Local immunotherapy with INTASYL™ self-delivering RNAi targeting CTLA-4 provides robust tumor control in vivo

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Abstract

Background Immune checkpoint inhibition (ICI) of CTLA-4 with ipilimumab has proven effective in improving clinical responses for patients with advanced melanoma or other approved indications in combination with nivolumab. However, systemic treatment with ipilimumab is associated with serious adverse events (SAEs) for many (>25%) patients that can be life-threatening and/or result in discontinuation of treatment. As such, balancing efficacy with associated toxicities remains a challenge in treating patients with ipilimumab.

Local intratumoral (IT) immunotherapy may enhance local activity and decrease systemic toxicity. Additionally, by using the tumor as its own vaccine, IT immunotherapy can ignite tumor-specific immune responses well beyond the local site of administration. While clinical testing of IT antibody therapies is underway, the high molecular weight properties of therapeutic antibodies may limit their local diffusion and retention time within tumors.

RNAi therapy is an emerging modality well-positioned to optimize local clinical application of ICI. We have previously demonstrated that self-delivering RNAi (INTASYL™) therapeutic compounds built on proprietary INTASYL technology silence targets with high specificity and without need for specialized formulations or drug delivery systems to provide robust antitumor efficacy to both directly-treated and to non-directly treated distal tumors when delivered IT in vivo.

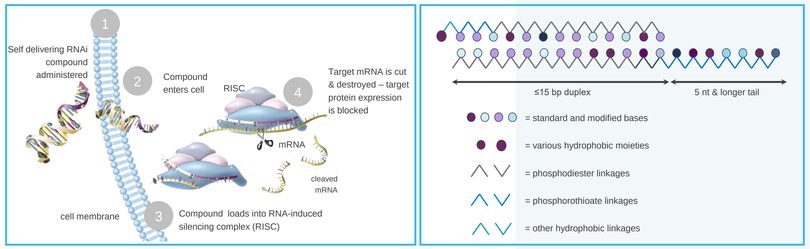
Here we present proof-of-concept (POC) in vivo data showing IT efficacy of a novel INTASYL targeting murine CTLA-4 (mCTLA-4; 27790) in two syngeneic mouse tumor models.

Methods CTLA4 mRNA silencing was validated in CHO K1 cells expressing murine CTLA-4 in vitro by RT-qPCR. For in vivo efficacy, Hepa1-6 or CT26 cells were implanted subcutaneously into the flanks of C57BL/6 or BALB/c mice, respectively. When tumors reached threshold volume (150 mm^3), animals were randomized into treatment groups; treatments were administered on Days 1, 4, 7, 10 and 13. Vehicle (PBS), a chemically identical non-targeting control (NTC) INTASYL, or INTASYL 27790 at two dose concentrations were administered IT; anti-CTLA-4 monoclonal antibody (mAb; clone 9D9) was administered intraperitoneally (IP). Tumor volumes and body weights were recorded longitudinally.

Results mCTLA-4-targeting INTASYL 27790 provided concentration-associated silencing of mCTLA-4 in vitro. When administered IT, mCTLA-4-targeting INTASYL 27790 elicited robust dose-associated antitumor efficacy in both in vivo tumor models compared with vehicle- or NTC-treated tumors, comparable to that observed under systemic IP treatment with anti-CTLA4 mAb.

Conclusions These data show IT INTASYL targeting mouse CTLA4 elicits robust on-target dose concentration-associated antitumor efficacy in two syngeneic tumor models in vivo and provide POC for targeting CTLA-4 IT with INTASYL.

Figure 1. INTASYL™ mechanism of silencing and structure



mCTLA-4-Targeting INTASYL 27790 Silences Mouse CTLA4 mRNA in vitro

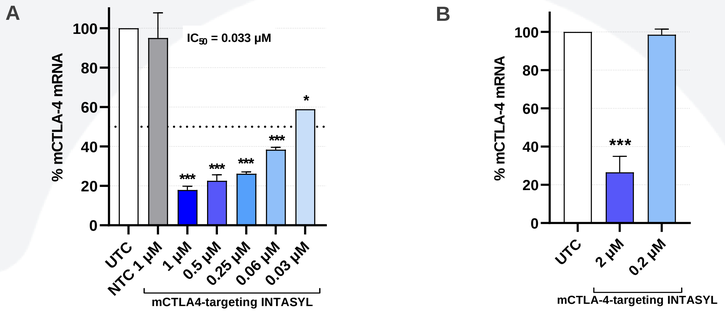


Figure 2. Mouse CTLA-4-targeting INTASYL 27790 silences murine CTLA4 mRNA in vitro

Mouse CTLA-4 (mCTLA4)-targeting INTASYL 27790 concentration associated silencing of mouse CTLA-4 mRNA compared to PBS vehicle treated control (UTC) or chemically identical but non-targeting INTASYL (NTC) in **A.** mCTLA4-expressing CHO K1 cells. **B.** Activated BALB/c mouse peripheral blood T cells. Means ± SEM (n = 2) are shown. Means were compared to UTC by one way ANOVA and Dunnett's post test. ***p<0.001; *p < 0.05.

