

# Long-Term Safety Evaluation of Continuous Intraocular Delivery of Aflibercept by the Intravitreal Gene Therapy Candidate ADVM-022 in Nonhuman Primates

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**Purpose:** To evaluate the long-term safety of vascular endothelial growth factor (VEGF) suppression with sustained aflibercept expression after a single intravitreal injection (IVI) of ADVM-022, an anti-VEGF gene therapy, in non-human primates (NHPs).

**Methods:** Non-human primates received bilateral IVI of ADVM-022, a gene therapy vector encoding aflibercept, a standard of care for the treatment of VEGF-based retinal disease. Aflibercept levels from ocular fluids and tissues were measured. Ocular inflammation was assessed by slit lamp biomicroscopy and funduscopy. The integrity of the retinal structure was analyzed by optical coherence tomography and blue light fundus autofluorescence and electroretinography was performed to determine retinal function. Histologic evaluation of the retina was performed at the longest time point measured (2.5 years after injection).

**Results:** Sustained expression of aflibercept was noted out to the last time point evaluated. Mild to moderate inflammatory responses were observed, which trended toward spontaneous resolution without anti-inflammatory treatment. No abnormalities in retinal structure or function were observed, as measured by optical coherence tomography and electroretinography, respectively. RPE integrity was maintained throughout the study; no histologic abnormalities were observed 2.5 years after ADVM-022 IVI.

**Conclusions:** In non-human primates, long-term, sustained aflibercept expression and the resulting continuous VEGF suppression by a single IVI of ADVM-022, appears to be safe, with no measurable adverse effects on normal retinal structure and function evaluated out to 2.5 years.

**Translational Relevance:** Together with the results from previous ADVM-022 preclinical studies, these data support the evaluation of this gene therapy candidate in clinical trials as a potential durable treatment for various VEGF-mediated ophthalmic disorders.

## Introduction

Inhibitors of vascular endothelial growth factor (VEGF) have revolutionized the treatment of retinal and choroidal vascular disorders, including wet or neovascular age-related macular degeneration (nAMD), diabetic retinopathy (DR), diabetic macular edema (DME), and macular edema associated with retinal vein occlusion (RVO). The current standard of

care for these conditions includes repeated intravitreal (IVT) administration of anti-VEGF therapeutics, including the US Food and Drug Administration–approved aflibercept (Eylea, Regeneron Pharmaceuticals, Tarrytown, NY), ranibizumab (Lucentis, Genentech/Roche, South San Francisco, CA), recently approved brolocizumab (Beovu, Novartis, Basel, Switzerland), and bevacizumab (Avastin, Genentech/Roche), which is used off label.<sup>1</sup> In clinical practice, intraocular injections of anti-VEGF therapy

are delivered as often as every 4 weeks, in many cases for the lifetime of the patient. With repeated, frequent anti-VEGF IVIs, patients with previously blinding VEGF-driven retinal disorders can expect to maintain and even improve visual function.<sup>2</sup> However, there is a substantial treatment burden associated with this clinical outcome. Despite commonly used treat-and-extend treatment regimens, patients still need at least six, and often times more,<sup>3,4</sup> injections in the first year after a diagnosis of nAMD.

As a direct consequence of the substantial treatment burden to the patient and their caregivers, numerous “real-world” studies indicate that patients receive considerably fewer anti-VEGF injections than what is recommended in product label or treatment guidelines, directly resulting in suboptimal anatomic and visual outcomes, especially when compared with the results noted in pivotal clinical trials.<sup>4-7</sup> Prolonged suppression of pathologic VEGF activity without the requirement for repeated IVIs has thus become a focus of several drug development efforts. The Port Delivery System (Genentech/Roche), for example, is an investigational approach that requires the surgical implantation of a transscleral device containing a reservoir that is refilled every 6 months to continuously release a special formulation of ranibizumab.<sup>8</sup> Although early clinical trial results are encouraging<sup>9</sup> and a phase III program was recently completed in nAMD, the Port Delivery System is implanted in the operating room and requires at least two refills each year, which potentially limits its broad use owing to health care capacity constraints.

Gene therapy has long been envisioned as a potential “one-and-done” transformational treatment approach to further decrease the treatment burden. Adeno-associated virus (AAV) vectors can be used as vehicles to deliver genetic material encoding an anti-VEGF molecule directly to the retina. The resulting endogenous production of the therapeutic molecule within the eye itself would thus eliminate the need for repeated injections of anti-VEGF therapies. This approach has the potential to significantly decrease the treatment burden associated with recurring IVIs and the opportunity to improve real-world visual outcomes. Early results from the most recent anti-VEGF gene therapy clinical studies, one using a surgical subretinal delivery carried out in the operating room (RGX-314) and the other an in-office IVI (ADVM-022), demonstrate the potential of a one-time gene therapy to provide long-lasting control of nAMD.

However, since the dawn of intraocular anti-VEGF pharmacotherapy two decades ago, there have been hypothetical concerns about potential adverse effects on retinal and choroidal physiology with long-term

VEGF suppression. VEGF expression in embryonic development is essential for survival<sup>10,11</sup> and targeted genetic deletion of VEGF in mice leads to abnormalities in the retinal pigment epithelium (RPE), increased cone apoptosis, and thinning of the choriocapillaris.<sup>12,13</sup> However, pharmacologic blockage of VEGF in an adult animal does not appear to have the same detrimental effects on the RPE and choroid.<sup>14-16</sup>

Although concerns of potential choriocapillaris thinning and RPE atrophy owing to long-term VEGF suppression remain, prolonged repeated IVT administration of anti-VEGF therapies in nAMD, DME, DR, and RVO appears to be safe and well-tolerated based on the real-world experience gained from millions of intraocular anti-VEGF injections over the past 20 years. Some caution is still warranted in long-term VEGF inhibition in patients with nAMD, because the long-term follow-up of clinical trial participants receiving anti-VEGF treatment for nAMD shows high rates of the development of macular atrophy. The Comparison of AMD Treatments Trials (CATT), for example, reported that 38% of eyes develop macular atrophy 5 years after starting anti-VEGF therapy.<sup>17</sup> However, the relative contribution of anti-VEGF therapy to the incidence of atrophy must be considered in the context of the occurrence of atrophy as a natural part of the disease process in all patients with AMD, independent of anti-VEGF treatment. In an analysis of participants in the Age-Related Eye Disease Study (AREDS) who progressed to nAMD during a follow-up period before the advent of anti-VEGF therapy, the incidence of macular atrophy was high, increased linearly over time and affected more than 60% of eyes by 10 years,<sup>18</sup> leading to the conclusion that factors other than anti-VEGF therapy may be involved in the development of the atrophy. Nonetheless, especially in the setting of sustained delivery of anti-VEGF treatment of nAMD, monitoring for the development and potentially accelerated progression of geographic atrophy (GA), macular atrophy, and nongeographic atrophy remains an imperative.<sup>19</sup>

ADVM-022 (AAV2-7m8-C11.CO.aflibercept) is a gene therapy vector using the novel viral capsid AAV2.7m8, which is able to transduce ocular structures when delivered via standard in-office IVI, resulting in the high-level expression of aflibercept,<sup>20</sup> an FDA-approved VEGF inhibitor for the treatment of nAMD, DR, DME, and RVO. To address any possible liability of long-term aflibercept gene therapy, we have evaluated the safety of prolonged, sustained intraocular expression of aflibercept following a single IVI of ADVM-022 in healthy, treatment-naïve non-human primates (NHPs). Sustained therapeutic levels of aflibercept expression were confirmed

from serial anterior chamber and vitreous taps out to 30 months after the ADMV-022 dose. Overall ocular health was comprehensively assessed by longitudinal anterior segment analysis by slit-lamp, fundus examinations, optical coherence tomography (OCT), and blue light fundus autofluorescence (FAF), as well as terminal histologic evaluation; the effect on retinal function was determined with full-field and multifocal electroretinography (ERG). Following these evaluations, ADVM-022 gene therapy designed for sustained delivery of aflibercept to the retina via a single IVI appears to be safe and well-tolerated, with no detectable adverse effects on normal retinal structure or function.

To the best of our knowledge, this study represents the first of long-term sustained therapeutic anti-VEGF expression in the NHP, which has a retinal structure that most closely resembles that of humans. Our findings have immediate clinical implications, not only with respect to the numerous ongoing sustained anti-VEGF therapies under development (including anti-VEGF gene therapy), but also in the context of the current suboptimal treatment paradigm of repeated, long-term IVIs of VEGF inhibitors for patients with nAMD, DR, DME, and RVO.

