Local modulation of T cell PD-1 using self-delivering RNAi as a potential immunotherapeutic

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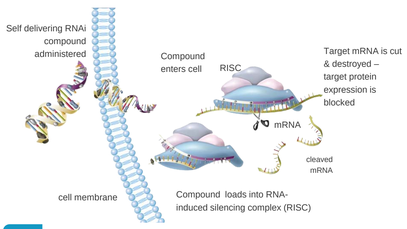
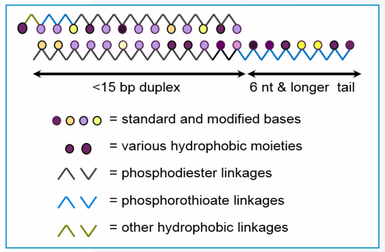
Introduction

The ability of tumor cells to evade the immune system (immune escape) as well as their acquired resistance to anti-cancer drugs constitute important barriers to the successful management of cancer. The interaction between the Programmed Death Ligand 1 (PD-L1) on the surface of tumor cells with the Programmed Death-1 (PD-1) receptor on cytotoxic T lymphocytes leads to inactivation of these immune effector cells and, consequently, immune escape. Restoration of anti-tumor immunity by blocking PD-1 signaling using antibodies has proven to be beneficial in cancer therapy. However, systemic anti-PD1 therapy has been shown to induce Grade 3-4 colitis, pneumonitis and hepatic toxicity. The ability to elicit local inhibition of the PD-1/PD-L1 axis should circumvent the systemic toxicity of current anti-PD-1 therapy. Phio Pharmaceuticals is developing PH-762, an RNAi compound based on the INTASYL™ platform (see figure below) that specifically targets PD-1 mRNA. Phio's INTASYL™ self-delivering RNAi technology allows efficient delivery of Ph-762 to the immune cells without the need for specialized formulations or mechanical transfection as is observed with current RNAi compounds.

PH-762 is being explored for intrinsic silencing of PD-1 for Adoptive T-Cell Transfer (ACT) with tumor infiltrating lymphocytes (TIL) (Poster #149). In addition, intratumoral injection of Ph-762 is being investigated as a mechanism of local inhibition of the PD-1/PD-L1 axis in the tumor microenvironment (TME). We have previously shown the feasibility of silencing PD-1 with self-delivering RNAi technology[1]. Here we demonstrate effective and efficient silencing of PD-1 in T cells by INTASYL™ PH-762.





USAGE

01 *Ex Vivo* transfection during adoptive cell therapy (ACT)

02 *In vivo* transfection via direct injection into tumor microenvironment (TME)

INTASYL™ compounds are RNAi compounds with an asymmetric duplex structure, a small duplex region (≤ 15 base pairs) and a single-stranded phosphorothioate tail. Furthermore these compounds are chemically modified with stabilizing and hydrophobic modifications which confer stability, efficient cellular uptake and reduced inflammatory response. These compounds are efficiently delivered to tissues, cancer cells, and immune cells via *in vivo* local administration or *in vitro* culture media.

PH-762 is rapidly and efficiently taken up by T cells resulting in potent silencing

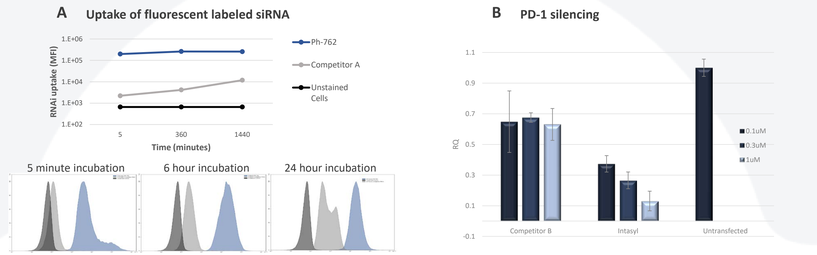


Figure 1. Activated T cells were incubated with fluorescently labeled PH-762 or a chemically modified siRNA (competitor A). siRNA uptake was monitored by flow cytometry (A) and PD-1 mRNA by RT-qPCR (B)

Concentration dependent silencing of PD-1 mRNA in human T cells by PH-762 with no effect on cell viability

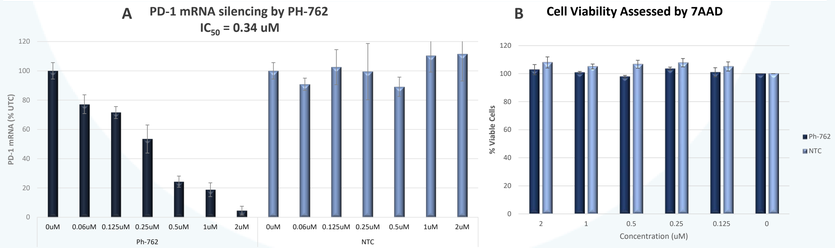


Figure 2. Human pan-T cells were activated with CD3⁺/CD28⁺ Dynabeads. Activated cells were incubated with either PH-762 or a non-targeting control (NTC) for 72 hours.PD-1 mRNA was assayed by RT-qPCR (A). Cell viability was assessed by 7-AAD (B).

