Novel Potent Antisense Oligonucleotides Targeting Transforming Growth Factor Beta 1 Isoform (TGF-β1)

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BACKGROUND: Transforming growth factor beta (TGF-β) is a key member of a large family of cytokines, which play critical, pleiotropic roles in the pathophysiology of various human diseases, such as cancer, inflammation, autoimmune disease, and cirrhosis/fibrosis. TGF-β1, -2, and -3 isoforms are cytokines encoded by different genes but sharing strong sequence and structure homology. They function as the primary mediators of TGF-β signaling via both non-canonical and canonical signaling pathways. In Oncology, TGF-β isoforms are associated with a wide range of biological processes such as tumor cell invasion and migration, angiogenesis, immunosuppression, as well as regulation of tumor stem cell properties. Hence, blocking the TGF-β signaling may have a multifold therapeutic benefit in Oncology, although therapeutic relevance of the respective TGF-β isoforms remains poorly documented. In order to evaluate the specific biological relevance of TGF-β1 isoform in cancer, we have initiated an extensive discovery program for identification of antisense oligodeoxynucleotides (ASO) constructs selectively inhibiting expression of the TGF-β3 ligand. Based on the sequence of the human TGF-β1 mRNA more than 150 Locked Nucleic Acid (LNA)-modified gapmers were designed, synthesized and tested in cell-based assays. Highly potent and selective TGF-β1 constructs were identified and selected based on efficient suppression of TGF-β1 mRNA/protein expression in different human and rodent tumor cell lines, and in human Peripheral Blood Mononuclear Cells (PBMCs). Effective target downregulation was demonstrated after lipofectamine-aided transfection (subμM concentration range), but also in the absence of any transfection agent (gymnotic delivery) at subμM concentrations. Human tumor cell viability was impaired after targeted suppression of TGF-β1 isoform mRNA/protein in cell-based assays, and surprisingly, in the Mouse, marked liver toxicity was induced following systemic administration of selected TGF-β1 specific ASOs.

Figure 1: TGF-β1 multi-modal tumor-promoting effects

- Tumor growth inhibition
- Increased tumor angiogenesis
- Reduced invasion & metastasis
- Increased local immune response
- Innate immune response

Figure 2: Downregulation of TGF-β1 and TGF-β2 mRNAs by LNA modified ASOs after transfection in human A172 glioma cells

Figure 3: TGF-β1 specific ASOs significantly suppress TGF-β1 mRNA and protein expression in human Pan0.1 pancreatic cancer cells at subμM concentrations after gymnotic delivery

Figure 4: TGF-β1 mRNA downregulation activity of the TGF-β3 specific ASO ASPH1047 in human PBMCs after incubation without transfection reagent (gymnotic delivery)

Figure 5: Effect of TGF-β1 specific ASOs on human Pan0.1 pancreatic tumor cell viability after incubation without transfection reagent (gymnotic delivery)

Figure 6: Effects of selected TGF-β1 specific ASOs on liver function following systemic administration in the Mouse

SCID, BALB/c, or BALB/c nude mice were treated with a cumulative weekly dose of 50 or 50 mg/kg of TGF-β1 specific ASOs (non-oligonucleotides tested) all cross-reacting with mouse TGF-β1 mRNA or unspecific control scrambled oligonucleotides (20 oligonucleotides tested) by subcutaneous injections. Albumin Ammonium Sulfate (ALS) levels in plasma were determined on Day 5, and were used as indicator of liver function.

- all tested TGF-β1 mRNA specific ASOs, but none of the control oligonucleotides, induced marked elevation of plasma ALT indicative liver toxicity in mice
- ASOs selectively targeting human, but not mouse TGF-β1 mRNA did not induce any elevation of plasma ALT under similar experimental setting (data not shown)
- Elevation of plasma ALT in mice was not observed with ASOs targeting TGF-β2 under similar experimental conditions (data not shown)
- Current active investigations are ongoing to explore if liver toxicity induced by TGF-β1 specific ASOs is limited to mice/rats

SUMMARY
- LNA-modified ASO gapmers have been designed based on human TGF-β1 mRNA sequence, and shown to potently and specifically suppress TGF-β1 mRNA/protein after delivery to human/mouse tumor cell lines and ex vivo in human PBMCs
- TGF-β1 specific ASOs, but not control oligonucleotides, impaired cell viability in human tumor cell lines
- In the Mouse, systemic treatment with specific TGF-β1 ASOs, but not unspecific control oligonucleotides (and TGF-β2 ASOs, data not shown) seems to induce liver toxicity

CONCLUSIONS & PERSPECTIVES
1. Although not yet therapeutically successfully exploited, TGF-β1 isoform represents an attractive molecular target for therapeutic intervention in Oncology (multi-modal cancer-promoting effects).
2. We have designed highly selective LNA-modified ASO gapmers targeting TGF-β1 in tumor cell lines and human PBMC, and impairing cell viability in tumor cells
3. Further exploration of TGF-β1 specific ASOs for systemic treatment of cancer is currently limited by the acute liver toxicity observed in the Mouse (studies in non-human primates currently ongoing)

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b) Use of LNA-modified gapmers is performed under a license from Santaris Pharma.