Next-Generation Antisense Oligonucleotides Targeting TGF-β mRNAs

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BACKGROUND: Transforming Growth Factor beta isoforms (TGF-β1, -β2 and -β3) are cytokines encoded by different genes but sharing strong and structure homology (*Fig. 1A*). TGF-β has been shown to play critical and pleiotropic roles in the biology of several human diseases. In particular, the different TGF-β isoforms are overexpressed to various degrees in many cancers in a spatio-temporal manner. Correlations between expression, disease stage and clinical parameters have been reported and over-expression linked to poor prognosis. TGF-β is associated with a wide range of biological processes in oncology including tumor cell invasion and migration, angiogenesis, immunosuppression, as well as regulation of tumor stem cells properties (*Fig. 1B*). Hence, selectively blocking the TGF-β signaling pathways *via* antisense oligonucleotide-mediated inhibition of TGF-β expression may have a multifold therapeutic benefit.

Isarna Therapeutics[(§)] has launched an extensive discovery program for the identification of novel potent 'next-generation' antisense oligodeoxynucleotides (ASOs) based on the various TGF-β isoform mRNA sequences. Several hundreds of ASOs selectively targeting TGF-β1 or TGF-β2 mRNA, or simultaneously targeting both TGF-β1 and TGF-β2 (dual 'inhibitors') or all three TGF-β isoforms (*pan*-specific 'inhibitors') have been designed, produced (with a wide range of nucleotide chemical modifications[(§§)] and Gap-mer structures) and tested in various cell-based assays and animal xenograft models.

[(§)] : *formerly Antisense Pharma*

[(§§)] : *company is working under a license from Santaris Pharma*

Figure 1A : *TGF-β mRNA* ***(a)*** *and protein* ***(b)*** *sequence and structure homologies between isoforms and across species.*

Only three bonafide TGF-βs (TGF-β1, -β2 and –β3) that are distinguished from the rest of the TGF-β superfamily by being the only ligands that bind to and signal via the type II TGF-β receptor (TbRII)

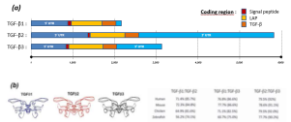


Figure 1B : *TGF-β represents an attractive therapeutic target that has multi-modal cancer-promoting effects.*

In human:
- Largely overlapping functions
- Different spatial and temporal expression patterns
- Overexpression in many tumors

EXPERIMENTAL DESIGN

- *In vitro* experiments: Human A-172 glioma or Panc1 pancreatic carcinoma cells – maintained in 2D cell culture - were treated with the indicated ASO concentrations by either lipofectamine-aided lipofection or gymnotic delivery for the indicated treatment periods. Subsequently cells were lyzed and analyzed for TGF-β mRNA expression using the bDNA assay. TGF-β mRNA levels were normalized to corresponding GAPDH mRNA levels. IC_{50} values from dose-response experiments were obtained using the program XIfit.
- *In vivo* experiments: Human 786-O renal cancer cells or Panc1 pancreatic cancer cells were implanted in the flank of CB17 SCID or Balb/c nude mice, respectively. Mice with established subcutaneous tumors (150-300 mm^3) were treated subcutaneously with the indicated doses of TGF-β2 specific ASOs daily for five consecutive days. Animals were sacrificed 24 hr after the last administration, and TGF-β mRNA levels were determined in tumors using the bDNA assay. TGF-β mRNA levels were normalized to corresponding GAPDH mRNA expression levels.

Figure 2 : *LNA-modified ASO gapmers are more potent than ENA-gapmer counterparts in suppressing TGF-β2 mRNA expression after lipofection as well as after gymnotic delivery in cell-based assays*

LNA- or ENA-modified ASO gapmers of different lengths (13mer, 14mer, 16mer and 17mer) directed against the TGF-β2 mRNA were tested in human A-172 glioma cells after lipofection (***Top***), and in human Panc-1 cells after gymnotic delivery (***Bottom***). TGF-β2 mRNA levels were determined 24 hr (lipofection) or 72 hr (gymnotic) after start of transfection. IC_{50} values for the 13mer and 16mer ENAs after gymnotic transfection could not be calculated from dose-response experiments (up to 10 µM)

