Off-the-Shelf CAR-NK cell Therapy co-Targeting GPRC5D and CD38 for the Treatment of Multiple Myeloma

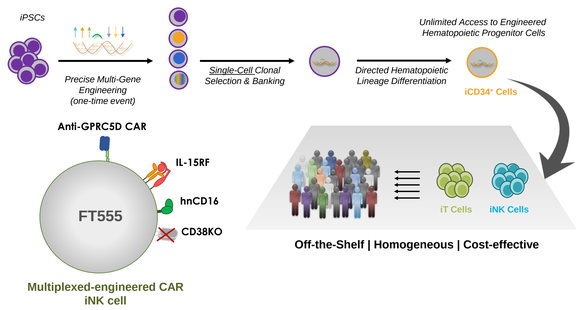
John Reiser[1], Szeman Ruby Chan[2], Ketan Mathavan[1], David Sillitti[2], Cristina Mottershead[2], Bethany Mattson[2], Melissandre Pache[1], Alma Gutierrez[1], Whitney Scoon[1], Yanni Zhu[1], Alison O'Connor[1], Andrew Gilder[1], Christine Chen[1], Kim Staquet[2], Michael Ports[2], Charles Drake[2], Joseph Erhardt[2], Ryan Bjordahl[1], Jode Goodridge[1], Bahram Valamehr[1]

[1]Fate Therapeutics Inc., San Diego, CA; [2]The Janssen Pharmaceutical Companies of Johnson & Johnson, Spring House, PA

Multi-Antigen Targeting in Multiple Myeloma

Recent improvement in patient outcomes with the use of novel cellular immunotherapies for multiple myeloma (MM) has raised the prospect for the emergence of a curative treatment. While BCMA-targeted chimeric antigen receptor (CAR)-T cell therapies have been successful in treating MM, CAR-T cell manufacturing challenges preventing broad patient access and treatment relapse drive the need for additional targeted therapies with emphasis on multi-antigen targeting and off-the-shelf availability. GPRC5D, a tumor-associated orphan G-protein-coupled receptor found to be highly expressed in MM, is an attractive target that has demonstrated promising clinical benefit when targeted via immunotherapy modalities. Here, we describe the development of FT555, an induced pluripotent stem cell (iPSC)-derived CAR-NK (CAR-iNK) cell product with the unique and effective ability to simultaneously co-target GPRC5D and CD38 (an additional tumor-associated antigen of MM) via combination with daratumumab, and which can be mass produced and made available off-the-shelf to support broad patient access.

Off-The-Shelf Application



Conclusion

- To create FT555, iPSC-derived iNK cell clones were engineered to uniformly express four anti-cancer modalities, including high-affinity non-cleavable CD16 (hnCD16), IL15 receptor fusion (IL-15RF), anti-GPRC5D CAR and deletion of CD38 expression
- FT555 cells demonstrate potent antigen-mediated cytokine responses against multiple tumor target lines in vitro with responses further enhanced with the addition of the anti-CD38 targeting monoclonal antibody (mAb) daratumumab
- FT555 maintains antigen-specific cytotoxicity and ADCC potential over multiple rounds against MM.1S targets in an in vitro restimulation assay
- FT555 exhibits robust and durable antigen-mediated tumor control in vivo against a highly aggressive, disseminated MM.1S multiple myeloma tumor model
- Combination of FT555 with daratumumab further enhanced in vivo tumor clearance and resulted in 25% complete tumor clearance when administered at 1x dose without cytokine support
- FT555 also demonstrated robust and durable antigen-mediated tumor regression in a distinct, disseminated OPM-2 myeloma tumor model when administered at 1x dose without cytokine support; activity improved when combined with daratumumab
- FT555 multi-dosing (3x doses) further improved tumor clearance and resulted in 86% complete tumor clearance when combined with daratumumab in the OPM-2 tumor model

