INTASYL PH-762: PD-1 Intratumoral Immunotherapy

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Intrasite Overview

Abstract

PH-762 is an INTASYL compound designed to precisely silence PD-1 mRNA. INTASYL™ is a self-delivering RNAi technology platform designed to impart specific properties to small interfering RNAs, providing extremely efficient delivery to a broad range of cell types and tissues without the need for specialized formulations or drug delivery systems. INTASYL’s innovative approach features a simplified chemical composition, potentially reducing toxicity and enhancing tolerability and efficacy.

In vitro investigations have demonstrated efficient uptake of PH-762 by human T cells, silencing of PD-1 mRNA, and subsequent protein reduction. Preclinical studies have shown that IT injections of murine targeted PH-762 (mPH-762) can silence PD-1 mRNA in the T cells within the tumor and increase the secretion of IFN-γ. mPH-762 was well tolerated at the maximum administered dose and treatment with mPH-762 provided robust and statistically significant inhibition of tumor growth.

Toxicokinetic studies conducted in marmoset monkeys demonstrated that PH-762, when administered intravenously at doses of up to 147 mg/kg, is well-tolerated. Importantly, no detectable cytokine-release associated cytokines were found in the plasma of treated monkeys.

PH-762 is currently being investigated in an open-label clinical study (NCT 06014086) to evaluate the safety and tolerability of neoadjuvant use of IT PH-762 in cutaneous squamous cell carcinoma (cSCC), melanoma, or Merkel cell carcinoma, to determine the pharmacokinetic profile of PH-762 after IT injection, to observe pathologic and immunologic tumor responses, and to determine the recommended dose for continued clinical development. Tumor changes are evaluated per iRECIST criteria and pathologic response. Immunologic response in tumor tissue and blood samples are assessed as secondary endpoints. This clinical study will establish the basis for continued clinical development of PH-762.

Efficient uptake of PH-762 with robust silencing of PD-1 mRNA and protein

PH-762 can be administered via intratumoral (IT) injection, which is anticipated to diminish the systemic side effects associated with conventional systemic antibody treatments. PH-762’s unique patented, self-delivering RNAi technology platform designed to impart specific properties to small interfering RNAs, providing extremely efficient delivery to a broad range of cell types and tissues without the need for specialized formulations or drug delivery systems. INTASYL’s innovative approach features a simplified chemical composition, potentially reducing toxicity and enhancing tolerability and efficacy.

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Conclusion

• PH-762 is rapidly taken up by cells and robustly silences PD-1 protein and mRNA in lymphocytes within the tumor microenvironment.
• Intratumoral injection of mPH-762 significantly inhibits tumor growth in murine tumor models and is well tolerated.
• mPH-762-mediated silencing of PD-1 within the TME may generate memory-specific T cells, promoting long-term memory.
• These findings support the ongoing clinical trial of PH-762’s safety and efficacy as a neoadjuvant therapy for treatment of cSCC, melanoma, or Merkel cell carcinoma and also supports study in other PD-1 expressing tumors.

**Figure 1. INTASYL™ mechanism of silencing and structure**

The biosynthetic instruction of INTASYL™ allows matrixdirect cellular uptake of the compounds into the tumor and immune cells, with high specificity to target genes.

**Figure 2. PH-762 is rapidly taken up by T cells and results in potent, concentration-associated silencing of PD-1 mRNA and protein**

Human pan T cells treated with PH-762 quantified by flow cytometry. Histograms depict count (Y-axis) and fluorescence intensity (X-axis) flow cytometry data at 6 hours post-treatment. Gray = untransfected, blue = PH-762, red = mPH-762. A: Human pan T cells treated with PH-762 quantified by flow cytometry. B: Human pan T cells treated with either PH-762 or a non-targeting control (NTC) vector quantified by flow cytometry. C: Human pan T cells treated with PH-762 quantified by flow cytometry. D: Human pan T cells treated with either PH-762 or a non-targeting control (NTC) vector quantified by flow cytometry.

**Figure 3. Intratumoral murine-targeted PH-762 (mPH-762) provides antitumor efficacy in a subcutaneous Hepa1-6 model of murine hepatocellular carcinoma**

Treatments were administered in a syngeneic Hepa1-6 model on Days 1, 3, 7, 10 and 14 by IT injection of 0.5 mL mPH-762, NTC or PBS vehicle, or by IP injection of anti-PD-1 mAb (200 µg/dose). Animals were weighed daily, and tumors measured every three days and volume calculated using the standard ellipsoid equation V = (L x W x T)/2. A: Treatment with mPH-762 robustly inhibited longitudinal and cumulative mean tumor growth in vivo compared to controls. Mean tumor volume is displayed on the X-axis and SEM. B: Treatment with mPH-762 did not significantly impact mean cumulative weight gain compared to controls and no evidence of local reaction was observed in the injection area over the course of treatment.

**Figure 4. PD-1 protein silencing on Tumor Infiltrating Lymphocytes (TILs) results in increase secretion of IFN-γ upon rechallenge, suggesting the generation of memory specific T cells**

An ex vivo characterization of the tumor microenvironment (TME) was performed in the Hepa-1-6 model. A. Treatment with mPH-762 significantly reduced the mean percentage of surface PD-1+ expression on CD8+ (TILs) compared to controls. B. mPH-762, administered intratumorally at a dose of 2 mg, generated systemic CD8+ T cells expanded vivo from peripheral lymphoid organs isolated on Day 14 of the study. Memory reactivity of CD8+ CD103+ T cells isolated from hepatic lymph nodes 3 weeks post-treatment was assessed ex vivo using flow cytometry. C. mPH-762 significantly reduced the mean percentage of surface PD-1+ expression on CD8+ (TILs) compared to controls.

**Figure 5. PH-762 administered IV at doses up to 147 mg/kg result in no detectable cytokine-release associated cytokines in the plasma of treated marmoset monkeys**

No detectable cytokine-release associated cytokines in the plasma of treated marmoset monkeys. Non-GFP analyses of plasma for cytokine release associated cytokines in the plasma of treated marmoset monkeys. Studies in non-human primates demonstrate that PH-762 is well-tolerated and does not induce release of cytokine-release associated cytokines (CRIS)-associated cytokines. A. IFN-γ, B. IL-2, C. TNF-α. Symbols represent individual animals, n=8 per condition (not shown if below limit of detection).