INTRODUCTION

- NK cells kill tumor cells through the recognition of stress ligands or loss of major histocompatibility complex Class I on tumor cells, making them attractive for use as cancer therapies.
- Effecter function of allomorphic NK cells can be diminished by the lack of functional persistence due to intrinsic metabolic programs and/or low levels of critical NK cell survival molecules such as interleukin-15, as well as tumor-intrinsic immunosuppressive mechanisms, such as high concentrations of transforming growth factor beta (TGF-β) within the tumor microenvironment.
- We hypothesized that knockout (KO) of the cytokine inducible SH2-containing protein (CISH) gene, a negative regulator of IL-2/IL-15 signaling, would improve NK cell effector function, while KO of the TGF-β receptor II (TGFBR2) gene would render NK cells resistant to TGF-β-mediated suppression.
- EDIT-201 is an allogeneic NK cell therapy that uses CRISPR-Cas12a gene editing to enhance NK cell effector function through double knockout of CISH and TGFBR2 genes.

METHODS

- CD8-depleted peripheral blood mononuclear cells were thawed into IL-15-containing NK MACS media and cultured for 14 days in GREX plates. CRISPR-Cas12a gene editing was performed by ribonucleoprotein electroporation and cells were cultured for an additional 72 hours prior to functional assays.
- Indel analysis was performed by polymerase chain reaction amplification of the genomic region surrounding the expected editing sites for each target followed by next-generation sequencing (NGS) and comparison to a reference genome to obtain percentage editing (indels).
- Spheroids were formed by seeding 5,000 NuLight Red labeled SK-OV-3 cells in 96-well ultra-low attachment plates. Spheroids were incubated at 37°C before addition of effector cells and 10 ng/mL TGF-β, followed by imaging every 2 hours on the Incucyte ZOOM (Essential) software. E:T ratio experiments with 10 unique NK cell donors for PC-Spheroids and minimum of five independent experiments with a representative of two independent experiments. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 by one-way ANOVA.

CONCLUSIONS

- EDIT-201 is a healthy donor-derived NK cell therapy with highly efficient CRISPR-Cas12a-mediated KO of CISH and TGFBR2.
- EDIT-201 experienced increased phosphorylation of STAT5 and decreased phosphorylation of Smad2/3, demonstrating increased sensitivity to IL-15 and resistance to TGF-β-mediated immunosuppression
- EDIT-201 demonstrated enhanced anti-tumor activity against multiple tumor spheroids and in an in vivo mouse model, suggesting that EDIT-201 is a potent and versatile cell-based medicine.
- Based on these results, EDIT-201 is being advanced to clinical development as an allogeneic cell-based medicine for cancer.