sd-rxRNA to Enhance NK Cell Activity for Adoptive Cell Transfer

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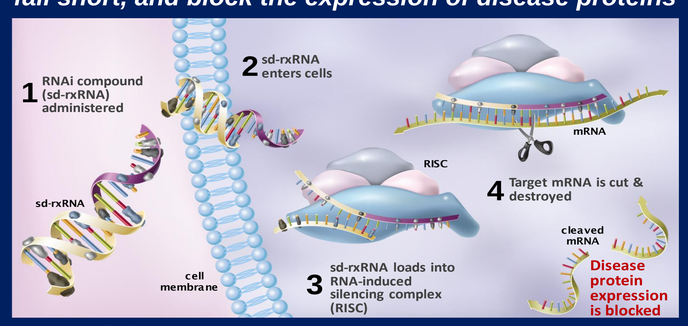
Background

NK cells are the body's first line of defense against cancer cells. They are able to rapidly recognize and kill tumor cells without prior sensitization. NK cells are an attractive candidate for use in adoptive cell therapy (ACT) because they are not required to be matched to a specific patient, making an off-the-shelf NK therapy product possible. Therapeutic use of NK cells against hematological cancers is limited by inhibitory receptors. Overexpression of such receptors, has been shown to reduce NK cell-mediated cytotoxicity. Overcoming this inhibition would allow for a more potent antitumor response following ACT. We have developed a new class of stable, self-delivering RNAi compounds (sd-rxRNAs®) that incorporate features of RNAi and antisense technology. sd-rxRNAs, demonstrate potent activity, stability, and are rapidly and efficiently taken up by cells. Using sd-rxRNA compounds against the inhibitory receptor TIGIT (RXI-804), we can suppress mRNA expression levels by greater than 95% in immune cells including T cells and NK cells. This reduction in mRNA results in reduced surface expression of TIGIT and an increased cytotoxic capacity of NK cells. Furthermore, we demonstrate that this potent activity in NK cells is maintained through cryopreservation. By treating NK cells *ex vivo*, prior to ACT with sd-rxRNA reducing the expression of proteins such as TIGIT, the anti-tumor response of these cells can be improved. Improved NK cytotoxic activity as a result of sd-rxRNA treatment during NK manufacturing is a promising approach towards more potent off-the-shelf therapy for hematological malignancies.

sd-rxRNA can penetrate immune cells, where antibodies fall short, and block the expression of disease proteins



RNA interference (RNAi) is a naturally occurring cellular process. Introduction of double stranded RNA into cells can result in association with the RNA-induced silencing complex (RISC) to target complementary mRNA sequences and degrade target genes. sd-rxRNAs are asymmetric RNAi compounds comprised of a small duplex region (≤ 15 base pairs) and a single-stranded phosphorothioate tail (4 to 12 nucleotides). In addition, sd-rxRNA compounds are chemically modified with stabilizing and hydrophobic modifications (e.g., sterol), which confer stability, efficient cellular uptake and reduced inflammatory response.

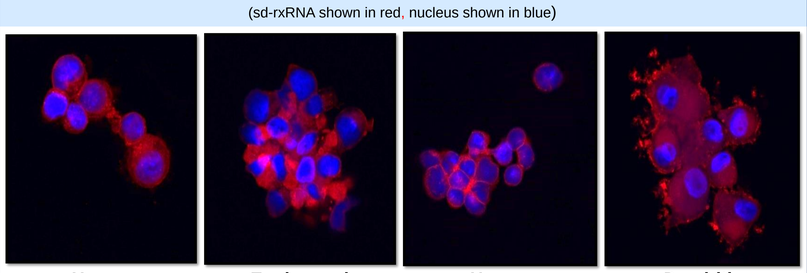
Figure 1: Mechanism of Cellular Gene Silencing using sd-rxRNA

Results

To test the uptake of sd-rxRNAs by immune effector cells without the use of transfection reagents, various human immune effector cells were transfected with 2uM fluorescently labeled sd-rxRNA in standard culture media for 24 hours. Cells were fixed with paraformaldehyde for 10 minutes and mounted for confocal microscopy. Results show rapid and efficient uptake in all cell types tested (Figure 2).

