Poster # 244

AFNT-111, a preclinically safe and effective TCR-engineered T cell therapy targeting the oncogenic driver KRAS G12V mutation

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Abstract

Mutations in the RAS family of genes are responsible for up to 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.

- Oncogenic driver mutations responsible for cancer are ideal targets
- KRAS – most frequent mutated oncogene in solid tumors
- 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 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 or KRAS wildtype 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 define the AFNT-111 TCR recognition motif and evaluate AFNT-111 self-peptide cross-reactivity, alloreactivity and cytokine-independent growth.

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

Cell lines used	Cancer derivation
CFPAC-1	Pancreatic
DAN-G	Pancreatic
SW620	Colon
SW480	Colon
COR-L23	Lung
NCI H441	Lung
SW527	Breast
OVCAR-5	Ovarian

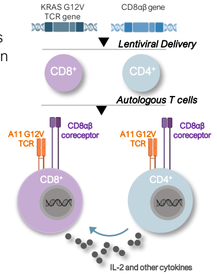
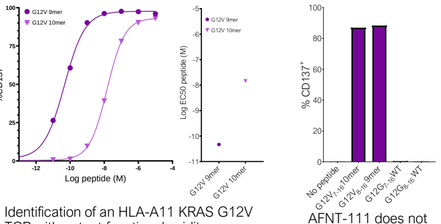


Fig 1: AFNT-111 T cell product

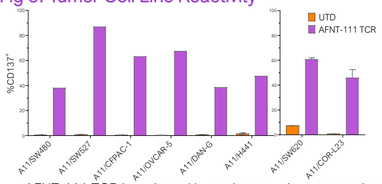
Fig 2: AFNT-111 TCR Discovery



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

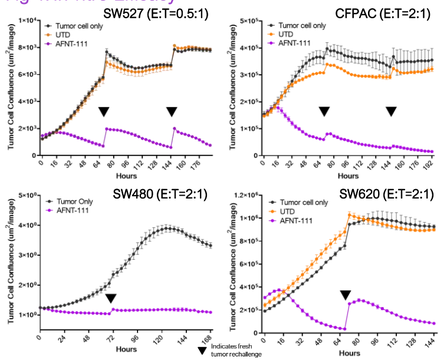
AFNT-111 does not recognize WT KRAS

Fig 3: Tumor Cell Line Reactivity



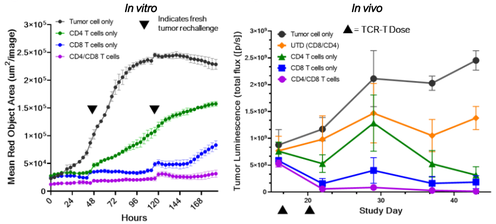
AFNT-111 TCR is activated by endogenously processed and presented KRAS G12V across diverse tumor cell lines

Fig 4: *In vitro* Efficacy



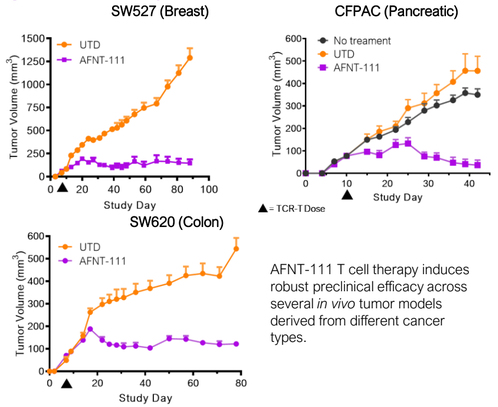
AFNT-111 TCR-T cells exhibit robust cancer killing in a repeat tumor challenge assay across diverse tumor cell lines

Fig 5: CD8α/β Enhanced Activity



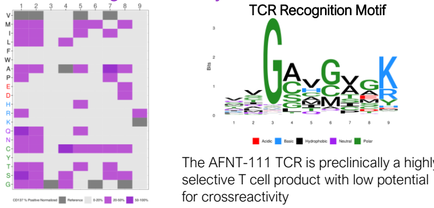
CD8α/β enhances AFNT-111 tumor control *in vitro* (CFPAC) and *in vivo* (CFPAC-Luc) by driving a coordinated CD4$^+$/CD8$^+$ T cell response

Fig 6: *In vivo* Efficacy



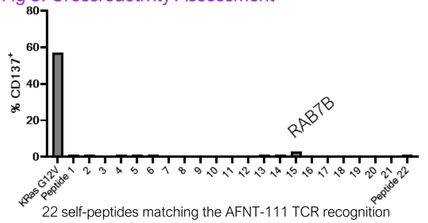
AFNT-111 T cell therapy induces robust preclinical efficacy across several *in vivo* tumor models derived from different cancer types.

Fig 7: XScan Mutagenesis Assay



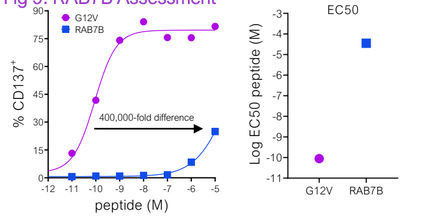
The AFNT-111 TCR is preclinically a highly selective T cell product with low potential for crossreactivity

Fig 8: Crossreactivity Assessment



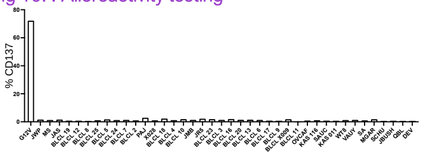
22 self-peptides matching the AFNT-111 TCR recognition motif do not induce significant AFNT-111 T cell activation. RAB7B tested comprehensively (below)

Fig 9: RAB7B Assessment



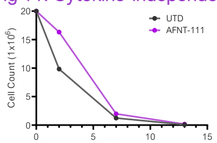
Dose response studies confirm that the AFNT-111TCR does not respond to physiological concentrations of RAB7B peptide

Fig 10: : Alloreactivity testing



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

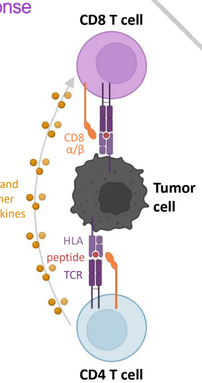
Fig 11: Cytokine-independent growth testing



Activated AFNT-111 T cells do not exhibit growth in the absence of cytokine

Coordinated CD4$^+$/CD8$^+$ Response

- AFNT-111: T cells transduced with high affinity TCR specifically recognizing 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 therapy that is cytotoxic to KRAS G12V-expressing tumor cells 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 1H'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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