FT522: A CAR NK Cell with the Unique Ability to Target Multiple Pathogenic Cell Types and Circumvent Lympho-conditioning in Systemic Autoimmunity

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Introduction

CD19-targeted chimeric antigen receptor (CD19 CAR) T cells represent a potential paradigm shift for the treatment of B-cell mediated autoimmune diseases (AID). In a recently published series, patients with refractory AID treated with Cy / Flu conditioning chemotherapy and autologous CD19 CAR T cells achieved drug-free remission with evidence of immunological reset. These results have led to the clinical investigation of a number of CD19 CAR-targeted cell therapies in AID. However, drivers of pathology can include additional immunological mediators including plasma cells and T cells. Also, the use of Cy / Flu conditioning chemotherapy, which has been necessary for effective treatment with cell therapy, is associated with significant toxicities including poor immune reconstitution, severe infections, and secondary malignancies. Therefore, a next-generation cell therapy would aim to maintain the same B-cell depletion activity, target additional pathogenic cell types, reduce or eliminate the need for conditioning chemotherapy and patient hospitalization, and enable combination with existing standard of care therapies.

To address these challenges, we developed FT522, a multiplexed-engineered, off-the-shelf CAR natural killer (NK) cell, which is derived from a clonal, CD38 knockout (KO) human induced pluripotent stem cell (iPSC) line that expresses a CD19 CAR; an alloimmune defense receptor (ADR) targeting 4-1BB; a high-affinity, non-cleavable CD16 (hnCD16) to maximize antibody-dependent cellular cytotoxicity (ADCC); and an interleukin (IL)-15/IL-15 receptor fusion protein (IL-15RF) to enhance effector cell function.

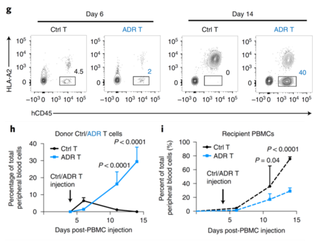
In preclinical studies, FT522 exhibited CD19-specific cytotoxicity toward CD19+ B cells through multiple rounds of target cell re-stimulation in a manner similar to primary CD19 CAR T cells. Additionally, in combination with rituximab (Rtx) or daratumumab (Dara), FT522 elicited potent ADCC against CD19- CD20+ and CD19-CD38+ B cell populations, respectively, while primary CD19 CAR T cells failed to eliminate these populations. Furthermore, in a 14-day mixed lymphocyte reaction assay using unmatched PBMCs from systemic lupus erythematosus (SLE)-diagnosed donors, FT522 eliminated both CD19+ B cells and alloreactive T cells, and exhibited functional persistence, as compared to ADR-null CD19 CAR NK and T cells, indicating that the functionality of FT522 can be uniquely maintained in the presence of an unmatched host immune system.

Our preclinical data and initial clinical data in B-cell lymphoma suggest that FT522 has the unique ability to elicit deep suppression of CD19+ B cells, target multiple pathogenic cell types, and overcome the requirement for conditioning chemotherapy with uncompromised effector function. Collectively, FT522 represents a promising off-the-shelf cell therapy for treatment of numerous autoimmune diseases while eliminating toxicities associated with patient conditioning.

Overview of Allo-immune Defense Receptor (ADR) as originally described by Mo et al. Nature Biotechnology 2020

Alloimmune Defense Receptor

ADR facilitates robust persistence in an allogenic setting



"The best defense is a good offense"

- ***ADR-armed CAR T cells specifically target activated host immune cells expressing 4-1BB, avoid host immune cell rejection, and retain potent and durable anti-tumor activity against CD19+ cells.***

Summary and Conclusions

FT522 is a novel off-the-shelf CAR NK cell therapy that has the unique potential to eliminate multiple pathogenic cell types and overcome the requirement of patient lympho-conditioning for the treatment of systemic autoimmunity.

