Combination intratumoral treatment with INTASYL™ self-delivering RNAi targeting TIGIT and PD-1/PD-L1 improves tumor control compared to monotherapy in a subcutaneous CT26 model of murine colorectal cancer.

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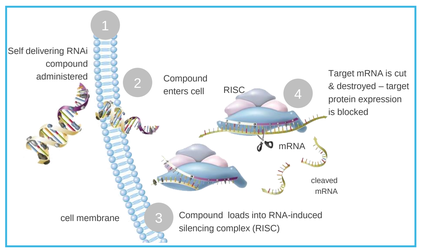
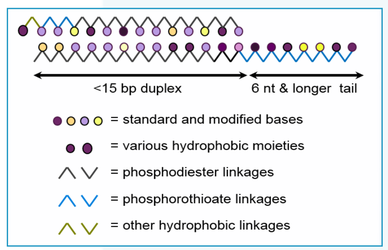
Introduction

Despite clinical successes of immune checkpoint blockade (ICB) antibodies blocking the inhibitory receptors CTLA-4, PD-1, or PD-L1, substantial challenges remain. Many patients do not respond, and ICB treatment is associated with serious immune-related adverse effects (irAEs) which are exacerbated by combination therapies. TIGIT blockade has been demonstrated to inhibit tumor growth kinetics in pre-clinical studies, prompting ongoing clinical trials, including those targeting TIGIT in combination with anti-PD-1 or anti-PD-L1.

The INTASYL™ platform is a self-delivering RNAi technology that (1) provides efficient delivery into target cells bypassing the need for specialized formulations, mechanical perturbation, or drug delivery systems; and (2) specifically and durably silence target gene expression when administered intratumorally (IT), providing *in vivo* tumor control. IT administration restricts pharmacokinetics to the tumor; an attractive strategy for mitigating ICB-mediated systemic irAEs. Additionally, using INTASYL, multiple targets can be silenced in combination. Previously, we have shown nearly complete attenuation of tumor growth *in vivo* under treatment with INTASYL™ compounds in an ICB-responsive murine model (Hepa1-6), and correlated efficacy with silencing of target mRNA / protein and modulation of immune effector cell composition and activation[1].

Here we demonstrate the *in vivo* efficacy of INTASYL specifically targeting TIGIT (PH-804), PD-1 (PH-762), PD-L1 (PH-790) alone or in combination in a model known to be somewhat refractory in responsive to ICB, namely a CT26 model of murine colorectal carcinoma.





INTASYL compounds

- **PH-804 (target: TIGIT mRNA)**
- **PH-762 (target: PD-1 mRNA)**
- **PH-790 (target: PD-L1 mRNA)**

USAGE

INTASYL "Self-delivering" features make them ideally suited for:

01 *In vivo* transfection via direct injection into tumor microenvironment (TME)

02 *Ex vivo* transfection during adoptive cell therapy (ACT)

Intratumoral INTASYL mono- or combination therapy targeting murine *Tigit* (mPH-804), *Pdcd1* (mPH-762), *or Cd274* (mPH-790) mRNA significantly improved tumor control in a subcutaneous CT26 BALB/c model

