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

Wosikowski, K.[1], Hasenbach, K.[1], Allais, L.[2], Fant, P.[2], Bellebeau, F.[2], Truchot, N.[2], Voute, H.[2], Krampert, M.[3], Roehl, I.[3], Vornlocher, HP.[3], Jaschinski, F.[1], Korhonen, H.[1], Vercruysse, K.[1], Fettes, P.[1], Mala, C.[1], Leo, E.[1], and Janicot, M.[1]

[1]Isarna Therapeutics GmbH, Munich, Germany, [2]WIL Research, Lyon, France, [3]Axolabs GmbH, Kulmbach, Germany

www.isarna-therapeutics.com
k.wosikowski@isarna-therapeutics.com

Presentation No.: 5714

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 phosphorothioate Locked Nucleic Acid-modified ASO gapmer 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 gymnotic delivery. Cells were lysed and TGF-β2 levels were quantified by bDNA 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 T_{MAX} of 24-48 h. The highest mean concentration (114 µg/g) of ISTH0036 was measured in the ciliary body & iris of the rabbit after 24 h of injection, followed by retina & choroid, optic nerve and sclera (30-40 µg/g). 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. Preclinical safety assessment of ISTH0036 in rabbits demonstrated good tolerability with only dose-related transient local inflammation and delayed lens opacification.

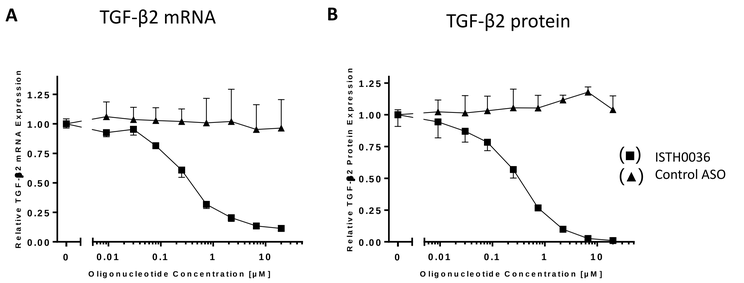
Conclusion: ISTH0036 demonstrated potent target TGF-β2 mRNA downregulation in cell-based assays and in relevant tissues of the eye in various preclinical species. Long-lasting posterior eye tissue distribution was consistent with the observed target engagement. This, combined with the limited toxicity 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***-gapmer pattern selectively targeting TGF-β2 mRNA

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

Concentration-dependent Downregulation of TGF-β2 mRNA and Protein

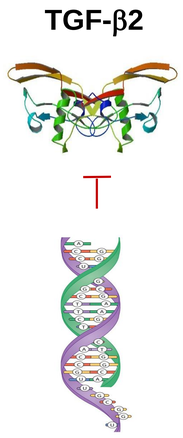


***Method**: Human Panc1 pancreatic cancer cells were incubated with ISTH0036 (■) or the scrambled control oligonucleotide C9_ASPH_0036 (▲) for 7 days.* **(A)** *TGF-β2 and GAPDH mRNA levels were measured in cell extracts by bDNA assay. Results are expressed as mean ± SD of 3 determinations, and are showing TGF-β/ GAPDH mRNA ratio relative to vehicle-treated cells.* **(B)** *TGF-β2 protein in cell supernatants was analyzed by ELISA. Results are expressed as mean and SD of quadruplicates and depicted relative to vehicle-treated cells.*

Results:

- ISTH0036 potently and specifically suppressed TGF-β2 mRNA and protein with IC_{50} values of 0.4 and 0.7 µM, respectively

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

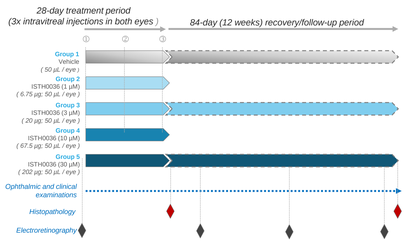


- One of the most important cytokines involved in the regulation of cell behavior in ocular tissues
- Predominant TGF-β isoform in the eye and found in large amounts in aqueous and vitreous humors and ocular tissues. Increased expression is reported in various ocular diseases (glaucoma, PVR, DR)
- Enhances gene expression related to tissue fibrosis, EMT, remodeling of ECM 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 axons

- Glaucoma
- Secondary cataract (PCO)
- Diabetic retinopathy
- Proliferative vitreoretinopathy
- Age-related macular degeneration
- Corneal diseases (pterygium, keratoconus)

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

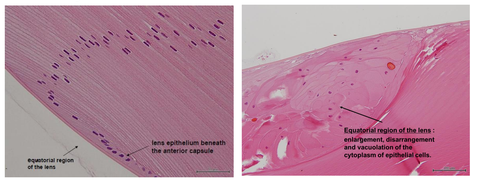
Design toxicity study:



