FT819-102: Clinical Translation of Off-the-Shelf TCR-less CD8αβ+ anti-CD19 CAR-T Cells for The Treatment of B cell-mediated Autoimmune Disorders

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Introduction

Autoreactive B cells are key drivers of various autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, and scleroderma. Management of autoimmune disease often includes prolonged use of immune suppressants and B cell-directed agents. Rituximab, an anti-CD20 antibody, has demonstrated the ability to deplete peripheral blood B cells. Despite this fact, the clinical efficacy of rituximab is limited and varies broadly among different autoimmune diseases due to incomplete clearance of autoreactive B cells, including those in secondary lymphoid tissues.

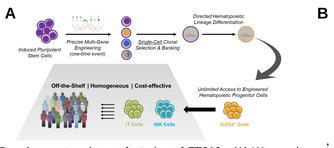
Recent clinical data has demonstrated the potential of autologous CD19-directed chimeric antigen receptor (CAR) T-cell therapy to deplete CD19+ B cells and induce durable, drug-free remission in refractory SLE patients (Mackensen, 2023). However, several obstacles may limit the potential application of autologous CAR T-cell therapy for treatment of patients with autoimmune diseases, including (1) the need for apheresis to collect patient T cells for manufacture, (2) the use of viral vectors for engineering, with associated risks for potential malignancies via insertional mutagenesis, and (3) lack of product consistency and availability, which are necessary to ensure patient safety and reach.

FT819 is an off-the-shelf, anti-CD19 CAR T-cell product candidate derived from a clonal master engineered induced pluripotent stem cell (iPSC) line. The use of a master engineered iPSC line as a starting cell source enables scaled production of a cell product which is well-defined and uniform in composition, can be stored in inventory for off-the-shelf availability, can be combined and administered with standard of care therapies, and can reach a broad patient population. Interim clinical data from a Phase 1 study of FT819 in relapsed / refractory B-cell malignancies (BCM Study) indicate a favorable safety profile and anti-tumor activity, with no events of any grade of immune effector cell-associated neurotoxicity (ICANS) or graft-versus-host disease (GvHD), low incidence of low-grade cytokine release syndrome (CRS), and partial and complete responses in heavily pre-treated patients (Mehta, 2022; NCT04629729).

Here, we present preclinical and clinical data from the Phase 1 BCM Study that support the development of FT819 in B cell-mediated autoimmune diseases. Preclinically, FT819 co-cultured with CD19+ WIL2S lymphoma cell line demonstrated potent and specific CD19+ B cell killing in an *in vitro* cytotoxicity assay. Similarly, in co-culture with peripheral blood from unmatched SLE donors, FT819 exhibited rapid, deep, and durable elimination of CD19+ B cells. In addition, blood samples obtained from patients treated in the Phase 1 BCM Study showed rapid, deep, and sustained depletion of CD19+ B cells in the periphery through the treatment cycle (Day 29). Importantly, significant B cell depletion was also observed in lymph node biopsies from the Phase 1 BCM Study, demonstrating the potential of FT819 to traffic to and function in secondary tissues. Furthermore, an analysis of hematopoietic reconstitution showed recovery of a naïve B-cell compartment occurring 1-3 months after FT819 infusion. Based on these preclinical and clinical observations, FT819 may offer an off-the-shelf approach to reset the immune system of patients with autoimmune diseases.

A Phase 1 study of FT819 for the treatment of patients with moderate-to-severe SLE is currently ongoing (Autoimmunity Study) and the first patient has recently been treated (NCT06308978). A pre-treatment sample from this first patient's peripheral blood was obtained and an *ex vivo* killing assay was conducted, which showed rapid and deep depletion of CD19+ B cells by FT819 at low E:T ratios.

Our proprietary iPSC product platform enables creation of a clonal master engineered iPSC line and mass production of multiplexed-engineered cell products, which are well-defined and uniform in composition and can be stored in inventory for off-the-shelf availability and broad patient reach



Development and manufacturing of FT819. (A) We used a multiplex-engineering platform to make a clonal master engineered iPSC line, which is the starting material for each manufactured batch of FT819 cell product. (B) FT819 expresses a transgene encoding for 1XX CAR19, an anti-CD19 CAR construct containing CD28 and modified CD3ζ signaling domains. In preclinical studies, fine tuning of the CAR signaling through CD3ζ enhances therapeutic potency with reduced propensity for exhaustion.

