PH-804, an INTASYL self-delivering RNAi compound that targets TIGIT, enhances NK cell cytotoxicity to tumor cells

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Abstract

Background: NK cells act as the body's first line of defense against cancer cells. They quickly recognize and kill tumor cells without prior exposure. Adoptive cell therapy (ACT) using NK cells shows promise against hematological cancers. Cytotoxic activity of these cells is restricted by inhibitory receptors that reduce NK cell-mediated cytotoxicity. Overcoming this inhibition would allow for a more potent antitumor response following ACT and potential application against solid tumors. We have developed a new class of stable, self-delivering RNAi compounds (INTASYL™) that incorporate features of RNAi and antisense technology. INTASYL compounds demonstrate potent activity, stability, and are rapidly and efficiently taken up by cells. INTASYL PH-804 targeting the inhibitory receptor TIGIT enhances the cytotoxic activity of expanded human NK cells in vitro.

Methods: Primary human CD56^{+} NK cells were expanded using the ImmunoCult™ NK Cell Expansion Kit from StemCell Technologies. Following the 14-day expansion protocol, cells were collected, and the cell density was adjusted to 0.5 x 10^{6} cells/mL in culture media containing IL-2. Cells were seeded directly into 24-well plates containing PH-804 ranging in final concentration from 1 µM to 5 µM. Taqman gene expression assays were used to determine expression levels of TIGIT following the RNA-to-Ct 1-step protocol. In addition, cells were stained using fluorescently labeled antibodies for flow cytometry. Cytotoxic capabilities of the PH-804 treated NK cells against the K562 (chronic myelogenous leukemia) cancer cell line were tested in a DELFIA cell cytotoxicity assay and IFN-γ release was assayed by ELISA.

Results: Treatment with PH-804 resulted in consistent mRNA and protein silencing without negative impact on NK cell viability. For example, treatment with 5 µM PH-804 resulted in a 60% reduction in TIGIT mRNA. The reduction was seen to at least 6 days post-treatment and resulted in a 45% reduction in surface expression of TIGIT by flow cytometry. Silencing of TIGIT with PH-804 resulted in increased expression of markers of NK cell activation and increased cytotoxic capabilities of NK cells against K562 cancer cells in the DELFIA cell cytotoxicity assay.

Conclusions: Here, we demonstrate the potential of PH-804 to improve NK cell potency in ACT. By treating NK cells with INTASYL targeting the inhibitory receptor TIGIT ex vivo, during NK cell expansion, the anti-tumor response of these cells was enhanced potentially resulting in a more effective cell therapy for hematological malignancies.

INTASYL Technology Overview

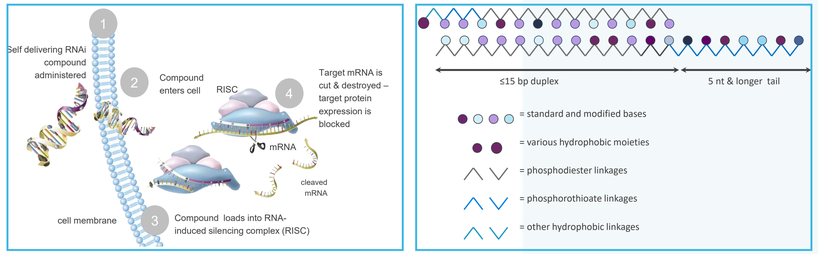

Figure 1. INTASYL™ mechanism of silencing and structure

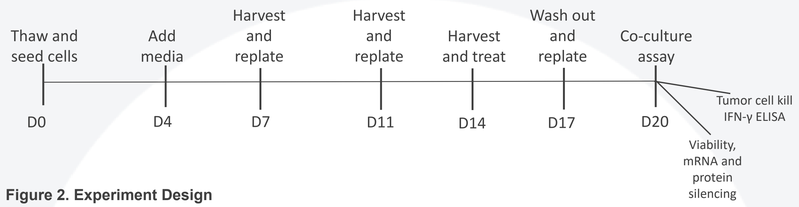

Figure 2. Experiment Design

Schematic of the primary NK expansion method with INTASYL treatment and readout timepoints indicated.

