INTASYL™ self-delivering RNAi therapy specifically dual-targeting BRD4 and PD-1 elicits complete tumor responses and evidence of synergy in a subcutaneous Hepa1-6 model of murine hepatoma in C57BL/6N mice

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Abstract: e14537

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Abstract

The therapeutic potential of targeting bromodomain and extra-terminal motif (BET) protein BRD4 is supported by promising preclinical data in multiple cancer contexts. However, clinical trials of BET inhibitors (BETi) have overall shown limited clinical efficacy as monotherapy. Insufficient specificity of available BETi also results in only passable safety profiles. BET proteins are now also understood to control a wide range of immunoregulatory pathways. Preclinical data suggests a synergistic impact with immune checkpoint blockade (ICB) targeting PD-1. Trials assessing the potential of BETi in rational combination with ICB are underway.

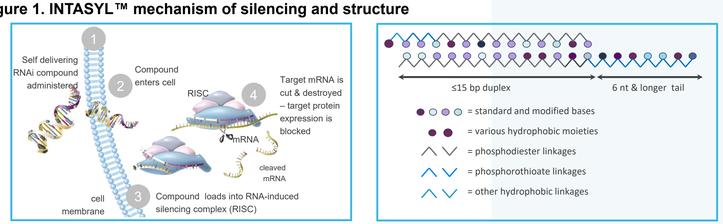
The INTASYL™ platform is a self-delivering RNAi technology that provides efficient delivery into target cells without need for specialized drug delivery systems. INTASYL provides specific, robust, and durable on-target gene silencing. Furthermore, INTASYL moieties targeting multiple specific targets can be easily formulated together, providing a highly versatile platform for providing multi-targeted combination therapy in a single drug substance.

Here we assessed the efficacy of mouse/human BRD4-targeting INTASYL PH-894 as monotherapy or co-formulated with mouse PD-1 targeting INTASYL mPH-762, in treating a subcutaneous Hepa1-6 model of murine hepatoma in C57BL/6N mice, at doses lower than the optimal dose in prior studies. On-target, specific silencing of BRD4 and PD-1 was demonstrated *in vitro*. *In vivo*, mice bearing tumors of ~150 mm^3 (N = 12/arm) were treated intratumorally on Days 1, 3, 7, 10 & 14 with PH-894 or mPH-762 as single-agent, or mPH-762/PH-894 dual-targeting INTASYL formulation at sub-optimal doses (0.1 mg/INTASYL/dose), or with mPH-762/PH-894 at half-suboptimal dose (0.05mg/INTASYL/dose) to assess potential synergistic impacts. Positive control groups received systemic (IP) (+)-JQ-1 (13.33mg/dose; qdx5), anti-mouse PD-1 antibody (75 µg; Days 1, 3, 7, 10, 14), or both treatments. Negative control animals received vehicle only. Tumor volumes and body weights were recorded longitudinally.

Compared to vehicle, suboptimal (+)-JQ-1, anti-PD-1, PH-894 or mPH-762 each elicited tumor control. Strikingly, treatment with suboptimal mPH-762/PH-894 elicited complete responses (CR; defined by completely resolving tumors that did not relapse) in 83% (10/12) mice, outperforming combination treatment with (+)-JQ-1 and anti-PD-1 mAb [CR for 75% (9/12) of mice]. Treatment with half-suboptimal doses of PH-894/mPH-762 resulted in CR for 50% (6/12) of mice and provided tumor control exceeding that elicited by each monotherapy, thus strongly suggesting a synergistic impact by the dual-targeting formulation. Tumors were isolated on Day 13 and *ex vivo* mechanistic analyses suggested mPH-762/PH-894 exerted impacts on both immune and tumor cells promoting antitumor efficacy.

These data demonstrate proof-of-concept of the therapeutic potential of dual-targeting INTASYL provided as a single formulation *in vivo,* suggesting potential for meaningful clinical therapeutic impact.

