Dual targeting PD-1 and BRD4 with INTASYL self-delivering RNAi elicits complete resolution of tumors and persistent anti-tumor immunity in vivo

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Abstract: 4154

Abstract

Background: Targeting the bromodomain and extra-terminal motif (BET) protein BRD4 is an attractive target for cancer immunotherapy, as it exerts impacts on both cancer cells and T cells. Preclinical data suggests added therapeutic potential in co-targeting the immune checkpoint PD-1.

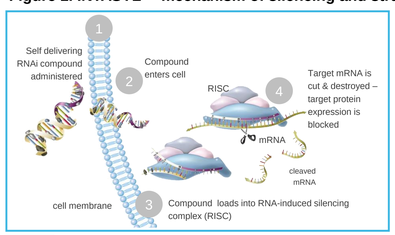
INTASYL™ is a self-delivering RNAi platform that provides both highly efficient delivery to target cells without need for specialized drug delivery systems and strong gene silencing of multiple targets in a single drug formulation. We have demonstrated strong anti-tumor efficacy of several INTASYL compounds *in vivo* after local administration, including PH-894 (targeting murine/human BRD4) and mPH-762, targeting murine PD-1. Synergistic activity was shown in a study with INTASYL co-targeting murine/human BRD4 and murine PD-1 (mPH-3861) in a preclinical Hepa1-6 model of murine hepatoma (HCC). Here we report the results of a rechallenge phase of that study for mice with stable complete resolution (CR, "cure") of tumors following mPH-3861 treatment, providing evidence of persistent anti-tumor immunity elicited by intratumoral (IT) INTASYL treatment.

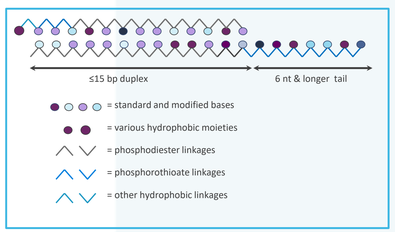
Methods: Subcutaneous Hepa1-6 tumors in C57BL/6N mice were treated with INTASYL mPH-3861 by IT injection. CRs were previously found to be induced by either mPH-762 and PH-894 when provided as monotherapy under optimal dose concentrations; therefore, in order to assess synergistic impacts of the dual targeting formulation, lower (suboptimal) doses were assessed. Cured mice or naïve controls were rechallenged 29 days post-treatment with an identical inoculum (1e07) of Hepa1-6 cells to the contralateral flank relative to the primary tumor. No additional treatment was provided. Longitudinal tumor measurements and body weights were recorded. A second tumor rechallenge was initiated on 177 days post-treatment; tumor cells were implanted on opposite flanks, in locations away from previous implantation sites. Hepa1-6 (1e07) cells were used again to show durability of the immune-response, and the LL/2 (1e05) cell line was used to determine the tumor specificity of the immune-response.

Results: Local treatment with mPH-3861 at low doses - suboptimal for monotherapy - resulted in stable CR of 83% (10/12) of the treated Hepa1-6 tumors and enhanced tumor control compared to mPH-762 or PH-894 monotherapy at suboptimal doses, suggesting target synergy. The treatment also resulted in a development of a systemic immune response: While rechallenge Hepa1-6 tumors (rTs) of naïve animals grew steadily as expected, 100% of rTs in mice previously cured by INTASYL mPH-3861 were cured again without requiring further treatment, after a brief short growth period. The systemic immune response after local mPH-3861 treatment seems both durable and tumor specific. Indeed, during a second tumor rechallenge without further treatment on Day 191, Hepa1-6 tumor cells were rejected in previously cured animals, while LL/2 tumors grew.

Conclusions: These data show that, in addition to eliciting stable complete resolution, local treatment with INTASYL mPH-3861 (dual targeting BRD4 and PD-1), engenders a systemic anti-tumor immunity that is both durable and tumor specific. Systemic immune response after local therapy with INTASYL compounds will also be investigated in an upcoming clinical study.

