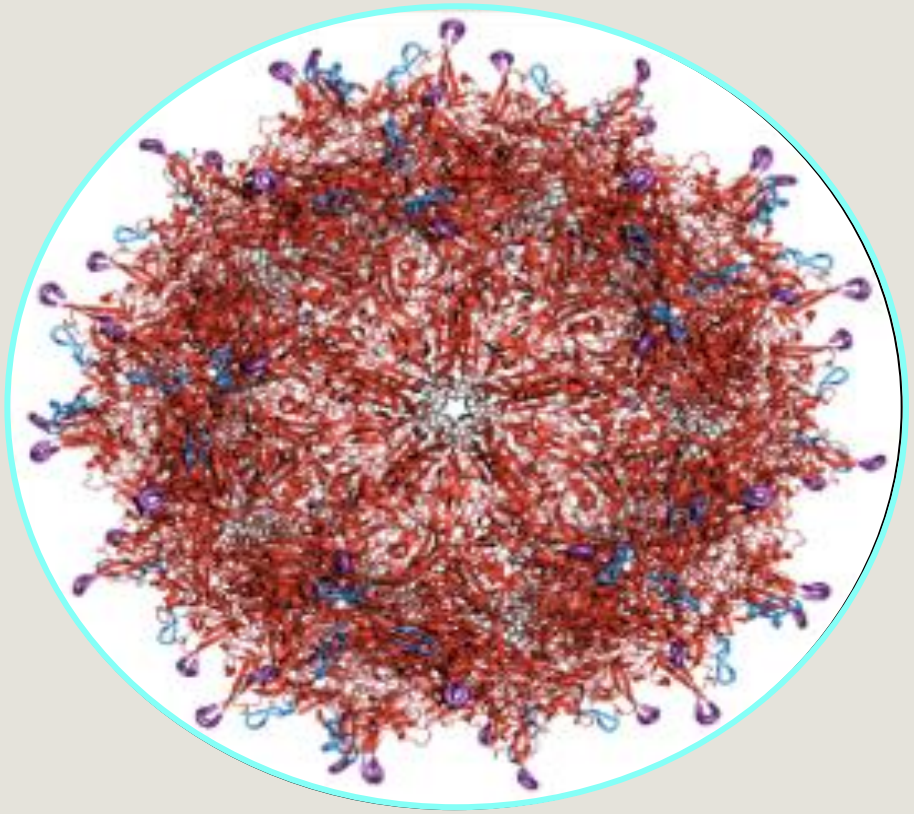
Gene Therapy for Neovascular AMD: Intravitreal Delivery of AAV-7m8 Vectors



