FT500, an Off-the-Shelf NK Cell Cancer Immunotherapy Derived From a Master Pluripotent Cell Line, Enhances T-cell Activation and Recruitment to Overcome Checkpoint Blockade Resistance

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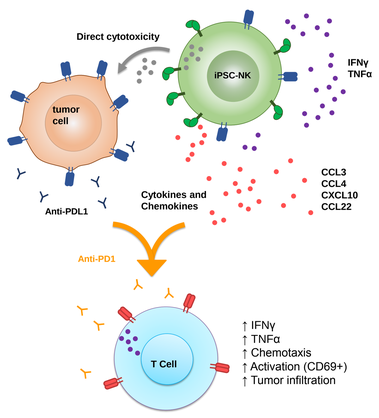
EXECUTIVE SUMMARY

- Induced pluripotent stem cells (iPSCs) are an ideal source for creating cell therapy products that are well-defined, uniform in composition, have a consistent and dose-dependent pharmacology profile, and **delivered off-the-shelf** for the treatment of large numbers of patients.
- Fate's unique iPSC technology allows for high throughput, **clonal selection of single input human iPSC** to create clonal iPSC master cell lines.
- **FT500** is an allogeneic, iPSC-derived, off-the-shelf NK cell immunotherapy (iPSC-NK) for solid tumor malignancy as a monotherapy and in combination with anti-PD1 and anti-PDL1 checkpoint inhibitor antibodies.
  - Allogeneic NK cells have been well tolerated clinically, without significant graft vs. host disease (GvHD) and other severe adverse effects
  - NK cells do not require prior antigen exposure for effector function
- iPSC-NK has potential to synergize with anti-PD1 and anti-PDL1 antibodies for combination immunotherapy:
  - Direct lysis of tumor cells reduces tumor load and releases tumor neoantigens
  - Secretion of inflammatory cytokine and chemokines enhances T cell activation and recruitment to the tumor site
- Down-regulation of HLA-I is a known tumor escape mechanism in response to checkpoint blockade, and loss of HLA-I enhances NK recognition and lysis of tumor cells.

GRAPHICAL ABSTRACT



CONCLUSIONS

- iPSC-NKs mediate direct cytotoxicity against solid tumor targets *in vitro* and *in vivo*, and demonstrate cytokine and chemokine production after tumor challenge
- iPSC-NKs enhance T cell migration and tumor spheroid infiltration, leading to a more rapid tumor killing kinetics
- iPSC-NK cells induce T cell migration out of circulation *in vivo* as demonstrated by the T cell recruitment assay
- Conditioned medium from iPSC-NKs induce T cell activation as shown through CD69 upregulation
- Co-culture of iPSC-NKs and T cells in a 3D tumor spheroid model led to tumor cell killing and enhanced production of IFNγ and TNFα
- FT500 has multi-point potential to synergize with checkpoint inhibitor therapy to overcome resistance and enhance function

RESULTS

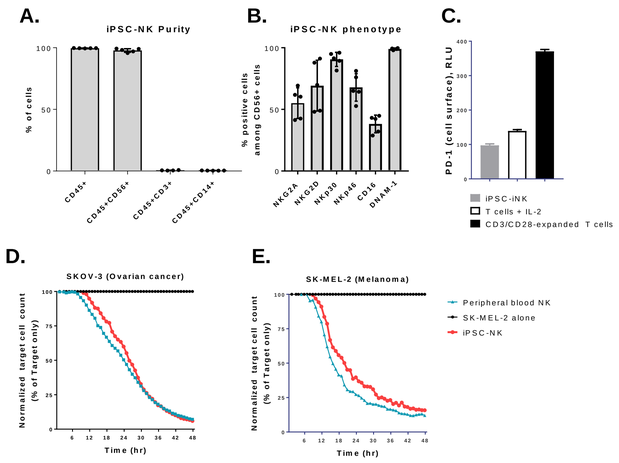


Figure 1. iPSC-NK production yields a population of mature NK cells with cytotoxic capacity against solid tumor targets. iPSC-NK are comprised of **(A)** CD45+CD56+ NK cells expressing the NK cell receptors **(B)** NKG2A, NKG2D, NKp30, NKp46, CD16, and DNAM-1, and are low/negative for PD-1 expression **(C)**. iPSC-NK are directly cytotoxic against solid tumor targets such as **(D)** SKOV-3 (ovarian cancer) and **(E)** SK-MEL-2 (malignant melanoma). Cytotoxicity is similar between iPSC-NK and in vitro expanded, peripheral blood derived NK cells.