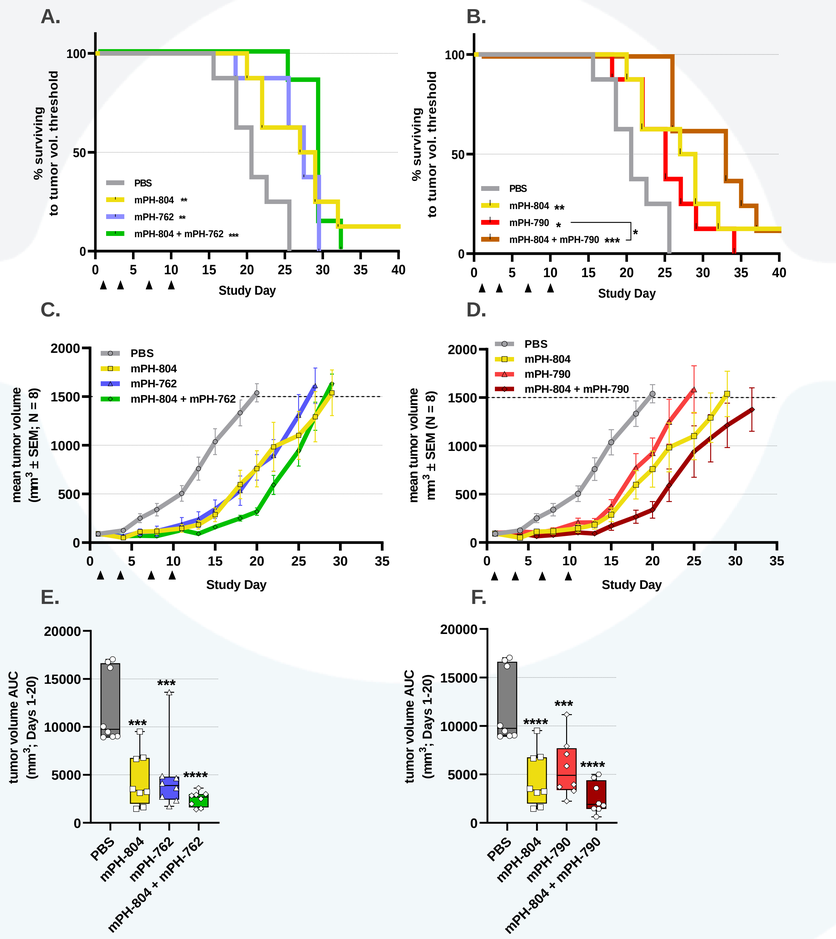


Figure 1. *In vivo* efficacy of INTASYL mono- vs. combination therapies in the subcutaneous CT26 Model in BALB/c mice.
INTASYLs™ were administered IT at 1 mg / INTASYL / dose on Days 1, 3, 7 & 10 (arrows). **A. & B.** Percentage of animals surviving to tumor volume threshold (1500 mm^3). Statistical significance assessed by Log-rank (Mantel-Cox) test. **C. & D.** Longitudinal mean tumor volume (mm3 ± SEM; N = 8). **E. & F.** Tumor volume area under the curve (AUC) calculated by trapezoidal transformation. Shown are box and whisker plots with medians indicated. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

Intratumoral INTASYL monotherapies provide tumor control analogous to that of systemically administered target-matched antibody therapies

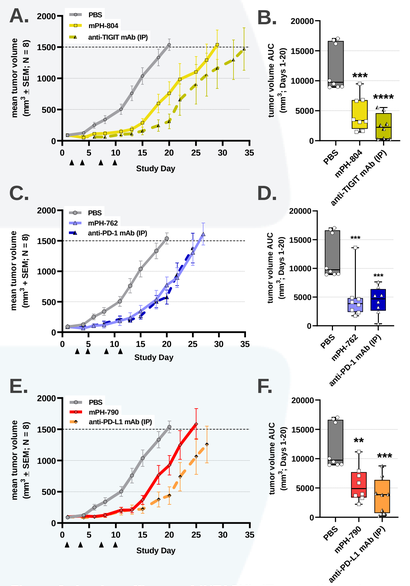


Figure 2. *In vivo* efficacy of INTASYL IT monotherapies vs. target-matched systemic (IP) antibody therapy in the CT26 model.
INTASYL (IT; 1 mg / dose) or target matched antibody therapies (IP; 200 µg / dose) were administered on Days 1, 3, 7 & 10 (arrows). **A. C. & E.** Longitudinal mean tumor volume (mm3 ± SEM; N = 8). **B. D. & F.** Tumor volume AUC calculated by trapezoidal transformation. Shown are box and whisker plots with medians indicated. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

Intratumoral INTASYL mono- or combination therapies are well tolerated *in vivo*

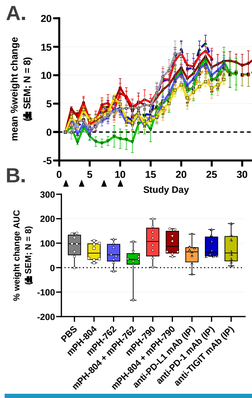


Figure 3. Percentage of weight change under treatment in the CT26 model.
A. Longitudinal mean percentage (%) body weight change (± SEM; N = 8). **B.** % weight change AUC. Box and whisker plots with median indicated.

On-target specific silencing of INTASYL mono- or combination therapies was validated in CD3/CD28 activated human pan T cells *in vitro*

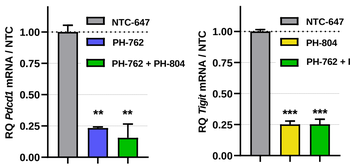


Figure 4. On-target silencing by INTASYLs in human T cells.
Human pan T cells were treated with 2 µM / INTASYL and activated with CD3/CD28 beads Mean relative quantity (RQ) of mRNA is shown ± SEM (N = 2) compared to that under treatment with non-targeting control (NTC) INTASYL. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

Conclusions

- These findings demonstrate the therapeutic potential of INTASYL combination therapies targeting TIGIT and PD-1 / PD-L1 checkpoint inhibition.
- *In vivo* efficacy of INTASYL monotherapy was analogous to that of systemically delivered antibody therapy for each target.
- INTASYL targeting TIGIT + PD-1 or TIGIT + PD-L1 in combination inhibited tumor growth kinetics vs. monotherapies.
- INTASYL combination therapies were well tolerated.
- As current systemic antibody therapies often produce serious immune-related adverse effects, especially in combination, INTASYL combination therapies administered IT represent an attractive alternative strategy, warranting further investigation in patients.

1. Dispersyn G et al., Journal of Clinical Oncology 38, no. 15_suppl; 2020 (DOI: 10.1200/JCO.2020.38.15_suppl.e15206)