Development of FT825/ONO-8250: an off-the-shelf CAR-T cell with preferential HER2 targeting and engineered to enable multi-antigen targeting, improve trafficking, and overcome immunosuppression

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Introduction

Chimeric antigen receptor (CAR)-T cells have limited efficacy in solid tumor settings, in part, because of challenges in:

- Differentiating tumor associated antigen expression between tumor and normal tissue
- Overcoming heterogeneity and/or loss of antigen expression
- Resistance to the tumor microenvironment
- Effective and sustained trafficking
- Effector cell fitness and persistence

Here we describe ***FT825/ONO-8250***, an off-the-shelf CAR-T cell therapy specifically engineered with seven functional elements to overcome barriers for effective cell therapy in solid tumors.

Results

Preferential targeting of HER2 expressed on tumor rather than normal cells is uniquely enabled by the novel H_2CasMab-2 binder

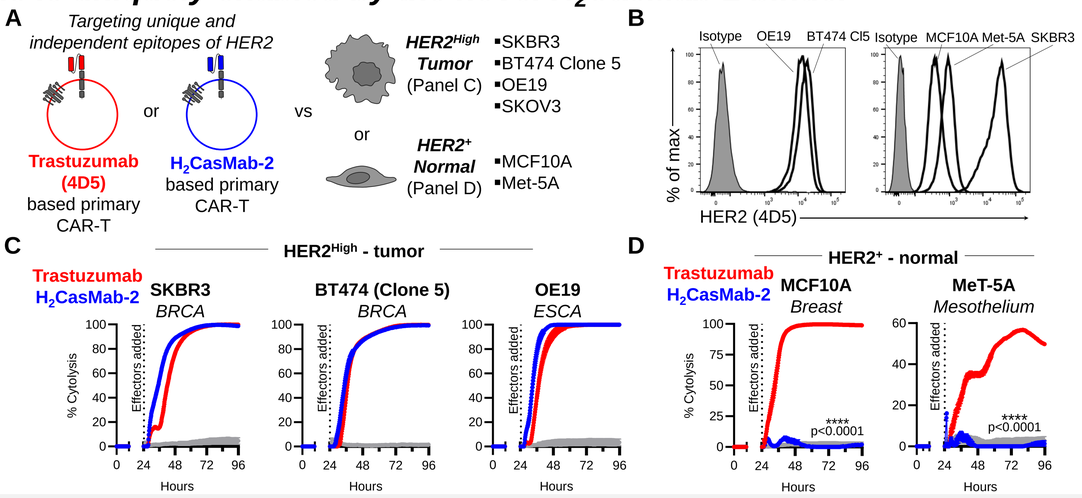

Figure 1. (A) Experimental system to evaluate CAR-mediated efficacy of Trastuzumab and H_2CasMab-2 based primary CAR-T cells against HER2-expressing tumor targets and normal cell lines. (B) Target lines were evaluated for HER2 expression by flow cytometry. (C) The cytolytic efficacy of primary CAR-T cells was evaluated in a xCELLigence assay on the indicated tumor target lines. (D) Similarly, primary CAR-T efficacy was determined on the indicated normal/non-tumorigenic cell lines. Significance was evaluated by AUC and unpaired two-tailed t test.

Mass production of multiplex-engineered CAR T cells made possible through a unique iPSC platform

