FT573: Preclinical Development of Multiplexed-Engineered iPSC-derived NK Cells Expressing a Novel Camelid Nanobody Chimeric Antigen Receptor (CAR) Targeting pan-Cancer Antigen B7-H3 (Abstract 5544)

Nicholas Zorko[1], Frank Cichocki[1], John Goulding[2], Bryan Hancock[2], Robert Blum[2], Mochtar Pribadi[2], Bjoern Gaertner[2], Tom Lee[2], Wen-I Yeh[2], Martin Felices[1], Ryan Bjordahl[2], Bahram Valamehr[2], Jeffrey S. Miller[1]

1. University of Minnesota, Masonic Cancer Center, Minneapolis, MN, USA 2. Fate Therapeutics, San Diego, CA, USA

Abstract

Introduction: B7-H3 (CD276) has gained significant clinical interest as a pan-tumor target antigen for development of various immuno-oncology agents. Due to its broad expression on a wide variety of solid tumors and minimal expression on normal tissues, B7-H3 is an ideal tumor antigen target. Additionally, high levels of B7-H3 are found on "immunologically cold" tumors such as glioblastoma multiforme, prostate cancer, head and neck cancer and soft tissue sarcomas, which typically have poor response to approved immune therapies. To effectively target B7-H3 with an off-the-shelf cellular therapy, we describe here the development of camB7-H3 CAR-NK cell utilizing our iPSC platform to engineer multiple modalities into a clonal iPSC line, which can serve as the starting cell source for mass production of off-the-shelf, iPSC-derived CAR-NK cells (CAR-iNK cells).

Methods: A camelid nanobody specific for human B7-H3 (camB7-H3) was discovered using a phage display library and validated in functional assays. camB7-H3 CAR-iNK cells were designed to 1) express membrane-bound IL-15/IL-15 receptor fusion for enhanced persistence, 2) have a CD38 knockout to improve metabolic fitness, 3) express a high-affinity non-cleavable CD16 to maximize ADCC when combined with a therapeutic antibody, and 4) express an anti-camB7-H3 CAR optimized for NK cell signaling. As the initial preclinical study, camB7-H3 CAR-iNK cells were assessed using flow cytometry-based functional assays evaluating CD107a and IFNg or xCelligence target killing assays against B7-H3 transgenic or naturally expressing tumor cell lines.

Results: The camelid single domain B7-H3 initially tested in CAR-T exhibited B7-H3 specific binding and specific activity against several solid tumor cell lines (breast, ovarian, prostate, lung). We next produced camB7-H3 CAR-iNK cells and demonstrated superior B7-H3-specific target elimination compared to untransduced iNK cells. These results were seen across multiple tumor lines representing various solid tumor indications. Further enhancement of anti-tumor efficacy was seen when combined with therapeutic antibodies, including trastuzumab and cetuximab.

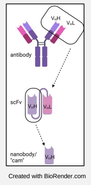
Conclusions: We have successfully produced and validated the specificity and function of an engineered camB7-H3 CAR-iNK cell exhibiting robust target killing and on-target specificity. To our knowledge, this is the first camelid nanobody antigen recognition domain reported in a CAR-NK cell to be used as an off-the-shelf immunotherapy. The combining camB7-H3 CAR-NK with monoclonal antibodies targeting HER2 and EGFR as a dual targeting approach will add additional tumor specificity, further increase the efficacy of tumor cell elimination and prevent antigen escape. Additional camB7-H3 CAR-iNK cell preclinical studies are in process and will be discussed.

B7-H3: An Optimal Pan-Tumor Immune Target

- B7-H3 (CD276) is a member of the PD-L1 superfamily
- Function is controversial, but typically viewed as immune-suppressive
- B7-H3 expression level correlates with grade and stage at diagnosis in addition to long-term patient outcomes
- Promotes metastasis, epithelial-mesenchymal transformation, and invasion
- Found on tumor neovasculature, but not normal vascular structures

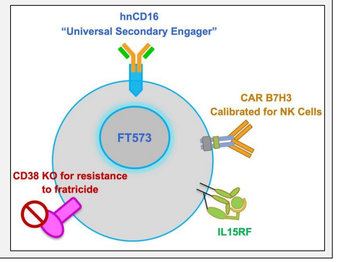
Advantages of Camelid Nanobodies

- Smaller than scFv (17 vs 25kDa)
- Generally higher affinity than scFv
- Improved binding to deeper grooves on antigen surface
- Highly resistant to denaturing under extreme conditions found in tumor microenvironment
- Low immunogenicity compared to other xenogeneic antibody sources