## Methods

### Vector

ADVM-022 (AAV2.7m8.C11.CO.aflibercept) uses a variant capsid of AAV2 denominated AAV2.7m8 with a 10 amino acid insertion in the loop IV within the AAV2 viral protein 3.<sup>21,22</sup> Aflibercept is a recombinant chimeric protein consisting of the VEGFA binding portion of human VEGF receptor-1 (domain 2) and VEGF receptor-2 (domain 3) fused to the Fc portion of human IgG1 immunoglobulin.<sup>23</sup> The expression cassette for aflibercept (C11.CO.aflibercept) carries regulatory elements including the ubiquitous human cytomegalovirus (CMV) immediate-early enhancer and promoter, an adenovirus tripartite leader sequence followed by an enhancer element from the adenovirus major late promoter, a synthetic intron, and a Kozak sequence driving expression of aflibercept. The cDNA sequence that encodes aflibercept in ADVM-022 has been codon optimized for expression in human cells and the resulting protein sequence is identical to that of the aflibercept recombinant protein (<https://go.drugbank.com/drugs/DB08885>). The aflibercept cDNA sequence is followed by a human scaffold attachment region and the human growth hormone polyadenylation site.

### Animals and Study Design

The data presented here were captured from two separate studies (Table), conducted in healthy, treatment-naïve adult (5–12 years old) African green monkeys (*Chlorocebus sabaeus*) of both sexes. A total of four animals received bilateral (OU) injections of ADVM-022 ( $2 \times 10^{12}$  vector genomes [vg]/eye); an additional three animals underwent OU injection with vehicle and were used as a control group. All animals were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The use of OU treatments and all procedural aspects of the animal study were approved by the Animal Care and Use Committee overseeing animal welfare at the primate facility (St. Kitts Biomedical Research Foundation, St. Kitts, West Indies) with which Virscio (New Haven, CT), previously known as RxGen maintains an exclusive facility agreement.

All animals were observed for retinal health for the duration of the study; analyses also included longitudinal monitoring of vitreous humor expression levels of aflibercept, as well as ocular tissue and aqueous and vitreous humor aflibercept levels at study conclusion. Before enrollment, all seven animals presented with normal slit lamp and fundus examinations, fundus images, Spectralis infrared and autofluorescence imaging, and OCT.

### Animal Care and Handling

Animals were anesthetized for all procedures and ophthalmic evaluations (8.0 mg/kg ketamine/1.6 mg/kg xylazine, intramuscularly to effect). General well-being was assessed before, during, and after sedation as well as twice daily on nonprocedure days. The amount of food biscuits consumed daily was monitored. Body weight was measured at the time of ophthalmic examinations throughout the course of the study.

### Test Articles Administration

All animals received OU IVT injections (IVIs) of  $2 \times 10^{12}$  vg/eye of ADVM-022 (animals A055, A070, A075, and K700) or vehicle (animals A118, A194, and K938). The IVIs were performed under local anesthesia (0.5% proparacaine) using a 31G 5/16-inch needle (Ulticare Vet RX U-100, # 09436) 2 mm posterior to the limbus in the inferior temporal quadrant, targeting the central vitreous.

The dose of  $2 \times 10^{12}$  vg/eye in NHPs evaluated in this study corresponds to a human equivalent dose of  $4 \times 10^{12}$  vg/eye when accounting for the

**Table.** Animal Treatment Groups

| Study               | Animal ID         | Last Assessment Time Point, Months | IVI Treatment | Dose (vg/eye), Volume                |
|---------------------|-------------------|------------------------------------|---------------|--------------------------------------|
| 1*                  | A194              | 21                                 | Vehicle       | N/A, 50 $\mu$ L, OU                  |
|                     | A118              |                                    |               |                                      |
|                     | K938              |                                    |               |                                      |
|                     | A055 <sup>1</sup> | 21                                 | ADVM-022      | $2 \times 10^{12}$ , 50 $\mu$ L, OU  |
|                     | A070 <sup>1</sup> |                                    |               |                                      |
| A075 <sup>1,2</sup> | 19                |                                    |               |                                      |
| 2**                 | K700              | 30                                 |               | $2 \times 10^{12}$ , 100 $\mu$ L, OU |

<sup>1</sup>Safety initially described in these animals out to 12.5 months after ADVM-022 injection.<sup>20</sup>

<sup>2</sup>Animal 075 died prematurely 19 months after ADVM-022 treatment owing to reasons unrelated to the test article (and all the assessments and sampling performed on the animal up to 19 months after treatment are included in the study).

\*Body weights, slit lamp examinations, funduscopy, IOP measurements assessed at baseline, week 2, months 1, 3, 6, 9, 12.5, 16, 17.5, 19, and 21; OCT imaging was performed at baseline, months 3, 6, 9, 12.5, 16, 17.5, 19, and 21; full-field ERG was performed at month 19; vitreous and serum sampling was performed at baseline, months 3, 6, 9, 12.5, 16, 19, and 21; and aqueous sampling was performed at baseline, months 7 and 16, and 21.

\*\*Body weights, slit lamp examinations, funduscopy, and IOP measurements assessed at baseline, day 2 and 7, week 2, months 1, 3, 6, 9, 12, 14.7, 20.2, 22.5, 28, 28.5, and 30. OCT imaging was performed at baseline, months 1, 2, 3, 6, 12, 15, 20, 22.5, 26, 28, and 30. Multifocal ERG was performed at month 30. Vitreous sampling was performed at months 1, 2, 3, 6, 12, 20, 22, 26, 28, and 30. Aqueous and serum sampling was performed at baseline, months 1, 2, 3, 6, 12, 15, 20, 23, 26, 28, and 30.

Animals were treated with a single IVI of ADVM-022 ( $2 \times 10^{12}$  vg/eye) or vehicle and assessed at different time points. The longest time point assessed in study 1 was 21 months after the dose, and 30 months for the single animal in study 2.

difference between the vitreous humor volumes of NHP and human eyes.<sup>24</sup>

### Ocular Inflammation Assessments by Slit Lamp Biomicroscopy and Funduscopy

Anterior segment cells and flare were examined by slit lamp biomicroscopy (SL-2E, Topcon, Tokyo, Japan) and scored using a modified Hackett–McDonald scale.<sup>25</sup> Examinations were performed at baseline, day 14, and months 1, 3, 6, 9, 12.5, 16, 17.5, 19, and 21 for animals A055 and A070; at baseline, day 14, and months 1, 3, 6, 9, 12.5, 16, 17.5, and 19 for animal A075; and at baseline, day 14, and months 1, 3, 6, 9, 12, 14.7, 20.2, 22.5, 28, 28.5, and 30 for animal K700.

Evaluation of the posterior wall for potential vitreous inflammation was performed by posterior segment slit lamp examination using a 90-diopter lens. Assessments included a detailed fundoscopic evaluation of the retina, blood vessels, and optic disc for any abnormalities.

### OCT and Blue Light Autofluorescence

OCT was performed at baseline and months 3, 6, 9, 12.5, 16, 17.5, 19, and 21 for animals A055 and A070;

at baseline and months 3, 6, 9, 12.5, 16, 17.5, and 19 for animal A075; and at baseline and months 1, 2, 3, 6, 12, 15, 20, 22.5, 26, 28, and 30 for animal K700 to evaluate retinal thickness and structural integrity using a Heidelberg Engineering Spectralis OCT Plus system (Vista, CA) and Heidelberg Eye Explorer (HEYEX) software (v1.6.1). The dense retinal volume scans consisted of 48 parallel scans of 30° in the horizontal plane positioned 50  $\mu$ m apart with image averaging more than 50 automatic retinal tracking frames with the scan grid centered on the fovea. The accuracy of the SD-OCT automated segmentation (i.e., internal limiting membrane and Bruch's membrane) was evaluated, and segmentation errors were corrected manually before computation of change maps and change in central retinal thickness.

