Preclinical Profile of ASPH_0047, a Potent and Selective Antisense Oligonucleotide Targeting Transforming Growth Factor beta 2 (TGF-β2)

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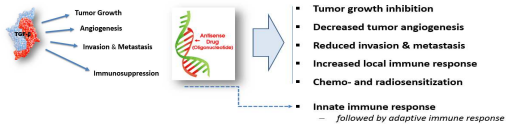
BACKGROUND: Transforming Growth Factor beta (TGF-β) proteins are members of a large family of related cytokines comprised of at least 33 members in mammals encoded by different genes, and which regulate a host of activities ranging from embryonic development to tissue homeostasis. The three bona fide TGF-β isoforms (TGF-β1, -β2 and -β3) play critical, pleiotropic roles in the pathophysiology of various human diseases. In cancer, correlations between TGF-β expression, disease 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.



More specifically, the TGF-β2 isoform has been reported to be a key molecular determinant of immunosuppression and invasiveness, and consequently playing a major role in metastasis. Therefore, inhibiting TGF-β2 appears as an attractive therapeutic intervention in Oncology. Based on the sequence of the human TGF-β2 mRNA, we have identified and engineered ASPH_0047, a 17-mer full phosphorothioate LNA-modified antisense oligodeoxynucleotide '4+4' gapmer, which shows potent and selective target mRNA and protein downregulation in various tumor cell-based assays, and promising anti-tumor activity in animal models.

ASPH_0047 : 5'-**CAAA**GTATTTGGT**CTCC**-3'

In preclinical species, ASPH_0047 features plasma and tissue pharmacokinetics profile similar to previously reported profiles for LNA gapmers, strong metabolic stability and long-lasting tissue distribution with marked tissue penetration in liver, kidney and spleen. Preliminary safety assessment of ASPH_0047 in rats and *Cynomolgus* monkeys upon repeated 30-min infusion consistently points at dose-related stimulation of the immune system in several organs, including accumulation of distended macrophages in lymph nodes, and degenerative renal and liver changes at high doses.

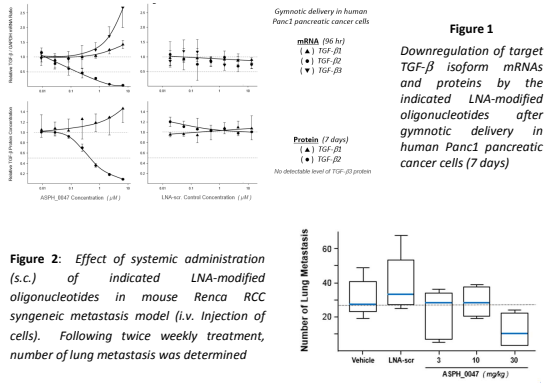


Figure 1

Downregulation of target TGF-β isoform mRNAs and proteins by the indicated LNA-modified oligonucleotides after gymnotic delivery in human Panc1 pancreatic cancer cells (7 days)

Figure 2: *Effect of systemic administration (s.c.) of indicated LNA-modified oligonucleotides in mouse Renca RCC syngeneic metastasis model (i.v. Injection of cells). Following twice weekly treatment, number of lung metastasis was determined*

Figure 3 : *Plasma PK profile following single s.c. administration of ASPH_0047 at indicated doses in tumor-naive immunocompetent mice.*

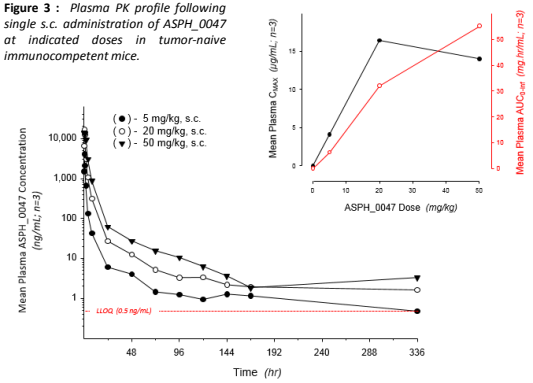


Figure 4 : *Tissue biodistribution profile following single s.c. administration of ASPH_0047 (20 mg/kg) in tumor-naive immunocompetent mice.*