FT573: Multiplexed-Engineered iPSC-derived CAR NK Cell Targeting pan-Cancer Antigen B7-H3

- Off-the-shelf allogeneic iPSC NK cell
- Novel camelid B7-H3 CAR optimized for function in NK cells
- High-affinity, non-cleavable hnCD16 to maximize ADCC when combined with therapeutic monoclonal antibodies
- IL-15/IL-15 receptor alpha fusion for enhanced persistence
- CD38 knockout to improve metabolic fitness



camB7-H3 Demonstrates Antigen Specificity Against Multiple B7-H3 WT/KO and B7-H3^{hi} Lines

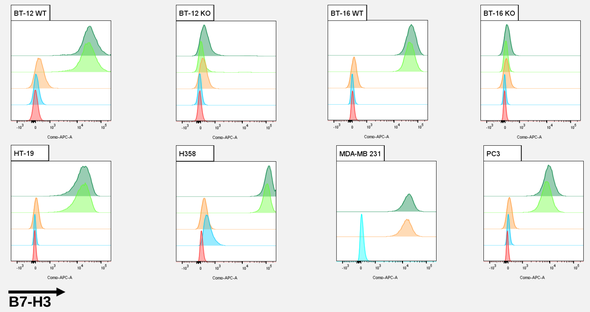


Figure 1. Single-domain camelid nanobody with a 10x His tag was incubated with target cell lines and then co-stained with anti-His APC or equimolar (16.67nM) commercial mAb B7-H3 APC (clone 7-157), isotype, anti-His APC alone, or unstained cells. BT-12 and BT-16 B7-H3 WT and KO cells were a gift from Dr. Crystal Mackall.

camB7-H3 CAR NK Demonstrates Increased Binding to B7-H3 Protein Compared to scFv B7-H3 CAR

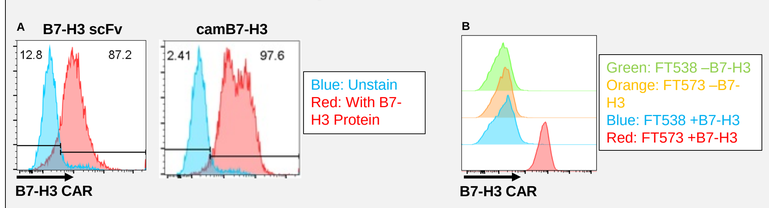


Figure 2. A) FT573 cells transduced with B7-H3 CAR demonstrate increased direct binding of recombinant B7-H3-streptavidin protein compared to scFv B7-H3 CAR. Cells were incubated with biotin-labeled fluorophore or unstained controls prior to analysis. B) CAR was assessed using a recombinant human B7-H3 protein stained with a secondary antibody. The two upper histograms did not have the protein in the staining cocktail while the bottom two did.

Optimizing camB7-H3 CAR Construct for Enhanced pan-targeting

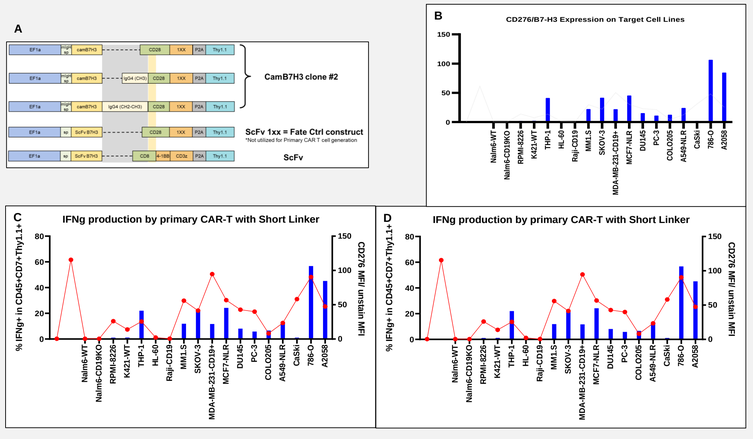


Figure 3. Optimization of CAR construct for NK cells with different linker lengths. A) Diagram of CAR constructs used to compare short, medium, and long linkers paired with camB7-H3 antigen recognition domains to scFv B7-H3 paired with Fate's control construct with 1xx domain or scFv and standard CAR construct. B) B7-H3 (CD276) MFI:unstain MFI for target cell lines. C) Interferon gamma production by CAR-T transduced with short linker constructs when stimulated with control or cell lines with varying levels of B7-H3 surface expression. D) TNF alpha production by CAR-T transduced with short linker constructs as described in (C).

