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. Tagman gene expression assays were used to determine expression levels of TIGIT following the RNA-to-Ci 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 negative impact on NK cell viability. For example, treatment with 5 µM PH-804 reduced viability of NK cells by 60% following 72 h treatment with 5 µM PH-804.

Conclusion: 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 4. Overview of DELFIA tumor cell killing assay

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.