Targeting Transforming Growth Factor beta 2 (TGF-β2) Isoform with ISTH0036 - a Potent and Selective Antisense Oligonucleotide - for the Treatment of Glaucoma

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Abstract

Purpose: Transforming growth factor beta (TGF-β) represents a family of cytokines, which function as primary mediators for TGF-β signaling involved in a wide range of biological processes in human diseases, like oncology, fibrosis and ocular diseases. Several different ocular diseases have been associated with TGF-β, including corneal diseases, proliferative vitreoretinopathy, posterior capsule opacification and glaucoma. In patients with primary open angle glaucoma (POAG) increased levels of TGF-β2 were found in the aqueous humor (AH). In POAG the AH outflow resistance is increased, leading to an elevated intraocular pressure. The changes in the outflow region are accompanied by alteration in the composition and amount of the extracellular matrix (ECM) and by changes in the actin cytoskeleton of the trabecular meshwork. The changes in the outflow region seem to be caused by TGF-β signaling and its downstream mediator connective-tissue growth factor (CTGF).

Methods: We have developed ISTH0036, a 14-mer phosphorothioate Locked Nucleic Acid-(LNA) modified antisense oligonucleotide gapmer targeting TGF-β2. In vitro studies have been conducted to characterize the effect of the ASO treatment in relevant primary cells, such as human trabecular meshwork (hTM) cells and murine astrocytes (mAC). To characterize the potential use of these ASOs in ocular diseases, studies have been performed to evaluate the effect of ISTH0036 *in vivo* after intravitreal injection in the mice eyes. The anterior eye segment has been dissected, RNA has been isolated and quantitative PCR has been done to measure the target mRNA downregulation compared to housekeeping genes.

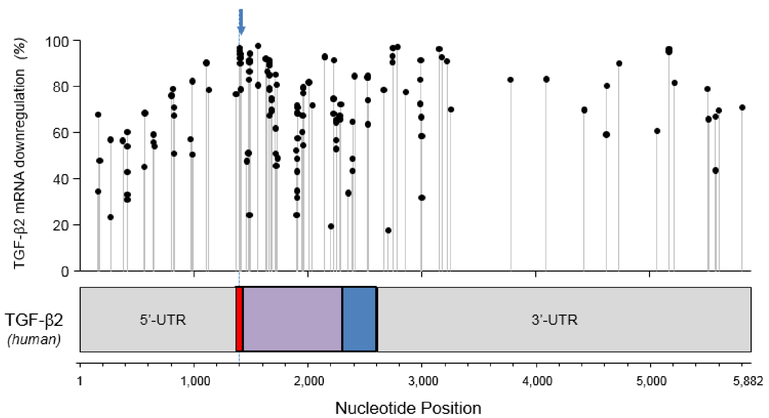
Results: Sequence-specific target downregulation and downstream pathway expression have been carefully analyzed and demonstrated in cell based assays. Intravitreal injection of ISTH0036 into the vitreous body of mice eyes leads to a sequence-specific downregulation of the target mRNA on day 2 and day 5 after injection in the anterior eye segment.

Conclusions: We have clearly demonstrated that ISTH0036 induces sequence-specific target and downstream pathway downregulation in relevant primary cells *in vitro* and in *tissues in vivo*, which makes ISTH0036 a powerful candidate for the treatment of ocular diseases.

ISTH0036: Compound Details

ISTH0036 represents a fully phosphorothioate 14-mer oligodeoxynucleotide with a 3+3 **LNA***-gapmer pattern selectively targeting the TGF-β2 mRNA

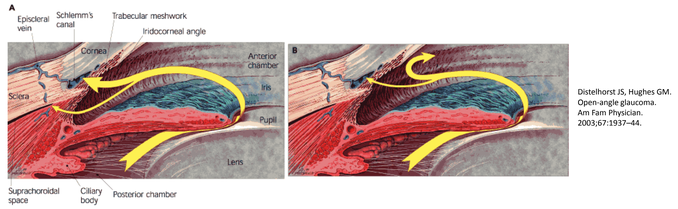
5'- **GA**$^{(Me)}$**C**CAGATGCA**GGA** -3'



Inhibition of TGF-β2 as Target for Multi-Modal Effects in Glaucoma

Various major ocular diseases with high medical need do exist that appear to be TGF-β pathway driven or dependent and could benefit greatly from effective treatment with TGF-β specific ASO, providing attractive development opportunities within the ophthalmic disease landscape.

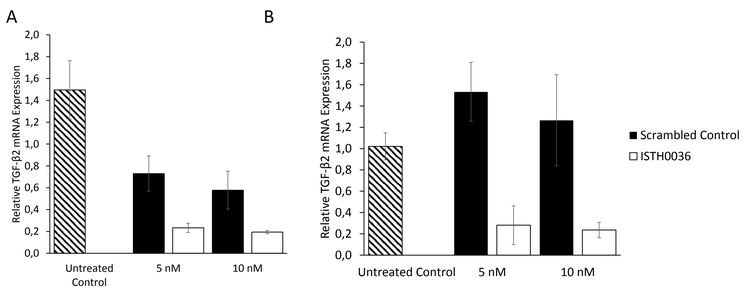
The link between TGF-β and glaucoma, glaucoma filtration surgery, proliferative vitreoretinopathy and posterior capsule opacification is scientifically well substantiated.



