INTASYL™ self-delivering RNAi therapeutic dual targeting PD-1 and CTLA-4 provides synergistic antitumor efficacy in the treatment of murine colon cancer in vivo

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Abstract
Combination immune checkpoint inhibition (ICI) with antibodies targeting PD-1 and CTLA-4 provides superior outcomes compared to either monotherapy alone. This combination has been approved for advanced melanoma, metastatic colon cancer and others. However, combination therapy with anti-PD-1/CTLA-4 antibodies often elicits serious immune related adverse events (irAEs), presenting an obstacle for effective treatment with combination systemic anti-PD and anti-CTLA4. Intratumoral (IT) immunotherapy is a strategy to enhance local activity while decreasing systemic irAEs. While clinical testing of IT antibodies is underway, antibodies’ high molecular weight limits their local diffusion and retention time within tumors.

RNA is an emerging therapeutic modality well-suited for local clinical application of ICI. We have demonstrated that self-delivering RNAi therapies built on proprietary INTASYL™ technology specifically silence their targets in tissues without need for specialized formulations, delivery systems and convey robust antitumor efficacy in vivo. Furthermore, multiple INTASYL® compounds can be easily co-formulated into multi-targeting therapeutics, providing specific silencing of multiple therapeutic targets in a single injection.

Here, we present proof-of-concept (POC) in vivo data demonstrating synergistic efficacy of a novel INTASYL® dual-targeting murine PD-1 and CTLA-4 in a syngeneic CT26 model of murine colon cancer. The dual-targeting INTASYL® is comprised of components mPH-762 (mPD-1) and 27790 (mCTLA-4), formulated in PBS. Dual on-target silencing was first validated in murine T cells in vitro. In vivo, CT26 cells were implanted subcutaneously into female BALB/c mice. Vehicle (PBS) or INTASYL® IT treatment commenced when tumors reached a mean threshold volume (150 mm³). Day 1) with doses given on Days 1, 4, 7, 10, and 13. Mice were administered 1 mg dose of each component compound, 1 mg dose of the dual-targeting INTASYL® (comprised of 0.5 μg of each component compound) or the dual-targeting INTASYL® at 2 mg/dose (comprised of 1 μg of each component compound). Tumor volumes and body weights were recorded longitudinally and analyzed by area under the curve (AUC). Tumors were isolated on Day 15 and mechanistic immunomodulation of the tumor microenvironment (TME) was assessed by flow cytometry.

IT INTASYL® dual-targeting PD-1 and CTLA-4 elicited robust dose-associated antitumor efficacy that was superior to the identical total dose of either single-targeting INTASYL®, demonstrating antitumor synergy by the dual-targeting coformulation. On-target mechanistic immunomodulatory effects were observed in the TME. These data demonstrate POC synergistic efficacy of IT INTASYL® dual-targeting CTLA-4/PD-1 in vivo, supporting further development to maximize efficacy and minimize irAEs.

Figure 1. INTASYL® mechanism of silencing and structure

A: Mechanism of silencing. B: INTASYL® structure. Property of Phio Pharmaceutica

Figure 2. mPH-762:27790 provides concentration-associated dual on-target silencing of PD-1 and CTLA4 surface protein in mouse EL4 cells. EL4 cells were treated with mPH-762:27790 (1:1 formulation) to achieve the total RNAi concentration as indicated. Impacts on target surface protein expression were assessed by flow cytometry six days post-start of treatment. A. Relative percentage (% ) of PD-1 EL4 cells compared to the untreated control condition. B. Relative % of CTLA-4 EL4 cells compared to the untreated control condition. Groups were compared by one way ANOVA and Tukey’s multiple comparison post hoc tests. **p<0.01; ***p<0.001; ****p<0.001. +p<0.05.

Figure 3. Intratumoral (IT) mouse PD-1/CTLA4 targeting INTASYL® provides synerstic antitumor efficacy in vivo in the CT26 mouse model of colon cancer

Intratumoral (IT) mouse PD-1/CTLA4 targeting INTASYL® is well tolerated in vivo

Figure 4. IT mPH-762:27790 does not significantly impact body weight gain in vivo. Body weight change was measured daily (in mm, n=8) from 1 day before to 15 days of treatment. Data are presented as mean ± SEM. **p<0.001; +p<0.05

Summary and Conclusions
Summary:
INTASYL is Phio Pharmaceuticals self-delivering RNAi therapeutic precision gene silencing platform. The objective of this proof-of-concept study was to evaluate the potential for synergistic efficacy of a mouse PD-1/CTLA4 dual targeting INTASYL when administered intratumorally (IT) in the syngeneic CT26 mouse model of colon cancer. The mouse PD-1/CTLA4 dual-targeting compound is comprised of 1:1 mPH-762 (PD-1 targeting) and 27790 (CTLA4 targeting). PH-762 (INTASYL silencing human PD-1) is under clinical investigation for treatment of advanced melanoma in the neoadjuvant setting (EudraCT number 2021-002859-10).

Conclusions:
• mPH-762:27790 provided dose-associated on-target silencing of both PD-1 and CTLA-4 in mouse EL4 cells in vitro.
• IT mPH-762:27790 provided dose-associated synergistic antitumor efficacy that was superior to the mono-targeting formulation of each component when provided at an identical total dose (1 mg/dose total for mono-treatment; 1 mg/dose total comprised of 0.5 mg/component dose for the dual-targeting formulation).
• IT mPH-762:27790 was well tolerated at dose levels tested.

These data demonstrate synergistic efficacy of a mouse PD-1/CTLA4 dual-targeting INTASYL self-delivering RNAi therapeutic in a proof-of-concept in vivo study. As a combination treatment with systemic anti-PD-1 and anti-CTLA4 therapeutic antibodies often leads to treatment-mediated severe immune related adverse events, dual-targeting IT INTASYL may represent a strategy to maximize antitumor efficacy while minimizing antibody treatment associated systemic toxicities.