camB7-H3 CAR-T cells Show Durable Control and Prevent Disease Progression *in vivo*

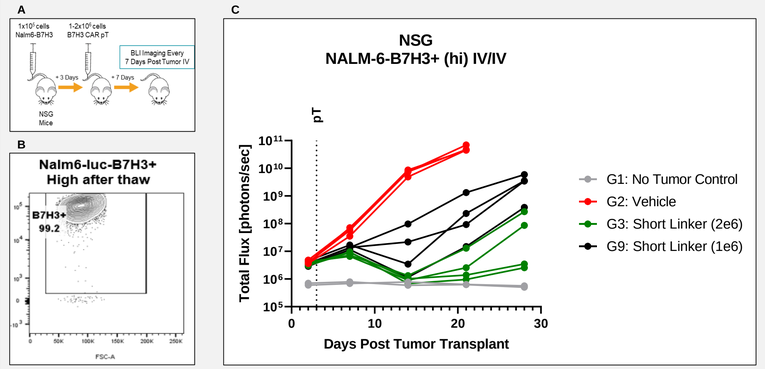


Figure 4. A) Schematic of in vivo model. NSG mice were injected with 1e5 Nalm6-Luc-B7-H3 High tumor cells on Day 0 followed by 1-2e6 CAR-T cells on Day +3. Luciferase intensity was followed weekly until Day +28 when remaining mice were sacrificed. B) B7-H3 Expression on transduced Nalm6 cells C) camB7-H3 CAR-T treated mice show reduced disease burden compared to treatment controls.

FT573 Exhibits camB7-H3 CAR specificity for B7-H3

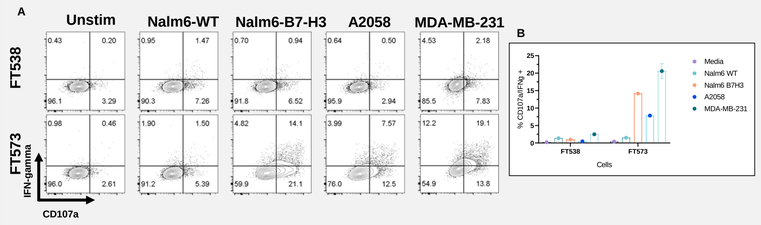


Figure 5. A) Representative flow cytometric analysis of CD107a and IFNγ expression for FT538 and FT573 cells upon stimulation with a variety of tumor targets in 4 hour stimulation. B) Percentage of cells double positive for CD107a and IFNγ after 4 hour stimulation.

Conclusions

- We have identified a novel camelid nanobody antigen-engager that is highly-specific for B7-H3
- Optimization of camB7-H3 CAR construct enhanced activity against B7-H3+ targets and uniquely demonstrated ubiquitous targeting of multiple solid tumor cancer cell lines
- Higher expression of camB7-H3 CAR expression on iPSC NK cells was observed compared to scFv B7-H3 CAR, suggestive of enhanced CAR-specific activity
- camB7-H3 CAR effectively controls disease progression in an aggressive disseminated xenograft model of B7-H3-transduced Nalm6 cells
- Initial studies show that FT573 CAR NK cells demonstrate specificity for B7-H3 via degranulation and inflammatory cytokine production *in vitro*

Citations: Yang et al., Int J Biol Sci., 2020
Zhou et al, Front. Immunol., 2021
Picarda et al., Clin. Can. Res., 2016
Asaadi et al., Biomarker Res., 2021
Theruvath et al, Nature Medicine, 2019

Funding: NAZ is supported by a Hematology Training grant (5T32HL007062-45, PI Vercellotti) and funding from the University of Minnesota's Academic Investment Education Program. JG, BH, MP, BG, TL, WY, RB and BV are employees of Fate Therapeutics. JSM receives research funding from Fate Therapeutics.