Figure 1. INTASYL™ mechanism of silencing and structure



INTASYL compound

mPH-762, INTASYL (self delivering RNAi) compound targeting murine PD-1 mRNA

PH-894, INTASYL compound targeting murine / human BRD4 mRNA

mPH-762/PH-894, dual targeting PD-1 and BRD4 INTASYL

USAGE

01 Direct injection into tumor microenvironment (TME)

02 Enhancement of adoptive cell therapies (ACT)[1,2]

[1] Cuiffo *et. al.* ASGCT Annual Meeting 2021, virtual
[2] Thalhofer *et.al.* SITC Annual Meeting 2020, virtual

PH-762/PH-894 mono- or dual-targeting INTASYL provided highly specific on-target silencing of PD-1 and/or BRD4 mRNA and protein to human T cells *in vitro*

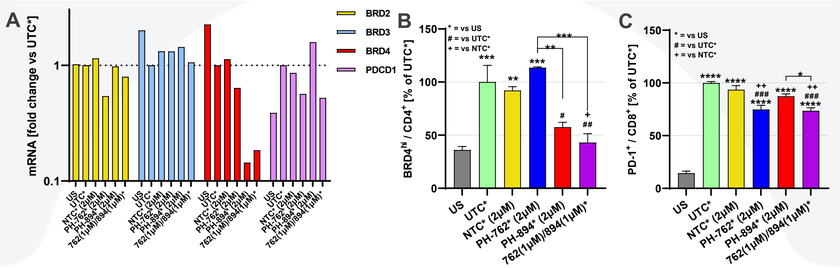


Figure 2. PH-762/PH-894 specific on-target mRNA and protein level silencing in activated pan T cells *in vitro*

Human pan T cells from multiple healthy donors (Stem Cell Technologies) were left unstimulated (US), stimulated (*) with 10 ng plate-bound OKT3 antibody (UTC*) or stimulated with OKT3 and simultaneously treated with 2 µM of INTASYL specifically targeting human PD-1 (PH-762*), BRD4 (PH-894*), both (762 (1 µM) / 894 (1 µM)*) or non-targeting control INTASYL (NTC*). **A.** Representative on-target mRNA silencing on Day 3 post-OKT3 stimulation / treatment as fold change relative to UTC*. PH-894 specifically silences BRD4, but not BET family members BRD2 or BRD3. **B.** Representative on-target protein silencing of BRD4 or **C.** PD-1 shown for CD4$^+$ or CD8$^+$ T cells respectively ± SEM (n = 3). Similar on-target impacts were observed across donors and T cell subtypes. Statistical significance was assessed by one way ANOVA and Dunnett's multiple comparisons *post-hoc* tests. *p < 0.05, **p < 0.01, ***p < 0.001, ****p<0.0001. * = vs US; # = vs UTC*; + = vs NTC* or as indicated by brackets.

Suboptimal dual-targeting IT mPH-762/PH-894 INTASYL cured 83% of tumors and showed evidence of target synergy in a Hepa1-6 model of murine hepatoma *in vivo*

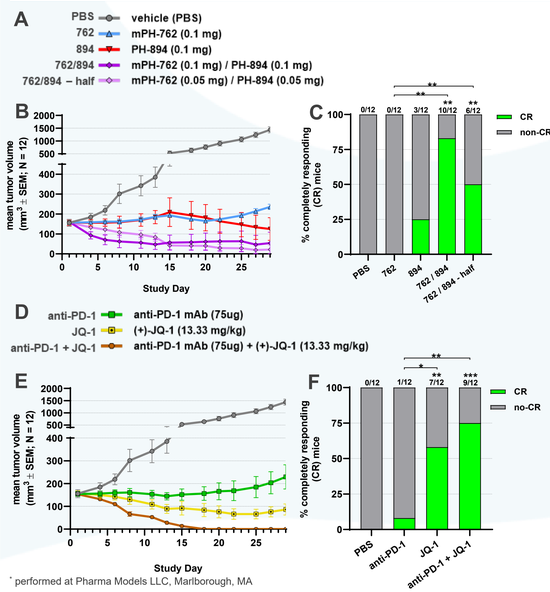


Figure 3. mPH-762/PH-894 synergizes to provide superior anti-tumor efficacy and cure rate at lower dose concentrations in a subcutaneous (SC) Hepa 1-6 model of murine hepatoma.