### Full-Field and Multifocal ERG

Full-field ERG was performed employing an Electro-Diagnostic Imaging full-field ERG apparatus and Visual Evoked Response Imaging System (VERIS 6.0) software (Electro-Diagnostic Imaging, Inc, Milpitas, CA). Monkey eyes were fully masked for 30 minutes for dark adaptation immediately after sedation for the examination and topical instillation of 10% phenylephrine and 1% cyclopentolate ophthalmic solutions. A rod-specific response was

elicited by a dim white flash after dark adaptation and pupil dilation. Mixed rod and cone responses were obtained using the standard bright white flashes under scotopic condition. To evaluate the function of the cone photoreceptors, monkeys were light adapted for 10 minutes at approximately 40 cd/m<sup>2</sup> and photopic flicker ERGs were elicited with a 30-Hz flickering white light (2.51 cd\*s/m<sup>2</sup>) on the background light at 40 cd/m<sup>2</sup>. The amplitude of the B-wave was measured from the A-wave trough to the peak of the B-wave or, if no A-wave is present, from the prestimulus baseline to the peak of the B-wave. The amplitude of the A-wave was measured from the prestimulus baseline to the peak of the A-wave.

Multifocal ERG was performed using an Electro-Diagnostic Imaging VERIS multifocal ERG instrument for stimulus generation and data collection, with visual stimulus consisting of 103 hexagonal elements displayed by projection onto the fundus by a fundus projector/camera calibrated by the VERIS autocalibration system. After achieving dilation and application of topical proparacaine (0.5%), contact lens electrodes were applied with hydroxypropyl methylcellulose (2.5%). Subdermal ground electrodes were placed in the brow for unilateral sequential testing. The sequence of eyes examined was determined randomly. The VERIS fast m-sequence was used with a frame rate of 75 Hz, with a sampling rate of 1200 Hz. Display luminance was 100 cd/m<sup>2</sup> with a minimum and maximum of 1 cd/m<sup>2</sup> and 200 cd/m<sup>2</sup>, respectively.

## Tonometry

Intraocular pressure (IOP) measurements were obtained with a TonoVet (iCare, Helsinki, Finland) tonometer at the dog setting. Under full sedation, the monkeys were placed in a supine position in the IOP testing apparatus. Three measurements were taken per eye at each time point and the mean value calculated.

## Vitreous and Aqueous Humor Collection

Vitreous humor (approximately 50 µL at each in-life time point; approximately 150 µL at termination) was sampled at baseline, months 3, 7, 9, 13, 15.5, 19, and 21 for animals A055, A075, A194, A118, and K938; at baseline and months 3, 7, 9, 13, 15.5, and 19 for animal A070; and at months 1, 2, 3, 6, 12, 15, 20, 22, 26, 28, and 30 for animal K700. Aqueous humor (approximately 50 µL at each time point) was sampled at baseline and months 7 and 16, and 21 for animals A055, A070, A194, A118, and K938; at baseline and months 7, 16, and 19 for animal A075; and at baseline and months 1, 2, 3, 6, 12, 20, 23, 26, 28, and 30 for animal K700.

Samples were collected using a 0.3-mL insulin syringe with a 27G needle introduced aseptically 3 mm posterior to the limbus.

## Termination, Tissue Collection, and Histology

All animals were euthanized with pentobarbital after completion of the final ophthalmic examinations and confirmation of the quality of imaging at terminal points. Before sacrifice, samples of vitreous humor, aqueous humor, serum, and plasma were collected. After sample collection, animals were euthanized, and globes enucleated. Right eye (OD) globes were dissected to isolate cornea, iris–ciliary body, lens, vitreous (full volume remaining after initial tap), choroid with RPE (further called *choroid*), neural retina (further called *retina*), sclera, and optic nerve. Choroid, retina, vitreous, aqueous, and ciliary body samples were analyzed for aflibercept levels. Left eyes (OS) were trimmed to remove the excess orbital tissue. Owing to inadequate fixation of ocular tissues from A055, A070, and A075, only sections from animal K700 (collected at 30 months after treatment) were available for hematoxylin and eosin (H&E) staining for histologic analysis. OD eye of K700 was immersed in 4% paraformaldehyde for 4 hours in a refrigerator after injection of 200 µL of 4% paraformaldehyde into the vitreous, then transferred to phosphate-buffered saline with 0.05% sodium azide and retained at 4°C. All segments were processed for paraffin sectioning. The posterior segment was cut in half from 12 o'clock to 6 o'clock through the center of the optic nerve head so that the posterior segment was divided into temporal and nasal portions. Serial 6-µm-thick sections of the retina and optic nerve head were vertically taken from the center of the optic nerve head to the peripheral retina. Sections were then stained using H&E.

## Aflibercept Analysis

Above lower limit of quantification levels of “free” aflibercept protein (i.e., unbound to VEGF) in the vitreous humor, aqueous humor, retina, choroid, and ciliary body were measured using a quantitative enzyme-linked immunosorbent assay (ELISA). ELISA plates (NUNC MaxiSorp, Thermo Fischer Scientific, Waltham, MA) were coated with 100 µL/well of recombinant human 165-VEGFA (rhVEGFA) (R&D Systems, Minneapolis, MN) at a concentration of 1 µg/mL in coating buffer (R&D Systems) and incubated overnight at 4°C. After washing with wash buffer (KPL), the plates were blocked with 300 µL/well of protein-free blocking buffer

(Pierce Biotechnology, Rockford, IL) for 2 hours. Afterward, the plates were washed and the samples were added (100  $\mu$ L/well) at following dilutions: 1:1000 for ADVM-022-treated samples and 1:50 for vehicle-treated samples) and incubated for 2 hours at room temperature. The plates were then washed again, and 100  $\mu$ L/well of anti-human Fc domain of IgG (Fc $\gamma$ )-specific antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch Labs, West Grove, PA) at 500 ng/mL in bovine serum albumin 1% in phosphate-buffered saline was added to the wells. After washing, 100  $\mu$ L/well of SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific) was added to the wells, and luminescence signal was measured using a microplate luminometer. The aflibercept ELISA lower limit of quantification was 7.8 ng/mL and the lower limit of detection was 156 pg/mL.

## Results

We previously published the initial expression, pharmacologic efficacy, and safety data related to ADVM-022 after IVI in NHPs.<sup>20</sup> The current study follows the three remaining ADVM-022-treated animals (A055, A070, and A075) and the three vehicle-treated animals (A194, A118, and K938) from that study out to 21 months (Table). An additional animal (K700) from a separate study that was treated with ADVM-022 in the same fashion was followed for a period of 30 months.

All animals were in the normal range at baseline ophthalmic screening, including tonometry, slit lamp biomicroscopy, funduscopy, color fundus photography, and OCT after IVI. All ADVM-022-treated animals received a single IVT dose of  $2 \times 10^{12}$  vg/eye; the vehicle-treated animals provided negative control data for specific assessments.

### ADVM-022 Mediated Long-Term Expression of Aflibercept

To assess the pharmacokinetics of ADVM-022, aqueous and vitreous humor samples were collected regularly during the observation period and analyzed by an ELISA method specific for free (unbound) aflibercept. No aflibercept expression was detected in the vehicle-treated control eyes (data not shown).

In the ADVM-022-treated animals, sustained robust levels of aflibercept were observed in vitre-

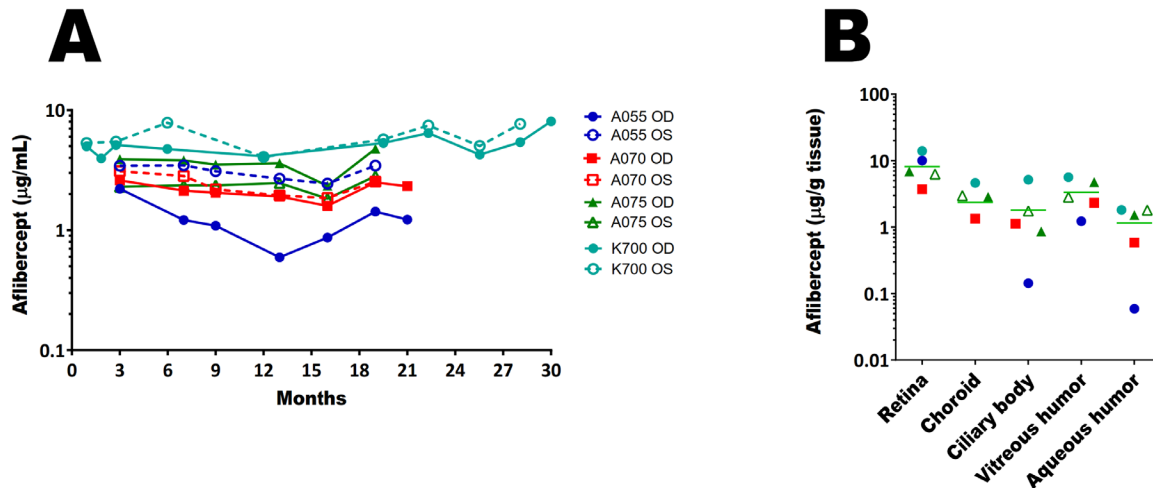
ous humor samples out to 30 months after the dose (latest time point assessed in the study), ranging between 1.2  $\mu$ g/mL (animal A055 OD, at month 21 terminal measurement) to 8.1  $\mu$ g/mL (animal K700, at month 30 terminal measurement) (Fig. 1A). These levels were previously shown to be pharmacologically active<sup>20</sup> and therapeutically meaningful.<sup>26</sup> Aqueous humor aflibercept protein levels, although generally lower than those measured in the vitreous, remained stable throughout the course of the study (Supplementary Fig. S1).

