Next Generation CAR-NK cell Therapy Leverages Alloimmune Defense Technology to Persist Without Conditioning Chemotherapy for the Treatment of Autoimmune Disease

Trever T. Greene[1], Seth Peng[1], Cara Bickers[1], Matthew Lunning[2], Premal Lulla[3], Veronika Bachanova[4], Alan Williams[1], Daniel Morales-Mantilla[1], Rina Mbofung[1], Allison Aguillar[1], Nick Brookhouser[1], Stuart Reeder[1], Wei Zhao[1], Deepa Patel[1], Ramzey Abujarour[1], Tom Lee[1], Raedun Clarke[1], Betsy Rezner[1], Jode Goodridge[1], Rebecca Elstrom[1], Debra Zack[1], Vaneet Sandhu[1], Bahram Valamehr[1], Lilly Wong[1]

[1]Fate Therapeutics, Inc., San Diego, CA; [2]University of Nebraska Medical Center, Omaha, NE; [3]Baylor College of Medicine, Houston, TX; [4]Department of Medicine, University of Minnesota, Minneapolis, MN

INTRODUCTION

Background: Autologous and allogeneic chimeric antigen receptor (CAR) T and NK cell therapies have shown promise for the treatment of a suite of autoimmune disorders. To date, cellular CAR therapies are reliant on pretreatment with conditioning chemotherapies (CCT) to support product activity. CCT supports CAR T and NK cell persistence through multiple mechanisms, including access to essential homeostatic cytokines and depletion of key immune populations that can attack and potentially eliminate CAR T-cell products, thereby limiting product persistence and activity. However, because CCTs are harsh treatments with extreme side-effects, and potential long-term consequences to reproductive health, therapies with a requirement for CCT, may not be ideal for all patients with autoimmune disorders. FT522 is an induced pluripotent stem cell (iPSC)-derived off-the-shelf, natural killer (NK) cell therapy designed to function without the need for CCT. FT522 incorporates a CD19- targeted CAR; CD38 knockout to enhance metabolic fitness; an alloimmune defense receptor (ADR) targeting 4- 1BB to prevent immune rejection; a high-affinity non-cleavable CD16 (hnCD16) Fc receptor to maximize antibody-dependent cellular cytotoxicity (ADCC) when cotreating with therapeutic antibodies; and an IL-15/IL15 receptor fusion protein (IL15RF) to offer cell-intrinsic cytokine support and function. Objectives: To determine whether FT522, in the absence of CCT, can function, persist, and deplete B cells for the effective treatment of autoimmune diseases.

Methods: Preclinical in vitro studies of FT522 were carried out using unmatched systemic lupus erythematosus (SLE) donor peripheral blood mononuclear cells (PBMC) to investigate the capacity of FT522 to eliminate SLE donor B cells. A clinical study of FT522 is being investigated in a multicenter Phase I clinical trial in patients with relapsed/refractory (R/R) B-cell lymphomas (BCL) (NCT05950334). FT522 is administered in three doses (Days 1, 4, and 8) either with CCT (fludarabine 30 mg/m2 and cyclophosphamide 500 mg/m2 , Days -5 to -3) or without CCT. Both regimens include rituximab(R) 375 mg/m2 (Day -4). Whole blood samples are collected at protocol defined timepoints to allow for comparison of product function and persistence with and without CCT.

FT522 is a next generation CAR-NK cell designed for synergy with monoclonal antibodies and self-sufficiency in a lympho-conditioning free environment

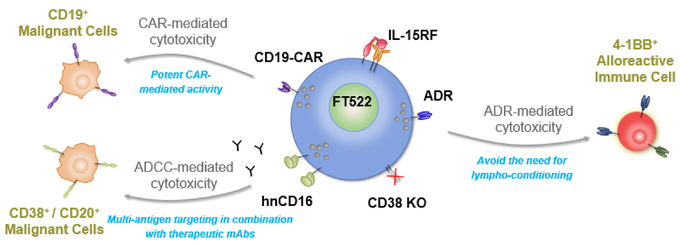

Figure 1. FT522 is a next generation CAR-NK cell with edits targeted to increase persistence and prevent allo-rejection. Illustration of FT522 genetic edits consisting of CD38 knockout (KO) to avoid fratricide and enhance metabolic fitness; a NK cell-specific CAR targeting CD19; an alloimmune defense 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.