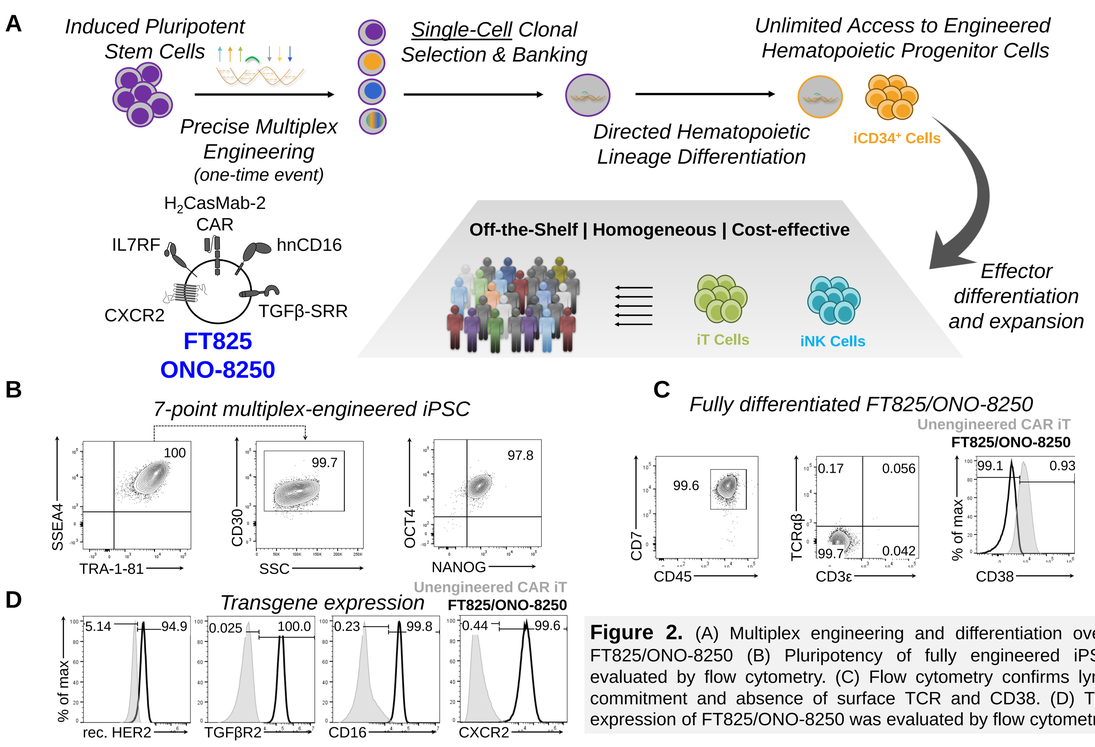

Figure 2. (A) Multiplex engineering and differentiation overview of FT825/ONO-8250 (B) Pluripotency of fully engineered iPSCs was evaluated by flow cytometry. (C) Flow cytometry confirms lymphocyte commitment and absence of surface TCR and CD38. (D) Transgene expression of FT825/ONO-8250 was evaluated by flow cytometry.

Conclusion

FT825/ONO-8250 is a multiplex-engineered CAR T cell designed to address and overcome challenges currently faced by cell therapies in solid tumors, including:

- ✓ *Consistency*: Derived from an iPSC master cell bank generated from a fully characterized multiplexed engineered clonal iPSC line to support uniform expression of all seven functional elements
- ✓ *Off-the-shelf*: Manufactured at large scale with a consistent starting point of iPSC MCB, to support on-demand availability of drug product
- ✓ *Selective*: Uniquely exhibits robust and preferential targeting of HER2 on tumor cells and not on normal cells, aided by novel H_2CasMab-2 binder and TRAC-mediated 1XX CAR activity
- ✓ *Allogeneic*: Complete elimination of TCR expression at the molecular level
- ✓ *Multi-targeted*: Multi-antigen targeting via hnCD16 potently mitigates tumor antigen heterogeneity and antigen escape
- ✓ *TME resistant*: Resistance to TGFβ led suppression
- ✓ *Fit*: Enhanced effector cell fitness and persistence through IL7-RF and CD38KO
- ✓ *Trafficking*: Enhanced solid tumor homing and trafficking through CXCR2

FT825/ONO-8250 uniquely exhibits potent specificity towards HER2 expressed by tumor cells and not by normal cells