Broad Applicability - Empower existing clinical treatment paradigms *and* expand applicability of engineered cells

(sd-rxRNA shown in red, nucleus shown in blue)

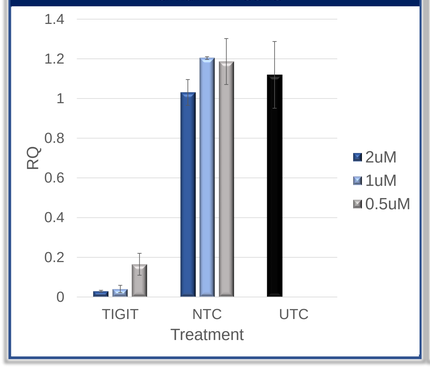


Human T Cells | ***Engineered T Cells*** | ***Human NK Cells*** | ***Dendritic Cells***

Figure 2: Fluorescently labeled sd-rxRNA (red) is taken up efficiently within 24 hours

NK cell work included tests with sd-rxRNA targeting TIGIT (RXI-804). Fresh human NK cells were isolated using negative selection and cultured in standard culture media containing IL-2. Twenty-four hours after isolation, cells were collected for transfection and the cell concentration was adjusted to ~1 x 10^6 cells/mL in RMPI media containing IL-2. Cells were seeded directly into 24-well plates containing chemically-optimized sd-rxRNA ranging in final concentration from 0.5 µM to 2 µM. Taqman gene expression assays were used to determine expression levels of TIGIT following the RNA to Ct 1-step protocol. TIGIT targeting sd-rxRNA in human NK cells causes potent and long-lasting dose dependent reduction of TIGIT mRNA (Figure 3). Surface expression of the target protein was analyzed by flow cytometry after 5 days (Figure 4).

A single dose of TIGIT targeting sd-rxRNA is able to reduce TIGIT mRNA levels by greater than 95% after 72 hours in freshly isolated human NK cells



The reduction of TIGIT mRNA by >60% persists for up to 7 days with a single dose of sd-rxRNA

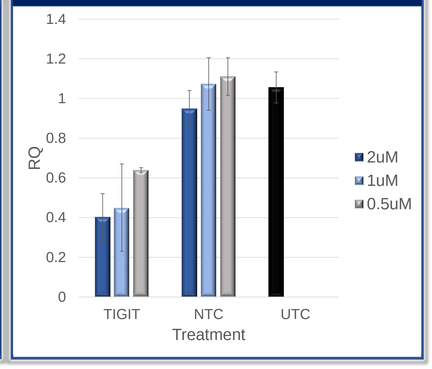


Figure 3: TIGIT targeting sd-rxRNA in human NK cells causes potent and long-lasting reduction of TIGIT mRNA

Surface reduction of the target protein is seen 5 days after transfection with a targeting siRNA

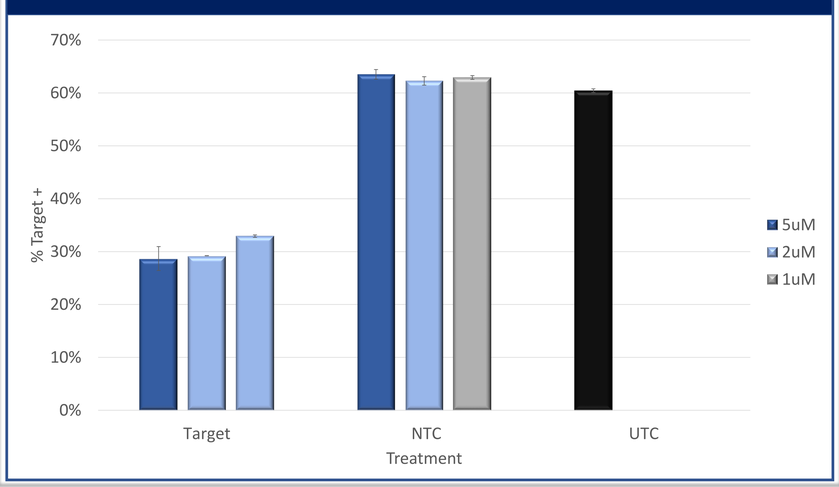


Figure 4: TIGIT targeting sd-rxRNA in human NK cells causes reduction of target mRNA which leads to a reduction in surface expression of the target protein

To assess the cytotoxic capacity of NK cells treated with TIGIT targeting sd-rxRNAs, a CD107a translocation assay was used. Translocation of CD107a to the surface of NK cells represents their degranulation activity and can be used to determine cytotoxic capacity. In this experiment, NK cells that were treated for 5 days with TIGIT targeting sd-rxRNA were cultured at a 10:1 ratio with K562 target cells. Following a 2 hour co-culture, the cells were stained for CD56 and CD107a and assessed by flow cytometry. $CD56^+CD107a^+$ cells were defined as degranulated NK cells and considered to be cytotoxic. NK cells in the absence of K562 cells were used as a negative control to determine baseline levels of CD107a expression. Our results show that treatment with TIGIT targeting sd-rxRNA significantly improves degranulation capacity of NK cells (Figure 5).

