Evaluation of Novel Antisense Oligonucleotides Targeting Transforming Growth Factor beta (TGF-β) Isoforms for the Treatment of Ocular Diseases

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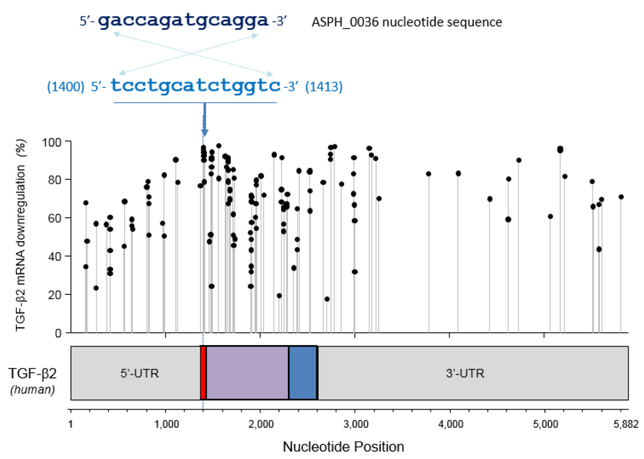
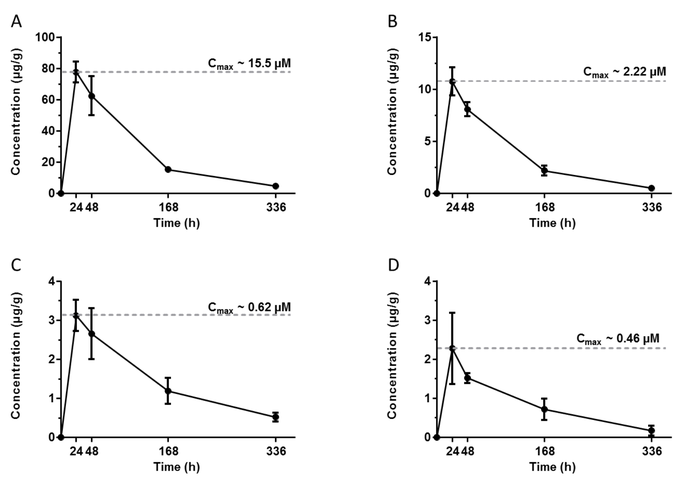
Transforming growth factor beta (TGF-β) represents a family of cytokines, which function as primary mediators for TGF-β signaling involved in a wide range of biological processes in human diseases, and in particular oncology, fibrosis and ocular diseases. Several different ocular diseases have been associated with TGF-β, including glaucoma, proliferative vitreoretinopathy (PVR), posterior capsule opacification (PCO; secondary cataract) and corneal diseases. Isarna Therapeutics has designed and developed highly potent and selective LNA-modified antisense oligonucleotide (ASO) gapmers targeting the various TGF-β isoforms, which have shown marked downregulation of target mRNAs in cell-based assays and tissues *in vivo*. In order to characterize the potential use of these ASOs in ocular diseases, we have initially conducted studies to evaluate the pharmacokinetic properties of the selected ASOs after intravitreal injection in the rabbit eye. In addition, we have analyzed the biodistribution and pharmacodynamic features of our compounds in aqueous humor, vitreous humor, lens, cornea, ciliary body & iris, choroid & retina, optic nerve, sclera tissue samples.

ASPH_0036 represents an LNA-modified antisense oligodeoxynucleotide targeting the human TGF-β2 mRNA



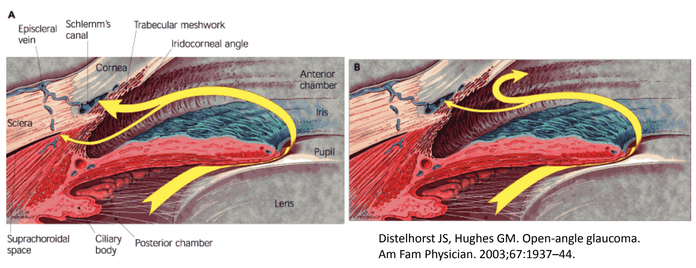
ASPH_0036 is an oligodeoxynucleotide molecule consisting of 14 nucleotides linked by phosphorothioate bonds, and locked nucleic acids in the 'first' (5'-end) and 'last' (3'-end) 3 nucleotide positions.

Pharmacokinetic study in Balb/c mice following single intravenous administration of ASPH_0036 at 20 mg/kg animal body weight



Mean concentrations (± SD; n=5) of ASPH_0036 after single i.v. bolus administration to mice in kidney (A), liver (B), skin (C) and lung (D). Grey dotted lines indicate corresponding C_{MAX} values, expressed in µM, assuming a 1:1 ratio between g and ml units.