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-3861, dual targeting PD-1 and BRD4 INTASYL

USAGE

01 Direct injection into tumor microenvironment (TME)[1,2]

02 Enhancement of adoptive cell therapies (ACT)[3,4]

[1] Cuiffo *et. al.* SITC Annual Meeting 2020
[2] Cuiffo *et. al.* AACR Annual Meeting 2021
[3] Cuiffo *et. al.* ASGCT Annual Meeting 2021
[4] Thalhofer *et.al.* SITC Annual Meeting 2020

Study Schematic: Subcutaneous multiple tumor rechallenge

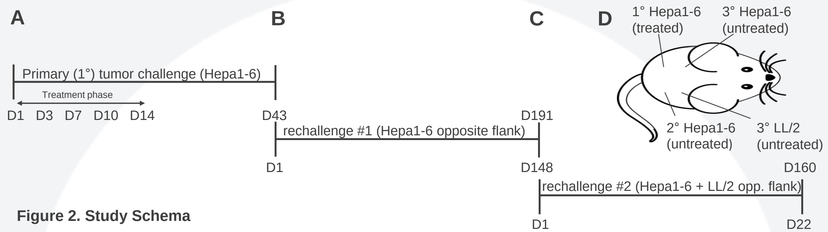


Figure 2. Study Schema

A. Treatment of primary (1°) unilateral subcutaneous (SC) Hepa1-6 tumor challenge was initiated at ~150 mm^3 mean tumor volume (Day 1 [D1]). The treatment phase extended through Day 14. Intratumoral (IT) INTASYL treatments or intraperitoneal (IP) antibody treatments were administered on D1, D3, D7, D10 and D14; no further treatment was provided after the initial treatment phase. **B.** On D43, animals with completely responding (CR) tumors (tumors that reached threshold volume of >50 mm^3 that responded to treatment such that they were unmeasurable and did not relapse and grow to any measurable volume), along with a cohort of naïve animals were rechallenged with an identical inoculum (1e07) of Hepa1-6 cells to the opposite flank. **C.** On Day 191 (Day 148 of rechallenge #1) the CR animals, along with another naïve cohort were inoculated with both a 2nd rechallenge of Hepa1-6 cells (1e07) and a primary challenge of LL/2 cells (1e05) to the opposite flank to assess specific antitumor immunity. **D.** Diagram of tumor type and inoculums over primary tumor challenge (1°), rechallenge #1 (2°) or rechallenge #2 (3°) phases of the study.

Dual targeting PD-1 and BRD4 with INTASYL cured 83% of tumors at a suboptimal dose concentration in the primary tumor challenge / treatment phase of the Hepa1-6 model *in vivo*

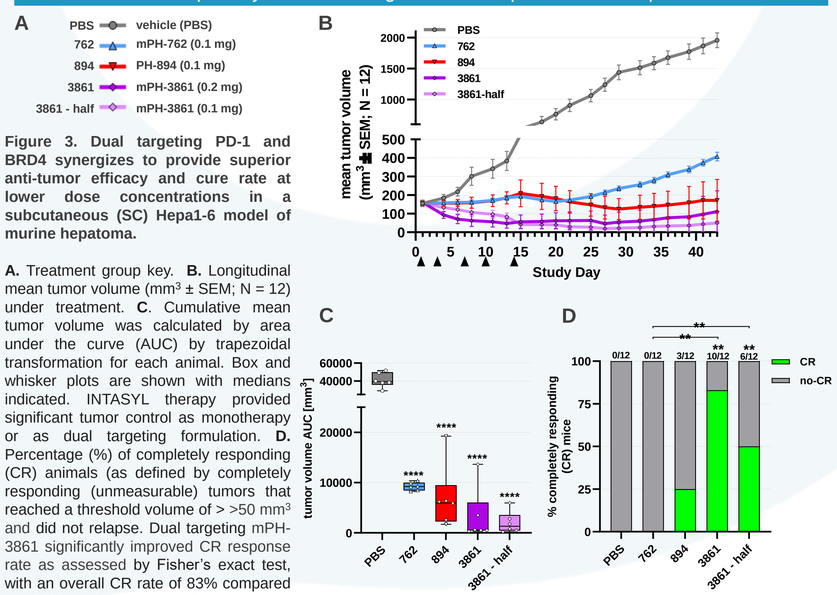


Figure 3. Dual targeting PD-1 and BRD4 synergizes to provide superior anti-tumor efficacy and cure rate at lower dose concentrations in a subcutaneous (SC) Hepa1-6 model of murine hepatoma.

