Poster Session A

AFNT-111: a novel TCR-engineered T cell therapy targeting the oncogenic driver KRAS G12V

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Introduction

Mutations in the RAS family are highly prevalent in human cancers. KRAS is the most frequently mutated RAS oncogene and patients with KRAS mutations have poor responses to standard treatment regimens. Therefore, successful KRAS-targeted therapies will meet a high unmet need. The AFNT-111 cellular therapy consists of autologous CD8+ and CD4+ T cells expressing a high avidity TCR specific for the prevalent KRAS G12V mutation presented by HLA-A*11:01, one of the most common HLA alleles worldwide. AFNT-111 is engineered to express the CD8α/β coreceptor, enabling a coordinated CD4+/CD8+ anti-tumor response to induce robust T cell activity and persistence while minimizing T cell exhaustion.

- Target: oncogenic driver mutations responsible for cancer
- Focus: mKRAS – most frequent oncogene in solid tumors
- Treat: TCR-T are an optimal modality to attack mKRAS with PoC demonstrated in multiple T cell trials[1,2]

Methods

Lentiviral vector was used to transduce CD4+ and CD8+ T cells from healthy HLA-A*11:01+ individuals with a KRAS G12V-specific human TCR and CD8α/β coreceptor. Engineered T cells were assessed against exogenous KRAS G12V peptide as well as a panel of tumor cell lines endogenously expressing KRAS G12V and probed for *in vitro* activation, proliferation, cytotoxicity and cytokine secretion. In parallel, *in vitro* safety studies were performed to evaluate autoantigen cross-reactivity and alloreactivity.

In vivo tumor efficacy studies were conducted using therapeutic administration of AFNT-111 and subcutaneous human KRAS G12V xenografts in NSG mice.

KRAS G12V+ cell lines

Cell lines used	Cancer derivation
CFPAC-1	Pancreatic
DAN-G	Pancreatic
SW620	Colon
SW480	Colon
COR-L23	Lung
NCI H441	Lung
SW527	Breast
PANC1 (KRAS G12V negative)	Pancreatic
HUCCT1 (KRAS G12V negative)	Cholangiocarcinoma

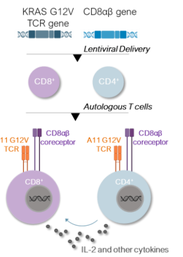
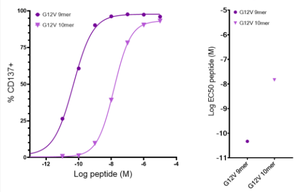
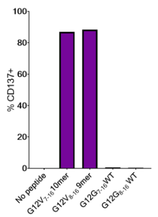


Fig 1: AFNT-111 T cell product

Fig 2: AFNT-111 TCR Discovery

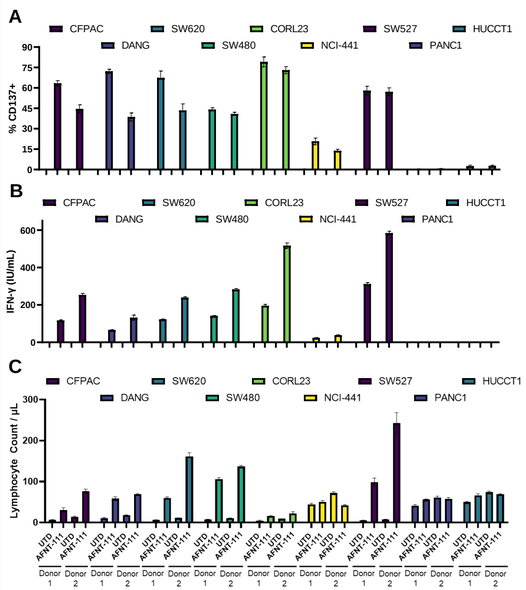


Identification of an HLA-A11 KRAS G12V TCR with potent functional avidity (EC50 in low pM)



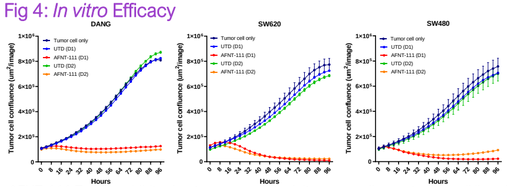
AFNT-111 does not recognize WT KRAS

Fig 3: Endogenous Tumor Cell Line Reactivity



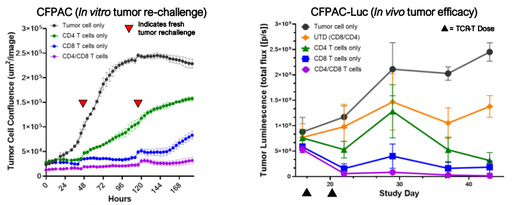
AFNT-111 T cells are (A) activated, (B) secrete cytokine, and (C) proliferate in response to endogenously processed and presented KRAS G12V across diverse tumor cell lines