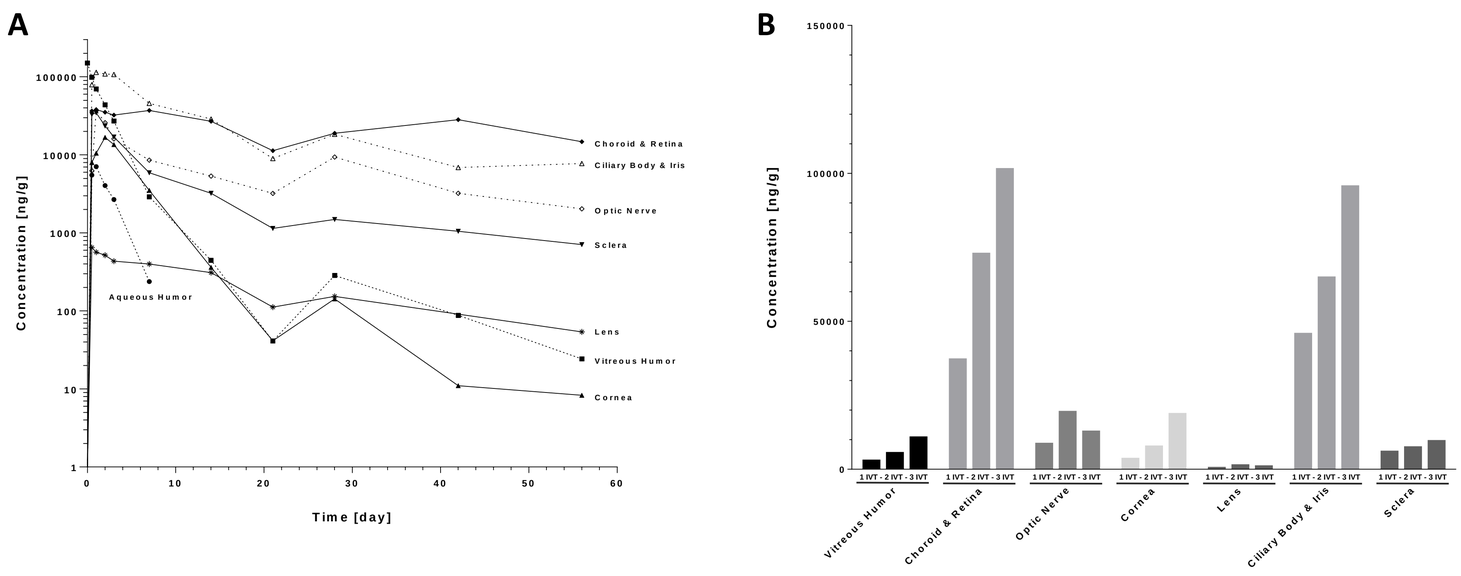
Various major ocular diseases with high medical need do exist that appear to be TGF-β pathway driven or dependent and could benefit greatly from effective treatment with TGF-β specific ASO, providing attractive development opportunities within the ophthalmic disease landscape.
The link between TGF-β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

Pharmacokinetic study in rabbit following single and repeated intravitreal administration of ASPH_0036