David M. Brown, MD

Retina Consultants of Houston

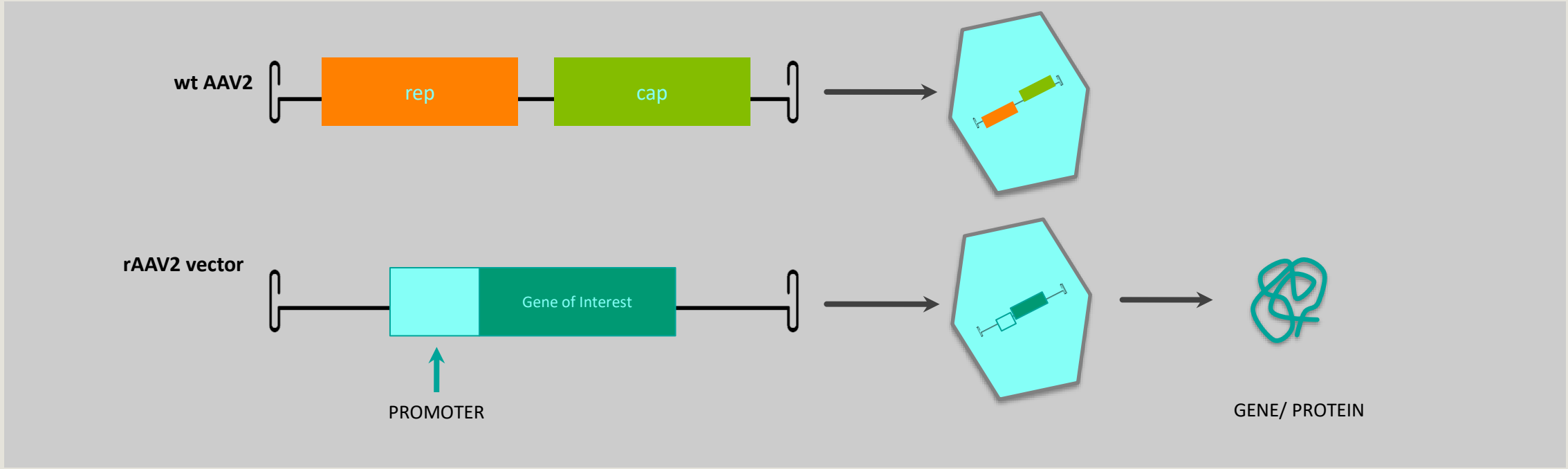
KEY POINTS

- Non Human Primate Models Demonstrate:
  - Robust expression of aflibercept sustained 22.5 months after single IVT injection
  - ADVM-022 delivered aflibercept is sustained at levels similar to the aflibercept recombinant protein-injected eyes 21-31 days post-dose.

- Human Phase 1 Trial ADVM-022 – OPTIC
  - IND Active – August 2018
  - Granted Fast Track – September 2018
  - First patient dosed November 2018 – Preliminary Data expected 1Q20.

Adeno-Associated Virus (AAV) as a Gene Therapy Vector

- Simple virus made safe for gene therapy
- Protein on outside, DNA on inside
- Non-pathogenic, non-replicating, non-integrating



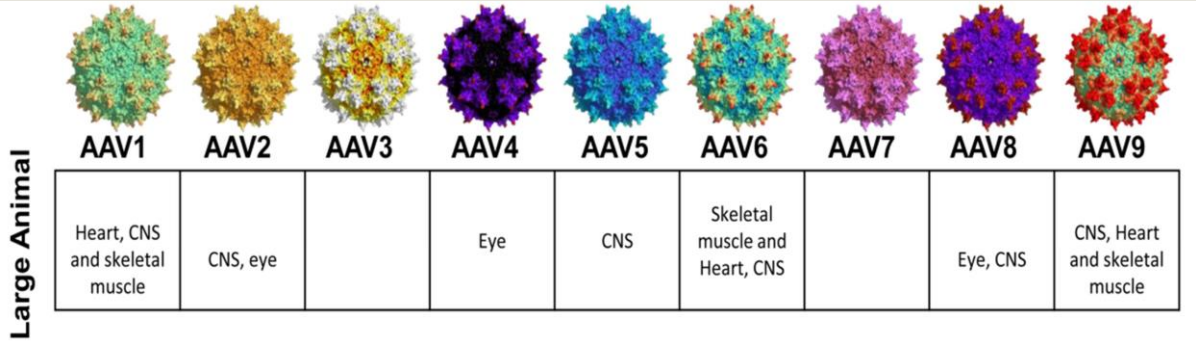
Gene Therapy rAAV Toolbox Options

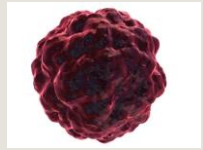
Capsid
- Improve transduction efficiency upon intravitreal delivery

cDNA: sFLT vs standard of care proteins
- sFLT
- Aflibercept
- Ranibizumab

Expression cassette
- Codon optimization
- Combination of various regulatory elements for enhanced protein expression

