Intratumoral mPH-762, a self-delivering RNAi therapeutic (INTASYL™) targeting mouse PD-1 generates systemic tumor-specific memory CD8⁺ T cells, providing a mechanism for abscopal efficacy toward untreated tumors in a murine hepatocarcinoma model

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Abstract

Intratumoral (IT) administration of immune checkpoint inhibition (ICI) holds potential to enhance local activity while decreasing systemic toxicity, however, high molecular weight antibodies are not well-suited for this application.

PH-762, a self-delivering RNAi compound targeting PD-1 built on proprietary INTASYL™ technology, rapidly enters tissues without need for further formulation or delivery vehicles. In preclinical studies, PH-762 and its murine-targeted analog (mPH-762) provide robust PD-1 silencing, anti-tumor efficacy and associated immunophenotypic changes in the tumor microenvironment (TME). PH-762 is currently under clinical investigation in a Phase 1b study as neoadjuvant therapy for advanced resectable melanoma (EudraCT number 2021-002859-10). We have previously shown that IT mPH-762 provides abscopal efficacy toward untreated distal tumors in a bilateral Hepa1-6 model of murine hepatocellular carcinoma. Here, we identify potential mechanisms underlying this abscopal efficacy.

Equivalent inoculums of Hepa1-6 cells were subcutaneously implanted into bilateral flanks of C57BL/6J mice (N = 12 / group). When tumors reached a mean volume of 150 mm^3, vehicle (PBS) or mPH-762 (0.5 mg/dose or 2 mg/dose) were administered to one of the two tumors (the directly treated tumor), on Days 1, 4, 7, 10 and 13. Tumor volumes and body weights were recorded longitudinally. mPH-762 provided efficacy both to the directly treated tumor (DT), as well as to the contralateral untreated distal tumor (UT). DT and UT tumors, mesenteric lymph nodes (mLNs) and spleens (SP) were isolated from a satellite group (N = 6) on Day 14. The remaining mice persisted on study through Day 17.

On-target immunomodulatory changes were assessed in DT and UT TME by immunostaining / flow cytometry. On-target immunomodulatory changes to TME appeared primarily confined to the DT only, suggesting that abscopal efficacy was mediated by indirect effects of mPH-762 treatment.

To elucidate the systemic antitumor effects, tumor reactive T cells were expanded from peripheral lymphoid organs (bulk mLN and SP) by 21-day culture with irradiated Hepa1-6 cells. Tumor specific memory reactivity was assessed by challenge with either intact Hepa1-6 cells or intact CT26 BALB/c colon cancer cells, PMA/ionomycin, or PBS controls: and assessed by intracellular IFN-γ, TNF-α and surface CD107a staining. IT mPH-762 increased both the frequency and Hepa1-6-specific reactivity of the CD8⁺ T cells expanded from mLN and SP compared to IT PBS.

These data indicate that IT mPH-762 generates a systemic immune response marked by the presence of durable tumor-specific reactive memory CD8⁺ T cells in systemic lymphoid organs, suggesting a mechanism underlying the abscopal efficacy of mPH-762. These data further support the clinical development of IT PH-762.

mPH-762 provides abscopal efficacy in the bilateral Hepa1-6 model – in vivo study design

A

Group	N	#Cells Tumor#1 (directly treated)	#Cells Tumor#2 contralateral (untreated)	Test Article	Dose	Dosing Days
1	12	1e07	1e07	PBS	--	1, 4, 7, 10, 13
3	12	1e07	1e07	mPH-762	0.5 mg	
4	12	1e07	1e07	mPH-762	2.0 mg	

B

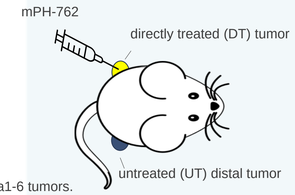

Figure 1. Bilateral Hepa1-6 in vivo study design

A. Study details overview table. **B.** Schematic of seeding and treatment of Hepa1-6 tumors.

Intratumoral (IT) treatment with mPH-762 provides both direct and abscopal antitumor efficacy in the bilateral Hepa1-6 murine hepatocellular carcinoma model