Results

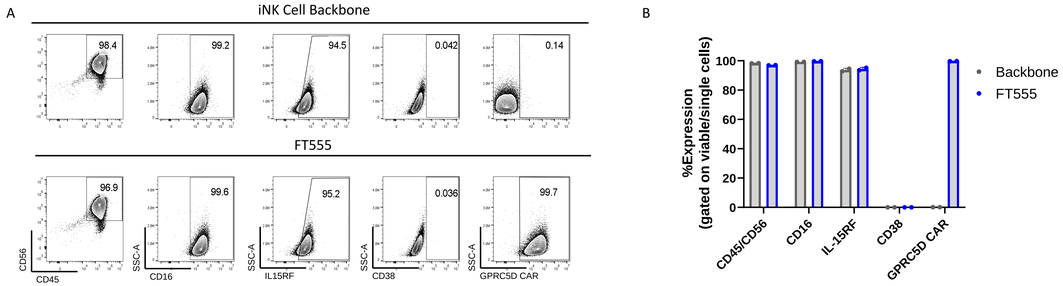


Figure 1. Uniform expression of NK markers and edits in differentiated iPSC-derived anti-GPRC5D CAR-NK cells. iPSC-derived NK cells were engineered to express hnCD16, IL-15RF, GPRC5D CAR and knocked out for CD38 to derive FT555. (A) FACS plots illustrating expression of NK markers and edited transgenes. Homogenous CD45/CD56 expression indicates hematopoietic lineage commitment to an NK cell. Uniform expression of transgenes CD16, IL-15RF and bi-allelic CD38 knockout. Expression of anti-GPRC5D CAR is shown relative to the CAR-negative, iNK cell backbone. (B) Representative bar chart displaying uniform expression of markers and engineered edits from (A). Data are representative of 2 independent assessments.

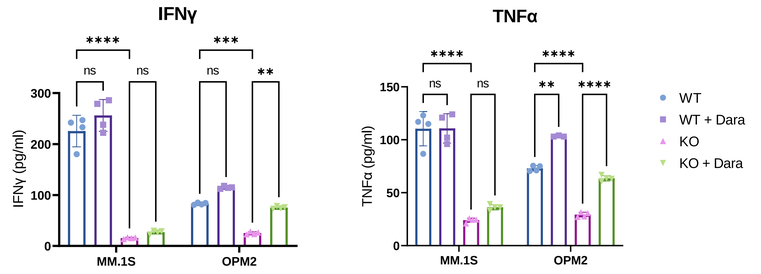


Figure 2. Antigen-specific cytokine secretion by FT555 against multiple MM lines. FT555 cells were thawed and co-cultured with MM.1S and OPM-2 wildtype (WT) and isogenic GPRC5D knockout (KO) lines +/- daratumumab at a 1:1 E:T ratio. After 24 hours, supernatants were harvested and analyzed for concentration of the cytokines interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) using the ELLA platform. Data represent 2 vials per line assessed in duplicate. **p value of <0.01, ****p value of <0.0001, ns = not significant.

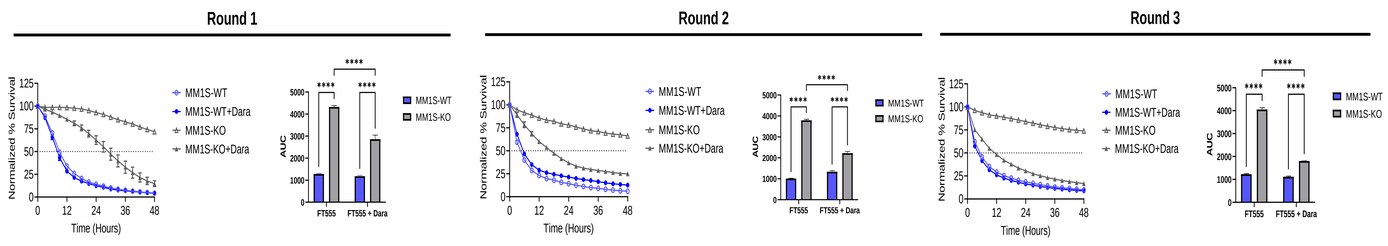


Figure 3. GPRC5D CAR-mediated cytotoxicity and ADCC against MM.1S target cells in a serial restimulation assay. Cytotoxicity by FT555 was assessed by IncuCyte imaging against MM.1S-WT-NLR and MM.1S-GPRC5D KO-NLR target cells at a 3:1 E:T ratio in three consecutive rounds of stimulation at 48 hours per round. Data are presented as the mean ± SD for each timepoint. Area under the curve (AUC) was calculated for each cytotoxicity curve at all three rounds of stimulation. Data are presented as the mean AUC of triplicate results ± SD. Statistics were performed using two-way ANOVA with Tukey's multiple comparison test. *p value of <0.05, **p value of <0.01, ***p value of <0.001, ****p value of <0.0001, ns = not significant.