rAAV Capsid Variants

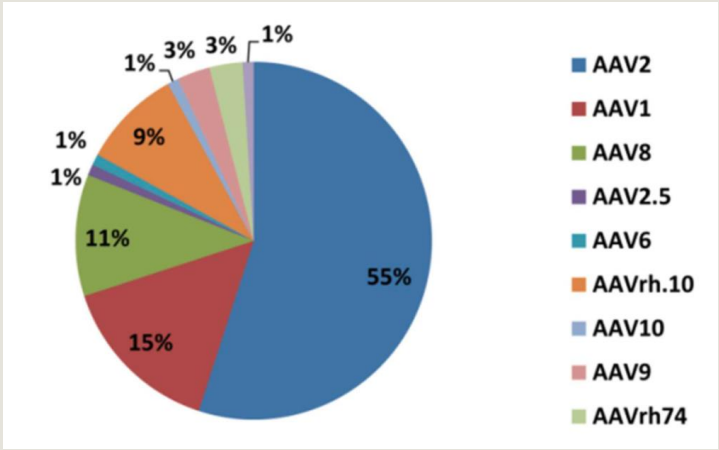




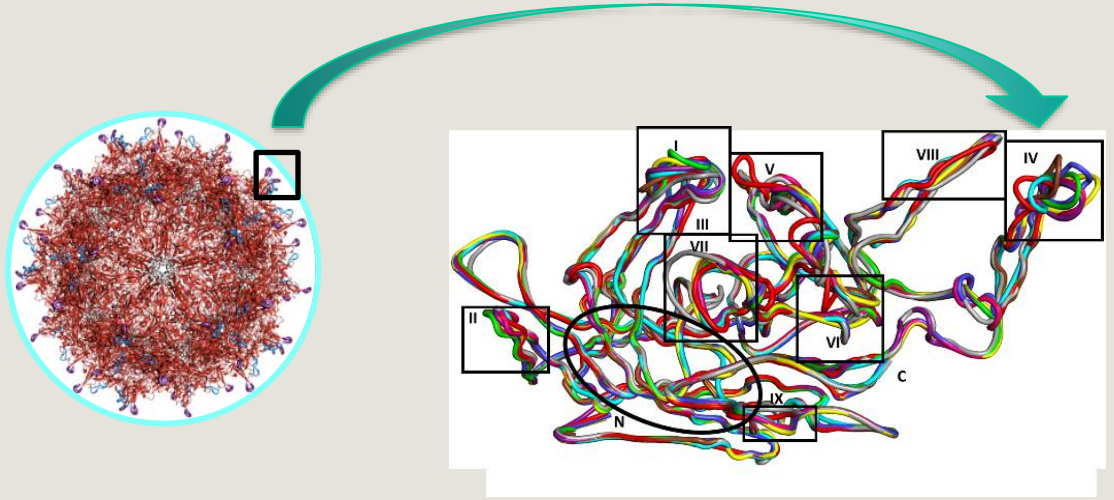
AAV150

	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9	AAV10	AAV11	AAV12
AAV1	100%	83%	86%	64%	59%	99%	85%	84%	82%	85%	67%	61%
AAV2	83%	100%	87%	61%	58%	83%	82%	83%	82%	84%	63%	60%
AAV3	86%	87%	100%	63%	58%	87%	84%	85%	83%	85%	65%	61%
AAV4	64%	61%	63%	100%	53%	63%	64%	64%	63%	64%	82%	79%
AAV5	59%	58%	58%	53%	100%	59%	59%	58%	57%	57%	53%	53%
AAV6	99%	83%	87%	63%	59%	100%	85%	84%	82%	85%	66%	61%
AAV7	85%	82%	84%	64%	59%	85%	100%	88%	81%	88%	67%	62%
AAV8	84%	83%	85%	64%	58%	84%	88%	100%	85%	93%	66%	62%
AAV9	82%	82%	83%	63%	57%	82%	81%	85%	100%	86%	64%	60%
AAV10	85%	84%	85%	64%	57%	85%	88%	93%	86%	100%	67%	61%
AAV11	67%	63%	65%	82%	53%	66%	67%	66%	64%	67%	100%	84%
AAV12	61%	60%	61%	79%	53%	61%	62%	62%	60%	61%	84%	100%

