A Multiplexed CRISPR-Cas12a Gene-Edited Healthy Donor-Derived NK Cell Therapy with Increased Granzyme B and Degranulation Supports Improved Serial Killing Capacity

POSTER
1536

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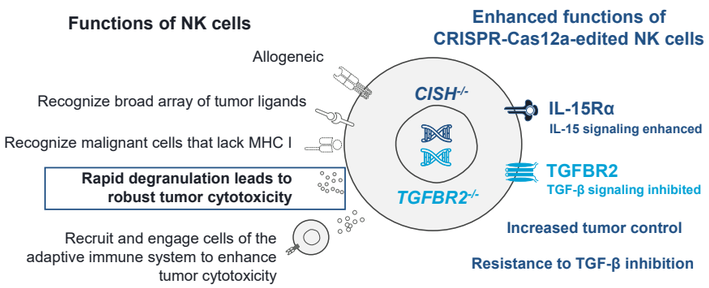
OBJECTIVE

To develop a platform to evaluate the serial killing capacity of CRISPR-Cas12a-engineered natural killer (NK) cells derived from induced pluripotent stem cells (iPSCs).

INTRODUCTION

- NK cells recognize a broad range of tumor cells and mediate robust tumor cytotoxicity directly and indirectly, making them an attractive cancer cell therapy.
- During NK cell-mediated cytotoxicity, granzyme B (GzmB) is released from cytotoxic granules with perforin to activate the apoptosis pathway of tumor cells, resulting in tumor cell death.[1]
- CRISPR-Cas12a-mediated knockout of cytokine-inducible SH2-containing protein (*CISH*) and transforming growth factor beta receptor II (*TGFBR2*) genes in NK cells (derived from healthy donors or iPSCs) have demonstrated resistance to transforming growth factor beta (TGF-β) inhibition and increased tumor control.[2–4]

Functions of unedited and CRISPR-Cas12a-edited NK cells



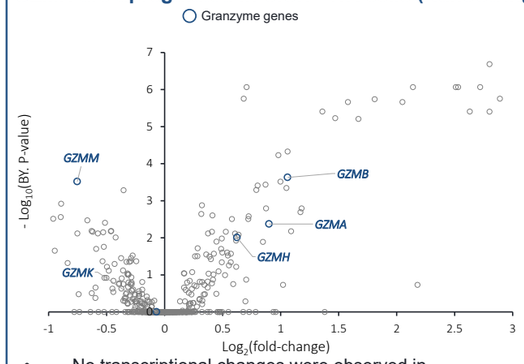
CISH, cytokine-inducible SH2-containing protein; IL-15, interleukin-15; MHC, major histocompatibility complex; NK, natural killer; TGF-β, transforming growth factor beta; TGFBR2, transforming growth factor beta receptor II.

METHODS

- *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells derived from CRISPR-Cas12a editing of healthy donor NK cells were used as a surrogate cell type for CRISPR-Cas12a-engineered NK cells derived from iPSCs (iNK cells).
- NK cells were co-cultured with IL-15 (10 ng/mL) for 3 days post-electroporation; post-edit transcriptional changes were assessed using NanoString analysis and independently verified using real-time polymerase chain reaction (RT-qPCR).
- To visualize GzmB activity in tumor cells, a novel GzmB reporter gene was developed and introduced to SK-OV-3 ovarian tumor cell lines using lentiviral vectors (SK-OV-3::GzmB).
- 25000 NK cells were co-cultured with 5000 SK-OV-3::GzmB cells labeled with NucLight Red and imaged every hour on the Incucyte S3 system for up to 36 hours.

RESULTS

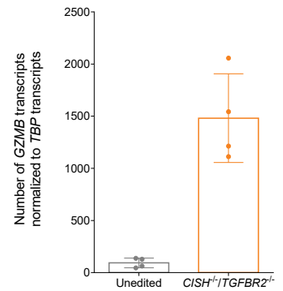
Figure 1. Granzyme transcripts *GZMB, GZMA*, and *GZMH* are upregulated in *CISH*$^{-/-}$ NK cells (NanoString)



- No transcriptional changes were observed in *TGFBR2*-edited NK cells or controls.

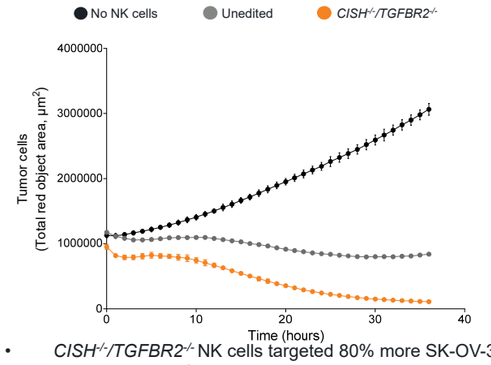
n=4 independent experiments from n=2 donors.
CISH, cytokine-inducible SH2-containing protein; NK, natural killer; TGFBR2, transforming growth factor beta receptor II.

