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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#5113

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-1/CTLA-4. 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.

RNAi is an emerging therapeutic modality well-suited for local clinical application of ICI. We have demonstrated that self-delivering RNAi therapeutics built on proprietary INTASYL™ technology specifically silence their targets in tissues without need for specialized formulations or 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 mm^3; Day 1) with doses given on Days 1, 4, 7, 10, and 13. Mice were administered either 1 mg/dose of each component compound, 1 mg/dose of the dual-targeting INTASYL (comprised of 0.5 mg of each component compound) or the dual-targeting INTASYL at 2 mg/dose (comprised of 1 mg 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.

PH-762 is currently under clinical investigation in a Phase 1b study for advanced melanoma.

INTASYL™ precision gene silencing platform

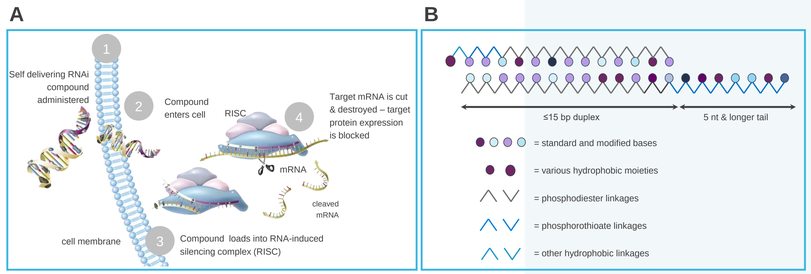

Figure 1. INTASYL mechanism of silencing and structure

A. Mechanism of silencing. **B.** INTASYL structure.

Dual mouse PD-1/CTLA-4 targeting INTASYL provides on-target protein silencing in vitro

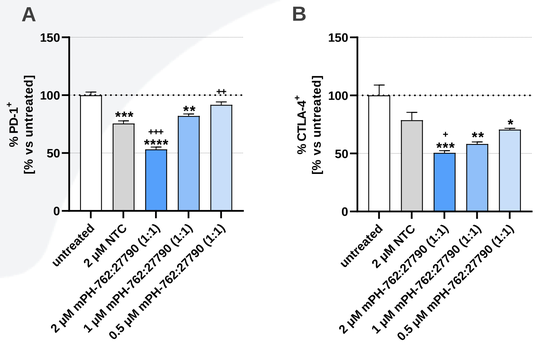

Figure 2. mPH-762:27790 provides concentration-associated dual on-target silencing of PD-1 and CTLA4 surface protein in mouse EL4 T cells.

EL4 cells were treated with mPH-762:27760 (1:1 formulation) to achieve the total INTASYL concentration as indicated. Impacts on target surface protein expression were assessed by flow cytometry six days post-start of treatment. **A.** Relative percentage (%) of $PD\text{-}1^+$ EL4 cells compared to the untreated control condition. **B.** Relative % of $CTLA\text{-}4^+$ EL4 cells compared to the untreated control condition. Groups were intercompared by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p<0.0001; ***p<0.001; **p<0.01; *p<0.05. * = vs untreated, + = vs NTC.

Intratumoral (IT) mouse PD-1/CTLA-4 targeting INTASYL provides synergistic antitumor efficacy in vivo in the CT26 mouse model of colon cancer

A

Group	N	#CT26 cells	Test Article	Dose	ROA	Schedule
1	8	5e05	PBS	--	IT	1, 4, 7, 10, 13
2	8	5e05	mPH-762 (mPD-1-targeting)	1 mg	IT	
3	8	5e05	INTASYL 27790 (mCTLA4-targeting)	1 mg	IT	
4	8	5e05	27790/mPH-762 coformulation (1:1 ratio)	1 mg	IT	
5	8	5e05	27790/mPH-762 coformulation (1:1 ratio)	2 mg	IT	

B

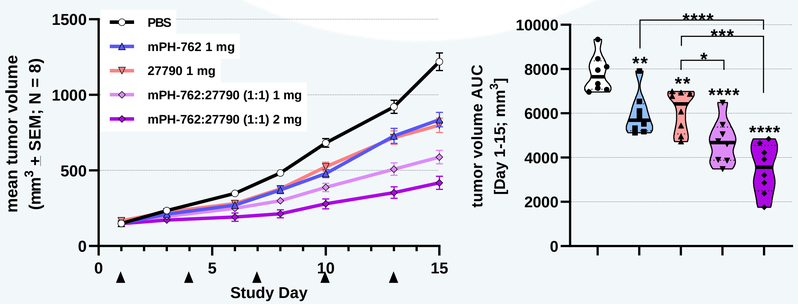

Figure 3. Intratumoral (IT) mPH-762:27790 provides synergistic antitumor efficacy in vivo in the CT26 tumor model

INTASYL or vehicle (PBS) were administered by IT injection of 50 µL volume. **A.** Mean tumor volume ± SEM (n = 8) over time. Treatments indicated by arrows. **B.** Tumor volume area under the curve (AUC) calculated by trapezoidal transformation over Days 1-15. Violin plots are shown with individual animals and medians indicated. Statistical significance of differences in mean AUC were assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p<0.0001, ***p<0.001, **p <0.01, *p<0.05.

Mouse PD-1/CTLA-4 targeting INTASYL (mPH-762:27790; 1:1 ratio of each component) provided superior antitumor efficacy compared to the equivalent total dose (1 mg) of either component alone, demonstrating synergistic efficacy of the dual targeting formulation.

Intratumoral (IT) mouse PD-1/CTLA-4 targeting INTASYL is well tolerated in vivo

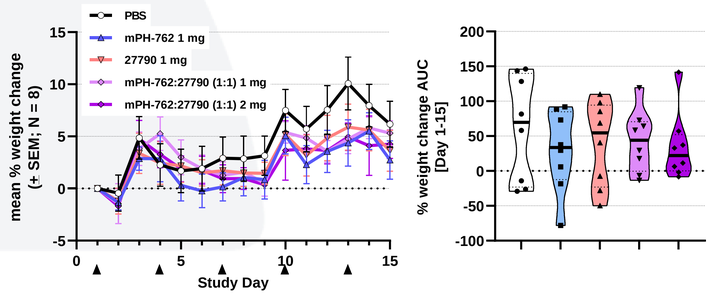

Figure 4. IT mPH-762:27790 does not significantly impact body weight gain in vivo.

Body weights were measured daily in the CT26 tumor model. **A.** Mean percentage (%) body weight change ± SEM (n = 8) over time. Treatments indicated by arrows. **B.** Mean body weight change area under the curve (AUC) calculated by trapezoidal transformation over Days 1-15. Violin plots are shown with individual animals and medians indicated. Statistical significance of differences in mean AUC were assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. There was no significant impact on body weight change compared to treatment with PBS vehicle for any compound tested.

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/CTLA-4 dual targeting INTASYL when administered intratumorally (IT) in the subcutaneous CT26 model of mouse colon cancer.

The mouse PD-1/CTLA-4 dual-targeting compound is comprised of 1:1 mPH-762 (PD-1-targeting) and 27790 (CTLA-4 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-targeting; 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/CTLA-4 dual-targeting INTASYL self-delivering RNAi therapeutic in a proof-of-concept in vivo study.
- As combination treatment with systemic anti-PD-1 / anti-CTLA-4 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.