Preclinical Studies

- Arming of NK cells with ADR uniquely enables cell proliferation and promotes functional persistence in an allogeneic host immune system
- FT522 demonstrates rapid and potent depletion of SLE donor CD19+ B cells. In addition, FT522 shows enhanced killing of additional pathogenic cell types, including CD38+ immune cells, in combination with mAbs as compared to primary CAR T cells
- FT522 shows dose-dependent trafficking and biodistribution to secondary and tertiary tissues
- FT522 uniquely exhibits (i) rapid and deep CD19+ B cell depletion, (ii) alloreactive T cell elimination, and (iii) enhanced persistence in a novel re-challenge assay comprised of SLE donor blood cells

Initial Clinical Data

- FT522 exhibited rapid, deep, and sustained B-cell depletion in the periphery, and enhanced persistence compared to a prior generation, CD19-targeted CAR NK cell, based on interim data from a Phase 1 study in relapsed / refractory B-cell lymphoma (NCT05950334)

Collectively, these data support the clinical development and unique therapeutic profile of FT522 as an off-the-shelf cell therapy for autoimmune diseases, with the potential to rapidly deplete CD19+ B cells and additional multiple pathogenic cell types as well as overcome the need for administration of conditioning chemotherapy to patients.

Results

FT522 is a CAR NK cell product candidate designed to eliminate multiple pathogenic cell types without requiring administration of conditioning chemotherapy to patients

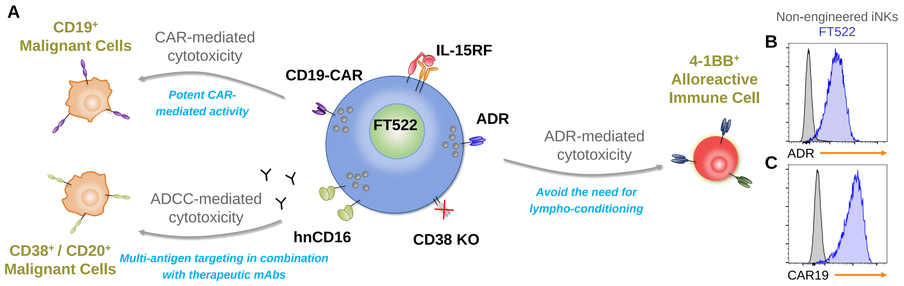


Figure 1. **(A)** Illustration of FT522 genetic edits consisting of CD38 knockout (KO) to avoid fratricide and enhance metabolic fitness; a NK cell-specific CAR targeting CD19; an alloimmune defense receptor (ADR) targeting 4-1BB; a high-affinity, non-cleavable CD16 (hnCD16) to maximize antibody-dependent cellular cytotoxicity (ADCC); and an interleukin (IL)-15/IL-15 receptor fusion protein (IL-15RF) for enhanced function. Representative FACS plots examining engineering of **(B)** ADR and **(C)** CAR19 on iNK cells.

FT522 demonstrates rapid and potent depletion of SLE donor CD19+ B cells and uniquely shows killing of additional pathogenic cell types in combination with mAbs

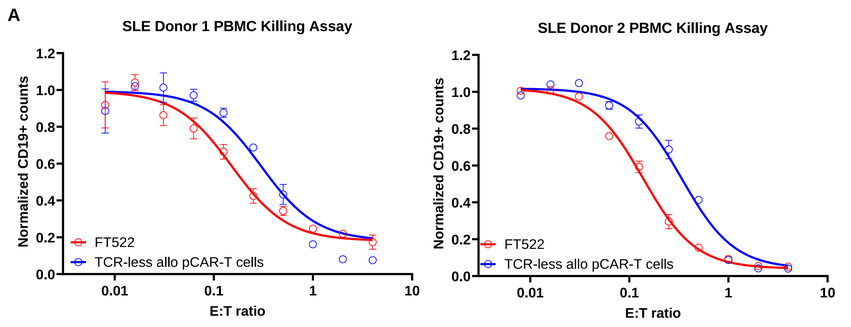


Figure 2. **(A)** FT522 demonstrates comparable-or-better CAR-mediated CD19+ B cell killing compared to TCR-less, allogeneic-like, primary CAR-T (pCAR-T) cells in a 24hr cytotoxicity assay with multiple E:T ratios and in two different SLE PBMC donors. **(B)** Left panel, an illustration of an in vitro dual targeting cytotoxicity assay where a 50:50 mixture of NALM6 WT (B cell surrogate) and NALM6 CD19KO (plasma cell surrogate) cells are co-cultured with either FT522 or pCAR-T cells in combination with Dara at an E:T ratio of 1:1. Right plot, FT522 in combination with Dara demonstrates superior dual targeting through CAR and ADCC of two different target cell types compared to pCAR-T cells over a 96-hour assay period in an in vitro dual targeting cytotoxicity.

