## 311 DISRUPTION OF TGF-B SIGNALING PATHWAY IS REQUIRED TO MEDIATE EFFECTIVE KILLING OF HEPATOCELLULAR CARCINOMA BY HUMAN IPSC-DERIVED NK CELLS

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**Background** Hepatocellular carcinoma (HCC) is common type of primary liver cancer with the third-highest mortality rate for all cancers.[1–3]Natural killer (NK) cell therapy has demonstrated efficacy for treatment of hematological malignancies, though their efficacy against solid tumors has been more limited.[4–6] Transforming growth factor beta (TGF-β) is highly expressed in the liver tumor microenvironment and is known to suppress NK cell-mediated activity.[7–9] Therefore, we hypothesized that disruption of TGF-β signaling plus expression of anti-HCC chimeric antigen receptors (CARs) can improve NK cell persistence, killing and proliferation in the HCC tumor microenvironment.

**Methods** Here, we generated human induced pluripotent stem cell (iPSCs) with either knock out (KO) of the TGF-β receptor 2 (TGFBR2-KO) or expression of a dominant negative (DN) form of the TGF-β receptor 2 (TGFBR2-DN) combined with CARs that target either GPC3 or AFP that are typically highly expressed on HCC. These anti-HCC CAR iPSCs were then effectively differentiated into NK cells and the function of these iPSC-NK cells tested against human HCC cell lines (HepG2, SNU-449) and K562 cells in the presence and absence of TGF-β.

**Results** As expected, we found that treatment with TGF-β reduced NK cell activity of the wild-type (WT) iPSC-derived NK cells, with or without anti-HCC CAR expression. However, both TGFBR2-KO and DN +/- anti-HCC CAR iPSC-NK cells are resistant to inhibition by TGF-β and mediated improved anti-HCC activity both *in vitro* and *in vivo*. Interestingly, expression of anti-HCC CARs on iPSC-NK cells did not lead to effective anti-HCC activity unless there was also inhibition of TGF-β activity. That is, the TGFBR2-KO-iPSC NK cells without CAR expression exhibited significantly improved anti-tumor activity and *in vivo* persistence when compared to WT iPSC-derived NK cells that expressed anti-HCC CARs, but were not resistant to TGF-β activity.

**Conclusions** Our findings demonstrate that TGF-β signaling blockade is required for effective NK cell function against HCC and inhibition of this pathway should be included to successful cell-based treatment of HCC and likely other malignancies that express high levels of TGF-β in the tumor microenvironment.

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**Ethics Approval** All mice were housed, treated, and handled in accordance with the guidelines set forth by the University of California, San Diego Institutional Animal Care and Use Committee and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals

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