Reduction of TIGIT protein by TIGIT targeting sd-rxRNA leads to an increase in cytotoxic capacity of NK cells

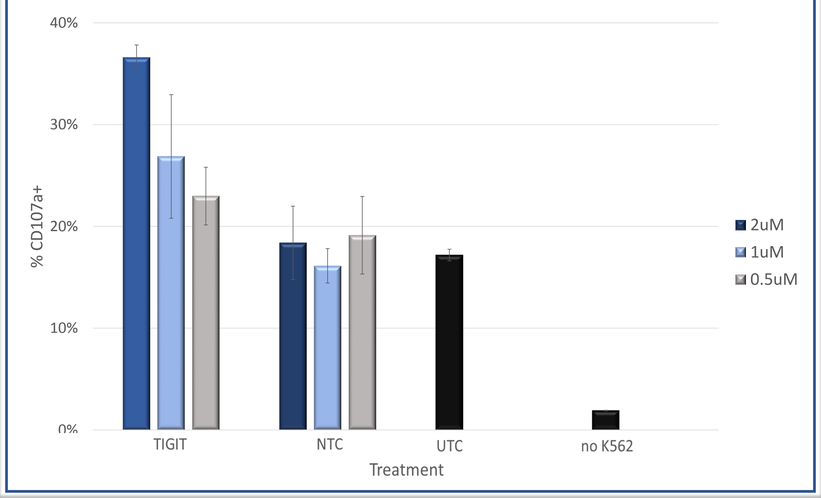
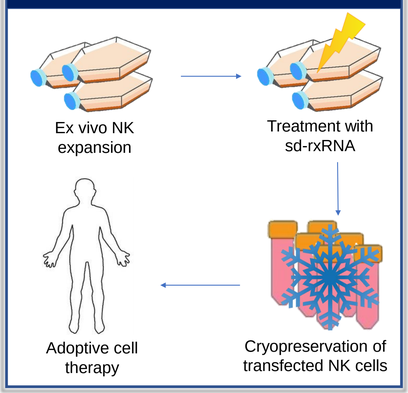


Figure 5: TIGIT targeting sd-rxRNA significantly increases the cytotoxic capacity of human NK cells.

To test whether a freeze/thaw cycle would negatively impact sd-rxRNA silencing of TIGIT, freshly isolated human NK cells were transfected with TIGIT-targeting sd-rxRNA for 48 hours. After 48 hours, cells were pelleted and resuspended in freeze medium containing 10% DMSO, cooled to -80ºC overnight then transferred to cryostorage for 72 hours. After 72 hours, the cells were thawed into standard culture medium and incubated an additional 24 hours. Silencing of TIGIT expression after a freeze/thaw cycle was similar to that of cells that had been transfected for 72 hours without freeze/thaw cycle (Figure 6).

sd-rxRNA treatment is compatible with "off-the-shelf" (i.e. cryopreserved) NK products for adoptive cell therapy



TIGIT silencing is not negatively impacted by freeze/thaw cycle

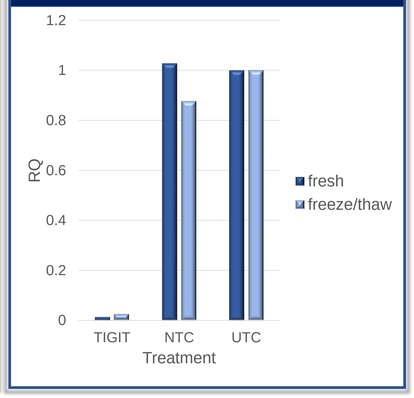


Figure 6: TIGIT silencing is seen after freeze/thaw cycle making sd-rxRNA treatment compatible with NK cell therapy protocols

Conclusion

These data demonstrate the potential of using sd-rxRNA to change NK cell phenotype for use in adoptive cell therapy. The use of these compounds is compatible with existing manufacturing paradigms (no need for transfection reagents, compatible with freeze/thaw cycles). Potent and long lasting reduction of target proteins can be achieved which results in an increase in killing capacity of the target cells. By treating NK cells with sd-rxRNA targeting immune checkpoints - such as TIGIT - or other inhibitory receptors, the anti-tumor response of these cells may be enhanced resulting in a more effective therapy for hematological malignancies.

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