In Situ Hybridization Demonstrates Ocular Distribution of Ixo-vec IVT Gene Therapy in Human Cadaver Eye with wAMD and Supports Durable Clinical Efficacy

4141

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BACKGROUND

- Ixoberogene soroparvovec (Ixo-vec) is an AAV gene therapy employing the best-in-class AAV.7m8 capsid for intravitreal delivery (IVT). This therapeutic approach has been shown to provide sustained intraocular aflibercept production for the treatment of neovascular age-related macular degeneration (nAMD). Clinical trials OPTIC (NCT03748784) and LUNA (NCT05536973) have demonstrated that a single IVT dose of Ixo-vec can substantially reduce or eliminate the need for repeated anti-VEGF injections.
- While the intraocular distribution of AAV.7m8 has been previously investigated in animal models, this report presents the first analysis of the biodistribution of the AAV.7m8-based Ixo-vec in a human eye 3.5 years after administration of a single IVT injection (2E11 vg/eye) to a study participant with nAMD in the OPTIC trial.

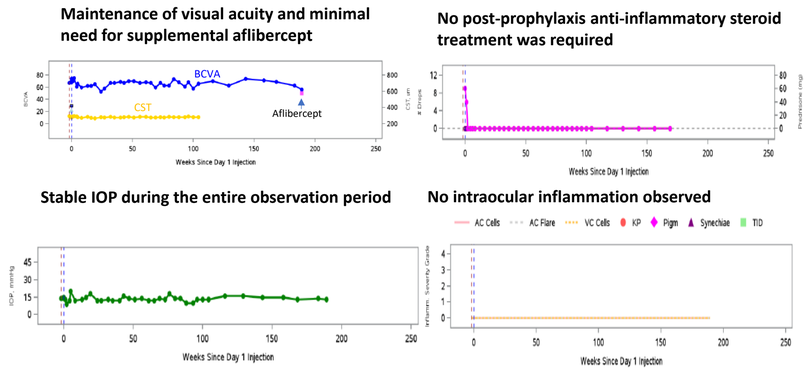
METHODS

- Tissues were fixed in 10% neutral buffered formalin for 24 hours upon receipt (48 hours postmortem) and embedded in paraffin.
- Ixo-vec vector genome DNA was detected using a probe designed against the engineered promoter.
- Aflibercept mRNA was detected using a 1-ZZ paired probe flanking the intron in 5′ UTR in the expression cassette. The probe generates a signal after the 5′ UTR intron has been spliced, which allows for the precise detection of transgene mRNA without vector DNA interference.
- Counter staining was performed using Gil's Hematoxylin.

CLINICAL OBSERVATIONS

- The patient exhibited a robust clinical response, as demonstrated by preservation of best-corrected visual acuity (63 ETDRS letters vs. 67 at baseline) and sustained anatomic control (central subfield thickness, 302 µm vs. 299 µm at baseline) at last study visit with only a single supplemental aflibercept injection (2 months prior to death) administered over 3.5 years (vs. 36 anti-VEGF injections in the previous 6.3 years) (Figure 1).
- No drug-related ocular or systemic adverse events were reported.

Figure 1. Single Ixo-vec Dose Resulted in Sustained Visual Acuity and Eliminated Need for Repeated Injections for 3.5 Years

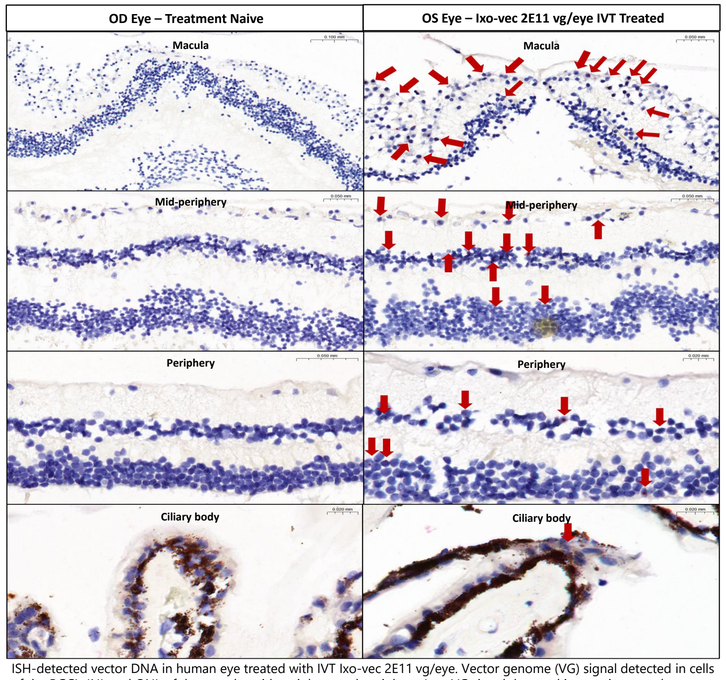


RESULTS

Ocular distribution of IVT-delivered Ixo-vec vector genome DNA:

- Analysis of vector biodistribution revealed widespread transduction of cells in the neural retina, specifically, in the macular, mid-peripheral, and peripheral regions.
- Transduction was primarily observed in retinal ganglion cells and cells of the inner nuclear layer with minimal transduction observed in photoreceptors and nonpigmented epithelium of the ciliary process (Figure 2).
- No transduction was observed in the retinal pigmented epithelium (RPE).
- No transduction was observed in treatment naïve OD eye (Figure 2).

