iPSC-derived NK cells Engineered with a Novel TGFβ signal Redirector Receptor Exhibit Enhanced Performance Against Solid Tumors

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Introduction

Transforming growth factor beta (TGFβ) is an immuno-suppressive cytokine commonly present in the tumor microenvironment (TME) that creates considerable challenges for the treatment of solid tumors. Here we describe a unique strategy where induced pluripotent stem cell (iPSC)-derived NK (iNK) and T (iT) cells engineered to express a chimeric TGFβ signal redirector receptor (TGFβ-SRR) block the TGFβ-mediated repressive signaling and redirect the signal to potentiate effector cell function and improve cell fitness to improve therapeutic efficacy in solid tumors.

Figure 1. Select cytokines can allow continued function of CAR-iNK cells even in the presence of TGFβ

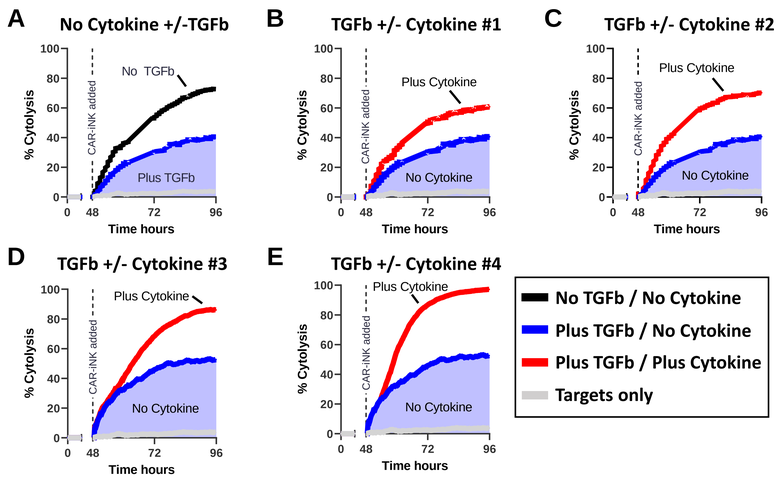

Figure 1. iPSC-derived CAR-NK cells co-cultured with Caski target cells and 20 ng/ml TGFβ. **(A)** xCelligence assay measuring the effect of TGFβ on CAR-dependent effector function. **(B-E)** Effect of select cytokines on attenuating the suppressive effect of TGFβ.

Figure 2. A TGFβ-Signal Redirector Receptor (TGFβ-SRR) can activate cytokine signaling pathways in the presence of TGFβ

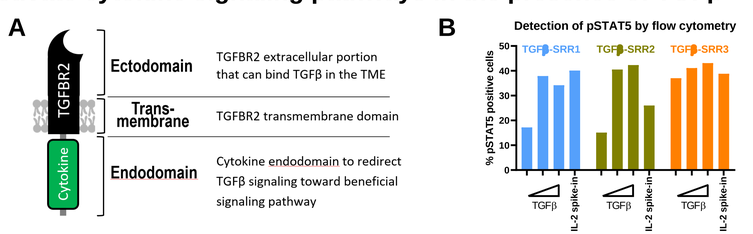

Figure 2. **(A)** Design of TGFβ-signal redirector receptor (TGFβ-SRR). **(B)** CAR-T cells expressing different TGFβ-SRR constructs were tested for a TGFβ-dependent increase in the proportion of phosphorylated STAT5 positive cells using flow cytometry. IL-2 spike-in was used as a positive control.

Figure 3. iPSCs engineered with a TGFβ-Signal Redirector Receptor (TGFβ-SRR) can be successfully differentiated into iNK cells

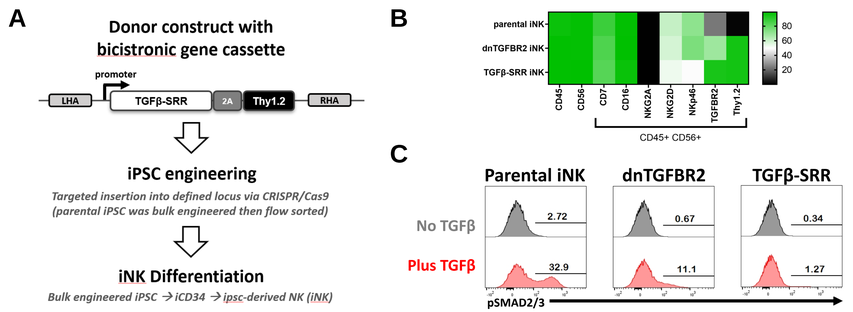

Figure 3. **(A)** Schematic showing the strategy used to generate iNK cells that express a TGFβ-SRR. **(B)** Phenotypic profile of mature iNK cells expressing TGFβ-SRR. **(C)** Representative histograms showing flow cytometric measurement of phosphorylated Smad2/3 in iNK cells after 1 hour of exposure to 20 ng/mL TGFβ.

