Development of Next Generation Multi-antigen Targeting Off-the-Shelf CAR T cells for Conditioning-free Treatment of B-cell Lymphoma

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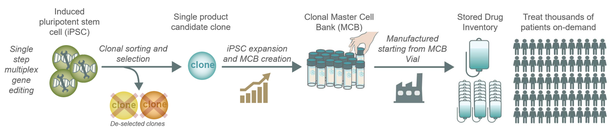
Introduction

Autologous chimeric antigen receptor (CAR) T-cell therapy has had success in the treatment of hematological malignancies, yet its clinical application remains hindered by several significant limitations. Major challenges include the high cost, complex manufacturing process, requirement for intense lymphodepleting chemotherapy prior to infusion, and the limited accessibility and scalability of the therapy. FT839 is an induced pluripotent stem cell (iPSC) derived CAR T cell designed to overcome these limitations and provide potent and versatile therapy against lymphocytic cancers. FT839 is a multiplex-engineered CAR T cell equipped with anti-CD19 and anti-CD38 dual-CAR expression to target a variety of cell types that lead to hematological malignancies including lineage specific or activated pathological cell subsets. FT839 also incorporates Sword and Shield™ technology, designed to both target and evade alloreactive immune cells and eliminate the need for intensive conditioning chemotherapy. Sword and Shield™ technology is the synergistic action of a novel Alloimmune Defense Receptor (ADR), which eliminates 4-1BB+ alloreactive immune cells, and the genetic ablation of CD58 ($CD58^{KO}$), which limits synapse formation with alloreactive cells. This strategy promotes functional persistence and evasion of host alloreactive immune responses. FT839 CAR T cells are also uniquely engineered to express a novel CD3-chimeric fusion receptor (CD3CFR) and a high-affinity, non-cleavable CD16 (hnCD16). These engineered attributes allow for flexible multi-antigen targeting in combination with clinically approved T cell engagers (TCEs) or monoclonal antibodies (mAbs). FT839 demonstrates versatility in targeting cancer cells via multiple antigen-receptor activation pathways, resulting in potent and flexible multi-antigen targeting. FT839 is engineered for success in treating challenging and heterogenous relapsed/refractory B cell lymphomas.

iPSC platform and multiplexed-engineering overcomes the challenges associated with existing approaches to traditional cellular therapy

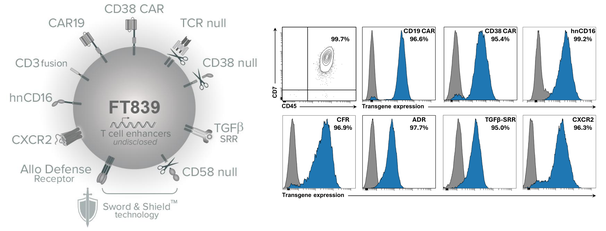


Platform Advantages

- ✓ **Defined Clonal Master Cell Bank (MCB)**: Single-cell derived, genetically uniform, selected for potency and genomic integrity.
- ✓ **Engineered MCB Starting Material**: One-time edit, highly scalable, donor-independent, and enables consistent high-quality products.
- ✓ **Modular Innovation**: Accelerates development through efficient, multiplexed engineering.

iPSC-derived Cell Therapy Products

- ✓ **Reliable, Scalable Drug Product**: Consistent, well-characterized, >5-year shelf stability; ~50,000-dose GMP-scale capacity at current site.
- ✓ **Cost-Effective & Consistent**: Low COGs (~$3,000/dose), inventory-based economics, and no donor variability.
- ✓ **Patient-Centered Therapy**: Off-the-shelf, antibody-like treatment with repeat dosing, low toxicity, and outpatient-friendly administration.



Summary & Conclusions

FT839 is a next generation iPSC derived CAR T-cell uniquely designed to target hematologic malignancies and overcome antigen escape mechanisms through multi-antigen targeting. FT839 incorporate novel synthetic functional elements designed to effectively eliminate the need for conditioning chemotherapy .

