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-β1 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 cell properties (Fig. 1B). Hence, selectively blocking the TGF-β1 signaling pathways via antisense oligonucleotide-mediated inhibition of TGF-β1 expression may have a multifactorial 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 Gapmer structures) and tested in various cell-based assays and animal xenograft models.

**Figure 1A:** TGF-β mRNA (a) and protein (b) sequence and structure homologies between isoforms and across species. Only three bona fide TGF-β1s (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 (TβRII).

**Figure 1B:** TGF-β1 represents an attractive therapeutic target that has multi-modal cancer-promoting effects.

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

**Experimental Design**

In vitro experiments: Human A-127 glioma or Panc1 pancreatic carcinoma cells – maintained in 2D cell culture – were treated with the indicated ASO concentrations by either lipofection-aided lipofection or gemytic delivery for the indicated treatment periods. Subsequently cells were lysed and analyzed for TGF-β1 mRNA expression using the qDNA assay. TGF-β1 mRNA levels were normalized to corresponding GAPDH mRNA levels. IC50 values from dose-response experiments were obtained using the program GraphPad Prism.

In vivo experiments: Human 786-O renal cancer cells or Panc1 pancreatic cancer cells were implanted in the flank of BALB/c nu/nu or nude mice, respectively. Mice with established tumors or subcutaneous tumors (50-300 mm3) 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-β1 mRNA levels were normalized to corresponding tumors by the qDNA assay. TGF-β1 mRNA levels were normalized to corresponding GAPDH mRNA expression levels.

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

LNA- or ENA-modified ASO gammers of different lengths (13mer, 14mer, 16mer and 17mer) directed against the TGF-β2 mRNA were tested in human A-127 glioma cells after lipofection (TOP) and in human Panc-1 cells after gemytic delivery (BOTTOM). TGF-β2 mRNA levels were determined 24 hr (lipofection) or 72 hr (gemytic) after start of transfection. IC50 values for the 13mer and 16mer ENAs after gemytic 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 TGFβ2a mRNA modified either using a 2+10x LNA-gapper, or a 3+11x modification pattern were transfected by lipofection (10 µM) into human A-127 glioma cells. TGF-β2a mRNA levels were determined 24 hr after start of lipofection.

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

**Figure 5:** TGF-β2 specific ASOs potently and specifically suppress TGF-β2 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-β2 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 IC50 values in the picomolar range (lipofection) and submicromolar range (gemytic 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-β1 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-β1 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