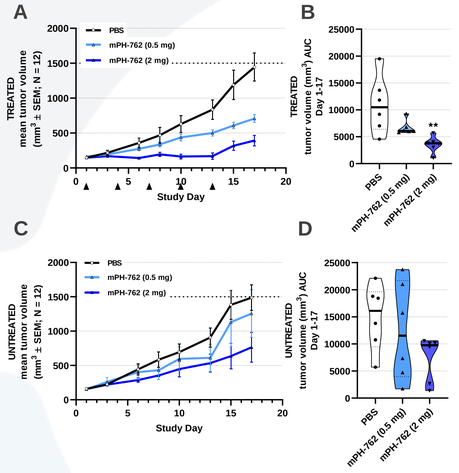

Figure 2. Intratumoral (IT) mPH-762 provides antitumor efficacy to both directly treated and untreated distal tumor in the bilateral Hepa1-6 model.

A. Mean tumor volume ± SEM (n = 12) of mPH-762-directly treated tumors over time. Treatment days are indicated by arrows. **B.** Directly-treated tumor volume area under the curve (AUC) calculated by trapezoidal transformation over Days 1-17. Violin plots are shown with individual animals and medians indicated. Statistical significance of differences in mean AUC was assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. **p <0.01. **C.** Mean tumor volume ± SEM (n = 12) of untreated contralateral tumors over time. **D.** Untreated tumor volume area under the curve (AUC) calculated by trapezoidal transformation over Days 1-17. 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 <0.01.

IT mPH-762 silences its target PD-1 across multiple immune cell populations of the tumor microenvironment (TME) and increases leukocytes and T cells in directly treated tumors

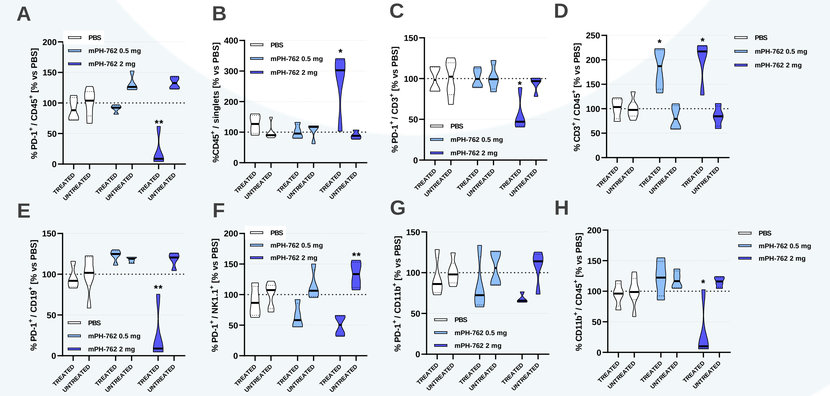

Figure 3. IT mPH-762 silences PD-1 and provides antitumor-associated modulation confined to the directly treated TME.

Immunophenotypic characterization of Day 14 directly treated vs untreated distal TME by flow cytometry (n = 6/group). Violin plots with medians indicated show relative percentages (%) vs the IT PBS-treated vehicle control group for treated and untreated tumors. **A.** %PD-1⁺ events of CD45⁺ leukocytes (%PD-1⁺ / CD45⁺). **B.** %CD45⁺ / singlet events. **C.** %PD-1⁺ / CD3⁺ T cells. **D.** %CD3⁺ / CD45⁺. **E.** %PD-1⁺ / CD19⁺ B cells. **F.** %PD-1⁺ / NK1.1⁺ NK cells. **G.** %PD-1⁺ / CD11b⁺ myeloid cells. **H.** %CD11b⁺ / CD45⁺. Statistical significance of differences in groups means intercompared within treated or untreated TME by one way ANOVA and Dunnett's multiple comparisons *post-hoc* tests. ***p<0.001; **p<0.01; *p<0.05.