- ✓ **Pan-hematologic malignancy targeting and flexible anti-tumor activity:** FT839 is equipped with anti-CD19 and anti-CD38 dual-CAR system to target lineage specific or activated pathological cell subsets, including cells of hematologic malignancies.
- ✓ **Multi-Antigen targeting:** FT839 is engineered to express novel CD3-chimeric fusion receptor (CD3CFR) and a high-affinity, non-cleavable CD16 (hnCD16) for versatile multi-antigen targeting in combination with clinically approved T cell engagers (TCEs) or monoclonal antibodies (mAbs), strategic options to combat tumor heterogeneity and antigen escape.
- ✓ **Engineered for efficient trafficking and enhanced persistence in tumor microenvironment**: FT839 is engineered with CXCR2 (C-X-C motif chemokine receptor 2) for enhanced trafficking to the tumor site and with TGFβ-SRR (Transforming growth factor signal redirector receptor) for enhanced persistence and resistance to immunosuppressive tumor microenvironment.
- ✓ **Resistance to host immune cell-mediated rejection:** Sword & Shield™ technology consisting of CD58 knockout ($CD58^{KO}$) for immune ***evasion*** and alloimmune defense receptor (ADR) to ***selectively eliminate*** allogeneic immune responses. Addition of CD38 CAR provides supplemental resistance by eliminating CD38+ alloreactive immune cells.
- ✓ **Elimination of lymphodepleting preconditioning**: Sword & Shield™ (ADR and $CD58^{KO}$) dual-resistance strategy to avoid pre-conditioning chemotherapy and eliminate the toxicity risks of lymphodepletion and increase utility.
- ✓ **Off-the-shelf**: Manufactured from an iPSC master cell bank that is derived from a fully characterized and multiplex-engineered clonal iPSC line, delivering uniform expression of functional elements at a large scale to support on-demand delivery.

Results

Through CAR-mediated co-targeting of CD19 and CD38, FT839 broadly and effectively eliminates a wide range of B cell leukemias and lymphomas with durable outcome

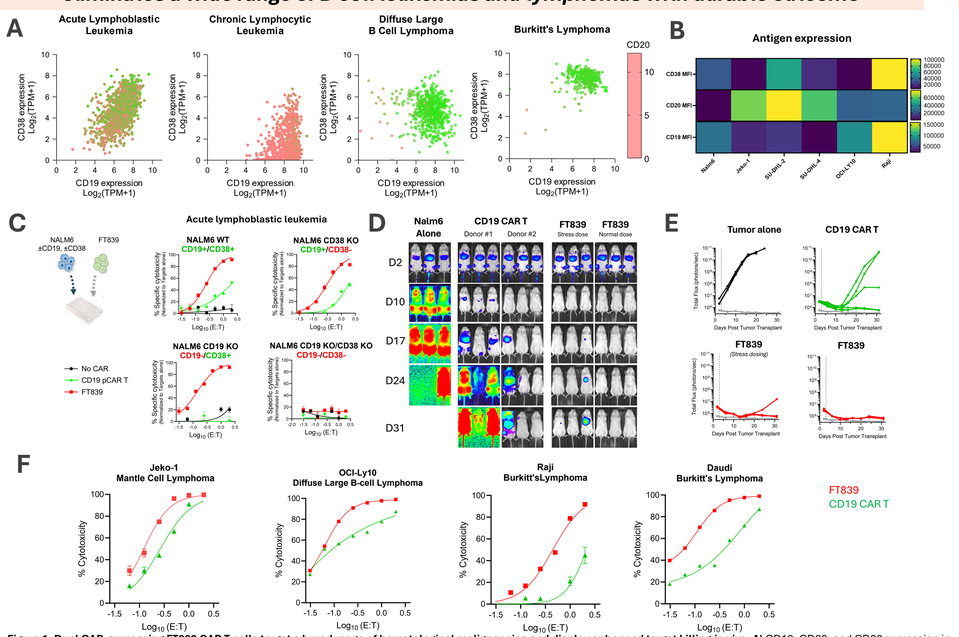


Figure 1. Dual CAR-expressing FT839 CAR T cells target a broad range of hematological malignancies and display enhanced target killing *in vivo*. **A)** CD19, CD38, and CD20 expression in B cell malignancies (mRNA) or, **B)** surface protein expression on commonly used lymphoma and leukemia lines. **C)** *In vitro* schematic; FT839 CAR T cells expressing both anti-CD19 and anti-CD38 CARs cells or CD19 CAR expressing primary T cells (CD19 pCAR T) were challenged with NALM6 lines with single, combination, or no expression of CD19 and CD38 antigens in a 24-hour cytotoxicity assay over a range of E:T (2:1 to 1:32). **D)** Mice were inoculated with luciferase expressing (CD19+CD38+) NALM6 leukemia B cells and treated with FT839 or CD19 pCAR T cells generated from two independent healthy donors. Tumor growth was monitored by bioluminescence-based imaging. **E)** Quantification of tumor burden; each line represents an individual mouse. Background luminescence is shown for non tumor bearing mice (gray). **F)** FT839 CAR T cells or CD19 CAR expressing primary T cells (CD19 CAR T) were challenged with the indicated lymphoma target lines with variable levels of CD19 or CD38 expression over the indicated E:T.