Figure 2. Ocular Distribution of IVT Administered Ixo-vec

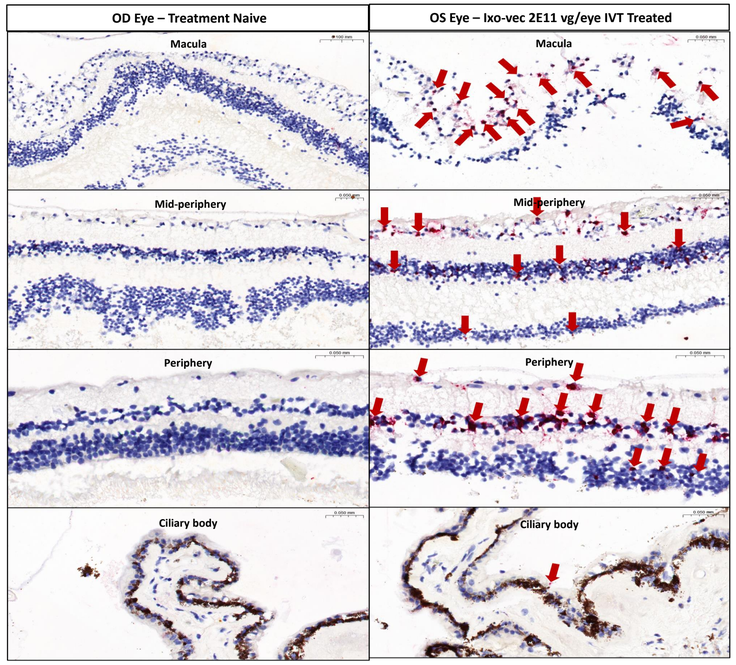


ISH-detected vector DNA in human eye treated with IVT Ixo-vec 2E11 vg/eye. Vector genome (VG) signal detected in cells of the RGCL, INL and ONL of the macula, mid-periphery and periphery. Low VG signal detected in nonpigmented epithelium of the ciliary body. RGCL: *retinal ganglion cell layer. INL: inner nuclear cell layer. ONL: outer nuclear layer. Red arrows: detected VG signal.. Red: vector DNA. Purple: nuclei.*

Ocular distribution of IVT-delivered Ixo-vec aflibercept mRNA:

- Aflibercept mRNA expression was identified primarily in retinal ganglion and inner-nuclear layer cells of the macula and far-periphery of the retina. Occasional expression was observed in retinal photoreceptors, and infrequently in the nonpigmented epithelium of the ciliary process.
- No expression was observed in the RPE.
- No aflibercept mRNA expression was observed in the Treatment Naïve OD eye (Figure 3).

Figure 3. Ixo-vec Drives Most Prominent Expression of Aflibercept in the Macula and Retina Periphery



ISH-detected aflibercept mRNA in human eye treated with IVT Ixo-vec 2E11 vg/eye. mRNA signal detected in cells of the RGCL, INL and ONL of the macula, mid-periphery, and periphery. Low mRNA signal detected in nonpigmented epithelium of the ciliary body. RGCL: *retinal ganglion cell layer. INL: inner nuclear cell layer. ONL: outer nuclear layer. Red arrows: detected mRNA signal. Red: aflibercept mRNA. Purple: nuclei.*

CONCLUSIONS

- This study presents the first ever cell-level mapping of intraocular transduction pattern and transgenic aflibercept expression in human retina following IVT gene therapy.
- The sustained presence of Ixo-vec vector DNA and aflibercept mRNA at 3.5 years post injection correlates with the durable suppression of fluid and maintained visual acuity in the patient.
- No vector transfer detected in the treatment naive eye, suggesting the absence of vector transfer between fellow eyes.
- Extensive transduction, with the most prominent aflibercept mRNA signals concentrated in the macula and peripheral retina. Notably, retinal transduction in humans was markedly broader than observed in NHP model (for NHP biodistribution data see poster presentation by Kris Poulsen, May 8, 2025, from 2:00 PM to 3:45 PM).
- The observed pattern of transduction and mRNA expression with prominent concentration in the central retina contributes to best-in-class efficacy of Ixo-vec in the OPTIC and LUNA clinical studies.

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Disclosures: ***The authors are employees at Adverum Biotechnologies, Inc., and hold shares at the company***