IT mPH-762 generates durable systemic tumor-specific memory CD8⁺ T cells in peripheral lymphoid organs

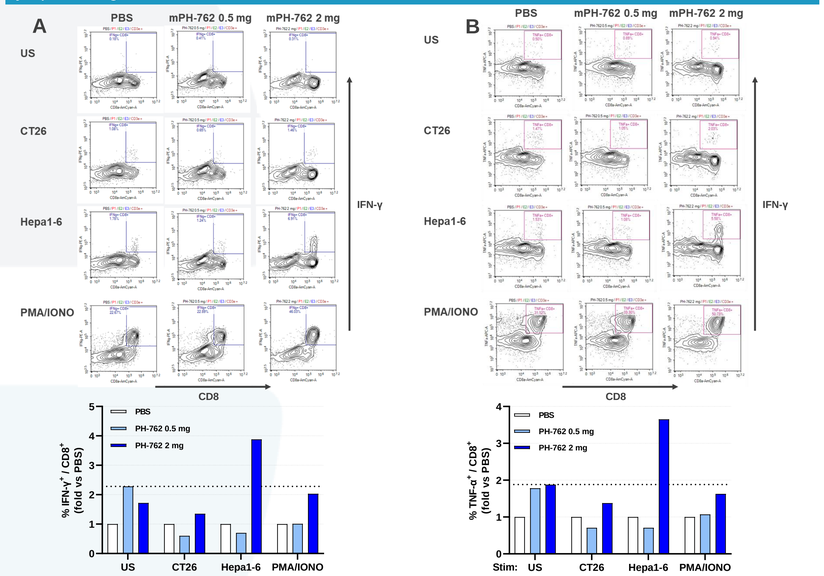

Figure 4. IT mPH-762 treatment generates durable tumor-specific memory CD8⁺ T cells in peripheral lymphoid organs

Pooled Day 14 tumor draining mesenteric lymph nodes (mLN) and spleens (SP) from the bilateral Hepa1-6 model were dissociated to single cell suspension and cultured ex vivo with irradiated Hepa1-6 cells and recombinant mouse IL-2 and IL-7 to expand tumor specific T cells. After 22 days of ex vivo expansion, T cells were challenged with either intact in vivo-matched (Hepa1-6) tumor cells or intact mismatched (CT26) tumor cells for 6 hours; PMA/ionomycin (PMA/IONO) or unstimulated (PBS) conditions were also included as positive and negative controls for nonspecific response, respectively. The tumor-specific reactivity of pooled systemic T cell derived from each treatment group was assessed by intracellular immunostaining/flow cytometry of viable (FSC/SSC, [P1]) singlet (FSC-H/FSC-A [E2]; SSC-H/SSC-A [E3]) CD8⁺ T cells for cytokine biomarkers of immune response IFN-γ and TNF-α. A tumor specific memory CD8⁺ T cell recall response was observed for animals previously treated with mPH-762 2 mg. **(top)** Flow cytometry plots showing group pooled **A.** %IFN-γ⁺ / CD8⁺ T cells or **B.** %TNF-α⁺ / CD8⁺ T cells across in vivo treatment groups and stim conditions; **(bottom)** Bar graphs show mean fold-change for each cytokine relative to the PBS-treated group by stim; line denotes level of nonspecific reactivity.

Conclusions

The objective of the study was to elucidate mechanisms underlying the abscopal antitumor response generated by intratumoral (IT) mPH-762, a self-delivering RNAi compound targeting mouse PD-1, toward untreated distal tumors.

PH-762 is under clinical investigation for treatment of advanced melanoma in the neoadjuvant setting (EudraCT number 2021-002859-10).

- Intratumoral (IT) mPH-762 provided antitumor efficacy to both directly-treated and untreated distal tumors in the bilateral Hepa1-6 murine hepatocarcinoma model.
- IT mPH-762 provided on-target silencing of PD-1 in TME across multiple populations of tumor infiltrating leukocytes (TILs) with associated increases in overall CD45⁺ and CD3⁺ T cell TILs and decreases in CD11b⁺ myeloid cells in directly-treated, but not untreated distal TME. These data suggest that IT mPH-762-induced abscopal antitumor efficacy is mediated through an indirect mechanism.
- IT mPH-762 2 mg generated systemic, durable (>22 days post final treatment) memory CD8⁺ T cells in peripheral lymphoid organs (pooled mLN/SP that were specifically reactive to matched (Hepa1-6) tumor challenge).
- These data suggest that mPH-762 provides abscopal efficacy via generation of systemic tumor specific immunity.
- These data further support clinical application of IT PH-762 in the neoadjuvant setting.