Distelhorst JS, Hughes GM. Open-angle glaucoma. Am Fam Physician. 2003;67:1937–44.

Glaucoma is a progressive optic neuropathy characterized by gradually increasing loss of retinal ganglion cells, which manifests clinically with loss of optic disc neuroretinal rim tissue, defects in the retinal nerve fiber layer, and deficits on functional visual field testing. Glaucoma is considered to be caused mainly by a chronical increase in intraocular pressure.

Target mRNA Suppression in Ocular Cells

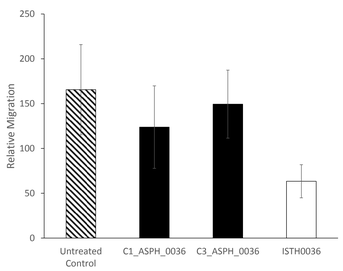


***Method**: Human trabecular meshwork cells (A) or murine astrocytes (B) were treated with 5 nM or 10 nM of either ISTH0036 or scrambled control oligonucleotides (C2_ASPH_0036 in human trabecular meshwork cells and C3_ASPH_0036 in murine astrocytes) by lipofectamine-mediated transfection. Forty eight hours after transfection, cells were lysed and TGF-β2 and GNBL2 mRNA levels were determined by RT-PCR. TGF-β2 mRNA values were normalized to GNB2L and are shown relative to lipofectamine control (set as 1). Results are represented as relative means +/- SD of 4-5 measurements.*

Relevant to the considered clinical indication (ISTH-01-111; first-in-human study), efficacy of ISTH0036 has been tested in primary ocular cells of human or murine origin.

ISTH0036 potently reduces TGF-β2 mRNA levels compared to untreated- or lipofectamine-treated cells in (A) hTM cells and (B) mACs.

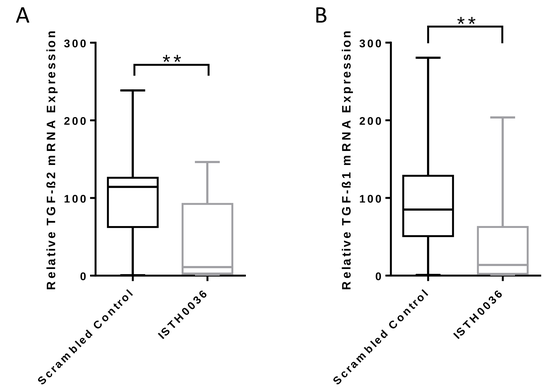
Inhibition of Migration of Murine Astrocytes



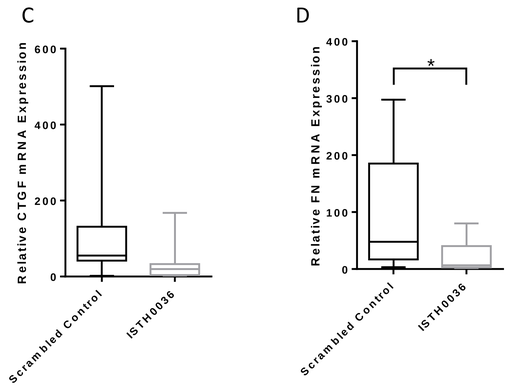
Treatment of mACs with ISTH0036 leads to a significant reduction of cell migration in an *in vitro* scratch assay, whereas scrambled control oligonucleotides had no or much less-pronounced effects.

***Method**: Murine astrocytes were treated with 5 nM of either ISTH0036 or scrambled control oligonucleotides (C1_ASPH_0036 or C3_ASPH_0036) by lipofectamine-mediated transfection. Twenty-four hours after transfection start, scratches of the cell monolayer were set and cellular migration was measured over a period of 6 h. Migration is shown relative to the lipofectamine control (set as 100%). Results are represented as relative means +/- SD of 3-7 measurements.*

Target and Downstream Signaling Pathway mRNA Downregulation in the Trabecular Meshwork after Intravitreal Treatment of Mice



→ ISTH0036 leads to a significant downregulation of TGF-β2 (A) and TGF-β1 (B) in the trabecular meshwork of wildtype CD-1 mice after intravitreal administration.



→ ISTH0036 leads to downregulation of POAG related TGF-β2 downstream signalling proteins like CTGF (C) and FN)(D) in the trabecular meshwork of wildtype CD-1 mice after intravitreal administration.

***Method (A-D)**: ISTH0036 or scrambled control were administered to CD-1 mouse eyes via a single IVT injection (0.3 µg), resulting in a calculated final test item concentration of 10 µM in the vitreous humor. The corneoscleral rim was collected for mRNA down-regulation analysis. TGF-β2 (A), TGF-β1 (B), CTGF (C) and FN (D) mRNA levels were quantified and normalized to corresponding GNB2L1 mRNA values. Data are represented as box plots, in which median values (line), upper and lower quartiles, and 95th and 5th percentiles are indicated (n=13-18).*

Conclusions

- ISTH0036 induces sequence-specific target downregulation in relevant primary cells *in vitro*
- ISTH0036 leads to a significant reduction of cell migration in murine astrocytes in an *in vitro* scratch assay
- ISTH0036 induces sequence-specific target and downstream pathway downregulation in the trabecular meshwork *in vivo*

→ ISTH0036 might be powerful candidate for the treatment of POAG

**Use of LNA-modified gapmers is performed under a license from Roche (formerly Santaris Pharma).*

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