259 IPSC-DERIVED NK CELLS ENGINEERED WITH A NOVEL TGFβ SIGNAL REDIRECTOR RECEPTOR EXHIBIT ENHANCED PERFORMANCE AGAINST SOLID TUMORS

Eigen Peralta*, Dan Lu, Hui-Yi Chu, Justin Rahman, Diana Galvan, Amit Mehta, Eric Sung, Jeffrey Chen, Masanao Tsuda, Elena Demeester, Earl Avramis, Alec Witty, Tom Lee, Bob Valamehr. *Fate Therapeutics Inc., San Diego, CA, USA*

Background 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.

Methods To identify iNK cell-specific pathways for TGFβ signal redirection, candidate cytokines were tested for their ability to mitigate suppression of iNK cell anti-tumor activity in the presence of recombinant TGFβ. Next, we developed TGFβ-SRR constructs where selected cytokine endodomains were fused to TGFBR2 ectodomain. TGFβ-SRR constructs were then engineered into iPSCs and differentiated into iNK cells. Phospho-flow for pSMAD2/3 was used to test for blockade of TGFβ signaling in recombinant TGFβ-treated cells. Antibody-dependent cellular cytotoxicity (ADCC) from TGFβ-SRR iNK cells was tested in co-cultures with SKOV-3, PC3, and MDA-MB-231 targets, then measured using xCELLigence readout. Innate killing mechanism was tested via serial restimulation assay with Raji targets and measured by flow cytometry. Co-cultures were performed in the presence of recombinant TGFβ.

Results Engineered iPSCs expressing candidate TGFβ-SRR constructs were successfully differentiated into iNK cells (>95% CD56+), uniformly expressing TGFβ-SRR transgene (TGFβ-SRR; >95% positive). Analysis for pSMAD2/3 in recombinant TGFβ-treated cells showed 95% reduction of SMAD2/3 phosphorylation in top performing TGFβ-SRR motif, indicating successful blockade of TGFβ signaling. Evaluation of ADCC toward multiple solid tumor lines and using various monoclonal antibodies (Herceptin, Cetuximab, and Avelumab) showed superiority of TGFβ-SRR iNK cells (>80% cytolysis) over parental iNK cell control (<40% cytolysis) in the presence of recombinant TGFβ. Innate killing mechanism was tested in the serial restimulation assay, where TGFβ-SRR iNK cells expanded 3.5-fold over parental iNK cells after the first round of co-culture. Notably, the TGFβ-SRR iNK cells exhibited enhanced functional persistence, completely controlling tumor growth through three rounds of co-culture despite the addition of suppressive quantities of recombinant TGFβ, unlike parental iNK cells which failed to control tumor growth after the first round.

Conclusions Collectively, the data illustrate that a customized TGFβ-SRR construct can redirect TGFβ-mediated suppression and potentiate effector cell function to enhance the anti-tumor activity of iNK cells. This novel synthetic receptor represents an innovative strategy to enable adoptively-transferred cell therapy to overcome the immunosuppressive TME for the successful treatment of bulky tumors.

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