GDA-301: ENGINEERED NAM-NK CELLS VIA CISH KNOCKOUT AND MEMBRANE-BOUND IL-15 EXPRESSION INCREASES CYTOTOXICITY AGAINST MALIGNANCIES

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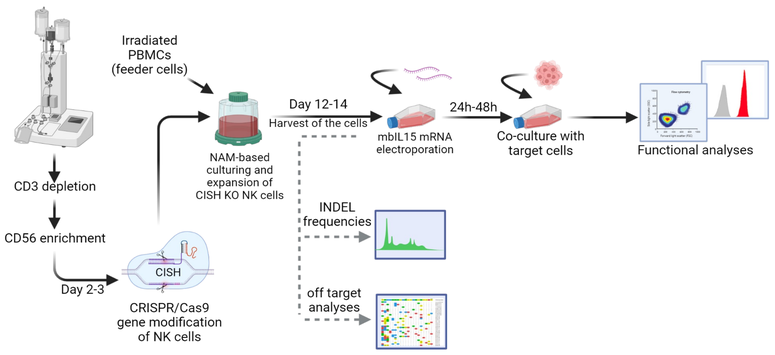
INTRODUCTION

- Gene modification strategies of allogeneic natural killer (NK) cells provide a promising next-generation immunotherapeutic tool. *Ex vivo* expansion of allogeneic NK cells using the nicotinamide (NAM) platform enhances NK cell functionality by (1) enhancing metabolic fitness, (2) increasing cytotoxic and potency activity, (3) generating a protective effect against oxidative stress, and (4) exhibiting improved homing to lymphoid tissues. These attributes provide opportunities to explore the therapeutic potential of NK cells in the clinic.
- The pleiotropic cytokine interleukin (IL)-15 is crucial for NK cell activity, proliferation, and persistence. Thus, NAM-NK cells were modified with mRNA electroporation to arm them with membrane-bound IL-15 (mbIL-15) to ensure continuous and autonomous signaling and increase the cells' potential persistence and survival.
- However, sustained IL-15 stimulation activates the cytokine-inducible SH2-containing protein (CISH), resulting in NK cell exhaustion. Thus, CRISPR-Cas9 technology was used to knockout (KO) the immune checkpoint CISH, which is involved in the negative regulation of IL-15 signaling.