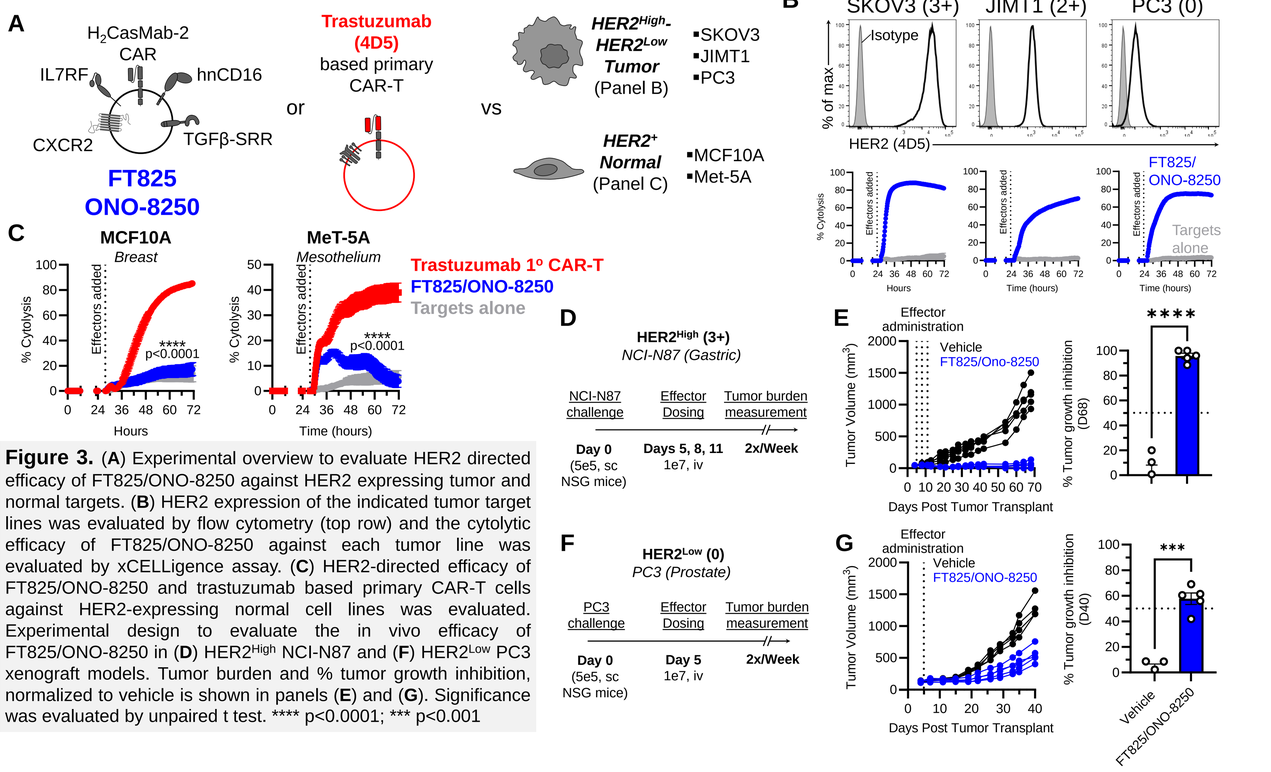

Figure 3. (**A**) Experimental overview to evaluate HER2 directed efficacy of FT825/ONO-8250 against HER2 expressing tumor and normal targets. (**B**) HER2 expression of the indicated tumor target lines was evaluated by flow cytometry (top row) and the cytolytic efficacy of FT825/ONO-8250 against each tumor line was evaluated by xCELLigence assay. (**C**) HER2-directed efficacy of FT825/ONO-8250 and trastuzumab based primary CAR-T cells against HER2-expressing normal cell lines was evaluated. Experimental design to evaluate the in vivo efficacy of FT825/ONO-8250 in (**D**) HER2^{High} NCI-N87 and (**F**) HER2^{Low} PC3 xenograft models. Tumor burden and % tumor growth inhibition, normalized to vehicle is shown in panels (**E**) and (**G**). Significance was evaluated by unpaired t test. **** p<0.0001; *** p<0.001

hnCD16 enables flexible and potent multi-antigen targeting by FT825/ONO-8250 through combination with various therapeutic mAbs