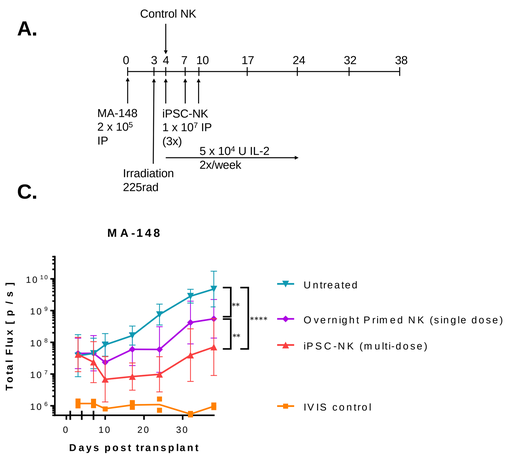


Figure 2. iPSC-NK mediate tumor regression and delayed progression in a xenograft model of ovarian cancer. The MA-148 ovarian cancer line was used as an in vivo solid tumor model for direct NK cell cytotoxicity with iPSC-NK. **(A)** 2 x 10^5 MA-148-luciferase cells (MA-148-Luc) were injected intraperitoneally. Three days later, mice were irradiated with 225rad. On day 4 after tumor transplant, mice were left untreated, treated with a single dose of CD3/CD19-depleted PBMC primed overnight in IL-15, or treated with doses of iPSC-NK on days 4, 7, and 10. IL-15 primed CD3/CD19-depleted PBMC represents a current clinical strategy and provides a comparator therapeutic regimen and NK cell positive control. **(B)** Individual IVIS images or **(C)** the geometric mean ± geometric SD are shown. N=8 mice per group. **p<.01, ****p<.0001, two-way RM-ANOVA with Tukey's multiple comparison test.

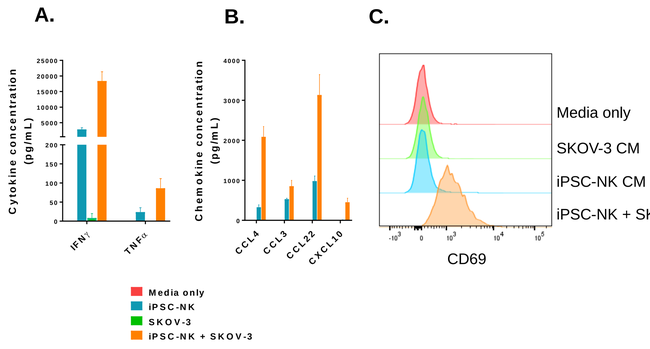


Figure 3. Activation of iPSC-NK produces soluble cytokines and chemokines that recruit and activate T cells in vitro. **(A&B)** Conditioned media (CM) was collected after 24 hour culture of iPSC-NK cells alone (blue), SKOV-3 alone (green), or iPSC-NK co-cultured with SKOV-3 cells at a 1:1 ratio (orange). CM was collected and the supernatant concentrations of **(A)** IFNγ and TNFα, or **(B)** CCL3, CCL4, CXCL10, and CCL22 were measured. **(C)** CM was transferred to allogeneic donor T cells, and CD69 expression on $CD3^+$ cells was evaluated after overnight culture. **(D)** CM was collected from the indicated conditions and used as a chemoattractant in the lower chamber of a transwell chemotaxis assay. Total T cells from 4 allogeneic donors were assessed for chemotaxis. **p<.01 from all other CM groups, one-way ANOVA with Tukey's multiple comparison test.