A comparative analysis of aflibercept expression in different ocular compartments (retina, choroid, iris/ciliary body, vitreous and aqueous humor) demonstrated the highest expression in retina ( $8.2 \pm 1.8$   $\mu$ g/g), followed by vitreous humor ( $3.4 \pm 0.8$   $\mu$ g/mL), choroid ( $2.4 \pm 0.8$   $\mu$ g/g), the ciliary body ( $1.8 \pm 0.9$   $\mu$ g/g), and aqueous humor ( $1.2 \pm 0.4$   $\mu$ g/mL) (mean  $\pm$  standard error of the mean) (Fig. 1B). The gradient of aflibercept concentration with highest levels observed in the retina and decreasing toward the front and back of the eye is consistent with what was observed in the previously reported efficacy and pharmacokinetics study.<sup>20,26</sup> One outlier was observed with respect to the level of aflibercept in the choroidal tissue in the OD of animal A055 that was below the limit of quantification, although the same eye showed high sustained levels of aflibercept in the other ocular compartments. Overall, the results of the analysis of long-term ADVM-022-mediated expression of aflibercept indicate robust sustained levels of the protein in the ocular compartments for the duration of the long-term study.

### Long-Term Ocular Safety of ADVM-022

ADVM-022 was well-tolerated throughout the study. There were no clinical signs indicative of ADVM-022-related systemic effects. Food consumption and body weight remained within levels normal for the monkey colony (Supplementary Fig. S2). No systemic (serum) aflibercept was detected at any time point (lower limit of quantification, 7.8 ng/mL; lower limit of detection, 156 pg/mL). Routine clinical observations did not show any abnormalities.

To evaluate the ocular safety and tolerability of ADVM-022 after IVT administration, eyes were assessed for possible long-term inflammatory responses using slit-lamp biomicroscopy and funduscopy, both before dosing and at different time points out to 30 months after the dose. In addition, retinal morphology and functional integrity were assessed by OCT and ERG, respectively.



**Figure 1.** A single IVI of ADVM-022 results in sustained levels of aflibercept in the vitreous humor throughout period out to 30 months that are comparable to the levels of aflibercept in the terminally collected ocular tissues. **(A)** Levels of aflibercept were quantified from the vitreous humor samples collected at indicated time points. **(B)** Levels of aflibercept were quantified from the terminally collected ocular tissues (retina, choroid, ciliary body, aqueous, and vitreous humor). Aflibercept levels were measured by VEGF-165 anti-human Fc sandwich ELISA assay. Green bars show mean levels of aflibercept. A055 OD sample showed no measurable levels of aflibercept in choroid. Levels of aflibercept in vitreous and aqueous humor in Figure 1B are expressed as  $\mu\text{g/g}$  based on the assumption that density of the tissues is approximately 1 g/mL. Please note that the error bars are smaller than the symbol. The values reported are above the lower limit of quantification.

## Slit Lamp Examinations and IOP Measurements

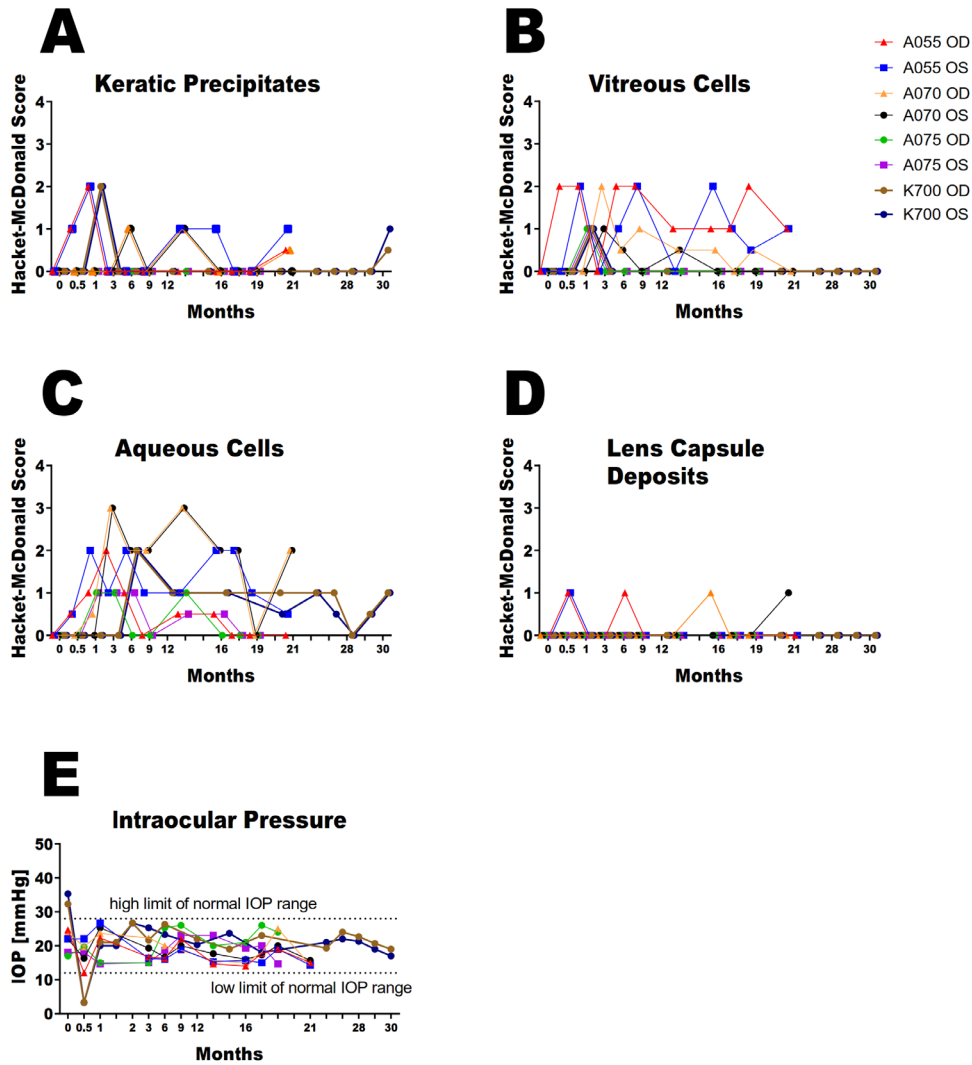
The long-term slit lamp observations in the animals treated with ADVM-022 were consistent with the 12.5 month-long observations in our previous study.<sup>20</sup> Inflammation signs were self-limiting and, in a majority of eyes, trended toward resolution by the end of the study, with no animals requiring anti-inflammatory treatment. Signs of inflammation were limited to mild aqueous cell infiltrates, mild vitreous cell infiltrates, mild to moderate keratic precipitates, and incidental mild lens capsule deposits (Fig. 2). The aqueous and vitreous cell response peaked at 1 month and, in most of the eyes, resolved by 3 months after the injection (Figs. 2B and 2C). Mild-to-moderate keratic precipitates were observed as early as 1 month after treatment, with an apparent decreasing trend at 16 months after treatment (Fig. 2A). Lens capsule deposits were characterized as mild and resolved in most eyes by the end of the observation period (Fig. 2D). There was no aqueous flare observed throughout the study in any of the eyes. A single case of mild vitreous haze was detected in one eye (A055 OD) at 12.5 months (grade 1+, that is, slight opacities without obscuration of retinal details) that resolved without anti-inflammatory treatment by 17.5 months as assessed by slit lamp examination and color fundus imaging.