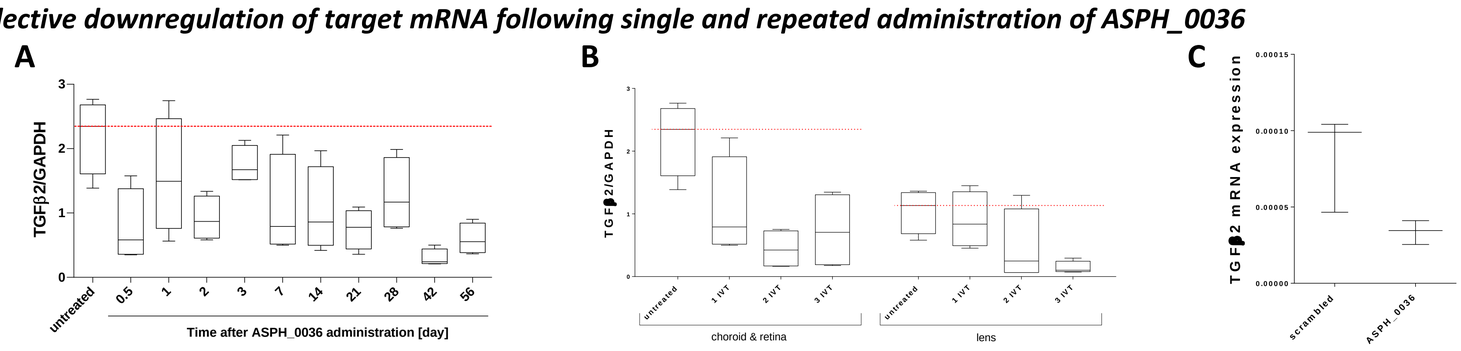


Method: *Healthy New Zealand White rabbits have been treated with either one, two or three i.vt. injections of ASPH_0036 (two weeks apart from each other) in both eyes to reach a final concentration of 30 µM in the vitreous humor. Timepoint of injection was designated as 0 h / day 0.*
Concentration of ASPH_0036 was measured in aqueous humor, vitreous humor, lens, cornea, ciliary body & iris, choroid & retina, optic nerve, sclera tissue samples. The level of TGF-β2 mRNA was measured in lens, cornea, ciliary body & iris, choroid & retina, optic nerve and sclera tissue samples. Time points of analysis have been day 0.5, 1, 2, 3, 7, 14, 21, 28, 42, 56 after single i.vt. injection and day 7 after the second or third i.vt. injection.

Results:

- ASPH_0036 has been injected intravitreally to achieve a final concentration of 30 µM in the vitreous humor. ASPH_0036 was detectable above LLOQ for 12 h, 24 h, 48 h and 72 h in all sampled humors and tissues.
- ASPH_0036 has a very rapid clearance from the vitreous humor and only a limited transfer and delivery to anterior eye tissues (aqueous humor, lens, cornea). There is a fast and prominent distribution in the posterior eye tissues (choroid/retina, ciliary body, optic nerve and sclera) and C_{MAX} appears after 24 h or 48 h.
- The pharmacokinetic of ASPH_0036 in vitreous humor shows a classical biphasic profile with rapid initial clearance (extensive tissue distribution) from the humor ($T_{1/2}\alpha$ ~0.5 day), and much longer terminal half-life ($T_{1/2}\beta$)
- The highest mean concentration (114 µg/g) of ASPH_0036 was measured in the ciliary body & iris after 24 h of injection, followed by retina & choroid (38 µg/g), optic nerve (37 µg/g) and sclera (34 µg/g).
- Multiple injections of ASPH_0036 (two weeks apart from each other) in both eyes showed an accumulation of the compound in choroid & retina, ciliary body & iris, cornea and vitreous humor

Selective downregulation of target mRNA following single and repeated administration of ASPH_0036



Method: *(A, B) Healthy New Zealand White rabbits have been treated with either one, two or three i.vt. injections of ASPH_0036 (two weeks apart from each other) in both eyes to reach a final concentration of 30 µM in the vitreous humor. Timepoint of injection was designated as 0 h / day 0. Target mRNA downregulation was measured in lens, cornea, ciliary body & iris, choroid & retina, optic nerve, sclera tissue samples. Time points of analysis have been day 0.5, 1, 2, 3, 7, 14, 21, 28, 42, 56 after single i.vt. injection and day 7 after the second or third i.vt. injection. (C) Human ciliary body tissue has been isolated from donor eyes, cultured and treated for 48 h with 10 µM of ASPH_0036. The mRNA was isolated and human TGF-β2 levels have been detected via quantitative qPCR and normalized to a housekeeping gene.*

Results:

- Sequence specific target mRNA downregulation was visible in choroid &retina (A), lens and in the optic nerve after single intravitreal administration of ASPH_0036. The target mRNA downregulation was detectable throughout the whole study period for choroid & retina (A), whereas the mRNA reached normal levels after 56 days again in lens and optic nerve (data not shown). Multiple injections increase the downregulation compared to untreated animals in choroid & retina and lens (B).
- Sequence specific target mRNA downregulation was visible human ciliary body primary tissue after a gymnotic delivery with 10 µM ASPH_0036 (C).

Conclusions:

1. The highest concentration of ASPH_0036 was measured in ciliary body & iris, followed by choroid & retina and optic nerve.
2. The highest concentrations of ASPH_0036 were detected 24 h after oligonucleotide administration.
3. Multiple intravitreal administrations lead to accumulation of the ASO in choroid & retina, ciliary body & iris, cornea and vitreous humor.
4. Long-lasting downregulation of target mRNA expression was observed in choroid & retina, lens and optic nerve after ASPH_0036 treatment when compared to vehicle-treated animals. Multiple injections increase the downregulation compared to untreated animals.

ASPH_0036 is a powerful candidate for the evaluation in various ocular diseases!

- *The authors wish to acknowledge Axolabs (Kulmbach, Germany), WIL Research (Lyon, France) and Oncodesign (Dijon, France) for the quality of their technical contribution in the presented studies.*
- *Use of LNA-modified gapmers is performed under a license from Santaris Pharma.*

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