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

Benjamin G. Cullfo, Andrew Boone, Dingxue Yan, Melissa Maxwell, Brianna Rivest, James Cardia, Simon P. Fricker

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 concept 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-targeting (mPH-762) provide robust PD-1 silencing, antitumor 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: T11507110). We have previously shown that mPH-762 provides abscopal efficacy toward untreated distal tumors in a bilateral Hepa-166 model of murine hepatoacellular carcinoma. Here, we identify potential mechanisms underlying this abscopal effect.

Equivalent inoculums of Hepa-166 cells were subcutaneously implanted into bilateral flanks of C57Bl/6J mice (N = 12 / group). When tumors reached a mean volume of 150 mm³, vehicle (PBS) or mPH-762 (0.5 or 2.0 mg/dose) was administered to one of the two tumors (tumor-bearing side) 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 the contralateral untreated distal tumor (UT). DT and UT tumors, mesenteric lymph nodes (mLN) and spleens (SP) were isolated from a saturated 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 immuno staining / 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 Hepa-166 cells. Tumor specific memory reactivity was assessed by challenge with either intact Hepa-166 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 Hepa-166-specific activity 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 Hepa-166 model – in vivo study design

Figure 1. Bilateral Hepa-166 in vivo study design
A. Study details overview table. B. Schematic of seeding and treatment of Hepa-166 tumors.

Intratumoral (IT) treatment with mPH-762 provides both direct and abscopal antitumor efficacy in the bilateral Hepa-166 murine hepatocellular carcinoma model

A. Mean tumor volume ± SEM (n= 12) of directly treated tumor (DT) and contralateral untreated distal tumor (UT) on Day 14. Directly treated tumor volumes are adjusted for the contralateral tumor volume (AUC) calculated by trapezoidal transformation over Days 8-14. 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 volumes are adjusted for 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<0.001. B. Directly treated tumor volume and contralateral tumor volume (AUC) calculated by trapezoidal transformation over Days 8-14. Violin plots are shown with individual animals and medians indicated.

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

Figure 4. IT mPH-762 treatment generates durable tumor-specific memory CD8⁺ T cells in peripheral lymphoid organs
Posed Day 14 tumor draining mesenteric lymph nodes (mLN) and spleens (SP) from the bilateral Hepa-166 model were dissociated to isolate viable CD8⁺ T cells and cultured ex vivo with irradiated Hepa-166 cells and antibiotic mouse IL-2 and IL-15 to expand tumor specific T cells. After 22 days of ex vivo expansion, T cells were challenged with either intact in vivo treated (Hepa-166) tumor cells or in unchallenged (CT26) tumor cells for 6 hours. PMA/Ionomycin or Contralateral (UT) untreated (PBS) conditions were also included as positive and negative controls for nonspecific responses, respectively. The tumor-specific reactivity of pooled systemic T cells, directly treated and UT of treated model was assessed by intracellular immunophenotypic analysis at Day 0 and Day 22. The stimulated T cells were stained for CD8, IFN-γ, TNF-α, PD-1, PD-L1, markers, and analyzed by flow cytometry. The frequency and IFN-γ expression of CD8⁺ T cells from the tumor-specific memory CD8⁺ T-cell recall response was observed for animals previously treated with mPH-762 (2 mg) reg. (reg) Flow cytometry plots showing group pooled A. UT; B. CT26; C. TILs on mLN or D. mLN-762; E. TILs-C26. Data across in vivo treatment groups and time points (conditions). (Bottom) Bar graphs show mean fold-change for each cytokine relative to the PBS-treated group by dtm; line denotes behavior over time.

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 concept targeting mouse PD-1, toward untreated distal tumors. PH-762 was in clinical investigation for treatment of advanced melanomas 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 Hepa-166 murine hepatocarcinoma model.
- IT mPH-762 provided on target silencing of PD-1 in TME across multiple populations of tumor infiltrating leukocytes (TLI) with associated increases in overall CD8⁺ and CD4⁺ T cell TIL and decreases in CD11b⁺ myeloid cells in directly treated and UT 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 (>2 days past final treatment) memory CD8⁺ T cells in peripheral lymphoid organs (positive IFN-γ that were specifically reactive in treated Hepa-166 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.