Combining FT536, a pan-Tumor Targeting CAR NK Cell Therapy, with CD16 Engagers Provides a Coordinated Targeting Strategy to Overcome Tumor Heterogeneity

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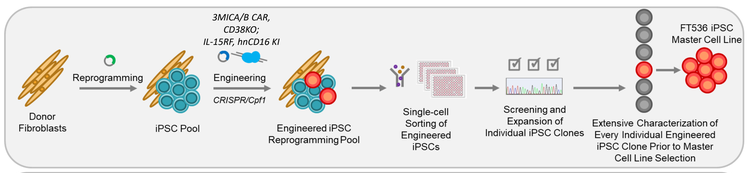
INTRODUCTION & GRAPHICAL ABSTRACT

- Tumor antigen heterogeneity, the paucity of tumor-specific antigens and pervasive immune evasion remain a significant challenge to the development of efficacious solid tumor immunotherapies.
- Immune checkpoint inhibition and bi-specific engagers are increasingly utilized in combination to enhance therapeutic applications against solid tumors.
- FT536 is a multiplexed-engineered clonal master induced pluripotent stem cell (iPSC)-derived NK cell product candidate that incorporates a novel CAR targeting the pan-tumor associated MICA and MICB (MICA/B) stress proteins (3MICA/B CAR).
- We have previously presented that FT536 can overcome multiple tumor immune evasion mechanisms; elicit significant and broad CAR-mediated anti-tumor cytotoxic effector function; and contains the ability for multi-antigen targeting through its expression of high-affinity, non-cleavable CD16 (hnCD16) Fc receptor.
- We here demonstrate that combining FT536 with monoclonal antibodies (mAbs) targeting EGFR, HER2, and a bi-specific c-met/EGFR mAb (amivantimab) results in potent antibody dependent cellular cytotoxicity (ADCC) and provides additional evidence that multi-antigen-specific tumor targeting affords potent cytotoxicity responses in models of tumor heterogeneity.
- We hypothesize that multi-antigen targeting of solid tumors could provide a novel approach to minimize antigen selection and immune escape through antigen-sculpting.
- To assess the potency of multi-antigen targeting and combinatorial therapeutic application in humans, a phase I first-in-human, dose-escalation clinical study of FT536 as monotherapy and in combination with tumor-targeting mAb therapy, including amivantamab, for the treatment of multiple solid tumor indications was designed and is currently enrolling (NCT05395052).

Off-the-shelf Multiplex-Engineered Cell Therapy Platform



FT536 iPSC Master Cell Line Expanded to generate FT536

Consistent and homogeneous NK cell manufacturing from renewable iPSC MCB

Uniform off-the-shelf NK Cell Products

- *Does not require patient or donor sourced cells*
- *Eliminates stochastic editing variability associated with pool editing*
- *Consistent, cost effective and highly scalable manufacture process*
- *Analogous to pharmaceutical drug product development*
- *Administered off-the-shelf in outpatient setting*

Lead Clone Selection Through Extensive Attribute Characterization

Desired Attributes

- Multiplexed Engineered iPSC clones selected
- Clones with copy number & locus-target verified
- Clones maintaining pluripotency
- Clones free of reprogramming vectors
- Clones demonstrating genomic stability
- Clones without off-target edits
- Clones with ideal propensity to become NK cells
- Clones with desired functional activity & specificity
- Selected FT536 MCB passing all test criteria

>500 clones → **Lead Clone**

Multiplexed Engineering
Incorporate multiple mechanisms of action to eradicate cancer

Mass Production
Reliable manufacturing process from MCB with high yield at low cost per dose

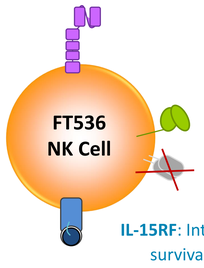
Uniform Products
Consistent identity, purity and potency of cell products

Off-the-Shelf
Stable, cryopreserved for on-demand treatment and expanded patient reach

Treatment Paradigm
Flexible out-patient treatment strategies to drive deep responses

Fate Therapeutics' induced pluripotent stem cell (iPSC) product platform enables mass production of off-the-shelf, engineered, homogeneous cell products that can be administered with multiple doses to deliver more effective pharmacologic activity, including in combination with cycles of other cancer treatments.

