Extended preclinical ocular biodistribution and pharmacodynamic profile of ISTH0036, an antisense oligonucleotide targeting transforming growth factor beta 2 (TGF-β2) for the treatment of ophthalmic diseases

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Abstract

Purpose: A critical role for TGF-β2 in the pathophysiology of several ocular diseases such as glaucoma, age-related macular degeneration or diabetic macular edema has been demonstrated, making this isoform a relevant therapeutic target. To support clinical development in these indications, the ocular biodistribution and pharmacodynamics profile of ISTH0036, a 14-mer phosphorothioate locked nucleic acid-modified antisense oligodeoxynucleotide-gamper, was evaluated in Cynomolgus monkeys following intravitreal administration.

Methods: To assess the time-dependent ocular tissue drug biodistribution and pharmacodynamics effects, ISTH0036 was administered (intravitreal injection) on Day 1 and Day 57 at a dose of 100 µg/eye into Cynomolgus monkey eyes. Furthermore, single administration of the compound at increasing doses of 30, 100 and 300 µg/eye was performed to assess the dose-dependency (on Day 29). An anion-exchange-HPLC method with fluorescence detection was used to analyze tissue concentrations. Target downregulation was determined using a branched DNA assay, and protein concentration in vitreous/aqueous humor was measured with a multiplex ELISA assay.

Results: Long-lasting and time-dependent biodistribution of ISTH0036 to the posterior eye tissues was observed. Similar high ISTH0036 concentrations were measured in the retina, choroid and ciliary body at 300-µg/eye administration (5-6 µg/g, on Day 29). High median drug concentrations in posterior eye tissues were still observed on the last day of measurement (Day 113). ISTH0036 induced in vivo long-lasting and dose-dependent TGF-β2 mRNA downregulation in retina and lens. TGF-β2 protein concentration decreased in the relevant vitreous humor after intravitreal injection of ISTH0036 and this effect was maintained up to Day 113. These results confirm previous findings in the rabbit eye.

Conclusion: ISTH0036 demonstrated potent target TGF-β2 mRNA downregulation in relevant tissues of the Cynomolgus monkey eye. Pronounced long-lasting posterior eye tissue distribution was consistent with observed target engagement. Demonstrated biodistribution and target engagement support further clinical development and provide rationale for Q2M or Q3M administration schedule of ISTH0036 for therapeutic intervention in ophthalmology.

Study Design

Table: 1 or 2 intravitreal (IVT) injection(s), on Day 1 and Day 57 (group 3 only) in both eyes

Group 1: saline (2)
Group 2: ISTH0036 (3 µM)
Group 3: ISTH0036 (10 µM)
Group 4: ISTH0036 (30 µM)
Group 5: ISTH0036 (100 µM)
Group 6: ISTH0036 (300 µM)
Group 7: ISTH0036 (1 µM monkey per group)

Fig. 1: Study design

Day 1 Day 29 Day 57 Day 85 Day 113

Fig. 2A: Time-dependency

Fig. 2B: Time-dependency

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
- Preclinical TGF-β2 isoform is 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, ECM remodeling of ECM and inflammation
- Stimulates ocular neo-vascular cell proliferation and therefore a role in neovascularization is proposed
- Involved in optic nerve head remodeling and demarcation of optic nerve scarring

Drug concentration

Target engagement

TGF-β2 multiplex

- Age-related macular degeneration
- Glaucoma
- Diabetic retinopathy
- Proliferative vitreoretinopathy

Pharmacodynamic Profile in Ocular Tissues of Cynomolgus Monkey after IVT injection

Fig. 3A: Dose-dependent target TGF-β2 mRNA downregulation in retina, lens and optic nerve head

Fig. 3B: Time-dependent target TGF-β2 protein downregulation in vitreous humor

Fig. 3C: Dose and time-dependent TGF-β2 protein downregulation in vitreous humor

Method: ISTH0036 was administered to monkey eyes via one or two IVT injections and ocular tissues collected according to the schema depicted in Fig. 1. (A)Ocular tissues were dissected and immediately snap-frozen for further analysis of target mRNA expression. TGF-β2 mRNA levels were quantified by qPCR assay and results were normalized to GAPDH values. (B) Aqueous and vitreous humor was collected and immediately snap-frozen for further analysis of TGF-β2, β-2 and β-2 protein concentration. Protein levels were determined by electrochemiluminescence-based multiple immunoassay (ECL TGF-β ELISA Kit R&DE, Mesoscale Development LCC). TGF-β2 and β-2 protein levels were not detectable under given experimental procedures. Data are represented as box plots, in which median values (line), upper and lower quartiles, and 10th and 90th percentiles are indicated. * p<0.05 compared to vehicle treated group. Statistical significance was analyzed using non-parametric 2-independent samples Wilcoxon-Mann-Whitney test.

Results:
- Significant time- and dose-dependent target engagement (TGF-β2 mRNA downregulation) in the retina and lens upon IVT administration(s) of ISTH0036
- Significant time- and dose-dependent target engagement (TGF-β2 protein expression) in vitreous humor. Minor effect in the aqueous humor

Conclusions:
- Long lasting tissue distribution in ocular tissues after IVT administration, with minor systemic exposure as indicated by low exposure in kidney
- Long-lasting, potent and selective in vivo target downregulation (TGF-β2 mRNA and protein)
- Data provide rationale for Q2M or Q3M administration schedule of ISTH0036 for therapeutic intervention in ophthalmology
- Data supportive of further clinical evaluation for treatment of patients with ocular diseases

*Use of LNA-modified gapmers is performed under a license from Roche.

Fig. 3: Day 113

Graph: ISTH0036 Concentrations in Ocular Tissues and Kidney Cortex Cynomolgus Monkey Following IVT ISTH0036 Administration

Dataset: ISTH0036 Concentrations in Ocular Tissues and Kidney Cortex Cynomolgus Monkey Following IVT ISTH0036 Administration

Graph: ISTH0036 Concentrations in Ocular Tissues and Kidney Cortex Cynomolgus Monkey Following IVT ISTH0036 Administration

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