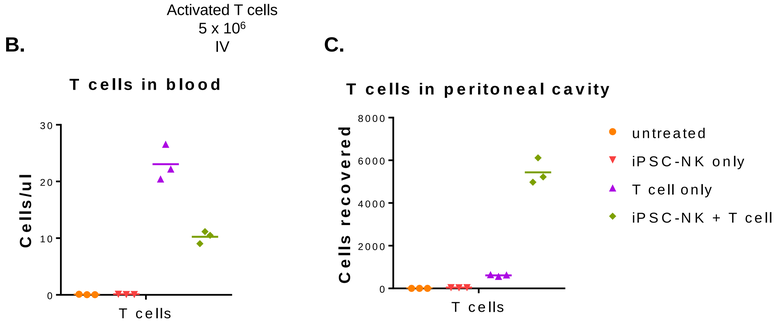


Figure 4. iPSC-NK can recruit activated T cells out of circulation in an in vivo recruitment model. **(A)** NSG mice were injected with 5 x 10^6 activated T cells intravenously, and 1 x 10^7 iPSC-NK cells intraperitoneally. Four days later, the peripheral blood and peritoneal cavity were assessed for the presence of T cells by flow cytometry. Compared with mice receiving T cells but no NK cells, mice that received iPSC-NK IP had **(B)** reduced T cell frequency in peripheral blood and **(C)** increased T cells in the peritoneal cavity.

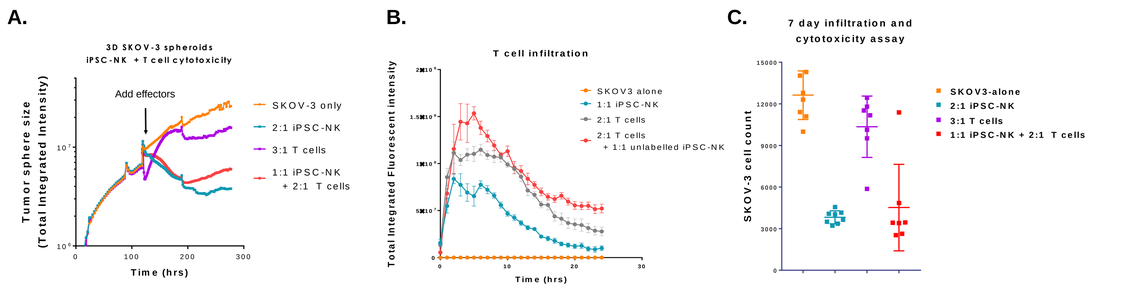


Figure 5. iPSC-NK co-culture enhances T cell infiltration of tumor spheroids and promotes enhanced cytotoxicity. **(A)** Infiltration of T cells into SKOV-3 spheroids was measured for 24 hours of co-culture with iPSC-NK (1:1 ratio), CD3+ T cells (2:1 ratio), or iPSC-NK + T cells (1:1 iPSC-NK, 2:1 T cell ratio). Infiltration was measured by total integrated green fluorescence intensity within the largest red object mask. **(B)** Cytotoxicity against SKOV-3 spheroids was measured by the total integrated fluorescence intensity. **(C)** 7 days after addition of effector cells, tumor spheroids were dissociated and SKOV-3 cells were counted for each condition shown. The mean ± SD is shown for 7 replicate wells.

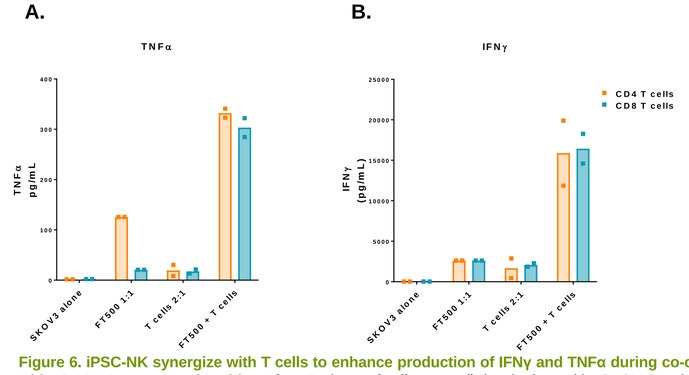


Figure 6. iPSC-NK synergize with T cells to enhance production of IFNγ and TNFα during co-culture with SKOV-3 tumor spheroids. After 7 days of effector cell incubation with SKOV-3 spheroids, supernatants were collected and assessed for **(A)** TNFα and **(B)** IFNγ production. Co-culture of T cells with iPSC-NK led to increased cytokine production for both $CD4^+$ and $CD8^+$ T cells.