Summary of observations:

Dose in µg per eye:	6.75 (1 µM)	20 (3 µM)	67.5 (10 µM)	202 (30 µM)
Ophthalmic and clinical observations:	No changes No examinations after Day 30	No changes	No changes No examinations after Day 30	Lens opacification as of Day 40 with time-dependent increase in incidence
Histopathology	No changes at Day 30 No examinations after Day 30	No changes at day 30 and Week 16	No changes at Day 30 No examinations after Day 30	Lesions at the equatorial region of the lens at Day 30 and Week 16
ERG	No ERG examinations	No changes in Weeks 6/7, 11 and 16	No ERG examinations	ERG changes observed in Week 6/7 No examination in Weeks 11 and 16

Normal histological structure of the lens



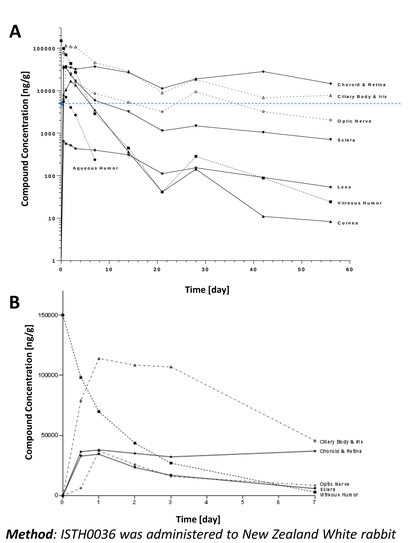
Day 30: Abnormal lens architecture at the equatorial region of the lens

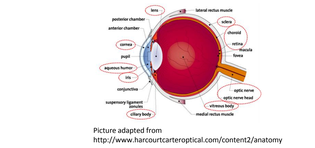
***Method:** Toxicity of ISTH0036 was assessed following three IVT administrations at 2-week intervals to Dutch-Belted rabbits. IVT injections of vehicle (sterile 0.9 % NaCl) or ISTH0036 at doses of 6.75, 20, 67.5 and 202 µg/eye, resulting in calculated test item concentrations of 1, 3, 10 and 30 µM in the vitreous humor, were performed. The main group animals (n=5/sex/group) were sacrificed 48 h after the final dose, recovery animals (n=6/sex/group) 12 weeks after the final dose.*

Results:

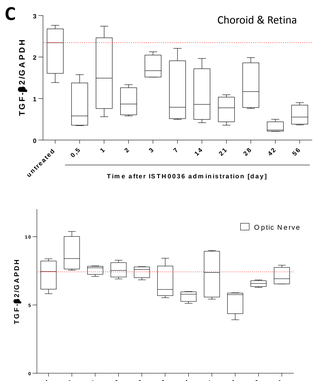
- Drug-related findings were <u>only</u> observed in the highest dose group (*i.e.*, 202 µg/eye; 30 µM group)
  - Histopathological changes of the lens at Day 30 (3 out of 10 animals) and in most animals at the end of the treatment-free period (9 out of 12 animals)
  - ERG changes at week 6/7
  - Opacification of the anterior part of the lens (capsule), starting from Day 40 with time-dependent increase in incidence (3 out of 12 animals on Day 40; and up to 11 out of 12 animals on Day 72 and later)
- NOAEL in the rabbit defined at 20 µg/eye (3 µM dose group)
- Very low plasma levels were observed following IVT injections of 202 µg/eye (30 µM group)

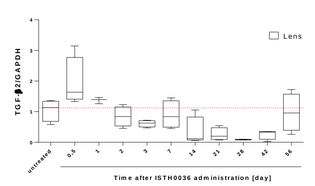
PK and PD Profile in Ocular Tissues of New Zealand White Rabbits after Single IVT Injection





Picture adapted from http://www.harcourtcarteroptical.com/content2/anatomy





***Method**: ISTH0036 was administered to New Zealand White rabbit eyes via a single IVT injection (190 µg/50 µL), resulting in a calculated final test item concentration of 30 µM in the vitreous humor. Aqueous and vitreous humors, cornea, lens, iris & ciliary body, choroid & retina, sclera and optic nerve were collected for tissue drug concentration and target TGF-β2 mRNA down-regulation analysis. Tissue biodistribution results are represented on (**A**) log- and (**B**) linear-scale as mean ISTH0036 concentrations (n=4). (**C**) TGF-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 90th and 10th percentiles are indicated (n=4).*

Results:

- ISTH0036 displayed a biphasic 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 one single IVT injection
- Effect was confirmed in dog: TGF-β2 mRNA downregulation in choroid & retina and lens on Day 35 after one single (300 µg/eye - 30 µM) 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 LNA-modified gapmers is performed under a license from Roche (formerly Santaris Pharma).*

ISARNA THERAPEUTICS