Preclinical Development of EDIT-201, a Multiplexed CRISPR-Cas12a Gene Edited Healthy Donor Derived NK Cell Therapy Demonstrating Improved Persistence and Resistance to the Tumor Microenvironment

Abstract ID: 145

Karrie K. Wong, Steven Sexton, Kelly Donahue, Lincy Prem Antony, Kevin Wasko, Jared Nasser, Amanda Pfautz, Owen Porth, William Pierce, Patricia Sousa, Sean N. Scott, Aaron C. Wilson, Kai-Hsin Chang, John A. Zuris, Christopher J. Wilson, Kate Zhang, Richard A. Morgan, and Christopher M. Borges

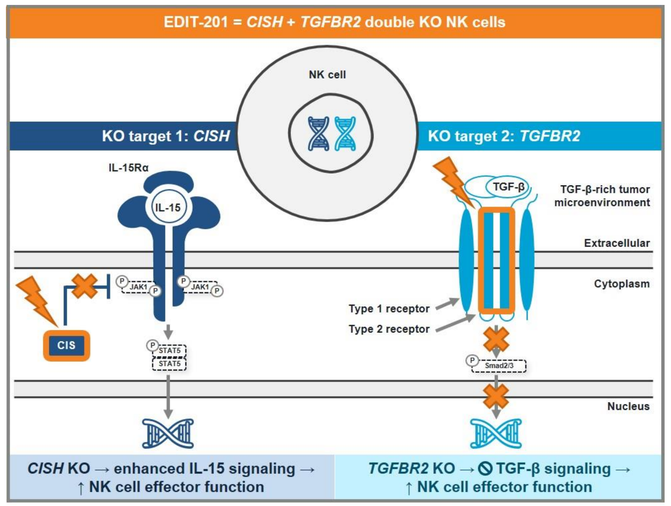
Editas Medicine, Inc., Cambridge, MA, USA

OBJECTIVE

- To evaluate the anti-tumor activity *in vitro* and in animal models of EDIT-201, a natural killer (NK) cell therapy derived from healthy human donor NK cells and edited using Cas12a

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
- The effector function of allogeneic 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 IL-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



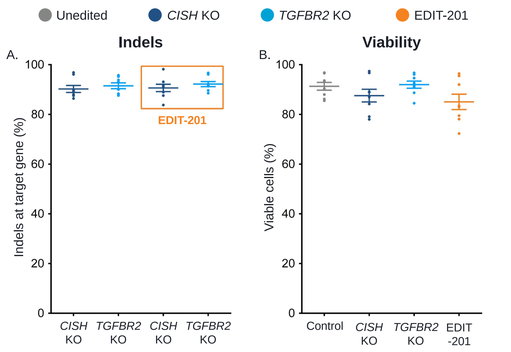
IL-15Rα: interleukin-15 receptor alpha; JAK: Janus kinase; P: phosphate; STAT: signal transducer and activator of transcription

METHODS

- CD3-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 site 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 NucLight 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 S3 system for up to 120 hours
- 0.5E6 or 1.0E6 fLuc-SK-OV-3 cells were infused intraperitoneal (i.p.) to NOD scid gamma (NSG) mice 7 days prior to i.p. infusion of 10E6 control or double KO NK cells. Bioluminescence imaging on the IVIS system was performed weekly

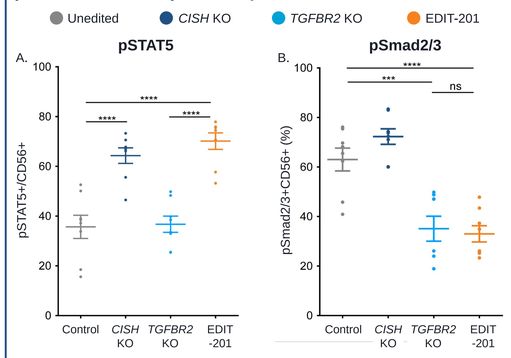
RESULTS

Figure 1. CRISPR-Cas12a demonstrated efficient editing (high percentage of indels) in viable healthy donor NK cells



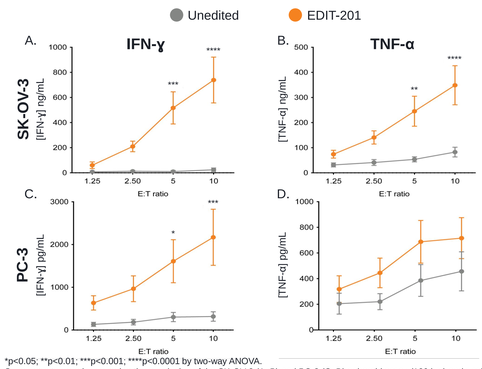
Editing at *CISH* and *TGFBR2* assessed by NGS (A) and viability assessed by AO/PI staining (B) 72 hours after CRISPR-Cas12a editing for each KO combination.
Three unique NK cell donors, representative of a minimum of five independent experiments.
AO: acridine orange; PI: propidium iodide

Figure 2. CRISPR-Cas12a editing of *CISH* and *TGFBR2* increased pSTAT5 levels upon IL-15 stimulation and reduced pSmad2/3 levels upon TGF-β stimulation



