Genetically Engineered Pluripotent Cell-Derived Natural Killer Cell Therapy Provides Enhanced Antibody Dependent Cellular Cytotoxicity Against Hematologic Malignancies and Solid tumors in Combination with Monoclonal Antibody Therapy

FT516: iPSC-derived hnCD16 NK Cell Cancer Immunotherapy

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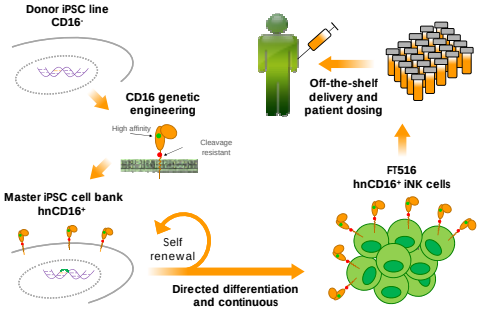
EXECUTIVE SUMMARY

- **FT516** is an allogeneic, off-the-shelf NK cell immunotherapy derived from a single cell derived master pluripotent cell line engineered to express a **high affinity, non-cleavable version of CD16 (hnCD16)** for enhanced antibody dependent cellular cytotoxicity (ADCC)
  - ADCC is a key natural killer (NK) cell effector mechanism that significantly contributes to the anti-tumor effect of therapeutic monoclonal antibodies (mAbs) including rituximab (anti-CD20), cetuximab (anti-EGFR) and trastuzumab (anti-ErbB2).
  - Engagement of CD16 on the surface of NK cells by the Fc portion of cell-bound mAbs activates NK cell activation, directed cytotoxicity, and cytokine production
  - Endogenous CD16 is cleaved upon NK cell activation, resulting in reduced surface expression
- FT516 is phenotypically mature, uniformly positive for CD16 expression, and demonstrate stable surface CD16 levels upon NK cell activation, in contrast to peripheral blood-derived NK cell
- FT516 is highly functional in vitro, exhibiting robust cytokine production in response to CD16 stimulation and **enhanced ADCC against multiple lymphoma targets** when combined with anti-CD20 antibody.
- **FT516 is functional in vivo and mediate superior ADCC-dependent regression in vivo lymphoma and ovarian cancer model**

GRAPHICAL ABSTRACT



RESULTS

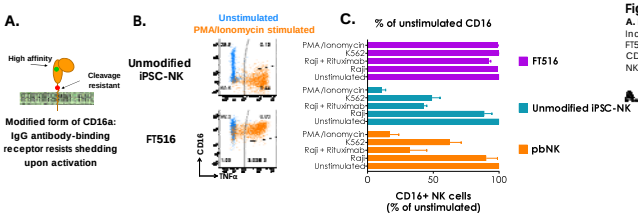


Figure 1. High affinity, non-cleavable CD16 (hnCD16) construct resists activation induced cleavage. **A.** CD16 construct used to make hnCD16 iNK utilizes high affinity (F158V) and non-cleavable version of CD16. **B.** Unmodified iNK or FT516 were left unstimulated (blue) or stimulated with PMA and ionomycin (orange). Activated FT516 produce TNFa, but resist CD16 cleavage. **C.** FT516, unmodified iPSC-NK, or peripheral blood NK were stimulated for 4 hours and CD16 shedding was determined by flow cytometry (n=4-6 per group).

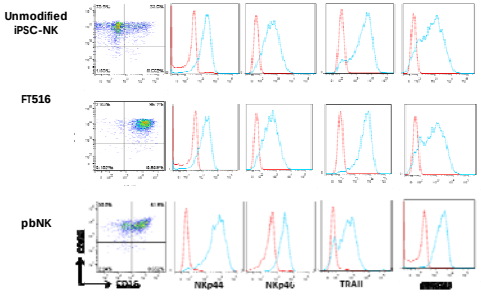


Figure 2. iPSC derived NK cells are phenotypically mature. WT iPSC-derived NK cells (WT-iNK), hnCD16-iPSC-derived NK cells (hnCD16-iNK), and adult peripheral blood NK cells were stained for a panel of NK cell receptors. Expression of each marker is shown by representative flow cytometry plots.