FT536: Off-the-Shelf Multi-Antigen-Targeting NK Cell Immunotherapy



3MICA/B CAR: Novel Chimeric antigen receptor targeting the membrane proximal α3 domain of MICA/B. *Ferrari de Andrade et al. Science 30 Mar 2018. DOI: 10.1126/science.aao0505*

hnCD16: High-affinity 158V, non-cleavable CD16 Fc receptor modified to augment ADCC. *Zhu et al. Blood 6 Feb 2020. DOI: 10.1182/blood.2019000621*

CD38 KO: Deletion of CD38 to eliminate anti-CD38 antibody mediated NK cell fratricide. *Woan et al. Cell Stem Cell Dec 2021. DOI: 10.1016/j.stem202108013*

IL-15RF: Interleukin-15 receptor fusion; a potent cytokine complex that promotes survival and persistence and reduces the dependency for exogenous cytokine support. *Woan et al. Cell Stem Cell Dec 2021. DOI: 10.1016/j.stem202108013*

RESULTS

Broad Expression Across Multiple Tumors

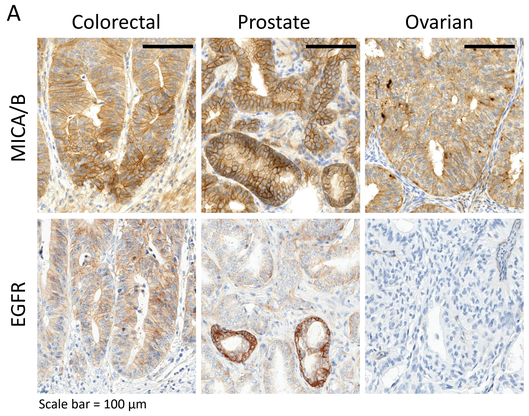


Figure 1. Tumor MICA/B and EGFR antigens are differentially expressed across distinct cancer indications. Treatment naïve patient tumor surgical sections were evaluated for MICA/B and epidermal growth factor receptor (EGFR) expression using established immunohistochemical (IHC) methods. Positive MICA/B expression was observed at different staining densities across all three tumors. In contrast EGFR demonstrated distinct staining patterns determined by tumor indication. The observed antigen heterogeneity and multiple antigen presence within the same tumor highlights an opportunity for multi-antigen targeting in order to maximize tumor cytotoxicity.

Preclinical Evaluation Shows Enhanced Anti-tumor Activity Across Multiple Cancers