Additional parameters of inflammation and safety were evaluated and received a score of 0, includ-

ing conjunctival congestion, conjunctival swelling, conjunctival discharge, pannus, corneal opacity severity, corneal opacity area, fibrin strands, aqueous flare, iris hyperemia, iris exfoliation, iris synechia, lens opacity, retinal vasculitis, and papillitis. The IOP remained within normal limits in all eyes, except the OD and OS of K700, which experienced a transient IOP decrease 14 days after treatment (Fig. 2E) that completely resolved by the next time point (1-month after the dose). After that time point, the IOP remained normal for all the eyes for the duration of the study.

## Structural Integrity of the Retina and RPE

OCT examination was performed, on average, every 3 months to evaluate retinal thickness and structural integrity post treatment (Fig. 3, Supplementary Fig. S3). The OCT image shown in Figure 3A is from the longest time point analyzed (30-month animal); individual OCTs from the other animals can be found in Supplementary Fig. S3. Anatomically, the retinal layers plus the underlying RPE and choroid showed no changes compared to baseline (Fig. 3B) up to latest time points assessed (21 months for A055 and A070, 19 months for A075, and 30 months for K700). Individual measurements of retinal thickness across nine sections of the retina (including the central, inner superior, inner temporal, inner nasal, inner inferior, outer superior, outer temporal, outer nasal, outer inferior retina) throughout the course of



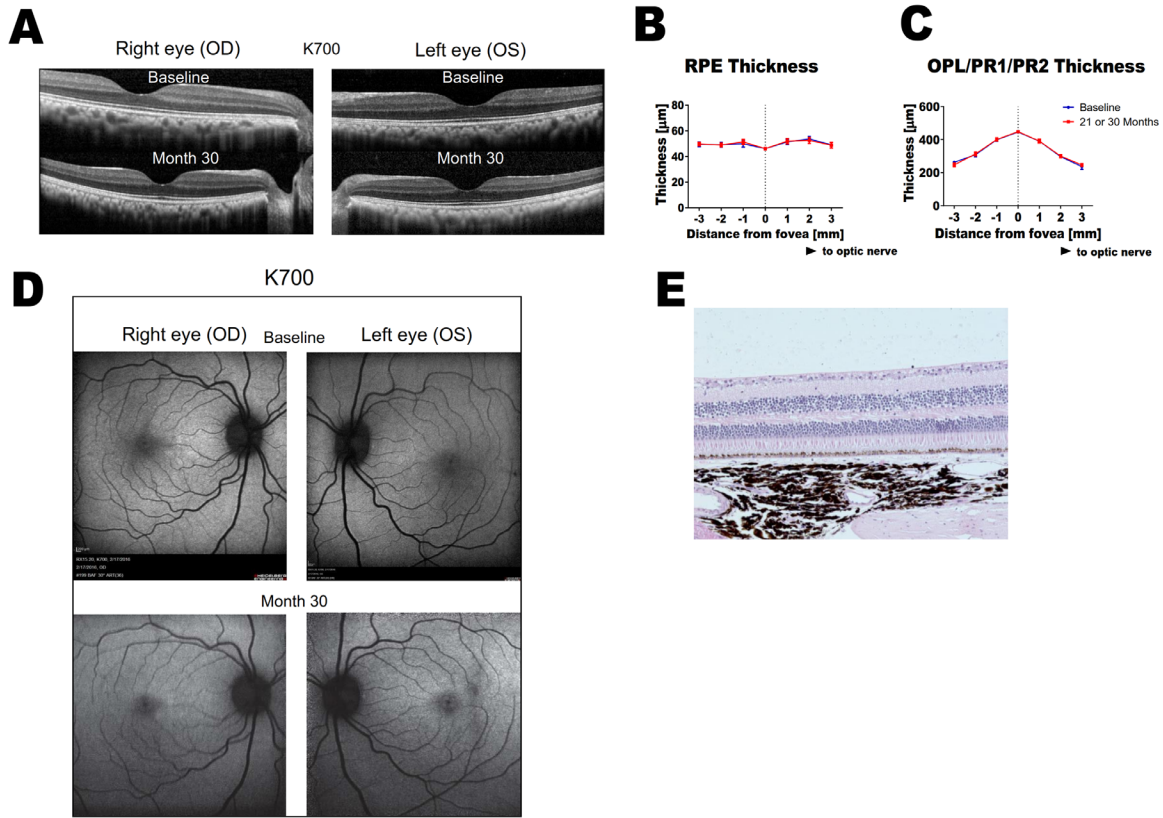
**Figure 2.** (A–D) Ocular clinical pathology and (E) IOP in the individual eyes treated with ADVN-022 were assessed throughout the course of two studies at the indicated time points and out to 30 months after IVI. Examinations revealed mild inflammation with a resolving trend throughout the time course. The parameters scored by the Hackett–McDonald irritation and inflammation scoring system are shown: (A) keratic precipitates, (B) vitreous cells, (C) aqueous cells, (D) lens capsule deposits, and (E) IOP. No vitreous haze has been detected except in one eye (A055, OD) at only one time point (12.5 months) at grade 1+. (E) The IOP was within the normal range except for both eyes in animal K700 on D14 (when it decreased to 3.3 mm Hg). The IOP in both eyes of K700 returned to normal at the next time point assessed (day 28). Normal IOP lower and upper IOP limits were determined based on the IOP measurements conducted on 148 eyes at baseline. The lower and upper IOP limits are designated as 2 standard deviations of the mean value ( $\mu \pm 2\sigma$ ). Scores are represented for each eye in the study for every time point assessed and each symbol represents one individual eye.

the study were conducted to identify atrophic changes. The longitudinal measurements did not find any significant variations, indicating long-term maintenance of retinal structural integrity (Supplementary Fig. S4).

To address the possible role of VEGF in the photoreceptor layer integrity,<sup>12,13</sup> we performed measurements of the thickness of the outer nuclear–photoreceptor 1–photoreceptor 2 (ONL–PR1–PR2) layer as well as the thickness of the RPE in segmented OCT sections, at baseline and at the end of the study

(Fig. 3C). Thickness of retinal ONL–PR1–PR2 layer is defined by photoreceptor layer (PR1) and the outer segment–RPE interdigitation (PR2).<sup>27</sup> An example of the segmentation analysis performed is shown in Supplementary Figure S5. The analysis revealed no changes in either the ONL–PR1–PR2 layers or the RPE layer. The absence of dystrophic changes in the retina was supported by blue laser FAF imaging (Fig. 3D, Supplementary Fig. S6). FAF allows mapping of the RPE status based on the lipofuscin autofluorescence,