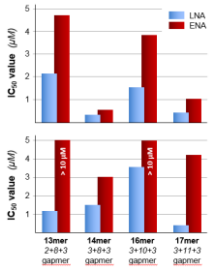
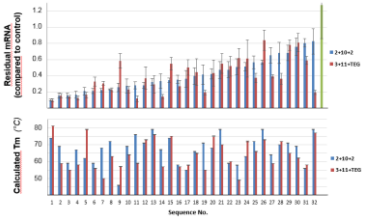


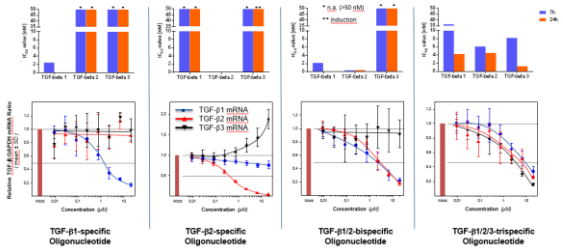
Figure 3 : *ASO sequence and modification pattern determine potency of LNA-modified ASOs in cell-based assays*

14mer ASO sequences specific for the TGF6b2 mRNA modified either using a 2+10+2 LNA-gapmer, or a 3+11+teg modification pattern were transfected by lipofection (10 nM) into human A-172 glioma cells. TGF-b2 mRNA levels were determined 24 hr after start of lipofection.



Theoretical melting temperatures (Tm) were calculated using the LNATM Oligo Tm Prediction Tool from Exiqon

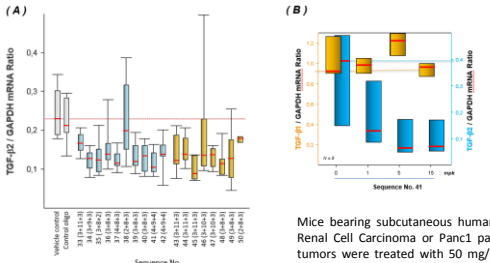
Figure 4 : *LNA-modified ASOs potently and specifically suppress mRNA of TGF-b isoforms after lipofection and after gymnotic delivery*



Top panels: Human A-172 glioma cells were treated with different concentrations of LNA-modified ASOs by lipofection. TGF-β mRNA levels were determined 7 or 24 hr after start of transfection. IC_{50} values are shown.

Lower panels: Human Panc-1 pancreatic cancer cells were treated with different concentrations of LNA-modified ASOs by gymnotic delivery. TGF-β mRNA levels were determined 96 hr after start of delivery.

Figure 5 : *TGF-β2 specific ASOs potently and specifically suppress TGF-b2 mRNA in human 786-O RCC* ***(A)*** *or Panc1 pancreatic* ***(B)*** *subcutaneous tumor models*



Mice bearing subcutaneous human 786-O Renal Cell Carcinoma or Panc1 pancreatic tumors were treated with 50 mg/kg body weight (***A***) or the indicated doses (***B***) of TGF-β2 specific ASOs daily, s.c., for five consecutive days. Animals were sacrificed 24 hr after last administration and tumor TGF-β mRNA levels were determined

SUMMARY

In cell-based assays:

- LNA-modified ASOs have higher potency (downregulation of target mRNA) than ENA-modified counterparts
- Comparison of different modification patterns show high contribution of sequence for efficacy with modulating effect of modification pattern in some cases
- Most active LNA-modified ASOs potently and specifically suppress the different TGF-β isoforms with IC_{50} values in the picomolar range (lipofection) and submicromolar range (gymnotic delivery)

In xenograft models:

- After systemic subcutaneous administration, LNA-modified ASOs targeting TGF-β2 potently and selectively suppress target mRNA expression in established subcutaneous tumors (and in kidneys, *data not shown*) at doses in the single-digit mg per kg body weight range

CONCLUSIONS & PERSPECTIVES

1. TGF-β isoforms represent attractive molecular targets for therapeutic intervention in Oncology (multi-modal cancer-promoting effects)
2. We have designed highly selective 'next-generation' ASOs targeting TGF-β isoforms with demonstrated high potency in cell-based assays and in several human xenograft models (TGF-β2)
3. Highly potent and selective TGF-β ASO molecules could provide tool agents and novel therapeutic opportunities for antagonism of the multiple tumor-promoting effects of TGF-β

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