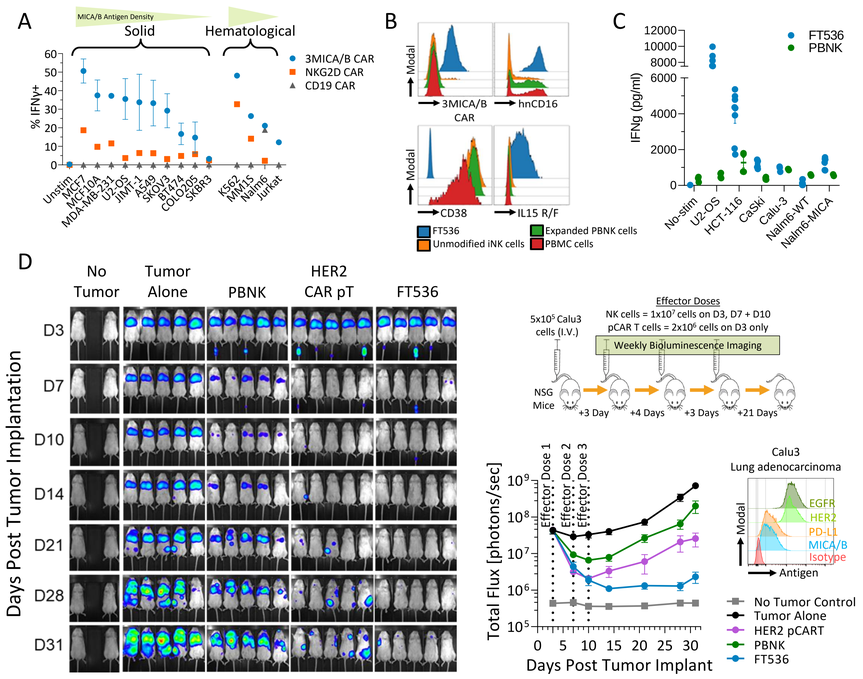


Figure 2. FT536 3MICA/B CAR expressing iNK cells provide broad tumor reactivity and in vivo efficacy. A) 3MICA/B CAR, NKG2D CAR and CD19 CAR expressing primary T cell IFNγ responses against a library of solid and hematological tumor cell lines expressing decreasing levels of surface MICA/B antigen. B) Transgene profile of FT536 compared to unmodified iNK, expanded peripheral blood (PB) NK and NK cells contained within a peripheral blood derived mononuclear cell (PBMC) fraction. NK cells were defined as CD45+/CD56+/CD3-. C) MICA/B specific cytokine release in response to a 4-hour co-incubation with high, medium, and low natively expressing MICA/B tumor cell lines at a 1:1 E:T ratio measured by MSD. D) Cryopreserved FT536 cells were adoptively transferred into mice bearing endogenous MICA/B expressing (low) Calu3 tumors and their ability to limit tumor growth was evaluated compared to PBNK and HER2 CAR expressing primary T cells.

Combining FT536 with Secondary Antigen Targeting CD16 Engagers Provides Broad Multi-Antigen Anti-Tumor Cytotoxicity

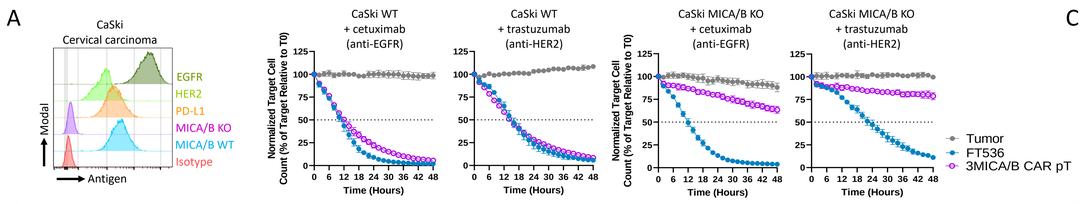
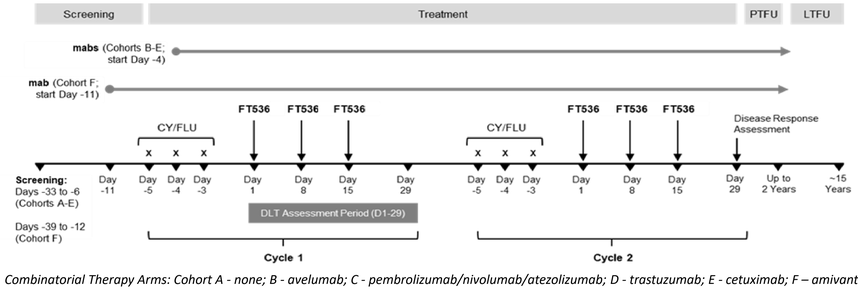


Figure 3: FT536 anti-tumor activity and antigen recognition is enhanced in combination with tumor targeting monoclonal antibodies. A) Cervical epidermoid carcinoma CaSki wild type (WT) and MICA/B knock out (KO) tumors were assessed for surface antigens and co-cultured with FT536 and 3MICA/B expressing primary T cells at a 3:1 effector:target (E:T) ratio ± 5µg/mL cetuximab (anti-EGFR) or trastuzumab (anti-HER2). Direct cytotoxicity and ADCC was evaluated via the detection of fluorescently labelled tumor cells and presented as the % of remaining tumor cells relative to tumor alone conditions. B) EGFR exon 20 insertion mutant NCI-H1975 non small cell lung cancer (NSCLC) were assessed for surface antigen expression and incubated with FT536 and PBNK cells at a 3:1 E:T ratio ± equimolar concentrations of amivantamab (anti-EGFR/c-MET), cetuximab (anti-EGFR), or trastuzumab (anti-HER2) and % cytolysis relative to tumor alone or tumor + relevant mAb condition was measured over time.