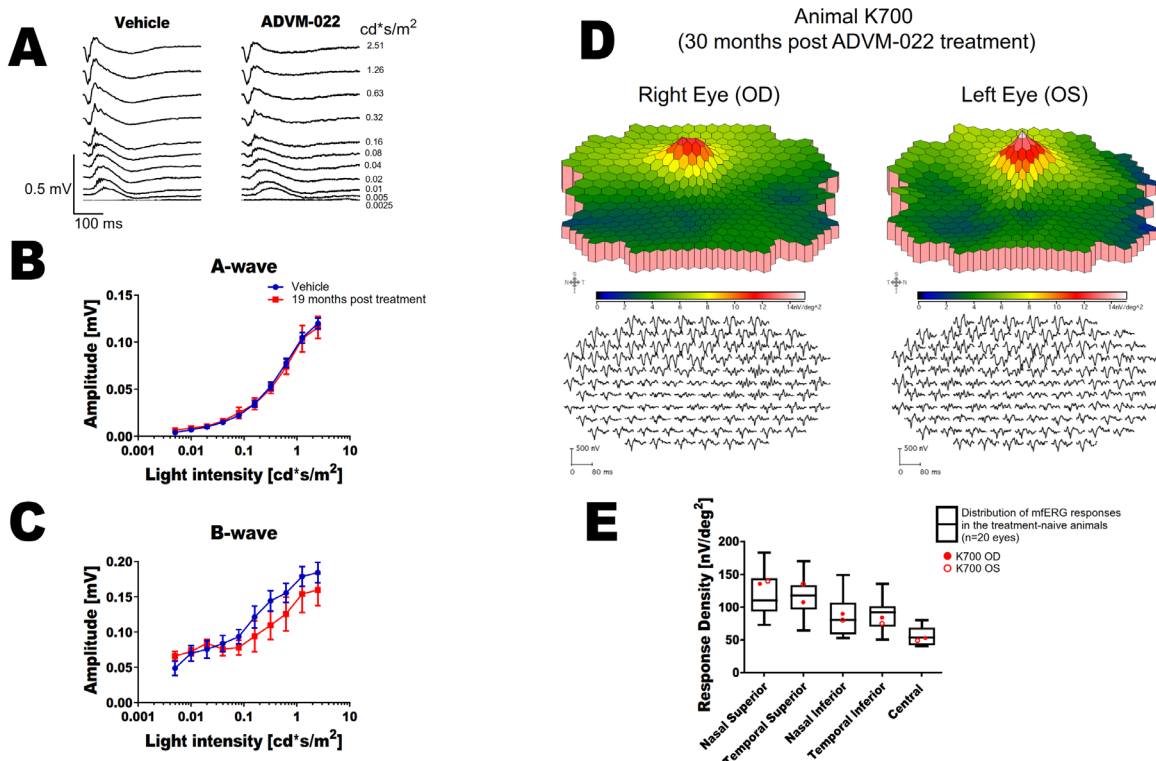


**Figure 3.** Long-term expression of aflibercept in the retina from ADVM-022-treated animals does not affect retinal morphology features as evaluated by the OCT or H&E staining. **(A)** OCT images of both eyes from a representative animal (K700) treated with ADVM-022 were analyzed at baseline and at 30 months after treatment. **(B)** RPE and **(C)** ONL-PR1-PR2 layer thickness measurement revealed no difference in any of the segments analyzed. Analysis is performed at baseline and at respective terminal time points (21 or 30 months after dosing) (Student *t* test,  $P = 0.99$  for RPE analysis and  $P = 0.79$  for ONL-PR1-PR2 analysis). Please note that some of the error bars are smaller than the symbol size. **(D)** FAF micrographs of animal K700 at baseline and 30 months after ADVM-022 treatment. **(E)** H&E staining of animal K700 OS retinal tissue 30 months after treatment. Note the integrity of all retinal layers and the absence of any abnormality in the morphology.

where excessive lipofuscin buildup detected by blue light as hyperfluorescence would indicate RPE cell dysfunction, and focal loss of autofluorescence would correspond to atrophic changes characteristic of GA. Blue laser FAF images demonstrated no signs of hypopigmentation or increased signal backscattering into the choroid that would be a characteristic of the retina affected by GA. Hyperpigmentation, indicative of pathologic lipofuscin buildup was also not observed. **Figure 3D** shows blue laser FAF at baseline and after 30 months after injection (the longest time point assessed). Images from additional animal scans can be found in Supplementary Figure S6. In agreement with the in-life assessments, H&E staining of the eye sections from animal K700 at the longest time point assessed (30 months after treatment with aflibercept concentration levels in vitreous at 8.0 μg/mL) did not reveal any histologic abnormalities (**Fig. 3E**, Supplementary Fig. S7).

### Functional Integrity of the Retina

The long-term effects of sustained aflibercept expression on photoreceptor function were assessed by ERG. Full-field scotopic ERG responses recorded at different intensities of light stimuli were compared to ERG responses in vehicle-treated animals (**Fig. 4A**). The a- and B-wave amplitudes were not statistically significantly different between vehicle-treated (A194, A118, and K938) and ADVM-022-treated animals (A050 and A075) at 19 months after treatment (repeated measures ANOVA, A-wave  $P = 0.95$ , B-wave  $P = 0.48$ ). In addition, cone function evaluated by 30 Hz photopic flicker ERG (Supplementary Fig. S8) was not statistically significantly different between vehicle-treated and ADVM-022-treated animals (repeated measures ANOVA,  $P = 0.3$ ). For the animal that has been followed for the longest time (K700), both eyes were assessed by multifocal ERG



**Figure 4.** ERG analysis of the ADVM-022 treated animals at the late time points reveals normal ERG responses. **(A)** Scotopic ERG recordings were performed on 2 animals (A055 and A075,  $n = 4$  eyes) treated with ADVM-022 19 months after injection and compared with vehicle-treated animals ( $n = 6$  eyes). **(B)** Analysis of scotopic A-wave and **(C)** B-wave revealed no statistically significant difference between ADVM-022 and vehicle treated animals (repeated measures ANOVA A-wave  $P = 0.95$ , B-wave  $P = 0.48$ ). **(D)** First order three-dimensional multifocal ERG topography density at 30 months after treatment (K700) revealed normal recordings. The stimulus configuration is an array of 103 hexagons scaled with retinal eccentricity. A local response was derived for each of the 103 stimulus elements. First-order ERG recording traces are shown in the lower panels. The multifocal ERG traces are shown equally spaced for clarity. **(E)** ERG response density at different regions of retina from both eyes for the animal treated with ADVM-022 (K700) at 30 months after the dose were plotted against the distribution of the ERG responses in treatment-naïve, healthy animals (white boxes,  $n = 20$  eyes) and were within the limits of responses recorded in the treatment-naïve animals.

at the terminal point of the study (30 months) and revealed normal recordings (Fig. 4D) with response density measurements within the normal distribution of multifocal ERG responses in healthy, treatment-naïve animals (Fig. 4E).

The results of the OCT imaging, ERG recordings, and slit-lamp examinations, along with histology at the latest time point evaluated, demonstrated that long-term expression of aflibercept after IVT administration of ADVM-022 did not induce morphologic or functional alternations in the NHP retina.

## Discussion

The introduction of IVI anti-VEGF pharmacotherapy nearly 20 years ago marked a momentous paradigm shift in the treatment of nAMD. Without

treatment, 1 year after diagnosis, patients with nAMD lose nearly three lines of vision; astonishingly, more than three-quarters of patients are legally blind (a visual acuity worse than 20/200) by 3 years after the diagnosis.<sup>28</sup> In contrast, with frequent IVT anti-VEGF injections (monthly injections with ranibizumab and slightly less frequently with aflibercept and brolucizumab), patients, on average, gain 7 to 10 ETDRS (Early Diabetic Retinopathy Study) letters in the first year after nAMD onset.<sup>29,30</sup> In prospective phase III pivotal clinical trials, the visual acuity gains are preserved as long as disease activity is monitored and followed with frequent anti-VEGF IVIs. If the follow-up and anti-VEGF treatment regimen is relaxed, visual acuity gains noted in pivotal trials may be lost.<sup>4</sup> Overall, in the pivotal trial extension studies (follow-up beyond the initial 2 years), the ability to maintain the visual acuity improvements from baseline was directly associated with the mean number of

anti-VEGF injections with more regular injections resulting in meaningfully better outcomes. Although in the real-world setting it is possible to maintain the best-corrected visual acuity with frequent and fixed interval dosing,<sup>31</sup> treat-and-extend regimens are more commonly used in clinical practice. The suboptimal visual acuity outcomes reported in numerous large real-world database analyses can, in great part, be directly attributed to patients receiving considerably fewer anti-VEGF injections than the number of injections administered in pivotal clinical trials. Factors responsible for the noteworthy undertreatment of patients with nAMD in the real world include the significant burden for patients, caregivers, and health care providers associated with both treatment and monitoring visits. Although anti-VEGF therapy transformed the nAMD management landscape, meaningful reduction in the treatment burden associated with repeated, discontinuous IVIs remains a vital unmet need to truly optimize patient outcomes.

The clinical success of Luxturna (voretigene neparovec-rzyl, Spark Therapeutics, Philadelphia, PA) in RPE65-associated inherited retinal degeneration<sup>32</sup> provides confidence that AAV ocular gene therapy can serve as a long-term approach for the treatment of monogenic inherited retinal disorders. Here, we show the potential of AAV vectors as a drug delivery platform to address more prevalent noninherited retinal disorders, including nAMD, DR, DME, and RVO, by providing the genetic material encoding an anti-VEGF molecule such as aflibercept directly to the eye. The resulting production of the therapeutic protein within and by the eye itself has the potential to substantially decrease, or possibly eliminate, the treatment burden associated with frequent IVIs and, given that the therapeutic protein is continuously being delivered to the tissues that are undergoing pathologic changes (retina and choroid), fewer anatomic fluctuations may occur, potentially resulting in improved visual outcomes. ADVN-022 is an AAV gene therapy product designed to deliver DNA sequences coding for the FDA-approved VEGF inhibitor aflibercept. ADVN-022 is being developed for the treatment of VEGF-driven ocular diseases. Unlike other ocular gene therapy products previously evaluated, ADVN-022 uses AAV2.7m8, a novel viral capsid that is able to efficiently transduce ocular structures when delivered via standard IVI, resulting in high levels of transgene expression and the secretion of therapeutically relevant levels of aflibercept. Because the IVT delivery of aflibercept and other anti-VEGF molecules has become standard clinical practice in office-based settings across the globe, we consider this path to be the optimal and most convenient route of administration

for the AAV-mediated anti-VEGF gene delivery to the retina.