Auto-CAR	Conventional Allogeneic CAR	iPSC CAR (off-the-shelf)
✓ Potent Targeted Therapy	✓ Potent Targeted Therapy	✓ Potent Targeted Therapy
✗ Requires Apheresis	✓ No Apheresis	✓ No Apheresis
× 2 Weeks of Engineering	✓ Pre-engineered	✓ Pre-engineered
× 7-10 Days Hospitalization	× 7-10 Days Hospitalization	✓ 0-3 Days Hospitalization
✗ Grade 3+ ICANS	✗ Grade 3+ ICANS	✓ No ICANS
✗ Limited Editing	✗ Limited Editing	✓ 10+ Edits (no upper limit)
✗ Variable Efficiency	✗ Heterogeneous and Inconsistent	✓ Homogenous and Consistent
✗ 1 Donor =1-2 Doses	✓ 1 Donor = 25-200 Doses	✓+ 1 Donor = 10^9 Doses

Figure 2. Comparison of Autologous, Conventional Allogenic, and iPSC strategies for CAR-T Cell Therapy.

RESULTS

FT522 effectively eliminates B cells from SLE patients, and this is functionally enhanced in combination with monoclonal antibodies

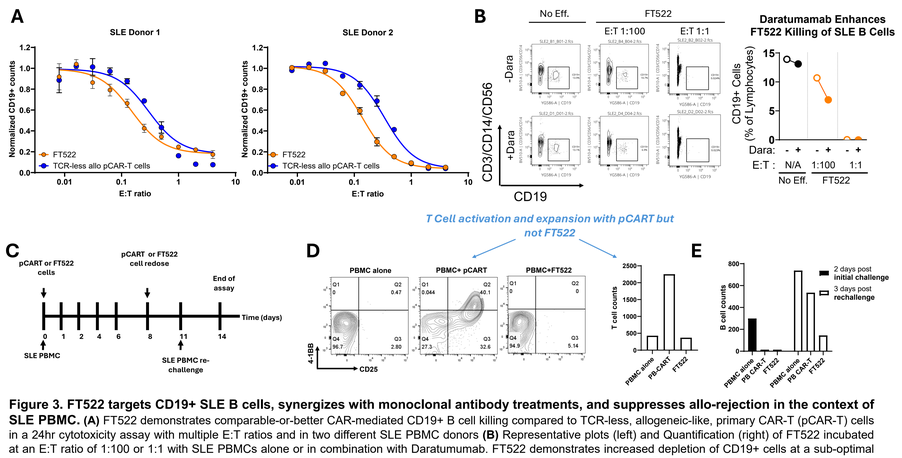

Figure 3. FT522 targets CD19+ SLE B cells, synergizes with monoclonal antibody treatments, and suppresses allo-rejection in the context of SLE PBMC. (A) FT522 demonstrates comparable-or-better CAR-mediated CD19+ B cell killing compared to TCR-less, allogeneic-like, primary CAR-T (pCAR-T) cells in a 24hr cytotoxicity assay with multiple E:T ratios and in two different SLE PBMC donors **(B)** Representative plots (left) and Quantification (right) of FT522 incubated at an E:T ratio of 1:100 or 1:1 with SLE PBMCs alone or in combination with Daratumumab. FT522 demonstrates increased depletion of CD19+ cells at a sub-optimal E:T ratio when combined with Daratumumab at 72hrs. **(C)** Illustration of an *in vitro* allogenic reaction rechallenge assay. SLE donor PBMCs are co-cultured with TCR-less, allogeneic-like, primary CAR-T (pCAR-T) cells or FT522 for 8 days, followed by redosing with effector cells and subsequent re-challenge with SLE donor PBMCs, and allowed to co-culture for a total of 14 days. **(D)** Example plot **(D, left)** of flow cytometry of CD3+ SLE donor PBMC T cells on Day 6 demonstrates T-cell activation and allo-response to pCAR-T cells, but not to FT522 cells concurrent with reduced T cell expansion in FT522 cultures (**D, right**). **(E)** pCAR-T cells are depleted upon completion of the allo-rechallenge assay, whereas FT522 cells continue to persist and deplete CD19+ cells at the end of the assay.

