fig 10: Potent Cytotoxic Activity of AFNT-211 Drug Product



AFNT-211 drug product kills all HLA-A11:01$^+$ KRAS^{G12V+} tumor cell lines tested. No non-specific cytotoxicity against non-KRAS^{G12V} tumor cells

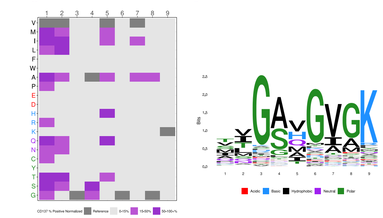
Fig 11: *In vivo* Efficacy



A single dose of AFNT-211 drives potent *in vivo* efficacy against large, well-established SW527 tumors in NSG mice

5x10^6 AFNT-211 cells administered IV on D33 post-tumor engraftment

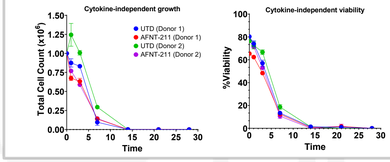
Fig 12: Cross-reactivity Assessment of AFNT-211



XScan mutagenesis studies demonstrate a highly KRAS^{G12V} specific AFNT-211 drug product

Human self-peptides matching the XScan recognition motif do not cross-react with AFNT-211

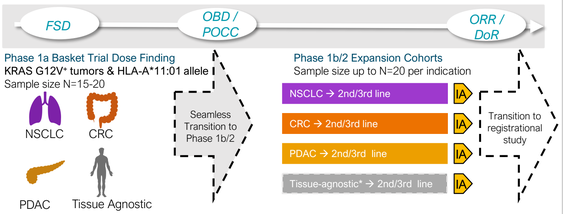
Fig 13: Cytokine-independent Growth



No *in vitro* cytokine-independent growth observed with AFNT-211

Summary and Clinical Trial Design

- The AFNT-211 TCR-T cell therapy is designed to generate durable therapeutic responses by utilizing a high avidity KRAS^{G12V} TCR, CD8α/β coreceptor and FAS-41BB switch receptor
- The THRIVE T cell manufacturing process generates high doses of drug product that exhibit naïve and central memory phenotypes
- Potent and specific pharmacological activity is observed in *in vitro* and *in vivo* studies with a preclinically safe profile
- AFNT-211 is ready for clinical translation with the following trial design:



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