Fig 4: *In vitro* Efficacy



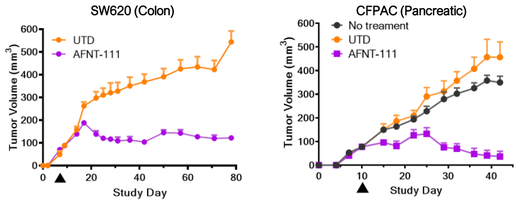
AFNT-111 T cells exhibit robust cancer cytotoxicity across diverse tumor cell lines in two donors (D = donor)

Fig 5: CD8α/β Enhanced Activity



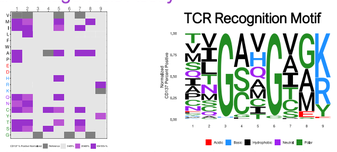
Inclusion of AFNT-111+ CD4 T cells enhances *in vitro* and *in vivo* efficacy by driving a coordinated CD4+/CD8+ T cell response

Fig 6: *In vivo* Efficacy



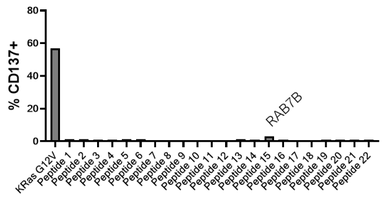
AFNT-111 T cell therapy induces robust preclinical efficacy across *in vivo* tumor models derived from different cancer types ▲= TCR-T Dose

Fig 7: XScan Mutagenesis Assay



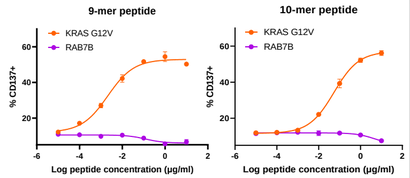
In vitro safety studies through site-directed scanning mutagenesis indicate that AFNT-111 is a highly selective T cell product with low potential for cross reactivity

Fig 8: Crossreactivity Assessment



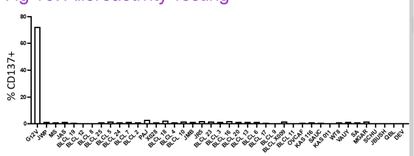
22 self-peptides matching the AFNT-111 TCR recognition motif do not induce significant AFNT-111 T cell activation. RAB7B tested comprehensively (below)

Fig 9: RAB7B Assessment



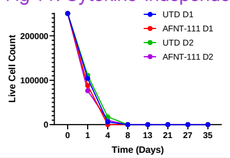
Peptide dose response studies confirm that AFNT-111 does not respond to a titration of either 9-mer or 10-mer RAB7B peptide

Fig 10: Alloreactivity Testing



No alloreactivity responses detected against a B-LCL library covering >95% of the most common HLA alleles

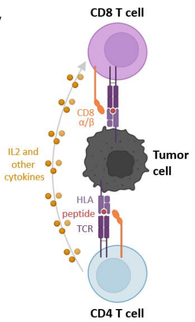
Fig 11: Cytokine-Independent Growth Testing



Activated AFNT-111 T cells do not exhibit growth in the absence of cytokine (D = Donor)

Coordinated CD4+/CD8+ Response

- AFNT-111: T cells transduced with a high avidity TCR and CD8 α/β specifically recognize KRAS G12V presented by HLA-A*11:01
- CD8 α/β co-receptor drives a coordinated CD8+/CD4+ T cell response by enabling CD4+ stimulation
  - CD4+ T cells provide helper activity to engage CD8+ T cells in the TME, promote effector activation and prevent CD8+ T cell exhaustion leading to enhanced CD8+ persistence
  - CD8 α/β-expressing CD4+ T cells also exhibit cytotoxic activity



Summary

- AFNT-111 is a potent and specific TCR-engineered T cell product that is cytotoxic to KRAS G12V-expressing tumor cells in both *in vitro* and *in vivo* preclinical studies
- Cross-reactivity and alloreactivity assessments establish a strong AFNT-111 preclinical safety profile
- AFNT-111 IND anticipated in 2H'23
- First-in-human clinical studies will initiate in collaboration with Fred Hutch Cancer Center
  - Phase I Objective: Safety, tolerability, maximum tolerated dose (MTD), and preliminary efficacy
  - Clinical Indications: advanced or metastatic Pancreatic ductal adenocarcinoma (PDAC), Colorectal adenocarcinoma (CRC), Non-small cell lung cancer (NSCLC)
  - Target population: HLA-A*11:01+; KRAS G12V+ patients

References

1. Leidner, R. et al. Neoantigen T-Cell Receptor Gene Therapy in Pancreatic Cancer. N Engl J Med 386, 2112–2119 (2022).
2. Tran, E. et al. T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. N Engl J Med 375, 2255–2262 (2016).

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