Interim Phase 1 translational data: Identification of persistent and viable FT522 product in the periphery of patients without rejection in a CCT-free environment

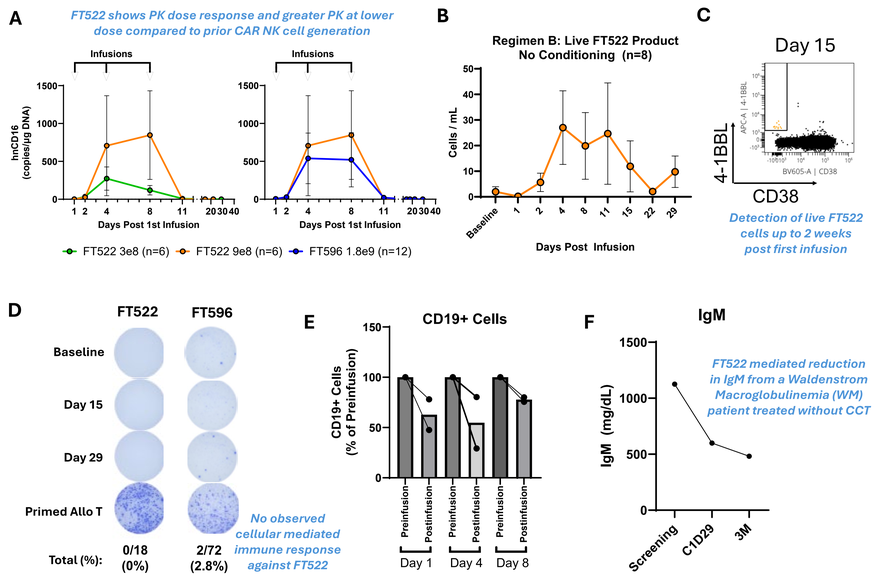

Figure 4. Interim Phase 1 translational data evinces FT522 persistence, lack of alloreactivity, and functional effectiveness of treatment without CCT. (A-F) Data collected from NCT05950334 Phase 1 study of FT522 in B-cell lymphoma (BCL). **(A)** PK determined by ddPCR from PBMCs for prior generation CAR NK (blue) or FT522 at 3e8 dose level (green) or FT522 at 9e8 dose level (orange) shows dose dependent increase in PK (left) as well as greater product abundance with FT522 at 2x lower dose (right). **(B)** Quantification and **(C)** representative plots of FT522 product detected in longitudinal blood samples from patients (n=8) treated without CCT. **(D)** Representative and tabulated data for cellular mediated alloreactivity against prior generation CAR NK (FT596) but no development of alloreactivity against FT522. **(E)** Quantification of B cells prior to and 30 minutes after FT522 infusion for all patients with >2 B cells/µL at pre-infusion timepoints (n=2). **(F)** IgM levels in a patient with Waldenström macroglobulinemia, dropped at D29 post treatment, and maintained this reduction out to 3 months.