RESULTS

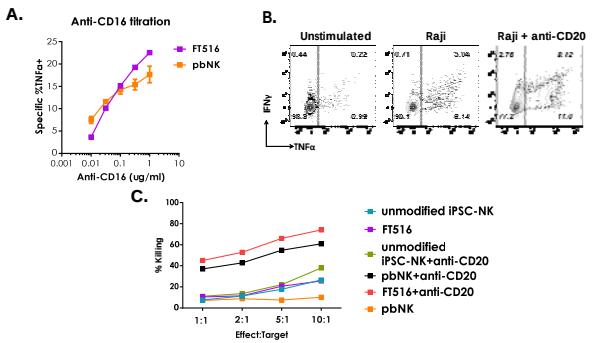


Figure 3. FT516 cells are functionally responsive to CD16 stimulation. **A.** FT516 responds to direct anti-CD16 stimulation. FT516 or peripheral blood NK (pbNK) from three donors were stimulated with P815 cells and increasing concentrations of anti-CD16 antibody. After 4 hours, NK cells were stained intracellularly for production of TNFa, and specific TNFa production was calculated by subtracting the no antibody control sample from the anti-CD16 sample for each condition. **B.** FT516 NK produce TNFa and IFNγ in response to Raji cells + anti-CD20 antibody. FT516 were left unstimulated or stimulated with a 1:1 ratio of Raji cells ± anti-CD20 antibody and stained for TNFα and IFNγ 4 hours later. **C.** ADCC against Raji cells was determined using CellEvent™ Caspase-3/7 Green Flow Cytometry Assay. Different amount of Raji cells were incubated with NK cells (effect to target ratio from 1:1 to 10:1) for 4 hours. Rituximab concentration was 10ug/ml.

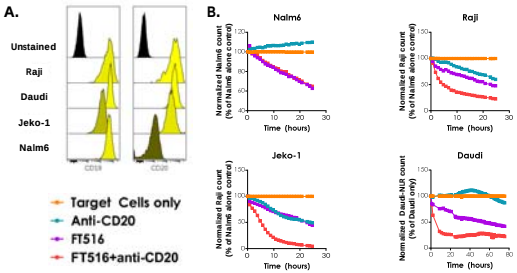


Figure 4. hnCD16-iNK cells have superior in vitro ADCC against multiple B cell lymphoma lines. **A.** Flow cytometry analysis of CD19 and CD20 levels on lymphoma cell line targets. **B.** Survival of target cells was quantified by Incucyte Zoom imaging for target cells only (orange), target cells plus anti-CD20 antibody (blue), target cells incubated with FT516 (purple), or target cells incubated with anti-CD20 and FT516 to induce ADCC (Red). FT516 mediated substantial ADCC in all CD20+ lymphoma lines. The data are presented as the normalized frequency of target cells remaining, where target cells without NK effectors = 100%.

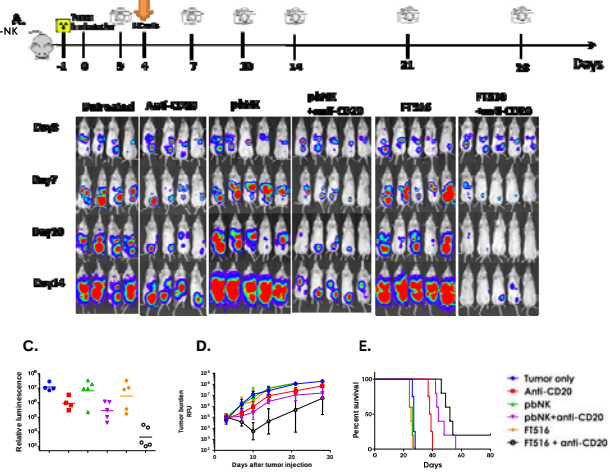


Figure 5. Single dose of FT516 effectively mediates ADCC in an *in vivo human lymphoma* cancer model. **A.** NSG mice were inoculated IP with 2E5 Raji cells expressing the firefly luciferase gene, and tumor engraftment was assessed by IVIS imaging 3 days later. On day 4 after transplant, mice were either left untreated or treated with 1E7 pbNK or FT516 alone or in combination with 300ug anti-CD20 antibody. NK cells were supported by injection of IL-15 for the first week and IL-2 for 3 weeks, and IVIS imaging was performed weekly to track tumor progression. **B.** IVIS images, **C.** tumor burden at day 14, and **D.** IVIS imaging timecourse are shown. The geometric mean±geometric SD for the mice shown in B. **E.** Survival curve of each group. The median survive time for untreated group, anti-CD20, pbNK + anti-CD20, and FT516 + anti-CD20 were 27, 39, 44, and 52 days, respectively.