A. Treatment group key. **B.** Longitudinal mean tumor volume (mm^3 ± SEM; N = 12) under treatment. **C**. Cumulative mean tumor volume was calculated by area under the curve (AUC) by trapezoidal transformation for each animal. Box and whisker plots are shown with medians indicated. INTASYL therapy provided significant tumor control as monotherapy or as dual targeting formulation. **D.** Percentage (%) of completely responding (CR) animals (as defined by completely responding (unmeasurable) tumors that reached a threshold volume of > >50 mm^3 and did not relapse. Dual targeting mPH-3861 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, ****p < 0.0001.

in vivo studies were performed at Pharma Models LLC, Marlborough, MA

Local INTASYL therapy with dual targeting mPH-3861 induces systemic anti-tumor immunity

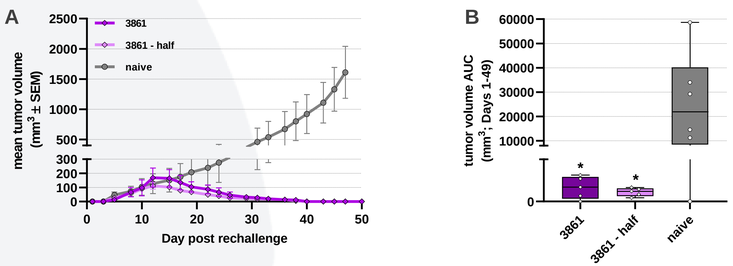


Figure 4. Mice previously cured by IT mPH-3861 treatment rejected identical Hepa1-6 rechallenge to the opposite flank, suggesting local mPH-3861 therapy induced systemic anti-tumor immunity

A. Longitudinal mean tumor volume (mm^3 ± SEM) for IT INTASYL-treated or positive (+) control systemic (IP) therapy (Tx) treated groups. Hepa1-6 cells seeded SC into the opposite flank of the 1° tumor challenge location initially grew in volume, but then fully regressed post ~D12 to become unmeasurable. Tumor regrowth was not observed for any animal. Cumulative mean tumor volume was calculated by area under the curve (AUC) by trapezoidal transformation for each animal. Box and whisker plots are shown with medians indicated. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. *p < 0.05, **p < 0.01.

Systemic anti-tumor immunity after local INTASYL therapy with dual targeting mPH-3861 is both durable and specific

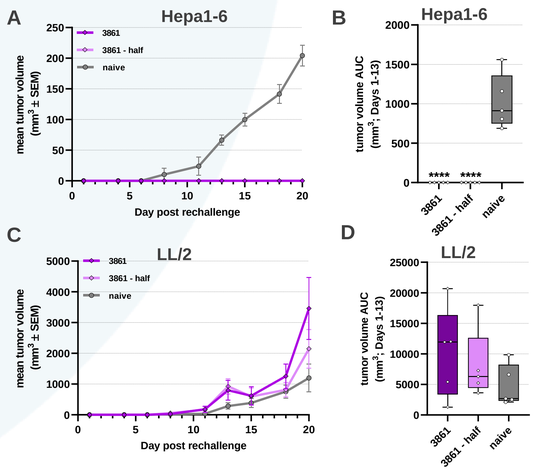


Figure 5. Mice previously bearing Hepa1-6 tumors cured by IT mPH-3861 treatment rejected a second Hepa1-6 rechallenge but not LL/2 ~2.5 months after treatment .

Previously cured mice were again rechallenged (rechallenge #2) with either **A. & B.** Hepa1-6 (1e07; opposite flank but different location of 1° challenge) or **C. & D.** LL/2 (5e05; same flank but different location of 1° Hepa1-6 challenge). **Left:** Longitudinal mean tumor volume (mm^3 ± SEM). **Right:** Cumulative tumor volume AUC with medians indicated. Statistical significance assessed by one way ANOVA and Dunnett's multiple comparisons *post-hoc* tests *p < 0.05, **p < 0.01.

Conclusions

- **Local treatment with dual targeting PD-1 and BRD-4 with INTASYL mPH-3861 cured 83% of Hepa1-6 tumor-bearing mice.**
- **Local INTASYL induced a durable and specific systemic anti-tumor immune response to the Hepa1-6 treated tumor type.**
- **This data suggests that locally administered INTASYL may provide durable tumor-specific systemic immune responses, warranting further evaluation in clinical studies.**