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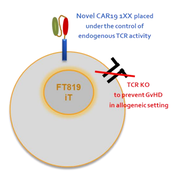
Summary and Conclusions

- Preclinical data demonstrate that FT819 exhibits rapid and deep depletion of pathogenic CD19+ B cells sourced from unmatched SLE donors
- Translational data from Phase 1 BCM Study demonstrate that a single dose of FT819 exhibits multiple mechanisms of action necessary for generating an immune reset in patients with B cell-mediated autoimmune disease:
  - Dose-dependent PK, with similar expansion kinetics to autologous CAR T cells
  - Rapid and deep CD19+ B cell depletion, with sustained suppression of B cells, in the periphery during the initial 30-day period following FT819 infusion
  - Secondary and tertiary tissue trafficking, infiltration, and activity, with complete elimination of CD19+ cells in tissue
  - B-cell reconstitution showing recovery of naïve and immature phenotype, with little to no recovery of memory B cells or plasmablasts
  - Capacity for rapid, deep, and sustained B-cell depletion without the use of fludarabine as a conditioning agent
- First SLE patient has been treated in Phase 1 Autoimmunity Study
  - Pre-treatment sample of the first patient's peripheral blood was obtained prior to conditioning chemotherapy and FT819 treatment; FT819 induced rapid and potent depletion of the patient's CD19+ B cells in *ex vivo* co-culture assay in "first-of-kind" translational assessment

Results

FT819 is an off-the-shelf, CD19-targeted, 1XX CAR T-cell product candidate that is comprised of CD8αβ+ T cells with a memory phenotype and high CXCR4 expression to promote tissue trafficking



1XX CAR19: Novel chimeric antigen receptor consisting of CD28 costimulatory domain and modified CD3z signaling domain for optimal effector cell persistence and anti-tumor potency

TRAC targeted CAR: Chimeric antigen receptor integrated into the T Cell Receptor Alpha Constant region to be regulated by endogenous control of TCR expression for optimal CAR performance

TCR null: Bi-allelic disruption of TRAC at the clonal level for complete removal of TCR expression and the elimination for the possibility of GvHD in allogeneic setting

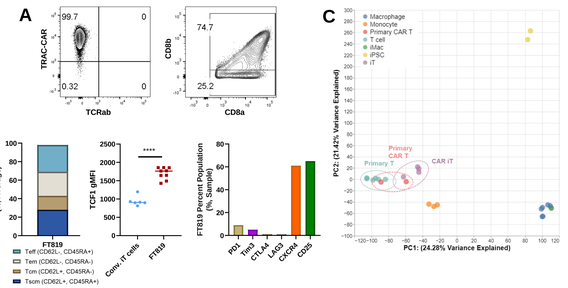
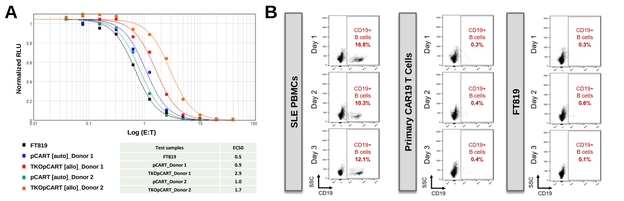


Figure 1. (A) FT819 consists of uniform and consistent TCR-null, CD8αβ+ CAR T-cells. (B) FT819 is comprised of memory T cells, with high expression of TCF1 and CXCR4 and low expression of exhaustion markers. (C) Global gene expression analysis shows FT819 clustering with primary T cells and primary CAR T cells.

FT819 demonstrates rapid, deep, and dose-dependent depletion of CD19+ B cells sourced from the peripheral blood of unmatched SLE donors in preclinical studies



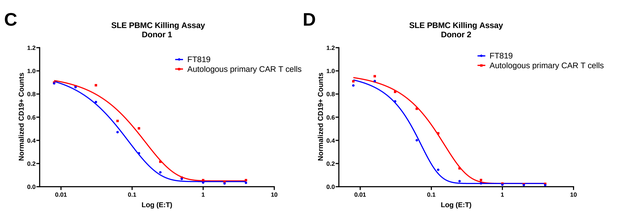
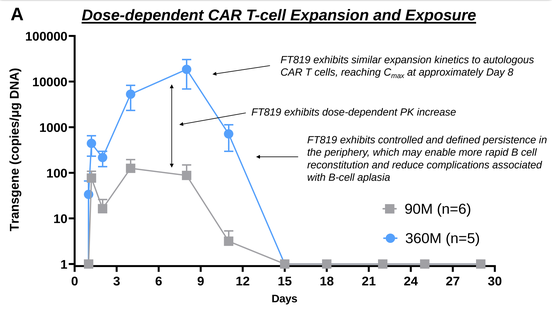
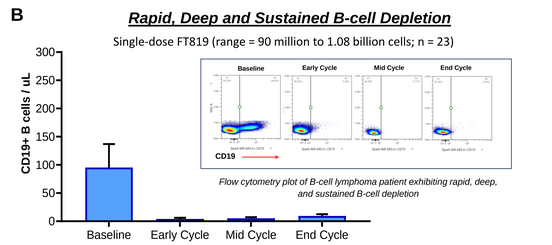