FT522 broadly distributes to secondary and tertiary tissues, supporting use in autoimmune diseases

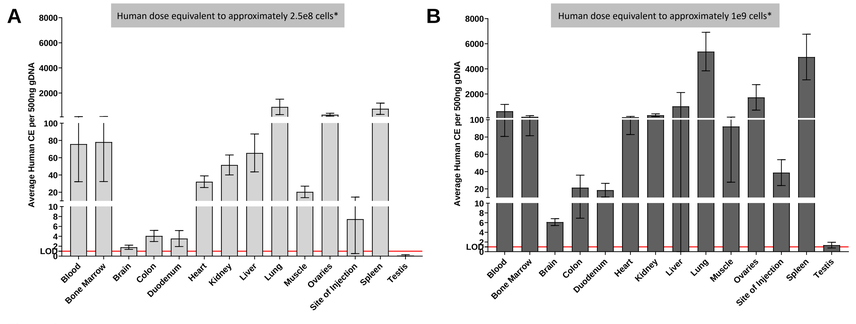


Figure 3. Preclinical in vivo biodistribution study looking at the presence of FT522 in various tissues. **(A)** NSG mice dosed with a human equivalency of 2.5e8 FT522 cells and **(B)** a human dose equivalency of 1e9 FT522 cells three times over 15 days and analyzed for biodistribution the day after the last dose. No cytokine support or target cells expressing CD19 antigen were provided in this study. Human dose equivalency was calculated based on allometric conversion between a 20g mouse and 65Kg human.

ADR-armed NK cells uniquely proliferate and persist in an allogeneic system

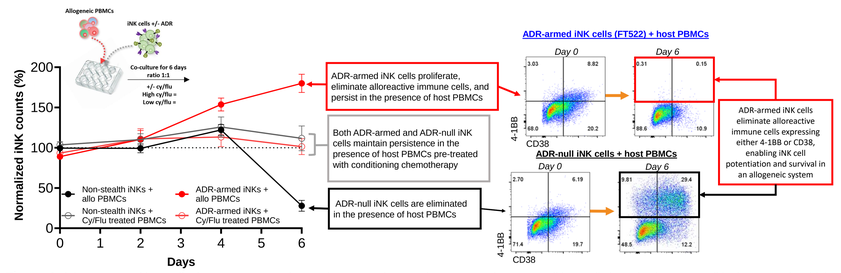


Figure 4. (A) ADR-armed iNK cells demonstrate superior persistence and expansion compared to ADR-null iNK cells in an in vitro preconditioning assay in which donor PBMCs were pre-treated with or without Cy/Flu, recapitulating the patient cell therapy preconditioning paradigm, prior to co-culture. This superior persistence and expansion is most acutely observed in the absence of Cy/Flu PBMC preconditioning.

FT522 exhibits rapid and deep CD19+ B cell depletion and eliminates alloreactive 4-1BB+ T cells, while maintaining functional persistence, against SLE donor PBMCs

