Identification and Characterization of Potent and Selective Antisense Oligodeoxynucleotides Targeting TGF-β1 or TGF-β2 mRNA

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BACKGROUND: Transforming Growth Factor beta (TGF-β) represents a family of key cytokines which have been shown to play critical and pleiotropic roles in the biology of several human diseases. In particular, TGF-β is overexpressed to various degrees in many cancers in a spatio-temporal manner, and correlation between expression, disease stage and clinical parameters has been reported. TGF-β, which over-expression has been linked to poor prognosis, is associated with a wide range of biological processes in oncology (***Fig. 1A***), including tumor cell invasion and migration, angiogenesis, immunosuppression, as well as regulation of tumor stem cells properties. Hence, blocking the TGF-β signaling pathways *via* antisense oligonucleotides (ASOs) directed against TGF-β mRNA expression may have a multifold therapeutic benefit by counteracting several key hallmarks of cancer (***Fig. 1B***). The three TGF-β isoforms (TGF-β1, -β2 and -β3) are encoded by different genes but sharing strong sequence and structure (***Fig. 2***), membrane receptor and downstream pathways homology. Despite substantial efforts from academic groups and pharmaceutical companies, relative relevance of each individual isoforms sustaining the overall biology of TGF-β still remains a controversial and poorly documented scientific field. We have then developed a rich pipeline of novel potent LNA-modified antisense oligodeoxynucleotide gapmers designed from the various TGF-β isoform mRNA sequences in order to generate appropriate tool agents for research purposes and potential therapeutic drug candidates. Several hundreds of molecules selectively targeting TGF-β1, -β2 or -β3 mRNA have been designed, produced and tested in various cell-based assays and animal tumor models.

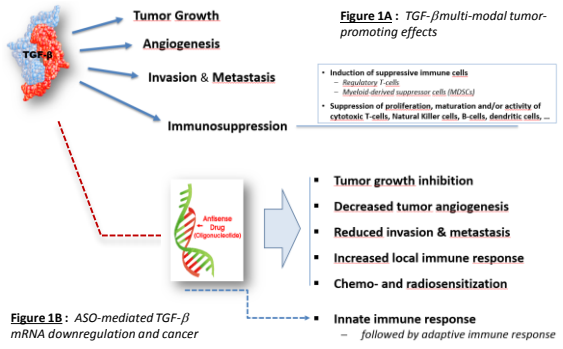
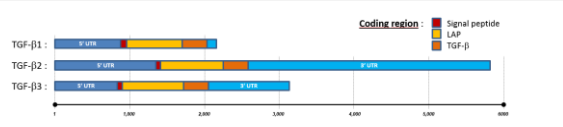


Figure 1A : *TGF-β multi-modal tumor-promoting effects*

Figure 1B : *ASO-mediated TGF-β mRNA downregulation and cancer*



Species	TGF-β1 vs TGF-β2	TGF-β1 vs TGF-β3	TGF-β2 vs TGF-β3
Human	71 (86)	77 (87)	80 (92)
Mouse	72 (85)	78 (87)	79 (91)
Chicken	65 (82)	71 (83)	80 (92)
Zebrafish	56 (74)	61 (75)	78 (90)

Overall % aminoacid sequence homology. Numbers in parenthesis represent sequence homology in the TGF-β protein sequence

Figure 2 : *Messenger RNA (TOP) and aminoacid (BOTTOM) structure and sequence homology between TGF-β isoforms*

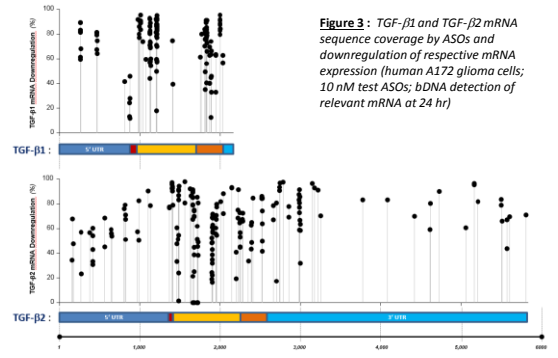


Figure 3 : *TGF-β1 and TGF-β2 mRNA sequence coverage by ASOs and downregulation of respective mRNA expression (human A172 glioma cells; 10 nM test ASOs; bDNA detection of relevant mRNA at 24 hr)*

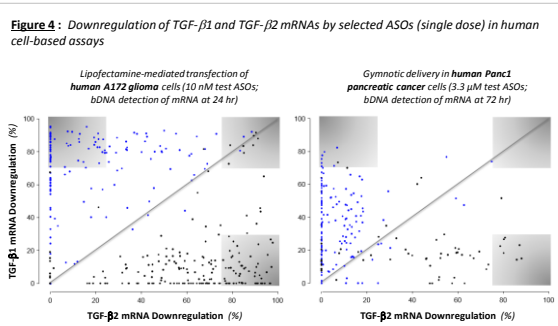


Figure 4 : *Downregulation of TGF-β1 and TGF-β2 mRNAs by selected ASOs (single dose) in human cell-based assays*

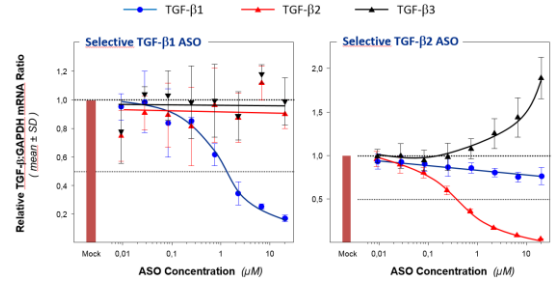


Figure 5 : *Dose-dependent downregulation of TGF-β isoform mRNAs upon gymnotic delivery of selective ASOs in human Panc1 pancreatic cell-based assays (bDNA detection of mRNA at 96 hr)*

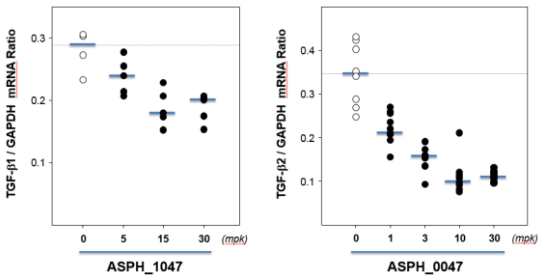


Figure 6 : *Downregulation of TGF-β1 (LEFT) and TGF-β2 (RIGHT) mRNAs by selected ASOs in Nude mouse kidneys[5]*

[5] : kidneys were used as surrogate organs for tumors. Target mRNA downregulation in tumors was confirmed with ASPH_0047 (data not shown) and analysis is ongoing with ASPH_1047

SUMMARY

- LNA-modified ASO gapmers have been designed from either human TGF-β1 or TGF-β2 mRNA sequence; covering both coding and untranslated regions
- Wide variety of potent ASOs have been identified with a wide spectrum of TGF-β isoform selectivity
- Potent target mRNA downregulation is achieved in cell-based assays and in animal models upon systemic administration

CONCLUSIONS & PERSPECTIVES

1. Although not yet therapeutically successfully exploited, TGF-β isoforms represent attractive molecular targets for therapeutic intervention in Oncology (multi-modal cancer-promoting effects)
2. We have designed highly selective LNA-modified ASO gapmers targeting the TGF-β isoforms (TGF-β3 program ongoing) with demonstrated high potency in cell-based assays and in several human xenograft models upon systemic administration
3. We are exquisitely positioned to explore potential therapeutic benefit of selective TGF-β isoform antagonists either as single agents, or in combination modalities

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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