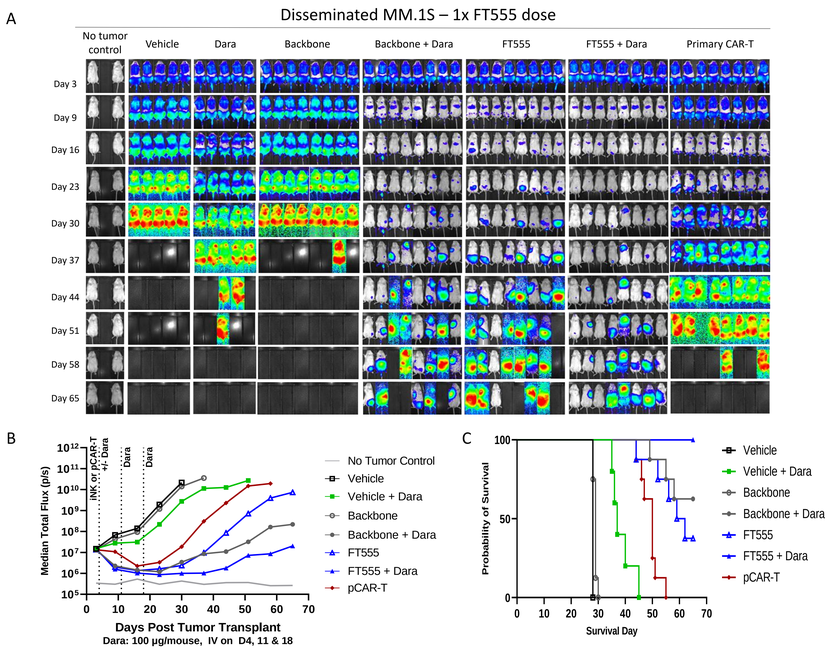


Figure 4. Tumor growth inhibition and increased survival by FT555 as a monotherapy and in combination with daratumumab against a disseminated MM.1S tumor model. Mice (n=8/group) were injected IV with 5×10^5 MM.1S target cells on Day 0. At Day 4, 1×10^7 iNK cells or 1×10^6 pCAR-T cells were injected IV. Daratumumab was injected IV on Days 4, 11, and 18. (A) Representative images depicting MM.1S tumor burden in all mice through Day 65. Tumor burden was assessed by IVIS imaging, conducted weekly. (B) Tumor growth curves depicted as median total flux values through Day 65. (C) Kaplan-Meier survival curve analysis indicating the probability of survival over the course of the study.

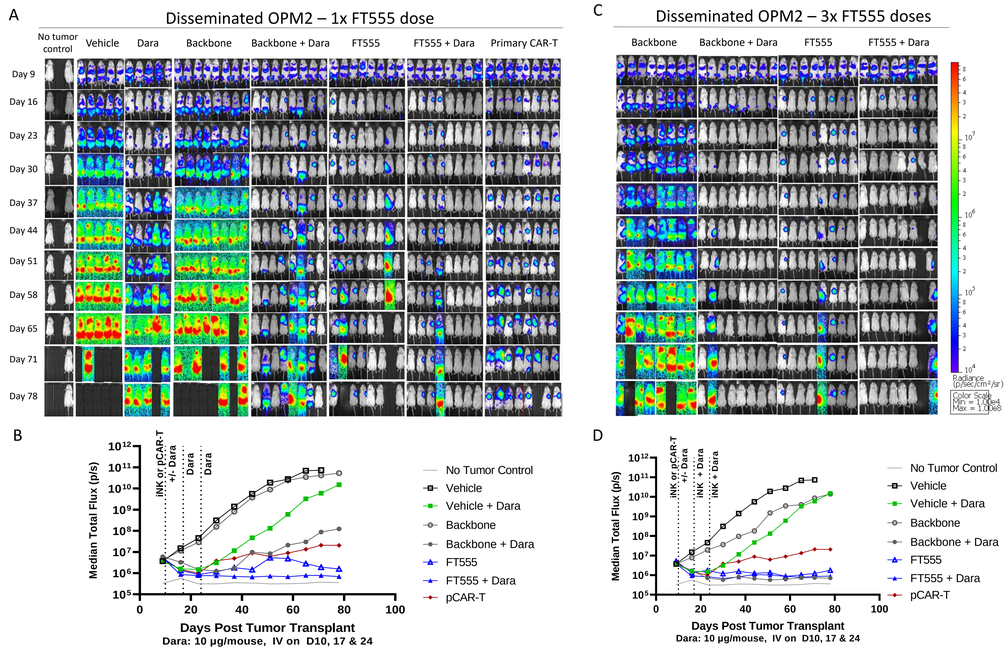


Figure 5. Multi-dose capacity of FT555 as a monotherapy and in combination with daratumumab against a disseminated OPM-2 tumor model. Mice (n=8/group) were injected IV with 5×10^6 OPM-2 target cells on Day 0. (A and B) At Day 10, one dose of 1×10^7 iNK cells or 1×10^6 pCAR-T cells were injected IV. (C and D) Two subsequent doses of 1×10^7 iNK cells were injected IV on Days 17 and 24 for a total of three doses. Daratumumab was injected IV on Days 10, 17, and 24 in both studies. (A and C) Representative IVIS images depict OPM-2 tumor burden in all mice through Day 78. Tumor burden was assessed by IVIS imaging, conducted weekly. (B and D) Tumor growth curves are depicted as median total flux values through Day 78.