Intratumoral mCTLA-4-Targeting INTASYL 27790 Provides Antitumor Efficacy in the Hepa1-6 Model of Murine Hepatocellular Carcinoma

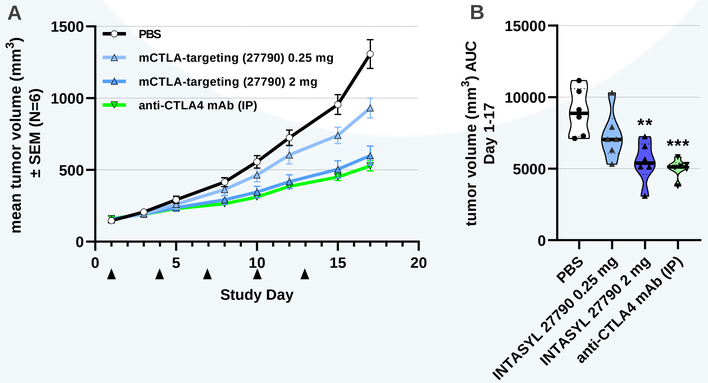


Figure 3. Intratumoral (IT) mCTLA-4-Targeting INTASYL 27790 provides dose associated tumor growth inhibition in the Hepa1-6 model of murine hepatocellular carcinoma

Subcutaneous Hepa1-6 model. IT INTASYL 27790 (0.25 or 2 mg / dose; IT); or anti-CTLA-4 mAb (BioXCell; 0.1 mg / dose; IP) were administered on Days 1, 4, 7, 10 & 13 (arrows)**. A.** Mean tumor volume (mm^3 ± SEM; N = 6) over time. **B.** Tumor volume area under the curve (AUC) over the course of the study (Days 1-17). Violin plots with medians and individual animals are indicated. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ***p < 0.001, **p < 0.01.

in vivo studies were performed at Pharma Models LLC, Marlborough, MA

Intratumoral mCTLA-4-Targeting INTASYL 27790 Provides Antitumor Efficacy Including a Complete Response in the CT26 Model of Murine Colon Carcinoma

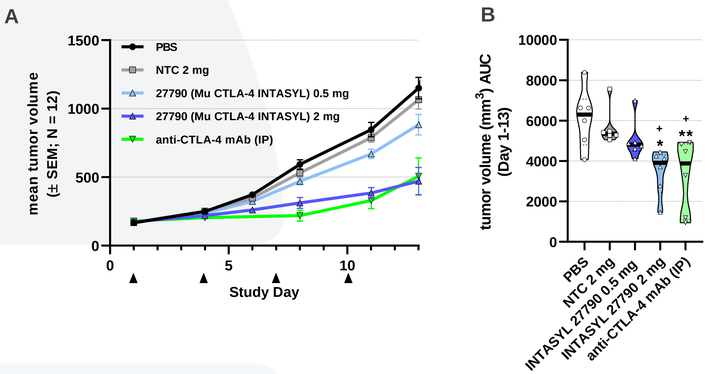


Figure 4. Intratumoral mCTLA-4-Targeting INTASYL 27790 provides dose-mediated tumor growth inhibition in the CT26 model of murine colon carcinoma

Subcutaneous CT26 model. IT INTASYL 27790 (0.5 or 2 mg / dose; IT); IT non-targeting control INTASYL (NTC), or anti-CTLA-4 mAb (BioXCell; 0.1 mg / dose; IP) were administered on Days 1, 4, 7, & 10 (arrows)**. A.** Mean tumor volume (mm^3 ± SEM; N = 12) over time. One tumor treated with 27790 at 2 mg completely responded (CR) so as to become unmeasurable; no CRs observed for other treatments. **B.** Tumor volume AUC (Day 1-13). Violin plots with medians and individual animals are indicated. Statistical significance assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. * = vs PBS; + = vs NTC ***p < 0.001, **p < 0.01.

Summary and Conclusions

- **Systemic antibody-mediated inhibition of CTLA-4 can be clinically effective, but use is currently limited by high incidence of immune related severe adverse events (irSAEs).**
- **Local therapy with INTASYL offers an approach to minimize systemic toxicity, while retaining local efficacy, an application not well suited to antibodies. Furthermore, intratumoral (IT) therapy with INTASYL has been demonstrated to ignite durable systemic and specific antitumor immune responses in preclinical mouse models.**
- **A novel INTASYL specifically designed to target murine CTLA-4 (27790) provided concentration associated silencing of murine CTLA-4 mRNA in vitro.**
- **Mouse CTLA-4-targeting INTASYL 27790 provided dose associated on-target antitumor efficacy in vivo syngeneic tumor models of both hepatocellular carcinoma (Hepa1-6) and colon carcinoma (CT26).**
- **These studies provide proof-of-concept in vivo antitumor efficacy for IT INTASYL targeting CTLA-4, supporting further development to maximize efficacy and minimize toxicity of CTLA-4 inhibition beyond the limitations of current antibody therapies.**

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