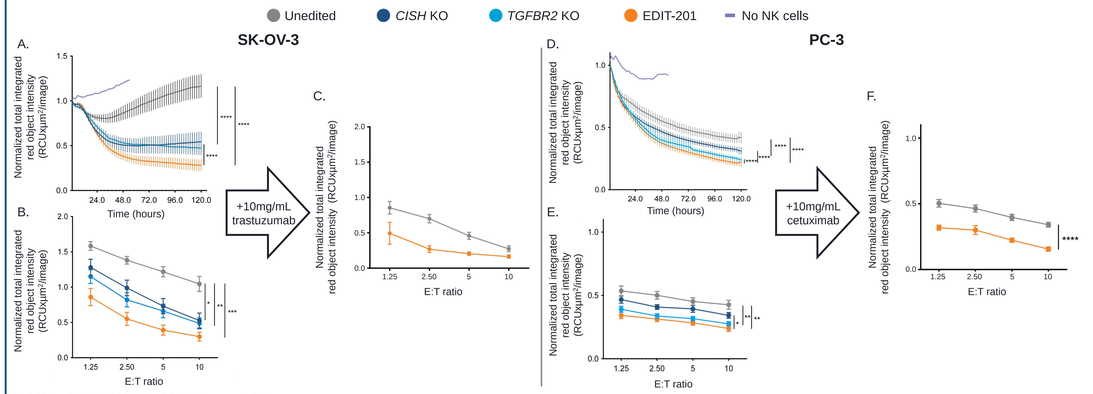
***p<0.001; ****p<0.0001 by one-way ANOVA.
Phosphoflow cytometry assay. NK cells cytokine starved for 18 hours, 72 hours after CRISPR-Cas12a editing, followed by re-stimulation for 120 min with IL-15 (A) or IL-15 + TGF-β (B).
Data are representative of four unique NK cell donors, two independent experiments.
ANOVA: analysis of variance

Figure 4. CRISPR-Cas12a editing increased inflammatory cytokine production after tumor spheroid co-culture compared with unedited control NK cells



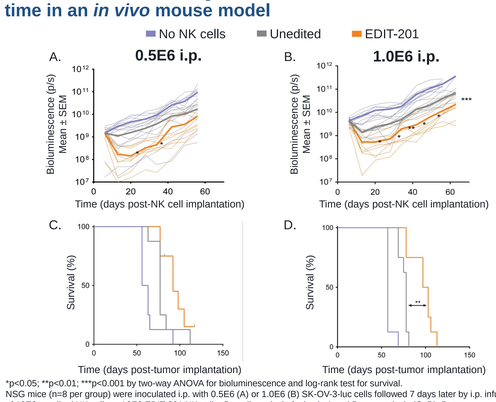
*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 by two-way ANOVA.
Supernatants were harvested at the conclusion of the SK-OV-3 (A, B) and PC-3 (C, D) spheroid assay (120 hrs) and analyzed for IFN-γ (A, C) and TNF-α (B, D) by AlphaLISA (+TGF-β conditions). SK-OV-3 analysis is six independent experiments with six unique donors; PC-3 analysis is five independent experiments with 10 unique donors.
IFN: interferon; TNF: tumor necrosis factor

Figure 3. CRISPR-Cas12a editing enhanced anti-tumor activity of NK cells against SK-OV-3 ovarian tumor and PC-3 prostate tumor spheroids compared with unedited control NK cells. EDIT-201 demonstrated increased anti-tumor activity vs single KO alone and amplified tumor killing effects through antibody-dependent cellular cytotoxicity



*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 by two-way ANOVA.
Spheroid analysis over time at 10:1 E:T (A, D) and at 100 hours (B, C, E, F). +10 ng/mL TGF-β (A, B, D, E). +10 ng/mL TGF-β and 10 mg/mL trastuzumab (C). +10 ng/mL TGF-β and 10 mg/mL cetuximab (F).
Minimum of three independent experiments with four unique NK cell donors for SK-OV-3 spheroids and minimum of five independent experiments with 10 unique NK cell donors for PC-3 spheroids.

Figure 5. CRISPR-Cas12a edited NK cells reduced SK-OV-3 ovarian tumor burden more effectively than unedited control NK cells, leading to an increased median survival time in an *in vivo* mouse model



*p<0.05; **p<0.01; ***p<0.001 by two-way ANOVA for bioluminescence and log-rank test for survival.
NSG mice (n=8 per group) were inoculated i.p. with 0.5E6 (A) or 1.0E6 (B) SK-OV-3-luc cells followed 7 days later by i.p. infusion of 10E6 unedited NK cells or 10E6 EDIT-201 NK cells. Overall survival of mice in A and B, respectively (C, D). Data are representative of two independent experiments.
SEM, standard error of mean

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

DISCLOSURES

Employees and shareholders of Editas Medicine:
K.K.W., S.S., K.D., L.P.M., K.W., J.N., A.P., O.P., W.P., P.S., S.N.S., A.C.W., K.C., J.A.Z., C.K.W., K.Z., R.A.M., C.M.B.

Acknowledgments:
The authors would like to thank all of their Editas colleagues for helping to plan, perform, analyze, and present this work. Editorial assistance was provided by Bethany Garnett of 2 the Nth (Cheshire, UK). This work was funded by Editas Medicine.