Improved anti-tumor immune function of TGFβR2 knock out and IL-15 knock in iPSC-derived NK (iNK) cells by TALEN® editing for use alone or in combination with CYT-303 Flex-NK engager

#3187

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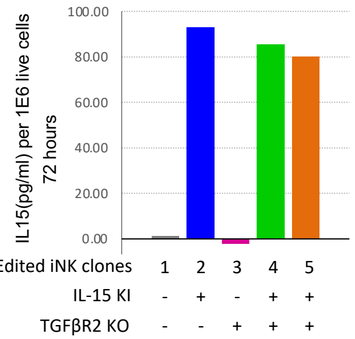
Background & Methods

Background: Induced Pluripotent Stem Cell (iPSC)-derived NK cells (iNK) offer an opportunity to generate unlimited homogenous NK cells for allogeneic off-the-shelf therapies. Interleukin-15 (IL-15) engineering in NK cells confer enhanced proliferation, persistence, cytotoxicity, and metabolic fitness. Activation of TGF-b signaling in NK cells may inhibit their anti-tumor functions in the tumor microenvironment (TME). Therefore, we hypothesized that iNK cells edited to knock-in IL-15 and knock-out TGFbR2 could exhibit improved immune function and overcome the immunosuppressive tumor microenvironment. Furthermore, the activity of these edited universal iNK cells could be enhanced when combined with CYT-303, a Flex-NK bispecific antibody NK engager targeting GPC3 expressed on hepatocellular carcinoma (HCC) tumor cells and NKp46 expressed on NK cells.

Methods: IL15 was knocked-in and/or the TGFβR2 was knocked out using Cellectis TALEN® technology in iPSCs and the verified edited iPSC clones were differentiated and expanded into CD56+ NK cells. The functional significance of these edits in iNKs were assessed in IL-15 and TGF-β dependent NK cell assays assessing proliferation, expression of activation receptors, and cytolysis of hepatocellular carcinoma tumors (HCC). Cytotoxic activity of these edited cells was also evaluated in the serial killing assay with or without CYT-303 NK engager in the absence or presence of TGF-β.

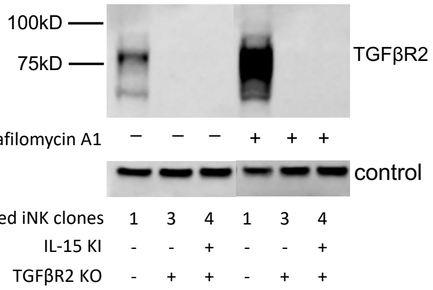
Results

1. The IL-15 expression level in IL15$^{+/+}$ knock-in iNK cells.



IL-15 release was measured by ELISA for the medium when the edited iNK cells were cultured about 72 hours. The densities of IL-15 were calculated by the standard curve of IL-15, and were normalized to the 1E6 iNK live cells for comparison.

2. The TGFβR2$^{-/-}$ knock-out iNK cells are verified by western blot.

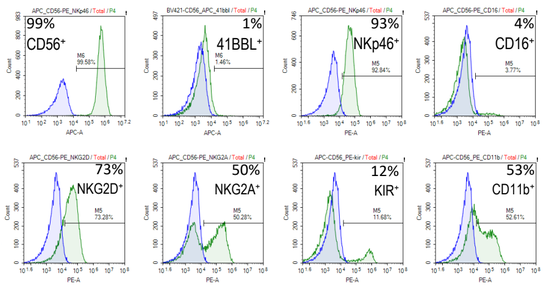


By using western blot assay for the wild type iNKs, the cellular pools of TGFβR2 accumulated after 24 hours treatment of Bafilomycin A1 which prevent the lysosome degradation. However, the expression of TGFβR2 could not be detected before or after the treatment of Bafilomycin A1 for the TGFβR2$^{-/-}$ iNK cells and IL15$^{+/+}$/TGFβR2$^{-/-}$ iNK cells, which implied that they are true knock out of TGFβR2.

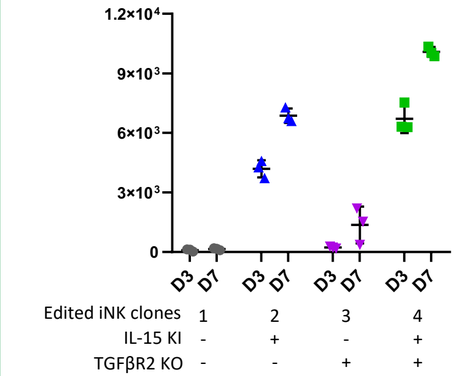
Results

3. Phenotype of IL15$^{+/+}$ & TGFβR2$^{-/-}$ double edited iNKs.

Flow cytometry results were displayed by histogram plots (blue: isotype control; green: detected markers). The IL15$^{+/+}$/TGFβR2$^{-/-}$ dual edited iNK cells consist of a homogeneous population of CD56+ NK cells (>99%). Several typical NK cell markers including NKp46, NKG2D, NKG2A, and CD11b are highly expressed, similar to their expression in PBNKs and wild type iNKs.