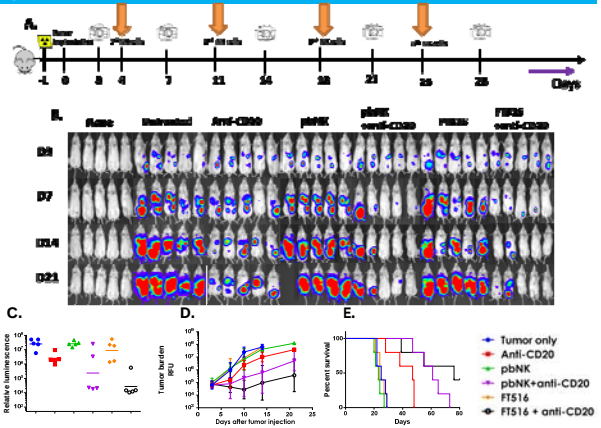


Figure 6. Multiple doses of hnCD16-iNK effectively mediate ADCC in an *in vivo human lymphoma* cancer model. **A.** NSG mice were inoculated IP with 2E5 Raji cells expressing the firefly luciferase gene, and tumor engraftment was assessed by IVIS imaging 3 days later. On day 4 after transplant, mice were either left untreated or treated with 1E7 pbNK or FT516 alone or in combination with 300ug Rituximab weekly for 4 weeks. NK cells were supported by injection of IL-15 and IL-2, and IVIS imaging was done weekly to monitor tumor progression. **B.** IVIS images, **C.** tumor burden at day 14, and **D.** IVIS imaging timecourse is shown. The geometric mean±geometric SD for the mice shown in B. **E.** Survive curve of each group. The median survive time for the untreated group, anti-CD20, pbNK+anti-CD20 and FT516+anti-CD20 were 25 , 47, 61and 76 days, respectively.

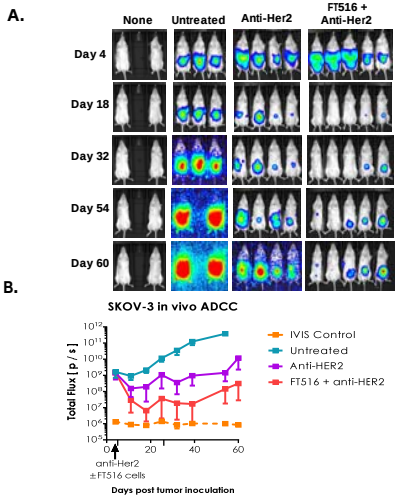


Figure 7. FT516 effectively mediates ADCC in an *in vivo* ovarian cancer model. NSG mice were inoculated IP with 1E5 SKOV-3 cells expressing the firefly luciferase gene, and tumor engraftment was assessed by IVIS imaging four days later. On day 5 after tumor transplant, mice were either left untreated or treated with anti-HER2 either alone or in combination with 5E6 FT516 cells. FT516 were supported by twice-weekly injections of IL-2, and IVIS imaging was done weekly to track tumor progression. **A.** IVIS images or **B.** the geometric mean - geometric SD for the mice shown in A.

CONCLUSIONS

- FT516 is an off-the-shelf NK cell product consisting of engineered expression of high affinity, non-cleavable CD16 (hnCD16) promotes enhanced NK cell responsiveness to CD16 stimulation and antibody dependent cellular cytotoxicity (ADCC)
- FT516 is derived from a master pluripotent cell line generated from a single engineered induced pluripotent stem cell
- FT516 is highly functional in vitro, exhibiting potent cytokine production and enhanced ADCC against multiple tumor targets
- FT516 is functional in vivo and mediate ADCC-dependent tumor regression in human lymphoma and ovarian cancer models
- FT516 is in preclinical analysis and is scheduled for IND filing during the second half of 2018

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