In earlier preclinical NHP studies, a single IVI of  $2 \times 10^{12}$  vg/eye ADVN-022 resulted in aflibercept expression levels that are comparable to the therapeutically active levels observed 3 to 4 weeks after IVT delivery of aflibercept recombinant protein.<sup>33</sup> Further, we were able to show that a single IVI of ADVN-022 was able to suppress laser-induced choroidal neovascularization in NHPs even when administered more than 1 year before laser treatment, demonstrating that the aflibercept protein expressed from ADVN-022 could efficiently inhibit VEGF activity in that acute model.<sup>34</sup>

In this study, we sought to evaluate the impact of long-term, continuous aflibercept expression and subsequent VEGF suppression on the structural and functional integrity on a normal primate retina up to 2.5 year after the IVI of  $2 \times 10^{12}$  vg/eye of ADVN-022.

Although other gene therapy approaches designed to deliver a VEGF inhibitor to treat nAMD have shown potential long-term structural and functional liabilities in NHPs,<sup>35–37</sup> our current study has not revealed any notable adverse effects on NHP retinal structure and function as measured by OCT and ERG, respectively. At the latest time points (21 months and 2.5 years) OCT scans from ADVN-022-injected animals seemed to be unchanged from baseline and were indistinguishable from those of control animals. Specifically, no significant changes were observed in the RPE, photoreceptors, or any other structural layers of the retina. The absence of dystrophic retinal alterations was supported by blue laser FAF imaging, a technique commonly used in clinical settings to evaluate signs of GA.<sup>38,39</sup> In addition, no hyperfluorescence owing to excessive lipofuscin buildup was detected, indicating normal RPE function. There were also no notable histologic changes observed by H&E staining 2.5 years after ADVN-022 injection. Normal ERG responses agreed with the preservation of structural integrity of the retina, after long-term expression of ADVN-022-delivered aflibercept. Although we did not evaluate the biological activity of ADVN-022-derived aflibercept in this specific study, there is indirect evidence that it can inhibit VEGF from a previous study using the same vector.<sup>34</sup> Moreover, the ELISA used to quantify the protein in the various ocular tissues and fluids in this study includes a VEGF-binding step. Therefore, our results showing the absence of negative effect on retinal structure or function after up to 2.5 years of continuous expression of a biologically active aflibercept molecule bring further evidence to support the concept that sustained VEGF inhibition with ADVN-022 provides a favorable safety profile.

The main limitation of our study is that it was conducted in healthy NHPs and it is conceivable that the safety profile of sustained VEGF suppression in eyes affected by nAMD may differ from that of normal, nondiseased eyes. However, the theoretical risks of VEGF suppression center around the nonpathologic role of VEGF in maintaining normal ocular structure and function. As such, in this study, we show that the expression of therapeutic levels of aflibercept (at concentrations within the range of the current every 2-month FDA-approved IVT injection-delivered aflibercept in nAMD<sup>26</sup>) do not appear to lead to any apparent adverse effects, even when VEGF levels are suppressed in eyes without elevated pathologic VEGF. The lack of any evidence of retinal damage from frequent anti-VEGF injections in patients with DME or RVO suggests that the suppression of VEGF does not directly damage retinal neurons and that any new-onset macular atrophy in patients with nAMD treated with anti-VEGF agents is likely to be owing to an interaction between the disease process and VEGF suppression. Nevertheless, theoretical risks of VEGF suppression based on the nonpathologic role of VEGF in maintaining a normal ocular structure and function remain. As such, in this study, we show that the expression of therapeutic levels of biologically active aflibercept at concentrations within the range of the current bi-monthly regimen indicated in the aflibercept label for the treatment of nAMD,<sup>33</sup> do not appear to lead to any adverse effects, even when VEGF levels are suppressed in eyes without elevated pathologic VEGF.

The only treatment-related observation of note in the NHP eyes that received ADV-022 was the mild to moderate inflammatory response manifested as mild aqueous and vitreous cell infiltrates, mild to moderate keratic precipitates, and mild lens capsule deposits. There was no involvement of the posterior structures, with no detectable vasculitis, papillitis, retinitis, or choroiditis. All inflammatory signals were self-limiting and trended toward resolution without the need for anti-inflammatory treatment. Other published preclinical studies of AAV-based gene therapies have also described vector-related inflammation.<sup>40–43</sup> Although it is generally accepted that the ocular inflammation observed is likely linked to a local immune response to the gene therapy,<sup>44</sup> it is difficult to pinpoint exactly which vector component is responsible because different AAV capsid serotypes and transgenes were used across these studies.

The antidrug antibody response to the AAV2.7m8 capsid, which is expected upon IVT administration of ADV-022, was not measured in this study; therefore, we cannot rule out a possible correlation, to some extent, between an immune response and the presence

of low levels of inflammation in the animals eyes. However, we<sup>45</sup> and others<sup>46</sup> have shown that, in NHPs, the readministration of vector in the fellow eye in the presence of preexisting neutralizing antibodies resulting from injection in the first eye, did not result in exacerbation of inflammation.

Further investigations are required to elucidate the specific mechanisms for the mild inflammatory reaction observed in the animals treated with ADV-022 and to understand how these findings can be translated into patients suffering from nAMD.

To the best of our knowledge, our study represents the longest serial follow-up of an evaluation of an NHP eye and the potential effects of sustained VEGF suppression.

Together with previous reports on the efficacy and pharmacokinetics of ADV-022–derived aflibercept,<sup>33,34</sup> the long-term safety results presented herein support the exploration of ADV-022 safety and efficacy in clinical trials. ADV-022 is currently being evaluated in two early human studies: (1) in a multicenter phase I clinical trial in patients with nAMD who have been previously treated with a high number of anti-VEGF IVIs (OPTIC trial NCT03748784), and (2) in a prospective, randomized, double-masked phase II clinical trial in patients with DME (INFINITY, NCT 04418427). The doses of ADV-022 being evaluated in the OPTIC and INFINITY trials,  $2 \times 10^{11}$  and  $6 \times 10^{11}$  vg/eye, are 20- and 6.7-fold lower, respectively, than the dose evaluated in this current NHP study. These early human trials will provide invaluable information toward the further development of ADV-022 as a one-time IVT treatment for VEGF-driven ocular diseases.

