Preclinical Profile of ISTH0036, a Potent and Selective Antisense Oligonucleotide Targeting Transforming Growth Factor beta 2 (TGF-β2) for the Treatment of Ophthalmic Diseases

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Abstract

Purpose: In ophthalmology, several diseases have been linked to the modulation of transforming growth factor beta (TGF-β) expression. Specifically for TGF-β2, a critical role in the pathophysiology of glaucoma has been demonstrated, making this isoform a relevant therapeutic target for a disease which is the leading cause for irreversible blindness in the world. ISTH0036, a 14-mer phosphorodiamidate Locked Nucleic Acid-modified ASO gapper against TGF-β2 mRNA was selected for further testing.

Methods: In vitro, cells were treated with increasing concentrations of ISTH0036 or scrambled control ASO by gynmastic delivery. Cells were lysed and TGF-β2 levels were quantified by ddPCR assay. TGF-β2 protein levels in cell supernatants were determined by ELISA. In vivo, ISTH0036 was administered via intravitreal (IVT) injection to eyes of several preclinical species. Ocular tissues were analyzed for tissue drug concentrations and target mRNA downregulation. The toxicity of ISTH0036 was tested in the rabbit following three IVT administrations at 2-week intervals.

Results: ISTH0036 shows potent and selective downregulation of target mRNA and protein in various cell-based assays. In vivo, fast and marked distribution of ISTH0036 to the posterior tissues was observed, with a Tmax of 24-48 h. The highest mean concentration (114 µg/g) of ISTH0036 was measured in the ciliary body 6 h into the rabbit after 24 h of injection, followed by retina & choroid, optic nerve and sclera. High drug concentrations in posterior eye tissues were observed up to 56 days after a single-IVT injection. ISTH0036 induced in vivo TGF-β2 mRNA downregulation in choroid & retina, optic nerve and lens. Predential safety assessment of ISTH0036 in rabbits demonstrated good tolerability with only dose-related transient local inflammation and delayed lens opacification.

Conclusion: ISTH0036 demonstrated potent target TGF-β2 mRNA downregulation in cell-based assays and in relevant tissues of the eye in various preclinical species. Long-lasting posterior eye tissue distribution was concluded as the observed target engagement. This, combined with the limited toxicity findings in preclinical testing, supported a rapid advancement of ISTH0036 into clinical development.

Compound

ISTH0036 is a fully phosphorothioate 14-mer oligodeoxynucleotide with a 3+3 LNA*-gapper pattern selectively targeting TGF-β2 mRNA

5’- GA(Me) CCAGATGCAAGA -3’

Concentration-dependent Downregulation of TGF-β2 mRNA and Protein

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>TGF-β2 mRNA</th>
<th>TGF-β2 protein</th>
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<tr>
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<tr>
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<tr>
<td>10.0</td>
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<td>0.40</td>
</tr>
<tr>
<td>100.0</td>
<td>0.20</td>
<td>0.20</td>
</tr>
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</table>

Method: Human Retinal pigment epithelial cells were co-cultured with ISTH0036 or the scrambled control oligonucleotide C<sub>15</sub>A<sub>15</sub>U<sub>15</sub>U<sub>15</sub> (A) for 7 days. (B) TGF-β2 and GAPDH mRNA levels were measured in cell extracts by ddPCR assay. Results are expressed as the mean and SD of determinations from a minimum of 3 replicate experiments.

Results: ISTH0036 potently and specifically suppressed TGF-β2 mRNA and protein with IC<sub>50</sub> values of 0.4 and 0.3 µM, respectively.

Inhibition of TGF-β2 as Target for Multi-modal Effects in Ophthalmic Diseases

TGF-β2

- One of the most important cytokines involved in the regulation of cell behavior in ocular tissues
- Predominant TGF-β isoform in the eye and found in large amounts in aqueous and vitreous humor and ocular tissues. Increased expression is reported in various ocular diseases (glaucoma, PXF, IR)
- Enhances gene expression related to tissue fibrosis, ECM remodeling of IOP and inflammation
- Stimulates vascular endothelial cell proliferation and therefore a role in neovascularization is proposed
- Involved in optic nerve head remodeling and deformation of optic nerve apex

4-week Intravitreal (IVT) Toxicity Study in Dutch-Belted Rabbits

Design toxicity study:

Summary of observations:

PK and PD Profile in Ocular Tissues of New Zealand White Rabbits after Single IVT Injection

Method: ISTH0036 was administered to New Zealand White rabbit eyes in a single IVT injection (0.05 µg/µL, 50 µL). Results in a calculated final mean concentration of 10 µg in the vitreous humor. Aqueous and vitreous humor, cornea, lens, iris & ciliary body, choroid & retina, irides, and optic nerve were collected for tissue drug concentration and target TGF-β2 mRNA regulation analysis. Tissue histopathological results are summarized in the Figure. Intraocular pressure (IOP) and β2-mRNA levels were quantified and normalized to corresponding GAPDH mRNA values. Data are represented as box plots, in which median values (line), upper and lower quartiles, and 10th and 90th percentiles are indicated (%).

Results:

- ISTH0036 displayed a biphase PK profile in vitreous humor, with rapid initial clearance from the vitreous humor and only a limited, if any, transfer and delivery to anterior eye tissues (aqueous humor and cornea).
- Fast and marked distribution to the posterior tissues (choroid & retina, ciliary body & iris, optic nerve and sclera) was observed.
- The highest mean concentration of ISTH0036 was measured in the ciliary body & iris (114 µg/g), followed by retina & choroid, optic nerve and sclera (30-40 µg/g).
- Long-lasting TGF-β2 mRNA down-regulation (Target engagement) in choroid & retina, optic nerve and lens up to Day 56 after a single IVT injection.
- Effect was confirmed in day 21 TGF-β2 mRNA down-regulation in choroid & retina and lens on Day 35 after a single injection (130 µg/g: 30-µg µg/mL IVT injection (data not shown).

Conclusions

- Long lasting, potent and selective target downregulation in vitro (TGF-β2 mRNA and protein) and in vivo (TGF-β2 mRNA)
- Long lasting tissue distribution in ocular tissues after IVT administration
- Minor systemic exposure in the blood compartment
- Data supportive of clinical evaluation for treatment of patients with advanced-stage glaucoma
- Clinical Phase I evaluation initiated in April 2015

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