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: CAAATTTGATGCTCC

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, including accumulation of distended macrophages in lymph nodes, and degenerative renal and liver changes at high doses.

Summary

1. 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.
2. ASPH_0047 exhibits ‘classical’ (LNA-modified oligonucleotides) pharmacokinetics and 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.

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 T1/2), 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.