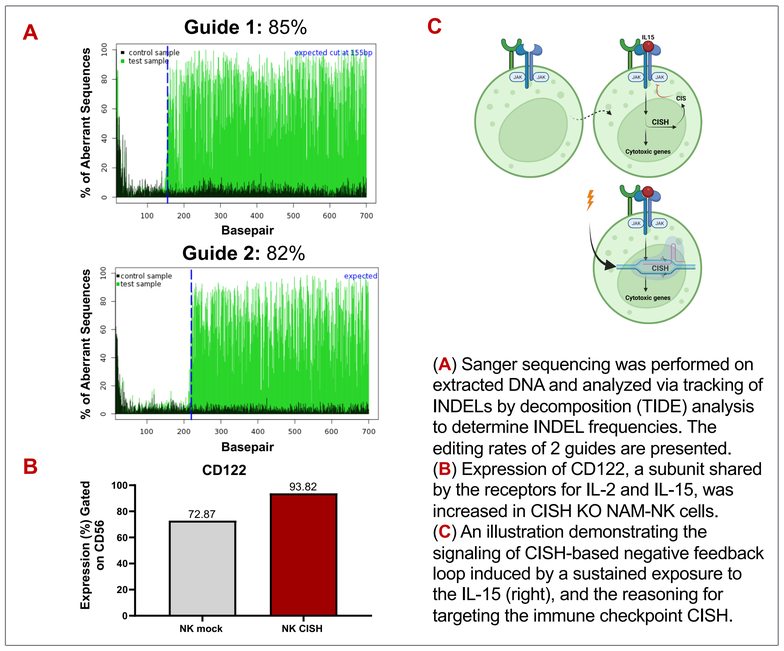
METHODS AND WORKFLOW



- Fresh apheresis samples from healthy donors were depleted of CD3 cells, followed by enrichment of CD56 and co-cultured with irradiated feeder cells (peripheral blood mononuclear cells [PBMCs]).
- Electroporation-based delivery of CRISPR/Cas9 system as a ribonucleoprotein was performed to target CISH, and editing was evaluated by quantifying insertions and deletions (INDEL) frequencies. Identification of off-target activity was done by GUIDE-seq.
- NAM-NK cells were cultured for 14 days, followed by electroporation with mRNA encoding a mbIL-15. The mbIL-15 expression was evaluated by flow cytometry.
- *In vitro* functional analyses of proliferation, potency, and cytotoxicity of modified NAM-NK cells were assessed by intracellular expression of proinflammatory cytokines and killing activity when co-cultured with tumor cell lines.

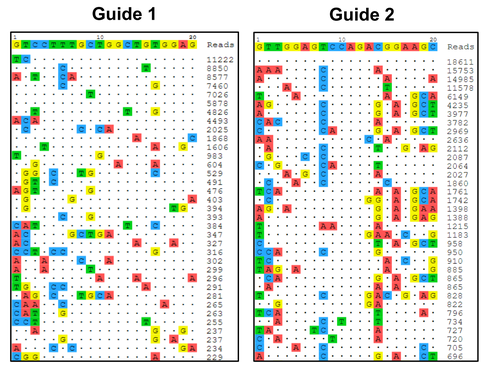
RESULTS

1. CISH KO EDITING



(A) Sanger sequencing was performed on extracted DNA and analyzed via tracking of INDELs by decomposition (TIDE) analysis to determine INDEL frequencies. The editing rates of 2 guides are presented. (B) Expression of CD122, a subunit shared by the receptors for IL-2 and IL-15, was increased in CISH KO NAM-NK cells. (C) An illustration demonstrating the signaling of CISH-based negative feedback loop induced by a sustained exposure to the IL-15 (right), and the reasoning for targeting the immune checkpoint CISH.

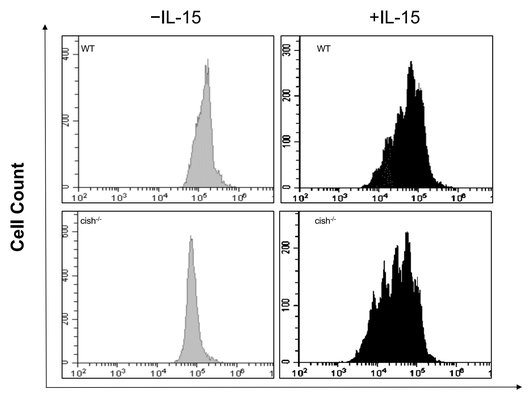
2. IDENTIFICATION OF OFF-TARGET SITES BY GUIDE-SEQ



Double-stranded oligodeoxynucleotides were co-delivered together with CISH XT 2-part gRNAs to a Cas9-expressing HEK293 stable cell line. The intended target sequences are shown in the top lines. Mismatches to the guide are shown underneath and highlighted in color. The number of GUIDE-seq sequencing reads are shown to the right of each site.

Guide 2 on-target read counts were higher than their respective off-target sites, and thus were selected for subsequent work.

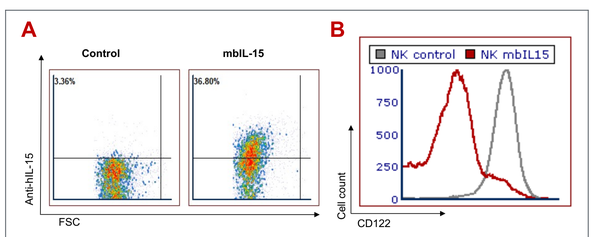
3. IMPROVED PROLIFERATION



Wild-type (WT) control (upper panels) and CISH$^{-/-}$ (CISH KO) NAM-NK cells (lower panels) were cultured for 4 days with or without soluble IL-15. The proliferative ability was detected by flow cytometry.

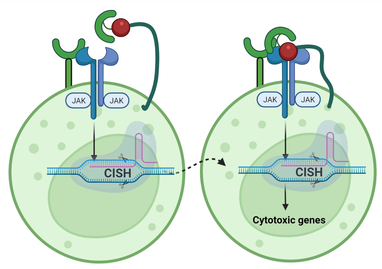
CISH KO NAM-NKs displayed improved proliferative ability.

