ASPH_0047: a Potent and Selective Antisense Oligonucleotide Targeting Transforming Growth Factor beta 2 Isoform (TGF-β2)

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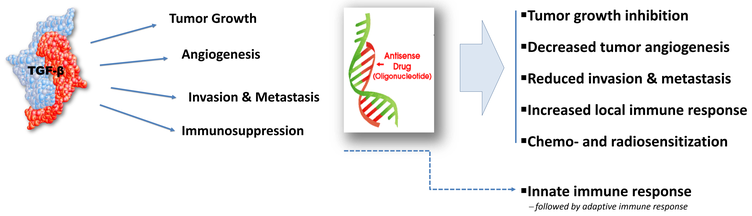
Abstract # 716

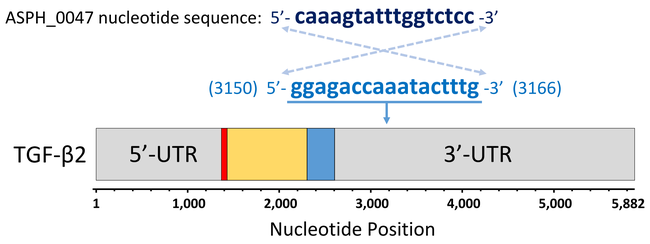
Abstract: Transforming growth factor beta (TGF-β) is a key member of a large family of cytokines, including bone morphogenetic protein (BMP), nodals, activins and others, which play critical, pleiotropic roles in the pathophysiology of various human diseases, such as cancer, inflammation, autoimmune disease, and cirrhosis/fibrosis. In particular, the different TGF-β isoforms (TGF-β1, -β2, and -β3, encoded by different genes but sharing high sequence and structure homology) are overexpressed in many human tumors. Correlations between TGF-β expression, cancer stage and clinical parameters have been reported and linked to poor clinical outcome. TGF-β has been associated with a wide range of tumor-promoting processes, including tumor cell invasion and migration, angiogenesis, immunosuppression, as well as tumor stem cell maintenance and protection. Therefore, blocking the TGF-β signaling pathway *via* inhibition of TGF-β expression appears as an attractive therapeutic intervention in Oncology.

We have previously reported the rational design and preliminary outcome of an extensive discovery program for identification of antisense oligodeoxynucleotides targeting the various TGF-β isoforms. We present here ASPH_0047, a 17-mer LNA-modified gapmer based on the sequence of the human TGF-β2 mRNA, as novel preclinical development candidate. ASPH_0047 shows potent and selective target downregulation (mRNA and protein) in various cell-based assays. Preliminary preclinical biodistribution studies in rodent point at tissue penetration in expected target organs (e.g., liver, kidney, spleen) and accumulation in tumor tissue consistent with observed target downregulation and efficacy in relevant *in vivo* experimental antitumor models. Key pharmacology properties and preclinical features of ASPH_0047 supporting rapid advancement to clinical development will be presented.

TGF-β multi-modal tumor-promoting effects

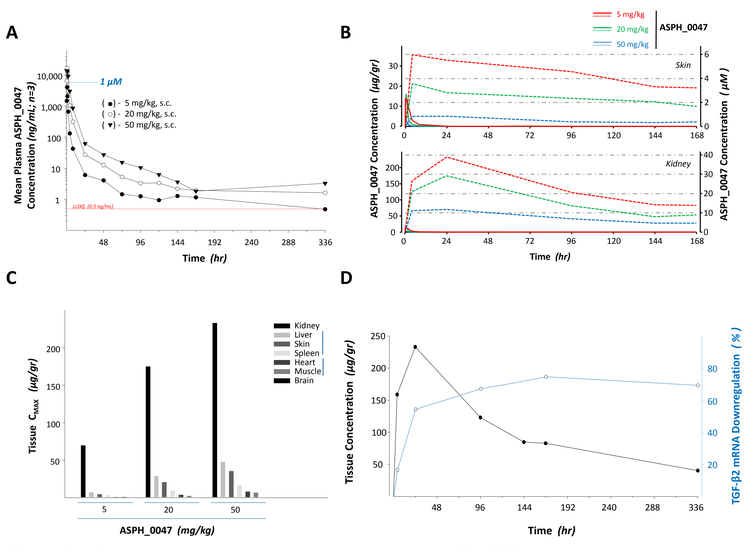


ASPH_0047: a LNA-modified antisense oligodeoxynucleotide targeting the human TGF- β2 mRNA



ASPH_0047 represents an oligodeoxynucleotide molecule consisting of 17 nucleotides linked by phosphorothioate bonds, and locked nucleic acids in the 'first' (5'-end) and 'last' (3'-end) 4 nucleotide positions.

Pharmacokinetic/pharmacodynamic study in mouse following single subcutaneous administration of ASPH_0047 at 5, 20 or 50 mg/kg



Method: *Balb/C mice were treated with a single subcutaneous injection of ASPH_0047 in sterile physiological saline at doses of 5, 20 and 50 mg/kg animal body weight. Samples of plasma, liver, kidney, spleen, skin, brain, leg muscle and heart were collected (from 3 individual animals), immediately snap-frozen and stored at -80°C until analysis with an AEX-HPLC method or for measurement of target TGF-β and GAPDH mRNA levels by bDNA assay. (**A**) depicts plasma pharmacokinetic profiles (mean plasma concentrations; µg/mL) following administration of ASPH_0047 at 5, 20 and 50 mg/kg; (**B**) illustrates the compared time- and dose-dependent profiles of plasma (solid line), kidney or skin tissue (dashed line) concentrations (in µM or µg/gr); (**C**) represents the compared tissue distribution (C_{MAX} values, µg/gr) in the indicated organs; and (**D**) presents a compared analysis of time-dependent tissue concentration (PK profile; with values expressed in µg/gr) and downregulation of TGF-β2 mRNA (PD profile) in kidney following administration of 50 mg/kg of ASPH_0047.*