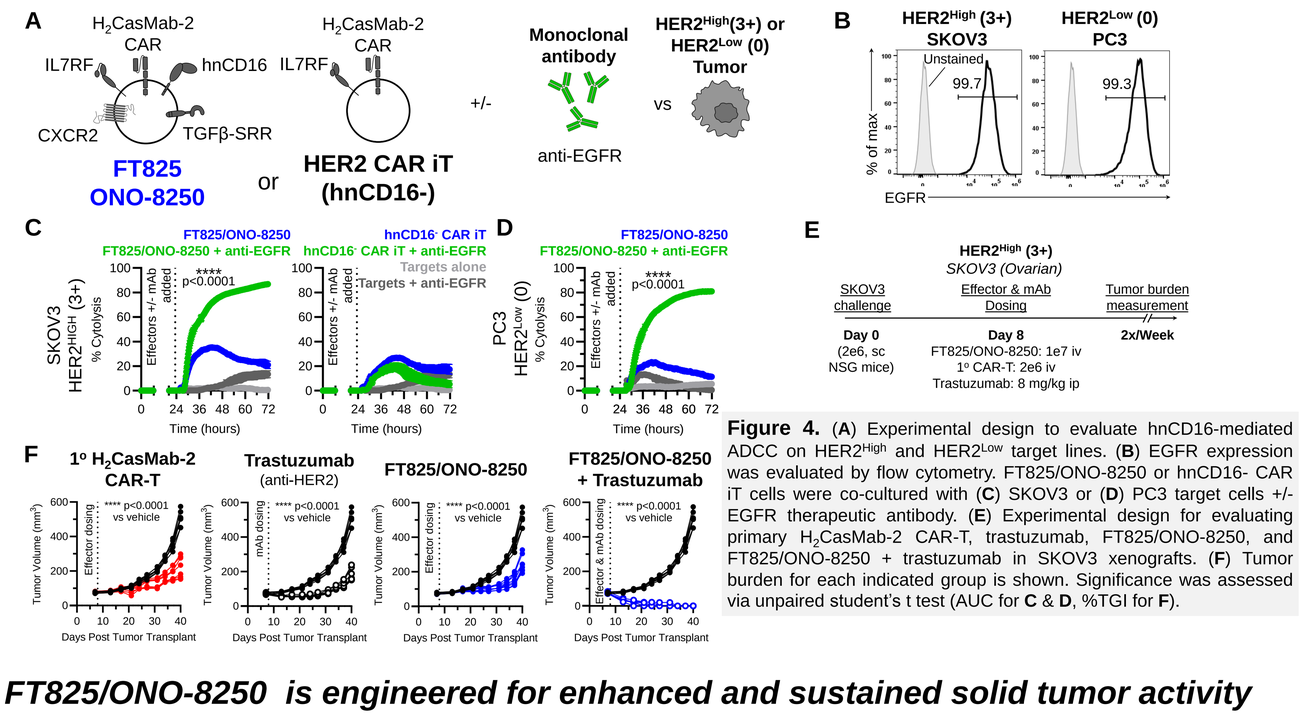

Figure 4. (**A**) Experimental design to evaluate hnCD16-mediated ADCC on HER2^{High} and HER2^{Low} target lines. (**B**) EGFR expression was evaluated by flow cytometry. FT825/ONO-8250 or hnCD16- CAR iT cells were co-cultured with (**C**) SKOV3 or (**D**) PC3 target cells +/- EGFR therapeutic antibody. (**E**) Experimental design for evaluating primary H_2CasMab-2 CAR-T, trastuzumab, FT825/ONO-8250, and FT825/ONO-8250 + trastuzumab in SKOV3 xenografts. (**F**) Tumor burden for each indicated group is shown. Significance was assessed via unpaired student's t test (AUC for **C** & **D**, %TGI for **F**).