Summary of Trials



Novel Capsid: AAV.7m8

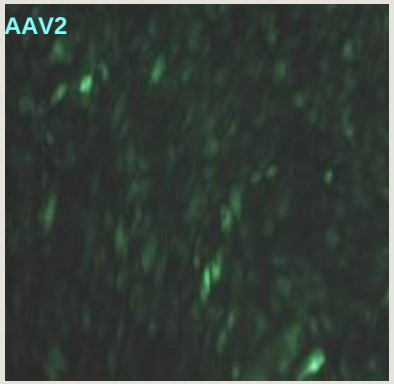


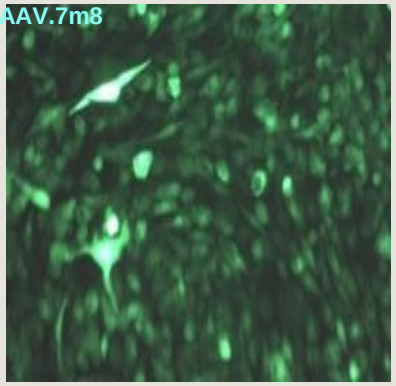
Variant of AAV2 with a 10-aa insertion in Loop IV of AAV2 VP3
Discovered by directed evolution (UC Berkeley)
Screened *in vivo* in murine retina
Exhibits robust tropism for photoreceptor via intravitreal injection

Dalkara et al. Sci. Transl. Med. (2013)