Figure 2. *GZMB* transcripts are upregulated 16-fold in *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells (RT-qPCR)



n=4 independent experiments from n=4 donors.
CISH, cytokine-inducible SH2-containing protein; GZMB, granzyme B; NK, natural killer; RT-qPCR, real-time polymerase chain reaction; TGFBR2, transforming growth factor beta receptor II.

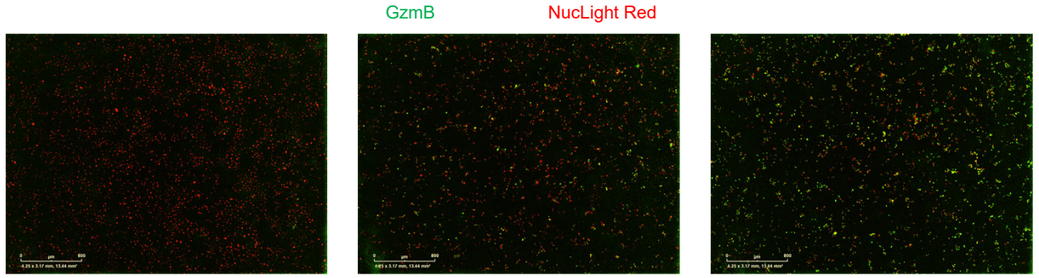
Figure 3. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells demonstrated enhanced tumor cytotoxicity vs unedited controls



- *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells targeted 80% more SK-OV-3 tumor cells with GzmB than unedited controls

SK-OV-3 tumor cells +10 ng/mL TGF-β over time at 5:1 E:T; tumor cells measured at 100 hours. Representative of 3 independent experiments from n=2 donors. Error bars are standard deviation.
CISH, cytokine-inducible SH2-containing protein; E:T, effector:target; GzmB, granzyme B; NK, natural killer; TGFBR2, transforming growth factor beta receptor II.

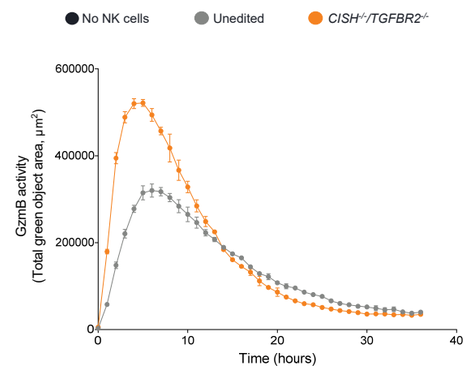
Figure 4. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells released more GzmB compared with unedited controls after 4 hours of co-culture with SK-OV-3 tumor cells



- *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells had greater anti-tumor cell GzmB activity (green) than unedited controls, which was detected 4 hours before that of unedited controls, suggesting an enhanced capacity for degranulation and GzmB-mediated cytotoxicity.
- After 14 hours of co-culture with SK-OV-3 tumor cells, *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells also expressed higher levels of CD107a (degranulation marker) compared with unedited controls.

Representative Incucyte images of SK-OV-3::GzmB cells co-cultured with NK cells for 4 hours. Green is the GzmB, red is NucLight Red (cell nuclei marker).
CISH, cytokine-inducible SH2-containing protein; GzmB, granzyme B; NK, natural killer; TGFBR2, transforming growth factor beta receptor II.

Figure 5. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells demonstrated faster and higher levels of GzmB degranulation compared with unedited controls



SK-OV-3 tumor cells +10 ng/mL TGF-β over time at 5:1 E:T; tumor cells measured at 100 hours. Representative of 3 independent experiments from n=2 donors. Error bars are standard deviation.
CISH, cytokine-inducible SH2-containing protein; E:T, effector:target; GzmB, granzyme B; NK, natural killer; TGFBR2, transforming growth factor beta receptor II.

CONCLUSIONS

- CRISPR-Cas12a-edited *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells expressed higher levels of GzmB and demonstrated more rapid and enhanced degranulation than unedited NK cells, suggesting this may be a potential mechanism for improved serial killing observed with *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells.
- This GzmB tumor cell-based reporter assay will be used to assess the serial killing capacity, including the speed and levels of degranulation, of CRISPR-Cas12a-engineered iNK cells.

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