Intratumoral use of self-delivering RNAi to reprogram the tumor microenvironment and boost the antitumor response

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Background

Immunotherapy targeting immune checkpoints has shown significant benefit in many cancers. However, systemic delivery can result in severe auto-immune toxicities, the risk increasing with the combination of checkpoint inhibiting antibodies. Local delivery of immunotherapies allows for multiple combination therapies, while potentially preventing significant systemic toxicities. One approach is to use self-delivering RNAi technology, INTASYL™, which has been shown to be safe and effective in clinical applications following local administration. Here we show the use of the INTASYL platform as a checkpoint inhibiting immunotherapy for intratumoral applications, through a series of preclinical *in vivo* studies.

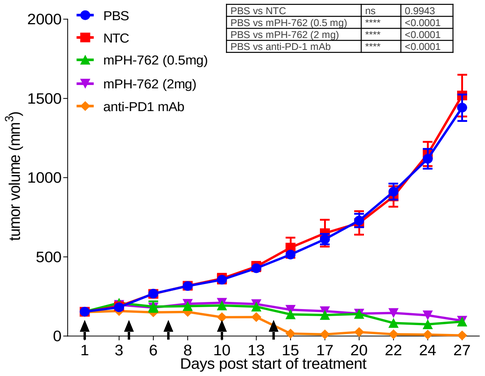
Methods

Self-delivering RNAi (termed INTASYL) compounds were made against mouse PD-1, TIGIT, and BRD4. INTASYL comprises an asymmetric duplex, a small duplex region (≤ 15 base pairs), a single-stranded phosphorothioate tail and chemical modifications to confer stability resulting in efficient cellular uptake allowing in situ delivery. The efficacy and immunomodulation of these INTASYL compounds was evaluated in the Hepa1-6 and CT26 murine syngeneic tumor models following intratumoral administration. Tumor volumes were recorded throughout the study. Tumor-Infiltrating Lymphocytes (TILs) were isolated twenty-four hours post the last dose and examined for gene silencing by RT-qPCR. Tumor microenvironment (TME) and T cell activation were analyzed by flow cytometry.

Results

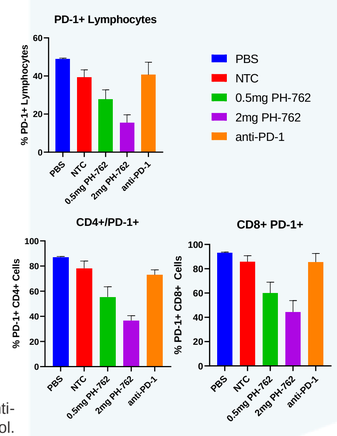
Results showed dose-dependent attenuated tumor growth compared to control groups. Relevant changes in the TME included an increase of CD8+ T cells and an increase of activation markers on TILs. Tumor growth reduction correlated with silencing of target mRNA and immune effector cell levels and activation.

Intratumoral injection of mPH-762 inhibits tumor growth



Hepa 1-6 tumor-bearing mice were treated with INTASYL targeting mouse PD-1 (mPH-762) intratumorally on Days 1, 4, 7, 10 and 14. Anti-PD-1 mAb was used as a positive control. NTC = Non-targeting control.

Intratumoral injection of mPH-762 silences PD-1 surface expression on isolated TILs

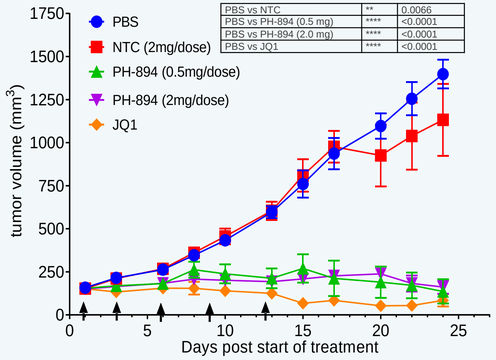


Key takeaways:

- Overcoming the immunosuppressive TME can be achieved by local administration of self-delivering RNAi (INTASYL™).
- Strong tumor growth attenuation correlated with silencing of target mRNA / protein and modulation of immune effector cell composition and activation.
- This provides a viable approach for locally delivered immunotherapy to overcome the shortcomings of systemic immunotherapy.