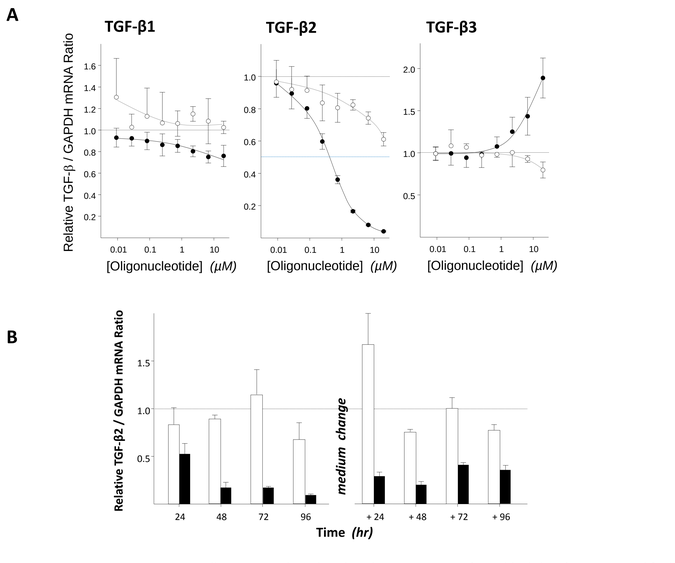
Results:

- Plasma PK after subcutaneous administration of ASPH_0047 displayed a classical biphasic profile, with T_{MAX} within the first 30 min, rapid elimination phase from the plasma (linked to marked tissue distribution), and longer terminal half-life.
- Dose-proportional plasma C_{MAX} at 5 and 20 mg/kg, and apparent 'saturation' at 50 mg/kg were observed (less pronounced when based on AUC values).
- The concentration of ASPH_0047 in all tissues of was dose dependent.
- The highest tissue exposure was observed in kidney, intermediate in liver, skin and spleen, and lowest in brain tissue.
- ASPH_0047 markedly suppressed TGF-β2 mRNA expression in kidney tissue, with effective downregulation observed for at least 14 days.

Conclusions:

1. ASPH_0047 specifically and potently suppressed TGF-β2 mRNA in cellular and xenograft models.
2. Plasma PK and tissue distribution profiles are consistent with the observed target mRNA downregulation in kidney and tumor tissue.
3. Pivotal toxicity and safety pharmacology program is ongoing to enable clinical exploration of ASPH_0047 as novel therapeutic opportunity based on antagonism of the multiple tumor-promoting effects of TGF-β2 isoform.

Concentration- and time-dependent selective downregulation of target mRNA following gymnotic delivery of ASPH_0047 in human Panc1 cells

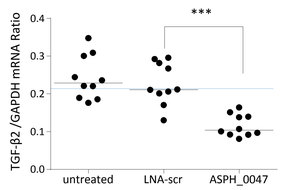


Method: *Human Panc1 pancreatic cancer cells were incubated with either ASPH_0047 (● , black bars) or LNA-modified control oligonucleotide (○ , white bars) and in the absence of any transfecting agent (gymnotic delivery) for 96 hr (**A**) or at indicated times (**B**, left side). After 96 hours, medium was changed and replaced by fresh oligonucleotide-free medium and cells were further incubated for the indicated times (up to 96 hr; **B**, right side). Then, cell extracts were prepared, and TGF-β1, TGF-β2, TGF-β3 and GAPDH mRNA levels were measured by bDNA assay. Results are expressed as mean ± SD of 3 determinations, and are showing TGF-β/ GAPDH mRNA ratio relative to mock transfected cells.*

Results:

- ASPH_0047 potently and specifically suppressed TGF-β2 mRNA with an IC_{50} value of 0.38 µM.
- Target mRNA downregulation was observed at 24 hours after gymnotic delivery of ASPH_0047, and continued to increase for up to 48-96 hours.
- After removal of ASPH_0047, target downregulation remained effective for at least another 96 hours.

Target mRNA downregulation in subcutaneous human 786-O renal cell carcinoma mouse model after ASPH_0047 treatment



Method: *Tumors were induced by a subcutaneous injection of human 786-O RCC cells into the right and left flank of CB17 SCID mice. Treatment started when the tumors reached volumes of 200-300 mm³. Subcutaneous injection of ASPH_0047 or scrambled control oligonucleotide at 50 mg/kg was administered for five consecutive days (QDx5). The study was terminated 24 hours after last treatment. The expression level of TGF-β2 and GAPDH mRNA in 786-O tumors was measured by bDNA assay. TGF-β2 mRNA expression was normalized to GAPDH. Statistical significance was analyzed using the Mann-Whitney test (***p = 0.0001)*

Results:

- ASPH_0047 selectively induced ~50 % downregulation of TGF-β2 mRNA expression in established 786-O subcutaneous tumors after systemic administration of 50 mg/kg for 5 consecutive days, as compared to saline or control oligonucleotide.

- *The authors wish to acknowledge Axolabs (Kulmbach, Germany) and Oncodesign (Dijon, France) for the quality of their technical contribution in the presented studies.*
- *Use of LNA-modified gapmers is performed under a license from Santaris Pharma.*

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