FT839 is engineered with CD3-chimeric fusion receptor (CD3CFR) and high-affinity, non-cleavable CD16 (hnCD16) to overcome antigen escape and tumor heterogeneity

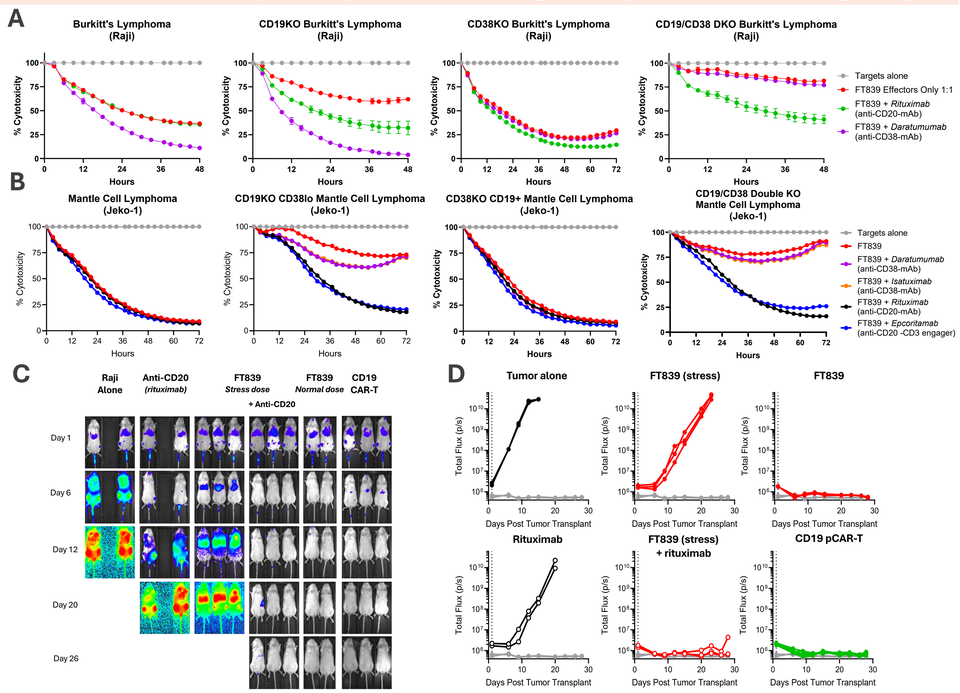


Figure 2. FT839 targets broad hematologic malignancies in combination with therapeutic antibodies (hnCD16 mediated ADCC) and clinically approved T cell engagers (via CD3-chimeric fusion receptor). FT839 CAR T cells or CD19 CAR expressing primary T cells (CD19 pCAR T) were challenged with the Burkitt's Lymphoma line, Raji **(A)** or Mantle cell lymphoma line, Jeko-1 **(B)** with single, combination, or no expression of CD19 and CD38 antigens. FT839 CAR T cells are also challenged with these tumors in combination with Rituximab, Daratumumab, Isatuximab, or Epcoritamab for 72-hour cytotoxicity assay. **C)** Mice were inoculated with luciferase expressing (CD19+CD38+) Raji lymphoma B cells and treated with FT839 CAR T cells alone or in combination with Rituximab or CD19 CAR T. Tumor growth was monitored by bioluminescence-based imaging. **D)** Quantification of tumor burden; each line represents an individual mouse. Background luminescence is shown for non tumor bearing mice (gray).

FT839 is engineered with Sword & Shield™ technology to support functional persistence in an allogeneic setting and eliminate the need for conditioning chemotherapy