Concomitant reduction of PD-1 surface protein expression

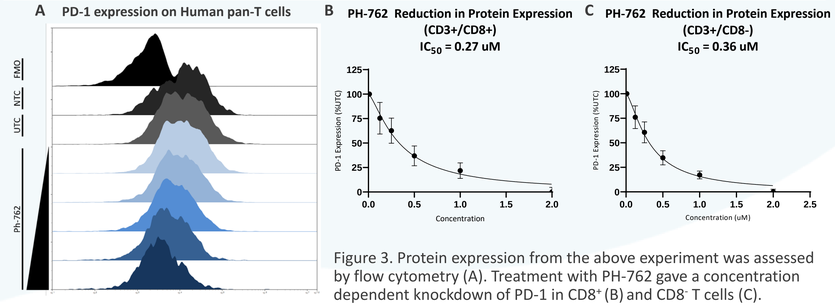


Figure 3. Protein expression from the above experiment was assessed by flow cytometry (A). Treatment with PH-762 gave a concentration dependent knockdown of PD-1 in CD8⁺ (B) and CD8⁻ T cells (C).

CD3/CD8 T cell phenotype is unaffected by treatment with PH-762

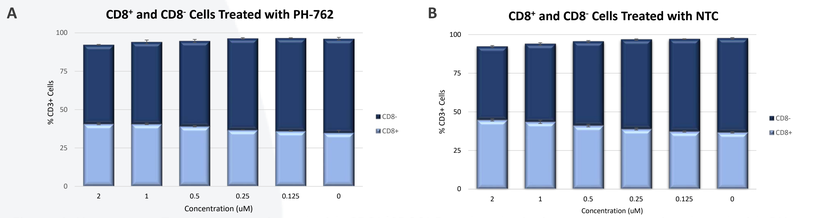


Figure 4. Human pan-T cells were activated with CD3⁺/CD28⁺ Dynabeads. Activated cells were incubated with PH-762 (A) or NTC (B) for 72 hours. CD3/CD8 expression was assessed by flow cytometry

Persistent reduction of PD-1

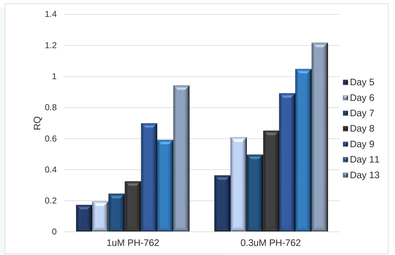


Figure 5. Activated human pan-T cells T cells were incubated with PH-762 for 72 hours. Cells washed and media replaced

Silencing of mouse PD-1

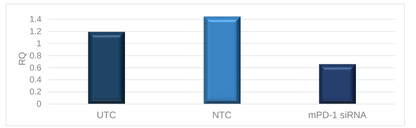


Figure 6. EL-4 mouse T lymphocyte cell line was incubated with murine siRNA for 72 hours

PH-762 increases function

PD-1 silenced TIL heightened IFN-g secretion in response to re-stimulation with autologous tumor cells.

See: Poster #149

Conclusion and Path Forward

- These findings support the hypothesis that local PD-1 mRNA silencing by PH-762 is a viable approach for overcoming tumor-induced immunosuppression
- Single administration of PH-762 effectively silences expression of PD-1 mRNA and protein in activated human T cells without effect on cell viability or CD8 effector cell phenotype
- PH-762 can effectively silence PD-1 in TILs resulting in enhanced function (Poster #149).
- PH-762 is being explored for intrinsic silencing of PD-1 for Adoptive T-Cell Transfer with TILs
- Knockdown of PD-1 demonstrated in mouse T cells with a construct targeted at murine PD-1
- Proof-of-concept in vivo studies have been initiated in mouse syngeneic tumor models utilizing intratumoral injection of a murine equivalent of PH-762
- Potential to move forward with studies to support a physician sponsored IND for intratumoral injection of PH-762

References

1. Self-delivering RNAi targeting PD-1 improves tumor-specific T cell functionality for Adoptive Cell Therapy of malignant melanoma. Ligtenberg MA. et al., Molecular Therapy (2018) 26: 1482-1493