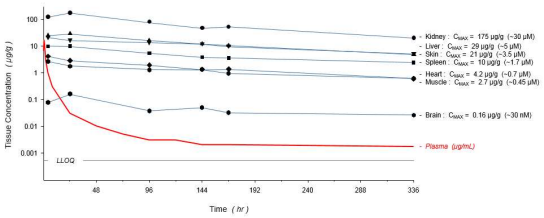


Figure 5 : *Plasma PK profile following multiple s.c. administration (days 0, 3 and 7) of ASPH_0047 (20 mg/kg) in tumor-naive immunocompetent mice.*

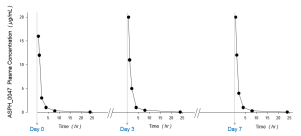


Figure 6 : *Tissue (kidney, skin and skeletal muscle) biodistribution profile following multiple s.c. administration (days 0, 3 and 7) of ASPH_0047 (20 mg/kg) in tumor-naive immunocompetent mice.*

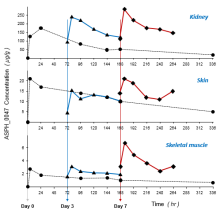


Figure 6 : *Dose-range finding studies in the Wistar Rat and Cynomolgus Monkey treated with the indicated increasing doses of ASPH_0047 (30-min infusion) twice a week for 2 weeks.*

Doses Rat : 0, 10, 30, 75 and 150 mg/kg/adm

Clinical signs
- *No mortality*
- *Reduced body weight gain*
- *Piloerection*
- *Reduced activity*

Histological changes
- *Heart : dose-dependent valvular thickening*
- *Liver and kidneys : dose dependent degenerative changes*
- *Lymph nodes and spleen : evidence of a stimulation of the immune system*

Blood cell count and blood chemistry
- *Increased liver enzyme activities, total bilirubin, creatinine, urea serum levels*
- *Elevated white blood cell counts*
- *Lower values in red blood cell parameters (red blood cell counts, haemoglobin and packed cell volume)*
- *Reduction in coagulation times.*

Doses Monkey : 10, 50 and 100 mg/kg/adm

Clinical signs
- *No mortality*
- *Body weight lost*

Histological changes
- *Kidneys : dose-dependent adverse events (e.g., tubular degeneration/regeneration, basophilia, dilatation and intratubular accumulation of cell debris, accumulation of basophilic granules) in the renal tubular epithelium.*
- *Liver : distended Kupffer cells with finely granular or vacuolated cytoplasm, not associated with other histopathological evidence of liver adverse effects*
- *Lymph nodes : accumulation of distended macrophages with granular/vacuolated cytoplasm similar to those seen in the kidneys*

Blood cell count and blood chemistry
- *no toxicologically relevant effects in haematology and coagulation parameters*

SUMMARY

- ASPH_0047 potently and selectively suppress expression of TGF-β2 mRNA and protein in cell-based assays, linked to anti-metastatic activity in experimental tumor models in vivo at well-tolerated doses
- Observed PK/PD profile and tissue biodistribution in the Mouse following single and multiple administrations consistent with drug-class (LNA-modified oligonucleotides). Main target organs: kidney, liver, spleen and skin
- Plasma PK profile and tissue biodistribution in the Rat and Monkey very similar to that observed with the Mouse (data not shown)
- Preliminary main toxicology observations point at inflammatory/immune reaction in target organs (kidney, liver and lymph nodes)

CONCLUSIONS & PERSPECTIVES

1. We have identified ASPH_0047 as highly potent and selective LNA-modified ASO gapmer targeting TGF-β2 in tumor cell lines and xenograft models (with demonstrated anti-metastatic effect).
2. ASPH_0047 exhibits 'classical' (LNA-modified oligonucleotides) PK/PD parameters in the Mouse with biphasic plasma PK profile (rapid clearance and long $T_{1/2}$), marked and long-lasting tissue biodistribution (main target organs: kidney, liver, spleen and skin), and long-lasting PD activity (in kidney)
3. Expected drug class-related findings have been observed in 2-week dose-range finding studies in the Wistar Rat and *Cynomolgus* Monkey allowing selection of dose range for the IND-/CTA-enabling GLP-toxicology studies

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