Next Generation Therapies Incorporate Multiple Allogenic Evasive Technologies

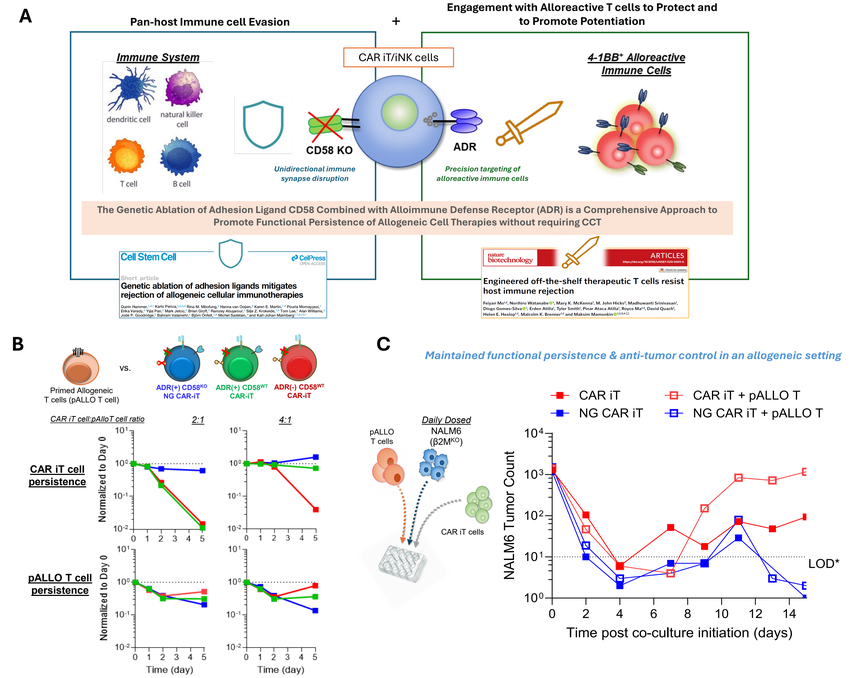

Figure 5: Next generation CAR iT cells combine ADR and CD58 KO to protect against alloreactive immune cells. (A) Graphic summary of next generation allo-defense strategies for future therapies **(B)** Schematic representation of a co-culture system that contains CAR iT cells, expressing +/- ADR or +/- ADR / CD58KO, at 2:1 or 4:1 E:T (CAR iT cells to primed allogeneic T (pAllo T) cells) for 5 days. pAllo T cells are generated following repeated-exposure to donor-specific iT cells. Cell counts were normalized to CAR iT and pAllo T alone wells. **(C)** Schematic representation (left) and daily tumor burden (right) resulting from tri-modal cultures containing CAR iT cells (+/- ADR / CD58KO), pAllo T cells, and β2M-deficient NALM6 leukemia B-cells challenged daily over 14 days.

SUMMARY AND CONCLUSIONS

FT522 is a next generation off-the-shelf CAR-NK cell uniformly consisting of 5 genetic edits, uniquely made possible using iPSC platform. Preclinical and translational data in R/R BCL demonstrate that FT522 can persist, function, and confer clinical benefit without the need for CCT. Furthermore, the data supports the application of FT522, without CCT, for B-cell mediated autoimmune diseases.

Preclinical Studies

- An iPSC-based approach to CAR therapy allows for the development of purpose-built cell therapies to solve essential problems in cell therapy such as scalability, safety, and synergy with existing therapeutics.
- FT522 function is enhanced by combination with monoclonal antibody and together, can drive better targeting and deeper killing than either alone.
- FT522 can target CD19+ B cells from SLE donors, and this capacity is uniquely enhanced through combination with Daratumumab.
- The allo-defense receptor (ADR) protects FT522 against host mediated allo-rejection and has been shown to do so more effectively than conventional MHC engineering.

Translational Data

- FT522 exhibits improved PK over prior generation CAR NK cell product.
- FT522 demonstrates persistence in the absence of CCT with no observed signals of host mediated allo-reactivity.
- FT522 mediates B cell depletion and reduction in disease associated antibodies as observed in a WM patient in the absence of CCT.
- Translational data from FT522 support ADR and cytokine autonomy as effective strategies to eliminate need for conditioning chemotherapy.

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, synergize with existing monoclonal antibody treatments, and overcome the need for administration of conditioning chemotherapy to patients.