Figure 4. iNK cells expressing a TGFβ-SRR exhibit enhanced innate killing capacity, persistence, and activation profile in the presence of TGFβ

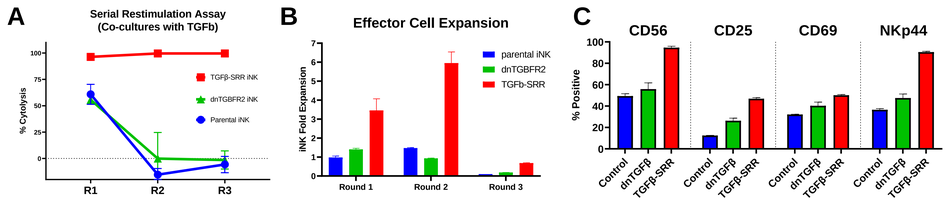

Figure 4. **(A)** Innate killing over three rounds of co-culture with Raji tumor cells in the presence of 20 ng/ml TGFβ. **(B)** Effector cell expansion at the end of each round of co-culture as determined by flow cytometry. **(C)** Proportion of iNK cells expressing the indicated activation markers at the end of round 2 as measured by flow cytometry.

Figure 5. TGFβ-SRR iNK cells exhibit enhanced ADCC and increased effector cytokine production toward solid tumor lines in the presence of TGFβ

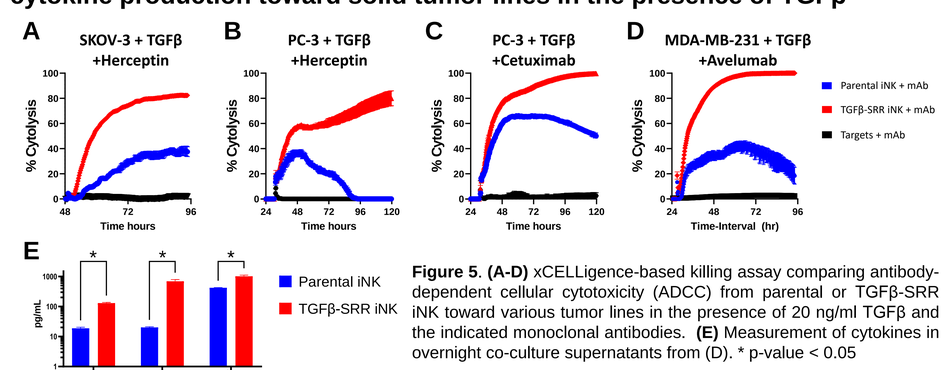

Figure 5. **(A-D)** xCELLigence-based killing assay comparing antibody-dependent cellular cytotoxicity (ADCC) from parental or TGFβ-SRR iNK toward various tumor lines in the presence of 20 ng/ml TGFβ and the indicated monoclonal antibodies. **(E)** Measurement of cytokines in overnight co-culture supernatants from (D). * p-value < 0.05

Figure 6. TGFβ-SRR iNK cells uniquely exhibit antitumor activity through sequential rounds of ADCC in a highly repressive culture condition saturated with TGFβ

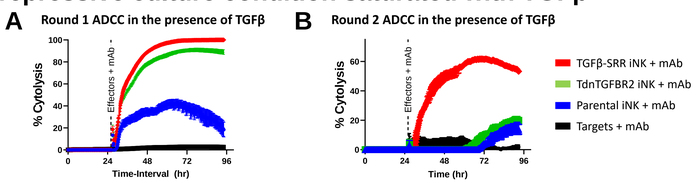

Figure 6. **(A)** xCelligence assay measuring ADCC from effector cells co-cultured with MDA-MB-231 targets in the presence of Avelumab and high-dose 20 ng/ml TGFβ. **(B)** Effector cells from the end of round 1 were co-cultured with target cells for a second round of ADCC measurement in the presence of Avelumab and TGFβ.

Figure 7. The Fate Therapeutics platform has multiple advantages over existing approaches to cellular therapy

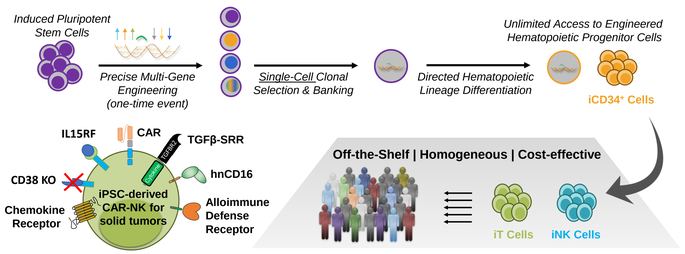

Figure 7. Overview of Fate's proprietary iPSC platform for the generation of precisely and uniformly engineered effector NK and T cells to express synthetic TGFβ-SRR.

Summary of Results

1. TGFβ can inhibit the anti-tumor activity of iPSC-derived NK cells (iNK). Certain cytokine signaling pathways when activated can attenuate the suppressive effects of TGFβ.
2. Synthetic TGFβ-signal redirector receptors (TGFβ-SRR) can enable activation of cytokine signaling in the presence of TGFβ.
3. iPSCs can be engineered with a TGFβ-SRR and subsequently differentiated into mature and phenotypically normal iNK cells.
4. iNK cells expressing the TGFβ-SRR exhibit enhanced innate killing capacity, proliferation, and activation profile in a serial restimulation assay with target tumor cells and in the presence of TGFβ.
5. iNK cells expressing a TGFβ-SRR exhibit enhanced ADCC and cytokine production in co-cultures with solid tumor lines in the presence of TGFβ.
6. iNK cells expressing a TGFβ-SRR exhibit superior ADCC function compared to iNK cells expressing a dominant negative TGFBR2 in sequential rounds of co-culture with target cells and TGFβ.

Conclusion

Synthetic redirection of TGFβ signaling is an effective strategy for enhancing the durable performance of NK cells in solid tumor settings and is compatible with an off-the-shelf iPSC-derived NK cell platform.