Treatment keys **A.** IT groups. **D.** Systemic groups. Longitudinal mean tumor volume (mm^3 ± SEM; N = 12) for **B.** IT INTASYL-treated or **E.** positive (+)control systemic (IP) therapy (Tx) treated groups. A sub-optimal dose of mPH-762(0.05mg)/PH-894(0.05mg) inhibited mean tumor growth better than either mPH-762(0.1 mg) or PH-894(0.1 mg), suggesting synergistic antitumor impact of the dual-targeting INTASYL. Similar results were seen under systemic +control combination treatment. Percentage (%) of completely responding (CR) animals (as defined by completely responding (unmeasurable) tumors that did not relapse for 29 days post final dose) for **C.** IT INTASYL-treated or **F.** +control systemic (IP) Tx treated groups. Dual-targeting mPH-762/PH-894 significantly improved CR response rate as assessed by Fisher's exact test, with an overall CR rate of 83% compared to 75% under combination systemic treatment.*p < 0.05, **p < 0.01, ***p < 0.001.

* performed at Pharma Models LLC, Marlborough, MA

Systemic (+)-JQ-1 + anti-PD-1 significantly inhibited weight gain, while IT mPH-762/PH-894 INTASYL was well tolerated, suggesting a superior safety profile for the dual targeting INTASYL while providing similar efficacy

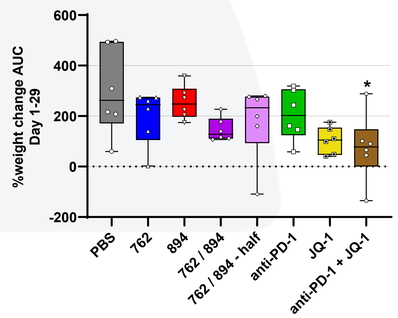


Figure 4. IT mPH-762/PH-894 is better tolerated than systemic combination (+)-JQ-1 + anti-PD-1 in the SC Hepa 1-6 murine hepatoma model.

Cumulative mean % weight change was calculated for each animal by group by calculating the area under the curve (AUC) by trapezoidal transformation. Box and whisker plots are shown with medians indicated. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. Only systemic (+)-JQ-1 + anti-PD-1 combination treatments produced a statistically significant inhibition of cumulative mean AUC compared to vehicle control treatment. *p < 0.05.

mPH-762/PH-894 elicits anti-tumor mechanistic impacts on both immune cells and tumor cells in the tumor microenvironment

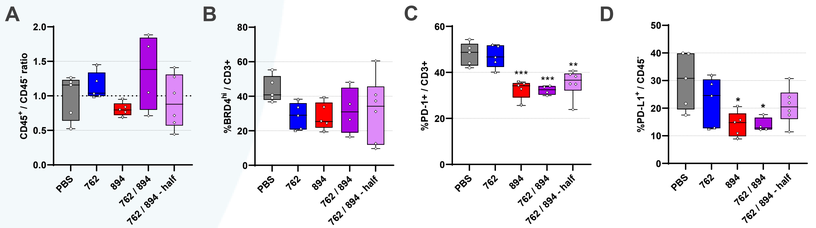


Figure 5. *Ex vivo* tumor microenvironment (TME) analysis suggests mPH-762/PH-894 elicits anti-tumor efficacy via mechanistic impacts on both tumor cells and immune cells.

Tumors were isolated on Day 13; phenotypic markers were assessed by immunostaining / flow cytometry. **A.** Ratio of tumor CD45$^+$ / CD45$^-$ cells. Dual-targeting INTASYL increased median lymphocyte / non-lymphocyte ratios in the TME. **B.** Tumor %BRD4^{hi} / CD3$^+$ CD45$^+$ T cells **C.** Tumor %PD-1$^+$ / CD3$^+$ CD45$^+$ T cells **D.** Tumor %PD-L1+ / CD45- cells. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests *p < 0.05, **p < 0.01, ***p < 0.001.

Conclusions

- **Single formulation PH-762/PH-894 INTASYL dual-targeting PD-1 and BRD4 provides highly specific mRNA and protein level silencing.**
- **At doses suboptimal in monotherapy, IT mPH-762/PH-894 INTASYL cured 83% of tumors *in vivo*, displaying evidence of target synergy.**
- **IT mPH-762/PH-894 INTASYL was well tolerated, while systemic combination (+)-JQ-1 + anti-PD-1 significantly inhibited weight gain, suggesting a superior safety profile for the dual targeting INTASYL with similar efficacy.**
- **mPH-762/PH-894 elicits anti-tumor mechanistic impacts on both immune cells and tumor cells in the tumor microenvironment.**