FT825/ONO-8250 is engineered for enhanced and sustained solid tumor activity

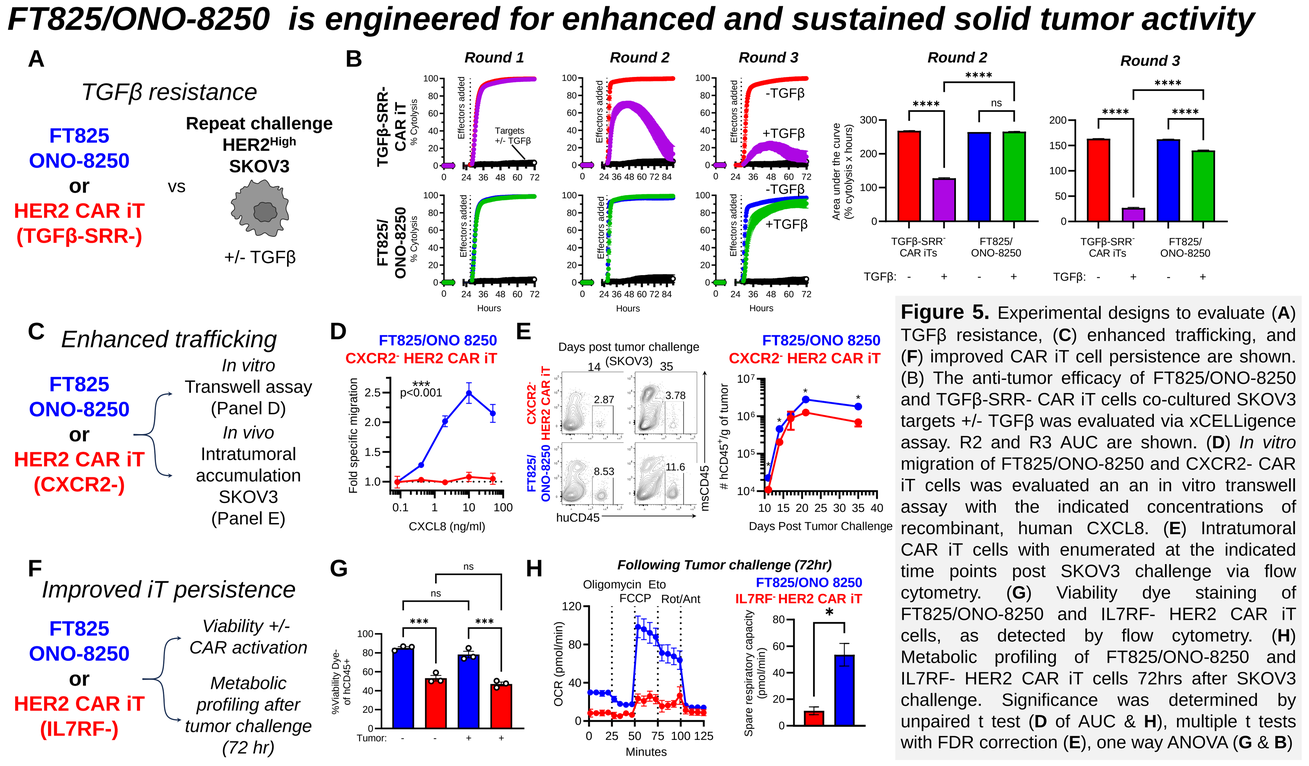

Figure 5. Experimental designs to evaluate (**A**) TGFβ resistance, (**C**) enhanced trafficking, and (**F**) improved CAR iT cell persistence are shown. (B) The anti-tumor efficacy of FT825/ONO-8250 and TGFβ-SRR- CAR iT cells co-cultured SKOV3 targets +/- TGFβ was evaluated via xCELLigence assay. R2 and R3 AUC are shown. (**D**) *In vitro* migration of FT825/ONO-8250 and CXCR2- CAR iT cells was evaluated an an in vitro transwell assay with the indicated concentrations of recombinant, human CXCL8. (**E**) Intratumoral CAR iT cells with enumerated at the indicated time points post SKOV3 challenge via flow cytometry. (**G**) Viability dye staining of FT825/ONO-8250 and IL7RF- HER2 CAR iT cells, as detected by flow cytometry. (**H**) Metabolic profiling of FT825/ONO-8250 and IL7RF- HER2 CAR iT cells 72hrs after SKOV3 challenge. Significance was determined by unpaired t test (**D** of AUC & **H**), multiple t tests with FDR correction (**E**), one way ANOVA (**G** & **B**)

Proposed Initial Clinical Development Plan

IND submission scheduled for 2H 2023

Monotherapy:
HER2-expressing solid tumors

Combination therapy:
ADCC enabled antibodies in solid tumors