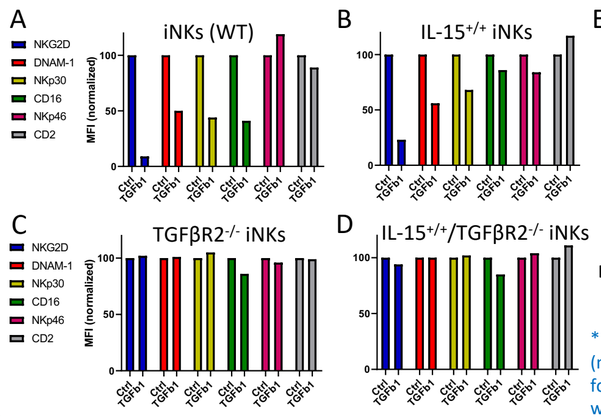


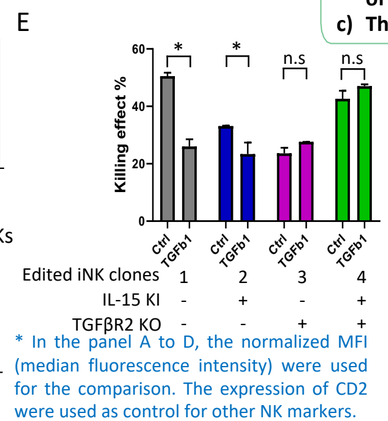
4. Compared to the non-edited iNKs, iNKs with IL-15 KI can extend persistence *in vitro* in the absence of exogeneous cytokine IL-2.



The wild type and engineered iNK cells were plated with the same number in the NK expansion media without IL-2 addition. After 3 days (D3) or 7 days (D7), cells were collected and analyzed by flow cytometry with quantitative beads.

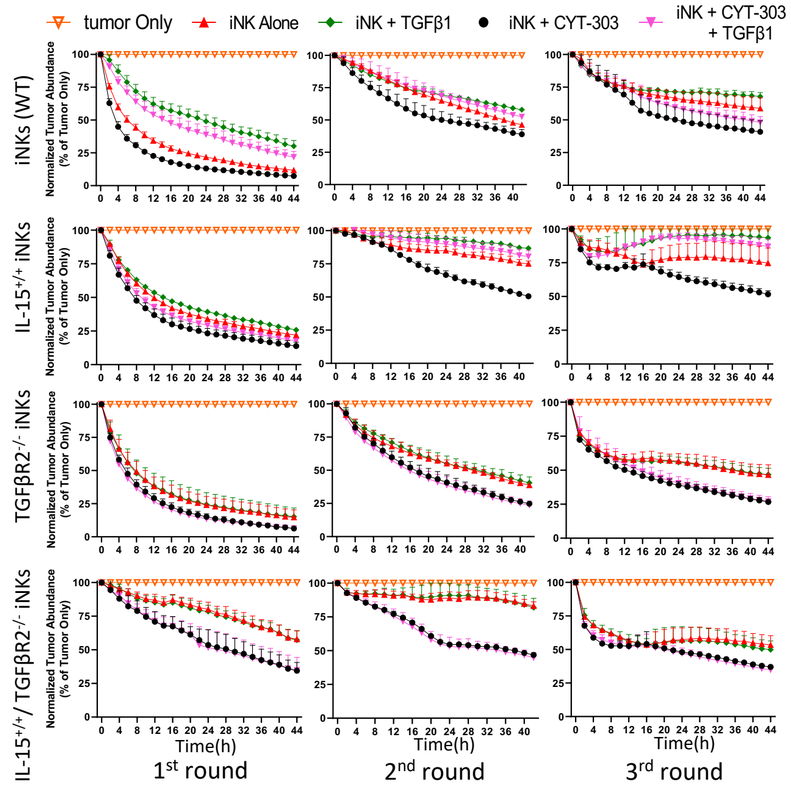
5. TGF-β, suppressed expression of a number of iNK (WT) cell activating receptors, such as NKG2D, DNAM-1, and NKp30, (A, B) and showed lower cytotoxicity against HCC tumors (E). iNKs with TGFβR2 KO are resistant to TGF-β-mediated suppression of activating receptors (C, D) and conferred resistance to TGF-β-mediated suppression of cytotoxicity against HCC tumor cells (E).





* In the panel A to D, the normalized MFI (median fluorescence intensity) were used for the comparison. The expression of CD2 were used as control for other NK markers.

6. Effects of CYT-303 on edited iNK cells w/o TGF-β.



a) iNKs with TGFβR2 KO w/o IL-15 KI were resistant to TGF-β mediated suppression during serial killing of HCC tumors.
b) In the presence of TGF-β, iNKs with TGFβR2 KO w/o IL-15 KI showed greater reversal of iNK dysfunction by CYT-303 NK engager compared to WT iNK cells.
c) These results show the greater potential of edited iNK cells to combine with CYT-303.

Conclusions

- These studies demonstrates KI of IL-15 and KO of TGFβR2 a promising strategy for TALEN®-engineered iNK cell therapies with improved persistence and effector function to overcome the immunosuppressive tumor microenvironment and mount potent anti-tumor immune responses.
- The data also provide a solid foundation for combining these edited iNK cells with CYT-303 to address the immunosuppressive tumor microenvironment to fight against HCC.