FT536-101: A Phase I, Open-Label, Multicenter Study of FT536 as Monotherapy and in Combination with Monoclonal Antibodies in Subjects with Advanced Solid Tumors

FT536-101 Treatment Schema (clinicaltrials.gov NCT05395052)



Combinatorial Therapy Arms: Cohort A - none; B - avelumab; C - pembrolizumab/nivolumab/atezolizumab; D - trastuzumab; E - cetuximab; F – amivantamab.

PTFU = Post-treatment follow-up, LTFU = Long term follow-up, CY = cyclophosphamide 500 mg/m²/day intravenously, FLU = fludarabine 30 mg/m²/day intravenously

Eligibility Criteria

Inclusion: Disease relapse or progression after at least one line of therapy; Measurable disease by RECIST
Cohort specific inclusion criteria:

Cohort/Combination	Criteria
Cohort A (monotherapy)	Locally advanced or metastatic non-small cell lung cancer (NSCLC), colorectal cancer (CRC), breast cancer, ovarian cancer or pancreatic cancer.
Cohorts B & C (+ anti-PD1/L1 mAb)	NSCLC, gastroesophageal adenocarcinoma, head and neck squamous cell carcinoma, or urothelial carcinoma with documented PD-L1 expression. Microsatellite unstable/mismatch repair deficient CRC.
Cohort D (+trastuzumab)	Any solid tumor cancer with HER2 IHC ≥2+; or Average HER2 copy number ≥4 per cell.
Cohort E (+cetuximab)	CRC that has relapsed or progressed following prior EGFR mAb treatment or has KRAS/NRAS mutation HNSCC that has relapsed or progressed following prior cetuximab treatment.
Cohort F (+amivantamab)	NSCLC with EGFR driver mutation and progressed or were not candidates for EGFR TKI, MET exon 14 skipping mutation progressed or were not candidates for MET TKI, MET amplification with MET/CEP7 ratio ≥ 1.8 or gene copy number ≥ 5.

Exclusion: ECOG ≥ 2; Insufficient hematologic, renal, hepatic, pulmonary, or cardiac function; Requiring systemic steroids or history of autoimmune disease; Uncontrolled infection

Table 1: First monotherapy FT536 escalation cohort (100 million cells/dose)

Patient	Tumor Type	Prior Lines of Therapy	Refractory to Last Prior Therapy	Number of FT536 doses	DLT, CRS, or ICANS	Change in Target Lesions	BOR
1	Pancreatic	1	Yes	3	None	+31.3%	PD
2	Pancreatic	2	Yes	3	None	-22.7%	PD*
3	Colon	5	Yes	3	None	NA	NA

DLT = dose limiting toxicity, CRS = cytokine release syndrome, ICANS = immune effector cell-associated neurotoxicity syndrome, BOR = best overall response, PD = progressive disease, NA = not available. Data as of 27Sep2022. *Disease progression due to new lesion.

Phase 1 Clinical Trial Interim Summary

- Three patients treated with one cycle of starting dose of 100 million cells/dose x 3 doses.
- No DLTs, CRS or ICANS were reported.
- Monotherapy escalation continuing at 300 million cells/dose.
- Dose escalation in combination with mAbs has been initiated at 100 million cells/dose.

SUMMARY

- MICA and MICB stress antigens are ubiquitously expressed in solid tumors and in combination with other tumor associated antigens, such as EGFR, represent a comprehensive approach to targeting bulky tumors that are often heterogenous.
- FT536 efficiently targets MICA and MICB stress antigens and provides broad anti-tumor reactivity and superior efficacy relative to peripheral blood NK cells and primary T cells expressing NKG2D or HER2 CAR.
- The combination of FT536 with multiple CD16 engagers, including therapeutic mAbs, results in additive functional activity between CAR and ADCC, eliciting durable tumor growth inhibition and mitigating antigen escape seen with heterogeneous tumors.
- FT536-101 Phase I Clinical Trial has safely administered 1 cycle of FT536 at 100 million cells/dose to 3 patients without any detected DLT, CRS or ICANS with plans to combine with ADCC-competent mAbs