PH-804 Treatment Resulted in mRNA and Protein Silencing Without Impact on Cell Viability

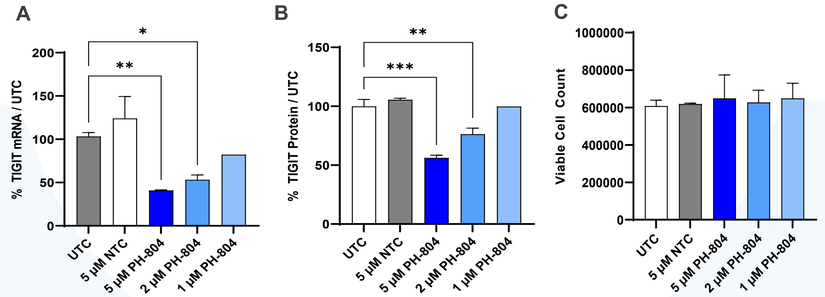

Figure 3. PH-804 suppressed expression of TIGIT mRNA and protein without a negative impact on viability

RT-qPCR, flow cytometry, and viable cell counts from Day 3 post treatment with PH-804. **A.** TIGIT mRNA is reduced by 60% following 72 h treatment with 5 µM PH-804, washout, and 72 h rest. **B.** TIGIT protein is reduced by 45% in primary NK cells after 5 µM PH-804 treatment for 72 h, washout, and 72h rest. **C.** Viable cell counts at the end of the culture period are not negatively impacted by treatment with PH-804. Means ± SEM (n = 3) are shown. Means were compared to UTC by one way ANOVA and Dunnett's post test. ***p <0.001; **p <0.01; *p <0.05.

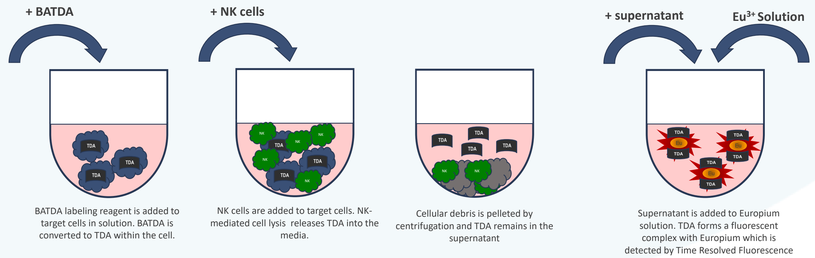

Figure 4. Overview of DELFIA tumor cell killing assay

Following expansion and treatment with PH-804, primary NK cells were cultured with TDA-loaded K562 tumor target cells at a ratio of 5:1 (NK:K562) for 4 hours. Supernatants were collected for analysis in a DELFIA assay and assayed for IFN-γ release by ELISA.

PH-804 Increased NK Mediated Cell Killing of K562 Tumor Cells and Release of IFN-γ

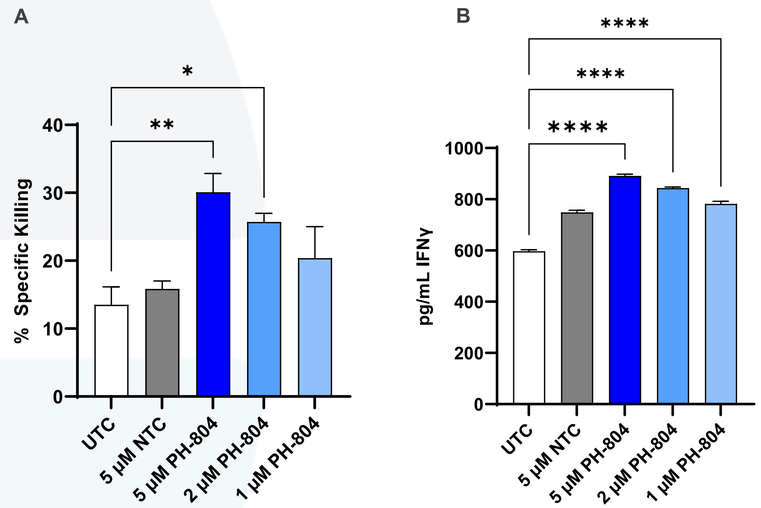

Figure 5. PH-804 increased specific cell lysis of tumor cells and enhanced IFN-γ secretion

DELFIA cell killing assay and IFN-γ ELISA data following 4-hour co-culture of primary NK cells and K562 tumor cells at a 5:1 E:T ratio. **A.** Percent specific killing of K562 tumor cells by PH-804 treated expanded primary NK cells is increased in a concentration dependent manner. **B.** IFN-γ release into the cell culture supernatant was significantly increased with PH-804 treatment of NK cells Means ± SEM (n = 3) are shown. Means were compared to UTC by one way ANOVA and Dunnett's post test. ****p<0.0001; ***p <0.001; **p <0.01; *p <0.05.

Summary and Conclusions

- **PH-804 treatment of expanded primary NK cells resulted in a concentration dependent reduction of TIGIT mRNA and protein that was potent and long lasting.**
- **PH-804 inhibition of TIGIT led to increased cytotoxic capacity of primary NK cells as evidenced by increased secretion of IFN-γ in a co-culture system.**
- **PH-804 treatment of primary NK cells more than doubled the cell killing of K562 tumor cells in co-culture.**
- **These data suggest that silencing of TIGIT with INTASYL PH-804 can improve the anti-tumor response of NK cells which provides a more effective cell therapy for hematological malignancies.**