Figure 2. FT819 exhibits potent activity against CD19+ B cells. (A) In a 24-hour killing assay against CD19+ WIL2S lymphoma cell line, FT819 demonstrates equipotent activity compared to an autologous-manufactured CAR T cell. (B) Rapid and deep depletion of CD19+ B cells was observed in a 72-hour killing assay against PBMCs sourced from a blood sample of an unmatched SLE donor (E:T ratio = 1:1). (C, D) FT819 displays dose-dependent CD19+ B cell depletion against PBMCs sourced from a blood sample of an unmatched SLE donor in a manner comparable to autologous-manufactured CAR T cells. E:T ratio analysis suggests both FT819 and autologous-manufactured CAR T cells have the potential to fully deplete the CD19+ B cell burden of patients with SLE at a dose of 100-300 million CAR T cells (diseased CD19+ B cell population in the periphery and tissue is estimated to be up to 300 million cells in patients with autoimmune disease; DOI: 10.4161/cc.8.3.7608, https://doi.org/10.1038/s41573-020-00092-2, DOI: 10.3389/fimmu.2019.01375). *Autologous-manufactured CAR T cells* = primary T cells transduced with lentivirus containing CAR transgene and cultured for 4 additional days for recovery and expansion. *Allogeneic-manufactured CAR T cells* = primary T cells engineered with CRISPR targeting TRAC locus with CAR transgene and cultured for 11 additional days for recovery and expansion.

Translational data from Phase 1 BCM Study demonstrate key therapeutic mechanisms in the periphery to enable immune reset in autoimmune diseases





Flow cytometry plot of B-cell lymphoma patient exhibiting rapid, deep, and sustained B-cell depletion

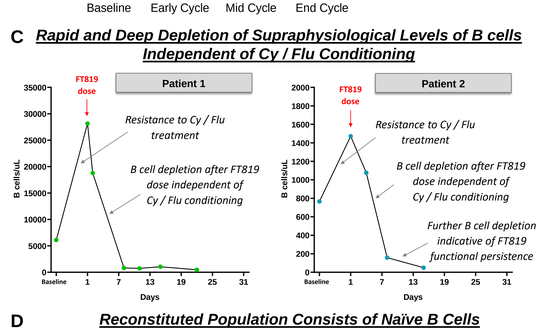


Figure 3. Data from Phase 1 BCM Study with single-dose administration of FT819 (NCT04629729). (A) FT819 PK (mean ± SEM) at 90 and 360 million cells in patients with r/r B-cell lymphoma (BCL) (n=11). In vivo CAR T-cell expansion and persistence were measured by a ddPCR assay according to the number of CAR transgene copies per microgram of genomic DNA in blood samples. (B) Rapid and deep B cell depletion, with sustained suppression of B cells, in the peripheral blood during FT819 treatment cycle in patients with r/r BCL (n=23; includes patients with baseline B cell counts in the range of normal (up to 1,000 cells/µL)). Baseline represents B cell counts in the peripheral blood prior to treatment with conditioning chemotherapy and single-dose FT819. Data represent the mean ± SEM. (C) Case study of two patients with r/r chronic lymphocytic leukemia, where B cell depletion was resistant to Cy / Flu conditioning chemotherapy. Rapid and deep B cell depletion was observed following a single dose of FT819. (D) Case study of plasma cell depletion and B cell reconstitution in 3 patients with diverse B-cell repertoire at baseline, with reconstitution population comprised of naïve B cells in 6-10 weeks following single-dose FT819.