4. mbIL-15 DETERMINATION



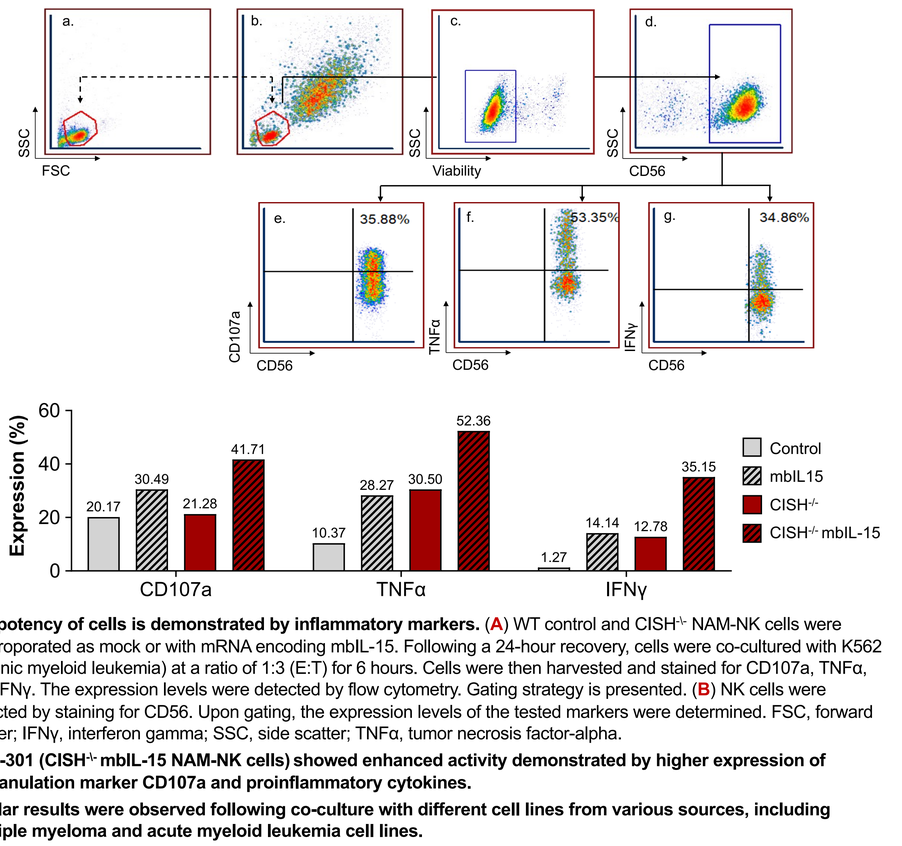
(A) The expression of IL-15 on the surface of the cells was detected 3 hours after electroporation. (B) Following the expression of the mbIL-15, the IL-15Rβ (CD122) was occupied and the expression levels were decreased. hIL-15, human interleukin-15.

5. ILLUSTRATION OF GDA-301



GDA-301: Schematic illustration of genetically modified NK cells that were CISH KO and expressed mbIL-15 (left), and where mbIL-15 was autonomously activated (right). By knocking out CISH, we were able to generate NK cells that were autonomously activated via mbIL-15 and sustained IL-15–based signaling, potentially leading to enhanced NK cell activity.

