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

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Abstract # 717

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 pathway 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 oligodeoxynucleotide (ASO) constructs selectively inhibiting expression of the TGF-β1 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 (sub-nM concentration range), but also in the absence of any transfection agent (gymnotic delivery) at sub-μM 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-β multi-modal tumor-promoting effects*

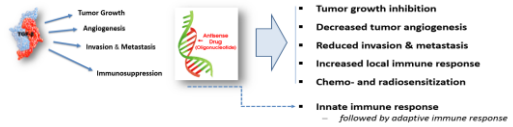
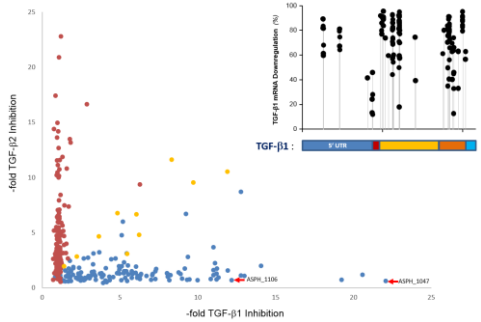
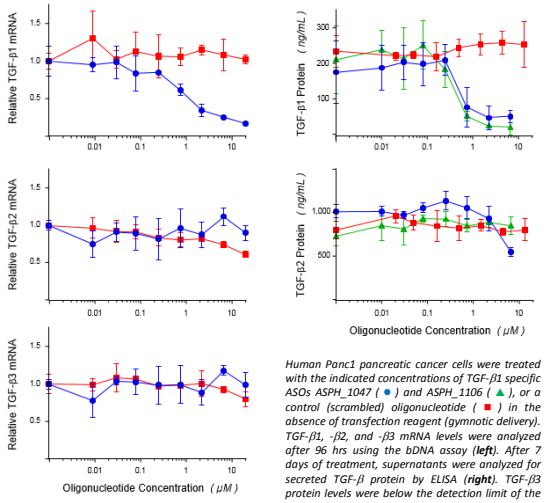


Figure 2 : *Downregulation of TGF-β1 and TGF-β2 mRNAs by LNA modified ASOs after transfection in human A172 glioma cells*



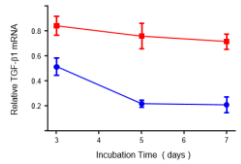
Human A172 glioma cells were transfected using Lipofectamine with 10 nM of LNA-modified antisense oligonucleotides designed to specifically suppress either TGF-β1 (●), TGF-β2 (●) or TGF-β1 and -β2 (●). TGF-β mRNA expression level was determined 24 hr after transfection using bDNA method. Expression values were normalized using GAPDH mRNA (housekeeping gene). Values are depicted as –fold inhibition which represents 1/residual mRNA values.

Figure 3 : *TGF-β1 specific ASOs specifically suppress TGF-β1 mRNA and protein expression in human Panc1 pancreatic cancer cells at sub-μM concentrations after gymnotic delivery*



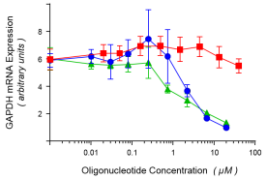
*Human Panc1 pancreatic cancer cells were treated with the indicated concentrations of TGF-β1 specific ASOs ASPH_1047 (●) and ASPH_1106 (▲), or a control (scrambled) oligonucleotide (■) in the absence of transfection reagent (gymnotic delivery). TGF-β1, -β2, and -β3 mRNA levels were analyzed after 96 hrs using the bDNA assay (**left**). After 7 days of treatment, supernatants were analyzed for secreted TGF-β protein by ELISA (**right**). TGF-β3 protein levels were below the detection limit of the ELISA. Mean values of 3 independent determinations ± SD are represented*

Figure 4 : *TGF-β1 mRNA downregulation activity of the TGF-β1 specific ASO ASPH_1047 in human PBMCs after incubation without transfection reagent (gymnotic delivery)*



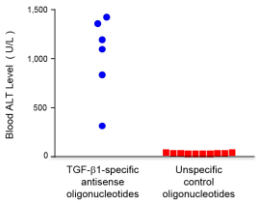
Isolated human Peripheral Blood Mononuclear Cells (PBMC) from two independent donors were treated with 10 μM of the TGF-β1 specific ASO ASPH_1047 (●) or an unspecific control (scrambled) oligonucleotide (■) in the absence of transfection reagent. TGF-β1 mRNA expression levels were analyzed at the indicated days using the bDNA assay. Mean values of 3 independent determinations ± SD are represented, and a representative result of one donor is shown.

Figure 5 : *Effect of TGF-β1 specific ASOs on human Panc1 pancreatic tumor cell viability after incubation without transfection reagent (gymnotic delivery)*



Human Panc1 pancreatic cancer cells were treated with the indicated concentrations of TGF-β1 specific ASOs ASPH_1047 (●) and ASPH_1106 (▲), or an unspecific control (scrambled) oligonucleotide (■) in the absence of transfection reagent (gymnotic delivery). After 7 days of treatment, GAPDH mRNA levels were determined by the bDNA assay as indicator of cell number and/or cell viability.

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 56 mg/kg of TGFβ1 specific ASOs (six oligonucleotides tested; all cross-reacting with mouse TGF-β1 mRNA) or unspecific control scrambled oligonucleotides (20 oligonucleotides tested) by subcutaneous injections. Alanine Aminotransferase (ALT) levels in plasma were determined on Day 5-6, 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/rodents

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 cells 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)

a) *The authors wish to acknowledge Axolabs (Kulmbach, Germany) and Oncodesign (Dijon, France) for the quality of their technical contribution in the presented studies.*

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

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