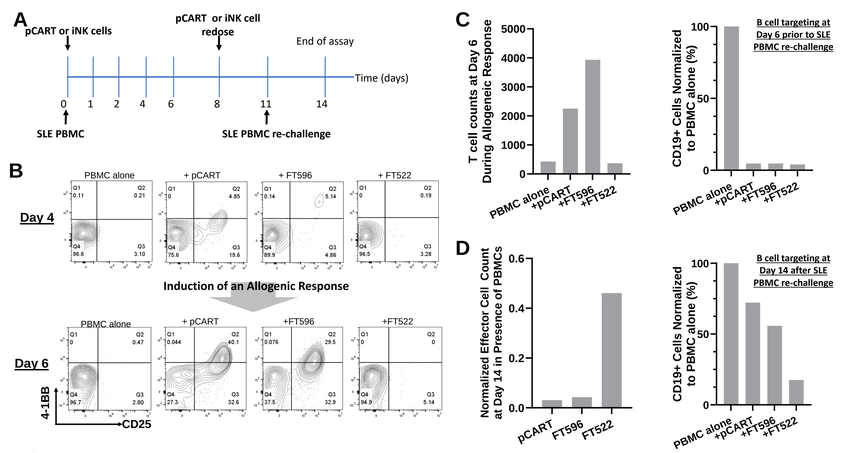


Figure 5. **(A)** Illustration of an in vitro allogenic reaction rechallenge assay in which SLE donor PBMCs are co-cultured with pCAR-T cells, FT596 (ADR-negative) or FT522 (ADR-armed) for 8 days, and redosed with effector cells and re-challenged with SLE donor PBMCs, and allowed to co-culture for a total of 14 days. **(B)** Flow cytometry of CD3+ SLE donor PBMC T cells on days 4 and 6 for CD25 and 4-1BB demonstrates T cell activation and allo-response to pCAR-T and FT596 cells, but not FT522 cells. **(C)** Total SLE donor PBMC-derived T cells expand in response to an allogeneic challenge on day 6 and CD19+ cell counts prior to re-challenge, normalized to PBMC alone. **(D)** pCAR-T and FT596 cells are depleted upon completion of the allo-rechallenge assay, whereas FT522 cells continue to persist and CD19+ cell counts at the end of the assay, normalized to PBMC alone.

Interim Phase 1 clinical data of FT522 in BCL demonstrates rapid, deep, and sustained depletion of CD19+ B cells

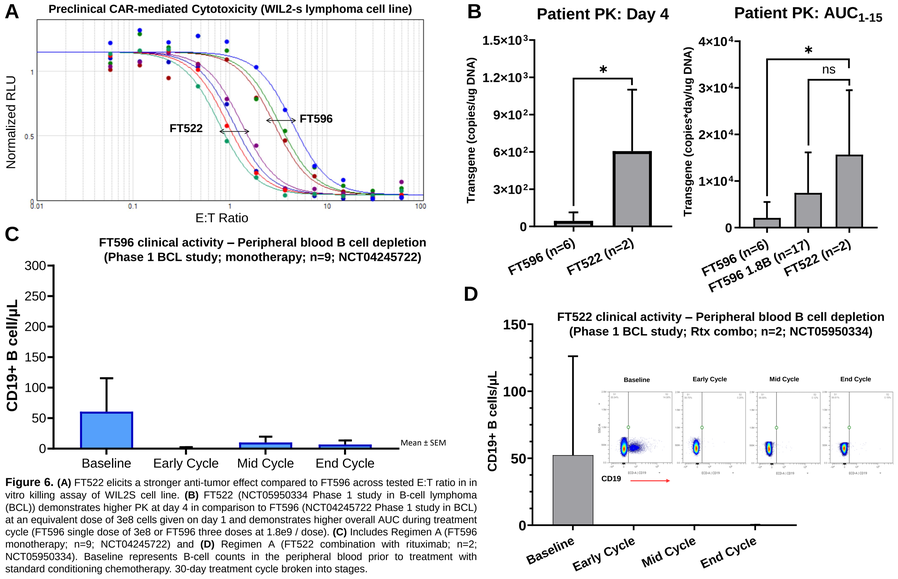


Figure 6. **(A)** FT522 elicits a stronger anti-tumor effect compared to FT596 across tested E:T ratio in in vitro killing assay of WIL2S cell line. **(B)** FT522 (NCT05950334 Phase 1 study in B-cell lymphoma (BCL)) demonstrates higher PK at day 4 in comparison to FT596 (NCT04245722 Phase 1 study in BCL) at an equivalent dose of 3e8 cells given on day 1 and demonstrates higher overall AUC during treatment cycle (FT596 single dose of 3e8 or FT596 three doses at 1.8e9 / dose). **(C)** Includes Regimen A (FT596 monotherapy; n=9; NCT04245722) and **(D)** Regimen A (FT522 combination with rituximab; n=2; NCT05950334). Baseline represents B-cell counts in the peripheral blood prior to treatment with standard conditioning chemotherapy. 30-day treatment cycle broken into stages.