Translational data from Phase 1 BCM Study demonstrate key therapeutic mechanisms in secondary and tertiary tissue to enable immune reset in autoimmune diseases

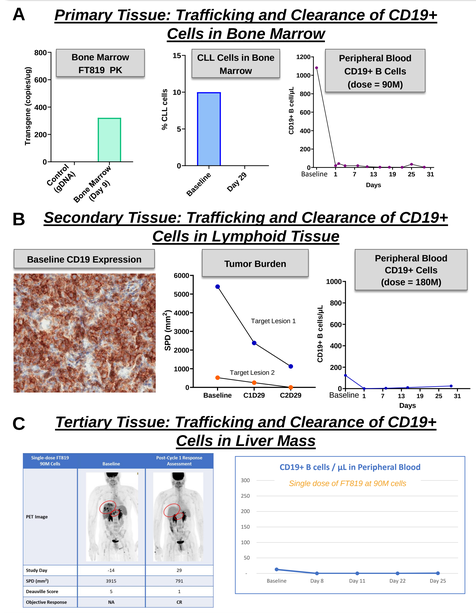


Figure 4. Data from Phase 1 BCM Study with single-dose administration of FT819 (NCT04629729). (A) Case study of chronic lymphocytic leukemia patient treated with conditioning chemotherapy followed by a single dose of FT819 at 90M cells. FT819 was detected and persisted in the bone marrow at Day 9, with complete elimination of CD19+ cells in the bone marrow and peripheral blood at the end of treatment cycle. (B) Case study of patient with r/r DLBCL treated with conditioning chemotherapy followed by a single dose of FT819 at 180M cells. Patient had 3 prior lines of therapy including autologous CD19-targeted CAR T-cell therapy. Baseline lymph node biopsy showed DLBCL cells with high CD19 expression. Single dose of FT819 resulted in rapid, deep, and sustained depletion of CD19+ B cells in the peripheral blood, and in >50% decrease in CD19+ tumor cells in secondary lymphoid tissue, after 1 cycle. Re-treatment with a single dose of FT819 resulted in complete metabolic response by FDG-PET with ~80% reduction of target lesion 1 and complete elimination of target lesion 2 at the end of cycle 2. (C) Case study of patient with r/r DLBCL treated with conditioning chemotherapy followed by a single dose of FT819 at 90M cells. Patient had 6 prior lines of therapy and was refractory to autologous CD19-targeted CAR T-cell therapy. Single dose of FT819 resulted in sustained depletion of CD19+ B cells in the peripheral blood and complete elimination of CD19+ masses in the liver indicating potential of FT819 to traffic, infiltrate, and function in secondary and tertiary tissue.

Translational data from Phase 1 BCM Study show capacity for rapid, deep, and sustained B-cell depletion without use of fludarabine as conditioning agent

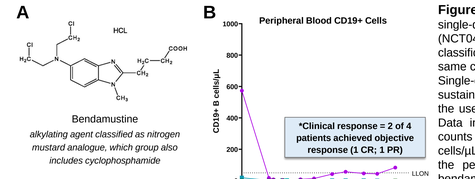


Figure 5. Data from Phase 1 BCM Study with single-dose administration of FT819 (NCT04629729). (A) Structural design and classification of bendamustine, which is in the same chemical class as cyclophosphamide. (B) Single-dose FT819 shows rapid, deep, and sustained depletion of CD19+ B cells without the use of fludarabine as a conditioning agent. Data include 4 patients* with baseline B-cell counts in the range of normal (up to 1,000 cells/µL). Baseline represents B-cell counts in the peripheral blood prior to treatment with bendamustine as an alternative to Cy / Flu conditioning chemotherapy and single-dose FT819. *LLON* = Lower limit of normal

First SLE patient treated in Phase 1 Autoimmunity Study

Pre-treatment sample of first patient's peripheral blood shows potent CD19+ B cell depletion with FT819 in ex vivo killing assay

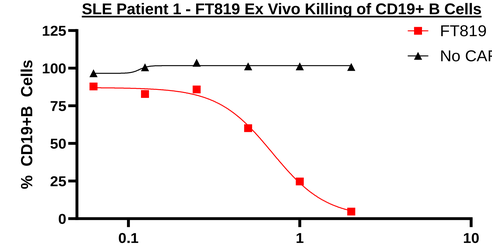


Figure 6. Data from Phase 1 Autoimmunity Study with single-dose administration of FT819 (NCT06398978). Using a pre-treatment sample of the first patient's peripheral blood obtained prior to clinical administration of conditioning chemotherapy and FT819, a 24-hour co-culture assay showed deep depletion of the patient's CD19+ B cells upon exposure to FT819 in a "first-of-kind" *ex vivo* translational assessment. The *ex vivo* translational assay did not include the use of cytokines to support the assay kinetics.