Long-term safety and efficacy of ISTH0036 – a selective TGF-β2 blocking antisense oligonucleotide in preclinical and Phase 1 clinical studies.

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Purpose

Transforming Growth Factor beta 2 (TGF-β2) has been widely described as a key cytokine involved in the pathophysiology of ocular disease. ISTH0036 is a 14-mer modified antisense oligodeoxynucleotide selectively targeting TGF-β2 mRNA. It exhibits potent activity in murine models of choroidal neovascularization (CNV). ISTH0036 induced a decrease in neovascularization, vascular leakage, fibrotic development, as well as blockage of epithelial-to-mesenchymal transition. The presented studies explored pharmacokinetics (PK), pharmacodynamics (PD), and toxicity in rabbits and non-human primates (NHP) and long-term safety in a first-in human Phase1 trial to support further developments.

ISTH0036

Fully phosphorothioate-modified 14-mer oligodeoxynucleotide
with a '3+3' **Locked Nucleic Acid** (**LNA**) gapmer pattern

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

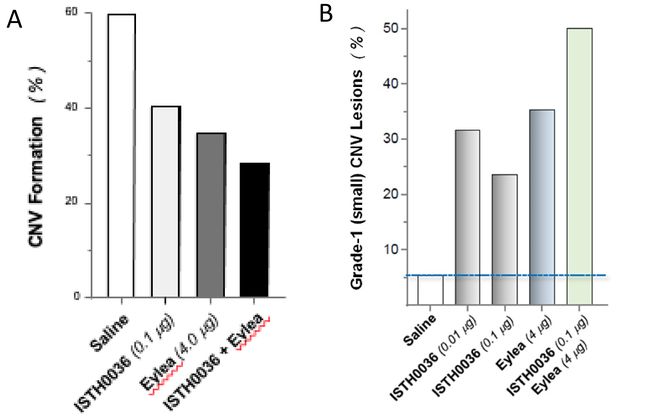
Methods

Long-term safety in Phase 1 was assessed after single ITV injection up to 12mo. PK, ocular tissue distribution, PD (target engagement), and long-term toxicity were studied upon single and repeated IVT administration(s) in rabbits and Cynomolgus monkeys. Results were compared with observations in the CNV model to support development in retinal diseases.

Long-Term Safety and Efficacy in Phase 1 study

- The Ph1 study population consisted of 12 pats with primary open-angle glaucoma (POAG) who were scheduled for trabeculectomy. They received a single IVT dose of ISTH0036 (6.75µg, 22.5µg, 67.5µg, or 225µg i.e., a concentration of 0.3, 1, 3 or 10 µM in the vitreous) at the end of trabeculectomy.
- Data on primary EP on safety and effects on intraocular pressure (IOP) at month 3 have been published before (1)
- Long-term follow-up to 12mo showed sustained efficacy, significantly more in the 2 higher doses and IOP remained clearly below pre-surgical /BL IOP values
- One of the 41 TEAEs was considered possibly related to ISTH0036, intravitreal injection and primary surgery: cataract (a known complication after trabeculectomy) in 1 pat treated with 10 µM, occurring during the safety FU
- There were no deaths or DLTs and no TEAE led to premature termination of the study participation.

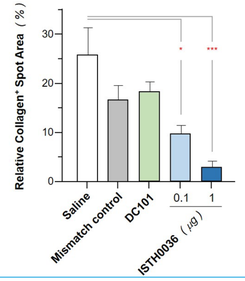
Efficacy of ISTH0036 and/or aflibercept on vascular leakage



CNV was induced in male C57BL/6JRj mice (8-10 animal/group). Animals were treated via single IVT injection in lasered eyes with either saline; 0.01-µg ISTH0036 (vitreous humor C_0~0.3 µM); 0.1-µg ISTH0036 (vitreous humor C_0~3 µM); 4-µg aflibercept (vitreous humor C_0~0.5 mg/mL); or ISTH0036 (0.1 µg)/aflibercept combination regimen. On Day 14, vascular leakage areas were determined, and **(A)** percentage of lesions and **(B)** percentage of grade-1 lesions was determined for each group.

- Administration (single IVT injection) of ISTH0036 or aflibercept markedly reduced the presence of CNV lesions **(A)**
- The percentage of grade-1 (small) lesions increased **(B)**, indicating that ISTH0036 not only decreased the lesion number but the remaining lesions were smaller
- Trend for better efficacy with ISTH0036/aflibercept combination treatment

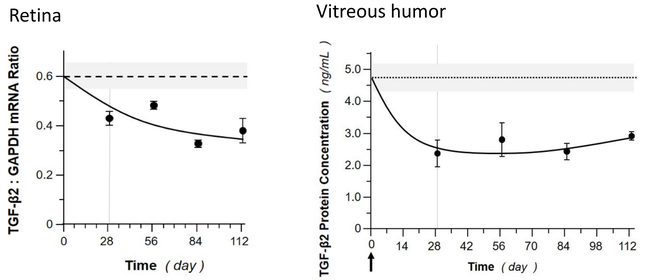
Effect on fibrosis development in the retinal lesions



Sirius Red positive area (± SD; n=4) after IVT injection of saline, ~6.0 µg DC101 (rat anti-mouse VEGFR2 antibody), ~1-µg of mismatch control oligonucleotide, or the indicated doses of ISTH0036 to mice following laser burns (performed on day 0) were determined. Collagen positive area (expressed as %) was measured on day 28. Student's t-test was used to determine the significance of the differences between vehicle control and test items (* $p < 0.05$).

- ISTH0036 inhibited dose dependently the development of fibrosis
- ISTH0036 more potently reduced the number of collagen positive areas than DC101

Long lasting target downregulation in retina and vitreous humor



Target mRNA and protein (TGF-β2) expression was analyzed - after single IVT injection of ISTH0036 in both eyes (100µg in 50-µL saline per eye, to achieve an approximate initial test item concentration of 10µM in the vitreous humor) of Cynomolgus monkeys (NHP)

- Long-lasting (up 4-months) sequence-specific downregulation of TGF-β2 mRNA in retina
- Long lasting TGF-β2 protein reduction in the vitreous humor

Preclinical safety evaluation

Pivotal GLP toxicology studies (up to 9-months) in DB rabbits and Cynomolgus monkeys identified cataractogenic processes as dose limiting toxicity. Histologically, lens disorganization, characterized by enlargement and fragmentation of the fibers at the equatorial region and beneath the posterior and anterior capsule were observed. ERG examination revealed ISTH0036-related changes at the high dose levels only (30-fold above the NOAEL).

Conclusions

- Compared tests of ISTH0036 in rabbits and NHPs resulted in similar toxicity profile, although with higher dose-dependent sensitivity observed in rabbits
- Pronounced and long-lasting time- and dose-dependent ocular tissue distribution and target engagement in retina and vitreous humor was observed, with longer effects observed in NHPs
- Aligned with biological efficacy demonstrated in preclinical models, and evidence of safety in a first-in-human study (1), these results strongly support the planned Phase 2 development of ISTH0036 in wAMD and DME which will start mid 2021

Financial Disclosure : *All authors are active consultants or have been consultants in the past for Isarna Therapeutics GmbH, i.e., the company that develops ISTH0036*

(1) Reference Pfeiffer et al. First-in-human phase I study of ISTH0036, an antisense oligonucleotide selectively targeting transforming growth factor beta 2 (TGF-β2), in subjects with open-angle glaucoma undergoing glaucoma filtration surgery. PLoS One. 2017 Nov 30;12(11):e0188899. doi: 10.1371/journal.pone.0188899.

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