6. GDA-301 SHOWS ENHANCED PROINFLAMMATORY ACTIVITY

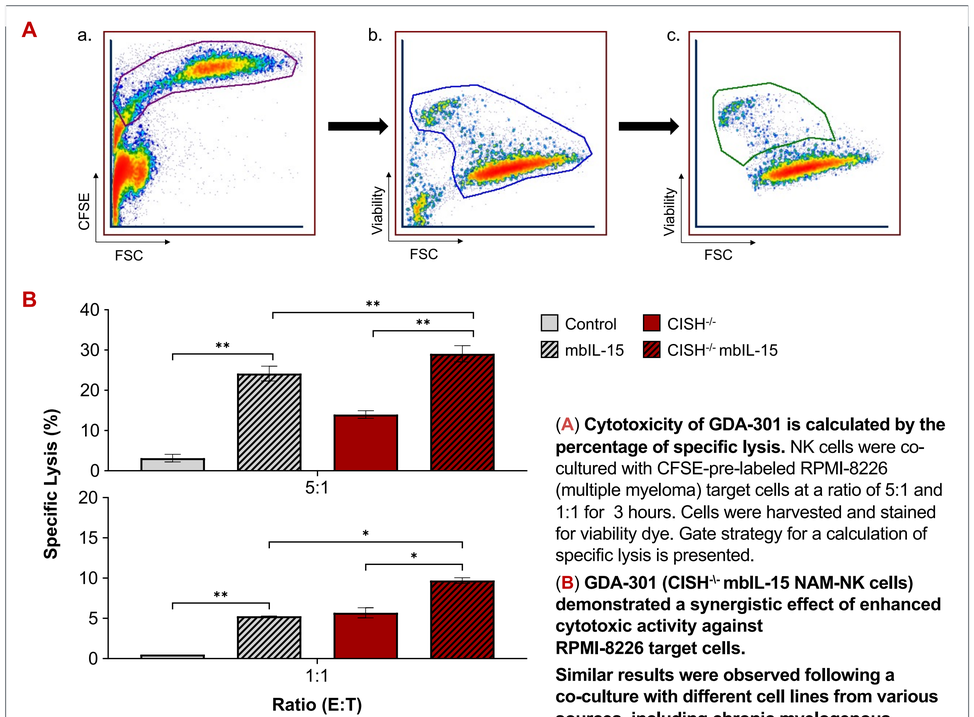


The potency of cells is demonstrated by inflammatory markers. (A) WT control and CISH$^{-/-}$ NAM-NK cells were electroporated as mock or with mRNA encoding mbIL-15. Following a 24-hour recovery, cells were co-cultured with K562 (chronic myeloid leukemia) at a ratio of 1:3 (E:T) for 6 hours. Cells were then harvested and stained for CD107a, TNFα, and IFNγ. The expression levels were detected by flow cytometry. Gating strategy is presented. (B) NK cells were detected by staining for CD56. Upon gating, the expression levels of the tested markers were determined. FSC, forward scatter; IFNγ, interferon gamma; SSC, side scatter; TNFα, tumor necrosis factor-alpha.

GDA-301 (CISH$^{-/-}$ mbIL-15 NAM-NK cells) showed enhanced activity demonstrated by higher expression of degranulation marker CD107a and proinflammatory cytokines.

Similar results were observed following co-culture with different cell lines from various sources, including multiple myeloma and acute myeloid leukemia cell lines.

7. GDA-301 DEMONSTRATES INCREASED KILLING ABILITY



(A) **Cytotoxicity of GDA-301 is calculated by the percentage of specific lysis.** NK cells were co-cultured with CFSE-pre-labeled RPMI-8226 (multiple myeloma) target cells at a ratio of 5:1 and 1:1 for 3 hours. Cells were harvested and stained for viability dye. Gate strategy for a calculation of specific lysis is presented.

(B) **GDA-301 (CISH$^{-/-}$ mbIL-15 NAM-NK cells) demonstrated a synergistic effect of enhanced cytotoxic activity against RPMI-8226 target cells.**

Similar results were observed following a co-culture with different cell lines from various sources, including chronic myelogenous leukemia and acute myeloid leukemia cell lines.

Values were determined as paired 2-tailed Student *t*-test. Data are shown as mean ± SD for statistical significance. *$P \leq 0.05$; **$P \leq 0.01$.
CFSE, carboxyfluorescein succinimidyl ester.

CONCLUSIONS

- The **mbIL-15** gene-modified NAM-NK cells represent a powerful tool that can target a variety of malignancies.
- **GDA-301:** The combined genetic manipulation of CISH and the engineered expression of mbIL-15 significantly enhances the potency and killing effect of NAM-NK cells, and represents a novel immunotherapeutic strategy for targeting of hematologic malignancies as well as solid tumors.

ACKNOWLEDGMENTS

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