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## References

1. Parikh R, Pirakitikulr N, Chhablani J, Sakurada Y, Singh RP, Modi YS. A multinational comparison of anti-vascular endothelial growth factor use: the United States, the United Kingdom, and Asia-Pacific. *Ophthalmol Retina*. 2019;3(1):16–26.
2. Rofagha S, Bhisitkul RB, Boyer DS, Sadda SR, Zhang K, Group S-US. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). *Ophthalmology*. 2013;120(11):2292–2299.
3. Chandra S, Arpa C, Menon D, et al. Ten-year outcomes of anti-vascular endothelial growth factor therapy in neovascular age-related macular degeneration. *Eye (Lond)*. 2020;34(10):1888–1896.
4. Ciulla TA, Hussain RM, Pollack JS, Williams DF. Visual acuity outcomes and anti-vascular endothelial growth factor therapy intensity in neovascular age-related macular degeneration patients: a real-world analysis of 49 485 eyes. *Ophthalmol Retina*. 2020;4(1):19–30.
5. Holz FG, Tadayoni R, Beatty S, et al. Multi-country real-life experience of anti-vascular endothelial growth factor therapy for wet age-related macular degeneration. *Br J Ophthalmol*. 2015;99(2):220–226.
6. Mones J, Singh RP, Bandello F, Souied E, Liu X, Gale R. Undertreatment of neovascular age-related macular degeneration after 10 years of anti-vascular endothelial growth factor therapy in the real world: the need for a change of mindset. *Ophthalmologica*. 2020;243(1):1–8.
7. Wecker T, Grundel B, Reichl S, et al. Anti-VEGF injection frequency correlates with visual acuity outcomes in pro re nata neovascular AMD treatment. *Sci Rep*. 2019;9(1):3301.
8. Sharma A, Kumar N, Kuppermann BD, Loewenstein A, Bandello F. Brolicizumab: is extended VEGF suppression on the horizon? *Eye (Lond)*. 2020;34(3):424–426.
9. Campochiaro PA, Marcus DM, Awh CC, et al. The port delivery system with ranibizumab for neovascular age-related macular degeneration: results from the randomized phase 2 ladder clinical trial. *Ophthalmology*. 2019;126(8):1141–1154.
10. Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature*. 1996;380(6573):435–439.
11. Ferrara N, Carver-Moore K, Chen H, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature*. 1996;380(6573):439–442.
12. Kurihara T, Westenskow PD, Bravo S, Aguilar E, Friedlander M. Targeted deletion of VEGFA in adult mice induces vision loss. *J Clin Invest*. 2012;122(11):4213–4217.
13. Saint-Geniez M, Kurihara T, Sekiyama E, Maldonado AE, D'Amore PA. An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. *Proc Natl Acad Sci USA*. 2009;106(44):18751–18756.
14. Long D, Kanan Y, Shen J, et al. VEGF/VEGFR2 blockade does not cause retinal atrophy in AMD-relevant models. *JCI Insight*. 2018;3(10):e120231.
15. Miki A, Miki K, Ueno S, et al. Prolonged blockade of VEGF receptors does not damage retinal photoreceptors or ganglion cells. *J Cell Physiol*. 2010;224(1):262–272.
16. Ueno S, Pease ME, Wersinger DM, et al. Prolonged blockade of VEGF family members does not cause identifiable damage to retinal neurons or vessels. *J Cell Physiol*. 2008;217(1):13–22.
17. Grunwald JE, Pistilli M, Daniel E, et al. Incidence and growth of geographic atrophy during 5 years of comparison of age-related macular degeneration treatments trials. *Ophthalmology*. 2017;124(1):97–104.
18. Christakis PG, Agron E, Klein ML, et al. Incidence of macular atrophy after untreated neovascular age-related macular degeneration: Age-Related Eye Disease Study Report 40. *Ophthalmology*. 2019;127(6):784–792.
19. Daniel E, Maguire MG, Grunwald JE, et al. Incidence and Progression of Nongeographic Atrophy in the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT) clinical trial. *JAMA Ophthalmol*. 2020;138(5):510–518.
20. Grishanin R, Vuilleminot B, Sharma P, et al. Pre-clinical evaluation of ADVIM-022, a novel gene therapy approach to treating wet age-related macular degeneration. *Mol Ther*. 2019;27(1):118–129.
21. Dalkara D, Byrne LC, Klimczak RR, et al. In vivo-directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous. *Sci Transl Med*. 2013;5(189):189ra176.
22. Khabou H, Desrosiers M, Winckler C, et al. Insight into the mechanisms of enhanced retinal transduction by the engineered AAV2 capsid variant -7m8. *Biotechnol Bioeng*. 2016;113(12):2712–2724.
23. Holash J, Davis S, Papadopoulos N, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci USA*. 2002;99(17):11393–11398.

24. Missel PJ. Simulating intravitreal injections in anatomically accurate models for rabbit, monkey, and human eyes. *Pharm Res.* 2012;29(12):3251–3272.
25. Goody R, Banerjee A, Lawrence M. *Validating a scoring methodology for drug induced anterior segment inflammation in non-human primate eyes.* Society of Toxicology Annual Meeting, San Antonio, TX, March 11–15. 2018.
26. Kiss S, Grishanin R, Nguyen A, et al. Analysis of aflibercept expression in NHPs following intravitreal administration of ADVIM-022, a Potential gene therapy for nAMD. *Mol Ther Methods Clin Dev.* 2020;18:345–353.
27. Soubrane G, Akinin I. Retinal layer thickness measurement using automated retinal segmentation with SD-OCT. *Invest Ophthalmol Vis Sci.* 2015;56(7):5923–5923.
28. Wong TY, Chakravarthy U, Klein R, et al. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. *Ophthalmology.* 2008;115(1):116–126.
29. Ho AC, Albin TA, Brown DM, Boyer DS, Regillo CD, Heier JS. The potential importance of detection of neovascular age-related macular degeneration when visual acuity is relatively good. *JAMA Ophthalmol.* 2017;135(3):268–273.
30. Holekamp NM. Review of neovascular age-related macular degeneration treatment options. *Am J Manag Care.* 2019;25(10 Suppl):S172–S181.
31. Peden MC, Suner IJ, Hammer ME, Grizzard WS. Long-term outcomes in eyes receiving fixed-interval dosing of anti-vascular endothelial growth factor agents for wet age-related macular degeneration. *Ophthalmology.* 2015;122(4):803–808.
32. Ciulla TA, Hussain RM, Berrocal AM, Nagiel A. Voretigene neparvovec-rzyl for treatment of RPE65-mediated inherited retinal diseases: a model for ocular gene therapy development. *Expert Opin Biol Ther.* 2020;20(6):565–578.
33. Kiss S, Grishanin R, Nguyen A, et al. Analysis of aflibercept expression in non-human primates following intravitreal administration of ADVIM-022, a potential gene therapy for neovascular age-related macular degeneration. *Mol Ther Methods Clin Dev.* 2020;18:345–353.
34. Grishanin R, Vuilleminot B, Sharma P, et al. Pre-clinical evaluation of ADVIM-022, a novel gene therapy approach to treating wet age-related macular degeneration. *Mol Ther.* 2019;27(1):118–129.
35. Ammar MJ, Zhou EJ, Morgan JI, et al. Safety of the subretinal delivery of RGX-314 AAV8-anti-VEGF Fab gene therapy in NHP: retinal structure over one year. *Invest Ophthalmol Vis Sci.* 2018;59(9):1422.
36. Lyubarsky A, Wielechowski E, Aleman TS, et al. Safety of subretinal delivery of RGX-314 (AAV8-anti-VEGF Fab) in the non-human primate as assessed by full-field ERG. *Invest Ophthalmol Vis Sci.* 2018;59:4546.
37. Lyubarsky A, Wielechowski E, Aleman TS, et al. Normal parameters of the full field ERG recorded with bipolar electrodes in Cynomolgus Macaque (Macaque fascicularis). *Invest Ophthalmol Vis Sci.* 2017;58(8):5344.
38. Guymer RH, Rosenfeld PJ, Curcio CA, et al. Incomplete retinal pigment epithelial and outer retinal atrophy in age-related macular degeneration: Classification of Atrophy Meeting Report 4. *Ophthalmology.* 2020;127(3):394–409.
39. Sadda SR, Guymer R, Holz FG, et al. Consensus definition for atrophy associated with age-related macular degeneration on OCT: Classification of Atrophy Report 3. *Ophthalmology.* 2018;125(4):537–548.
40. Maclachlan TK, Lukason M, Collins M, et al. Pre-clinical safety evaluation of AAV2-sFLT01- a gene therapy for age-related macular degeneration. *Mol Ther.* 2011;19(2):326–334.
41. Reichel FF, Dauletbekov DL, Klein R, et al. AAV8 can induce innate and adaptive immune response in the primate eye. *Mol Ther.* 2017;25(12):2648–2660.
42. Ye GJ, Budzynski E, Sonnentag P, et al. Safety and biodistribution evaluation in Cynomolgus Macaques of rAAV2tYF-CB-hRS1, a recombinant adeno-associated virus vector expressing retinoschisin. *Hum Gene Ther Clin Dev.* 2015;26(3):165–176.
43. Ye GJ, Budzynski E, Sonnentag P, et al. Safety and biodistribution evaluation in cynomolgus macaques of rAAV2tYF-PR1.7-hCNGB3, a recombinant AAV vector for treatment of achromatopsia. *Hum Gene Ther Clin Dev.* 2016;27(1):37–48.
44. Bouquet C, Vignal Clermont C, Galy A, et al. Immune response and intraocular inflammation in patients with Leber hereditary optic neuropathy treated with intravitreal injection of recombinant adeno-associated virus 2 carrying the ND4 gene: a secondary analysis of a phase 1/2 clinical trial. *JAMA Ophthalmol.* 2019;137(4):399–406.

45. Cepeda D, Yeh E, Nieves J, et al. Immunological response and durability of expression following sequential intravitreal administration of AAV2.7m8 gene therapy to the contralateral eye in non-human primates. *Invest Ophthalmol Vis Sci.* 2019;60(9):2910–2910.
46. Turunen H, Timmers AM, Newmark J, Pennock S, Shearman MS. Effect of neutralizing Anti-AAV antibodies on vector transduction following intravitreal administration of AAV in non-human primates. *Invest Ophthalmol Vis Sci.* 2019;60(9):2911–2911.