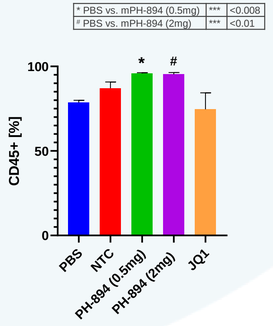
Results (cont.)

Intratumoral injection of PH-894 inhibits tumor growth



Hepa 1-6 tumor-bearing mice were treated with INTASYL targeting BRD4 (PH-894) intratumorally on Days 1, 4, 7, 10 and 14. JQ1, a non-specific inhibitor of bromodomain proteins, was used as a positive control. NTC = Non-targeting control (used as a negative control).

Intratumoral treatment with PH-894 increases TILs in the TME

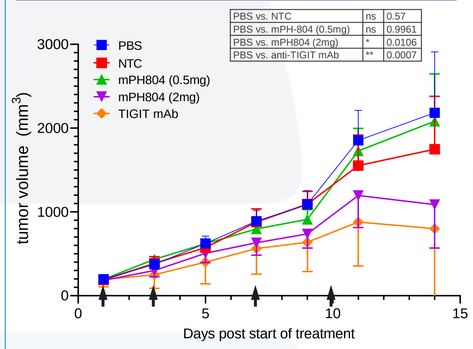


Mice were sacrificed on Day 14 after the last dose of PH-894.

Tumors were excised and TILs isolated and analyzed by flow cytometry.

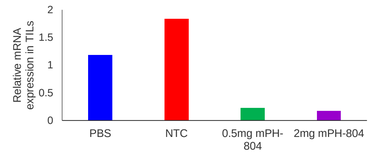
Results (cont.)

Intratumoral injection of mPH-804 inhibits tumor growth

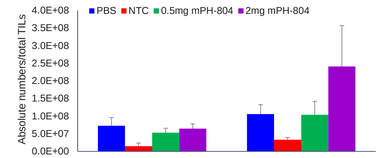


CT26 tumor-bearing mice were treated with INTASYL targeting mouse TIGIT (mPH-804) intratumorally on Days 1, 3, 7 and 10. Anti-TIGIT mAb was used as a positive control. NTC = Non-targeting control (used as a negative control).

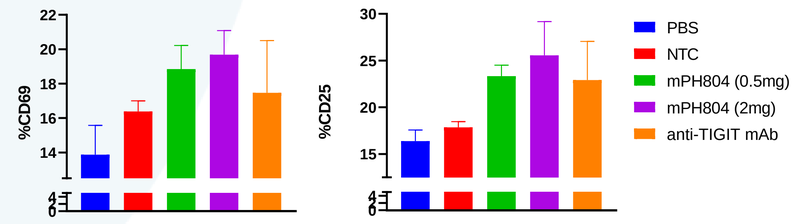
Intratumoral injection of mPH-804 silences TIGIT mRNA expression in the TME



Intratumoral injection of mPH-804 increases CD8+ effector T cells in the TME



Intratumoral injection of mPH-804 enhances T cell activation



Mice were sacrificed on Day 11 one day after the last dose of mPH-804.
Tumors were excised and TILs isolated and T cell activation markers CD25 and CD69 were analyzed by flow cytometry.

Directions for Future Research

- These studies demonstrate the feasibility of using INTASYL compounds intratumorally to overcome the immunosuppressive TME
- This is a viable novel approach to solid tumor immunotherapy and warrants further investigation in patients.
- IND enabling studies are ongoing.

Note: Error bars are S.E.M.