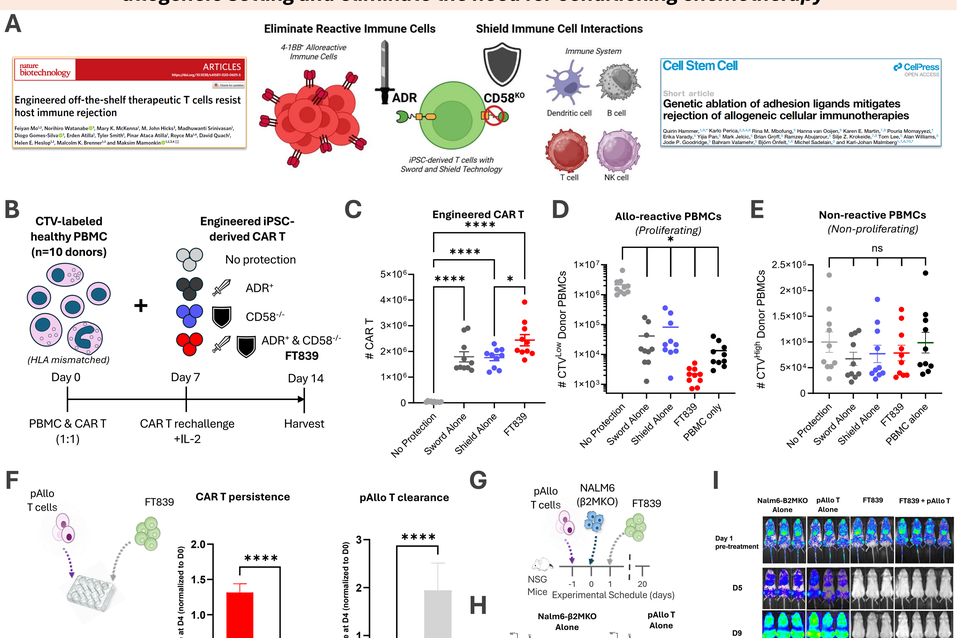


Figure 3. The Sword and Shield™ (ADR and CD58KO) technology protects iPSC-derived CAR T cells from allorejection, prevents expansion of highly reactive allo-PBMC while sparing non-reactive allo-PBMC. **A)** Illustration of Sword and Shield™ concept. **B)** Schematic of the *in vitro* mixed lymphocyte reaction assay performed with 10 different allogeneic PBMC donors and the indicated iPSC-derived T cells. PBMCs were labelled with cell trace violet (CTV). On day 14 of the MLR, flow cytometry was used to analyze **C)** total engineered CAR T cell remaining, **D)** total reactive allogeneic PBMC, **E)** total non-reactive PBMCs. **F)** Schematic of *in vitro* mixed lymphocyte reaction (MLR) consisting of primed alloreactive T cells (pAllo T cells), and FT839 CAR T cells or CAR T cells that lack ADR and CD58 KO technology (no protection). pAllo T cells are generated following repeated exposure to donor-specific T cells. Figure represents CAR T cell persistence and primed Allo T cell clearance at end of the assay. **G)** *In vivo* schematic, **H)** bioluminescence-based tumor burden quantification in mice inoculated with disseminated β2M-deficient Nalm6 leukemia B cells treated with FT839 CAR T cells in the presence or absence of pAllo T cells, background luminescence is shown for non tumor bearing mice (gray). **I)** Representative images depicting tumor burden. *RM one-way ANOVA, multiple comparison was used for statistical analysis of all graphs, showing indicated comparisons *p<0.05, ****p<0.0001, and ns = not significant.*

FT839 is engineered with CXCR2 and TGFβ-SRR for efficient trafficking to the tumor site and enhanced activity in the immune cell suppressive tumor microenvironment

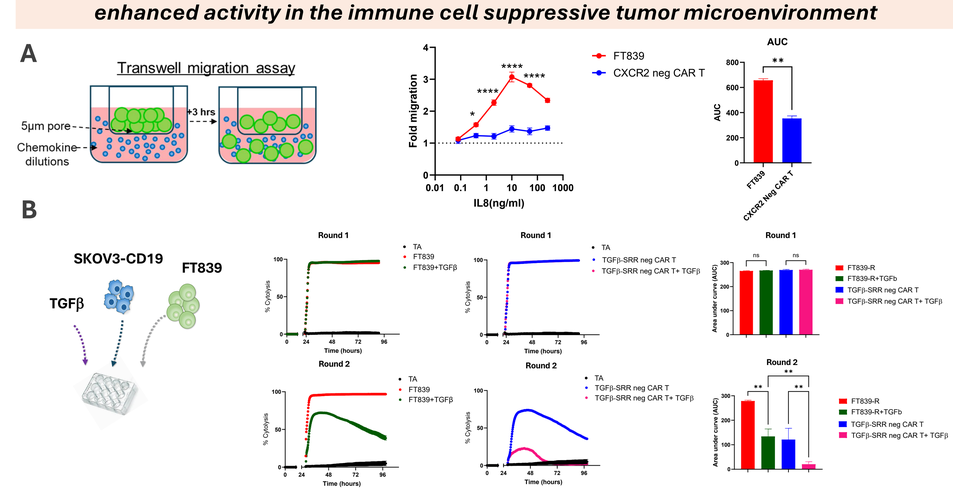


Figure 4. FT839 shows CXCR2 mediated migration towards CXCR2 ligand and robust anti-tumor activity in the presence of TGFβ. **A)** *In vitro* schematic; dose dependent migration of FT839 towards CXCR2 ligand, CXCL8, as compared to CXCR2 negative CAR T. Data are represented as mean ± SEM (Standard Error of the Mean), Two-way ANOVA and Šídák's multiple comparisons test were used for statistical analysis of dose response curve (**** p<0.0001 and *p<0.05. **B)** An xCelligence instrument was used to measure cytolysis of SK-OV3 targets by FT839 or TGFβ-SRR negative CAR T cell, with or without 10 ng/ml TGFβ. Representative cytolysis trace plots for each round by FT839 or TGFβ-SRR negative CAR T are shown. Tumor alone (TA) control is also shown for each round. For each round, area under the curve (AUC) was calculated for each sample (n=3), bars represent mean AUC ± SD (Standard deviation) of arbitrary units. An ordinary one-way ANOVA with a Tukey's multiple comparison test was performed to evaluate significance. ns= non-significant and p<0.005 (**).