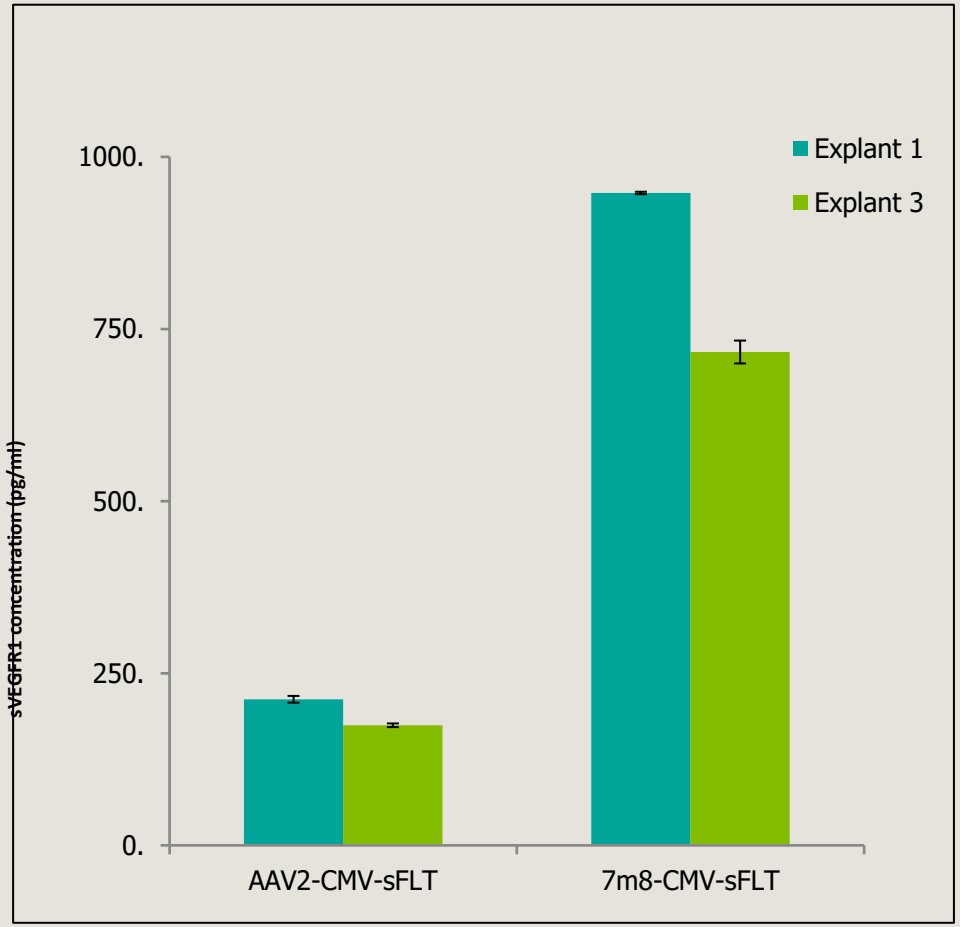
AAV.7m8 Improves Transduction

HEK293

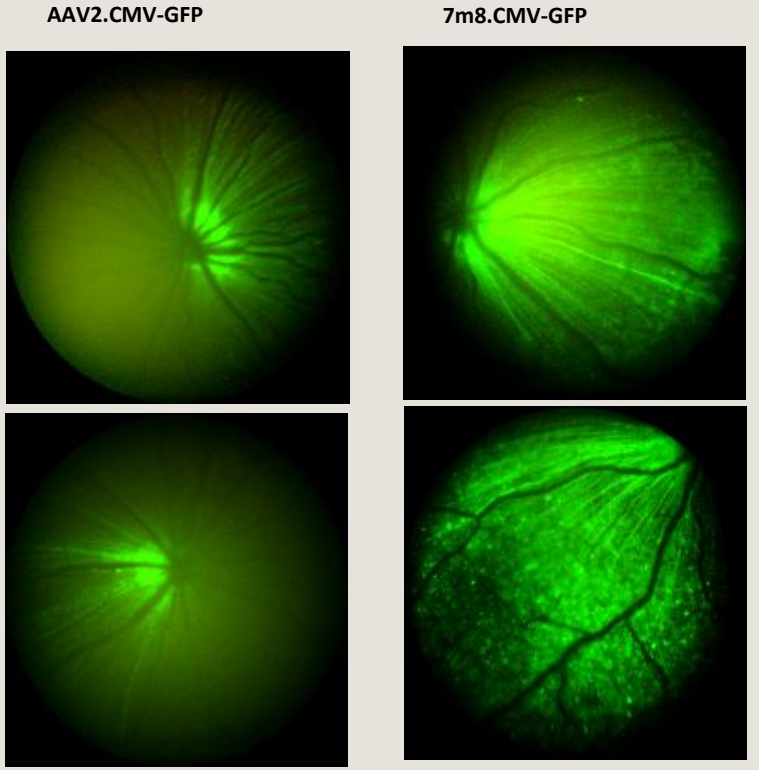




Pig retinal explant



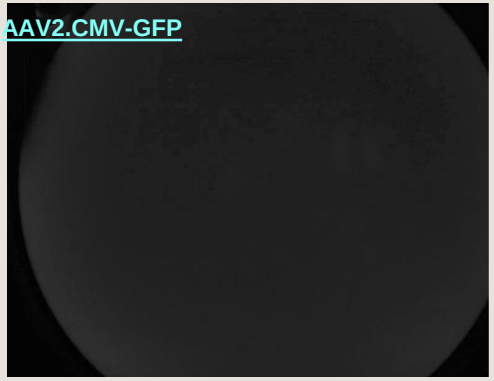
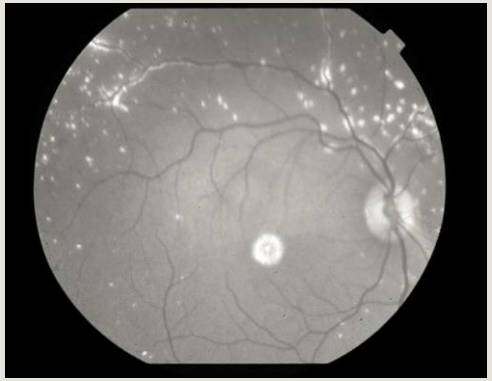
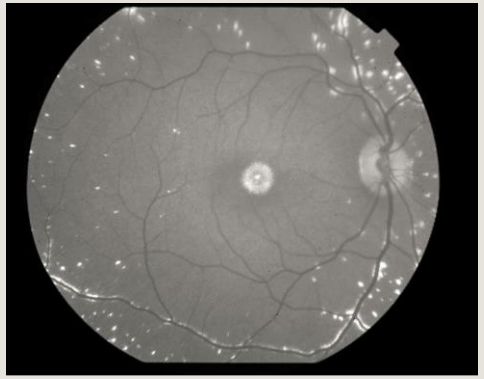
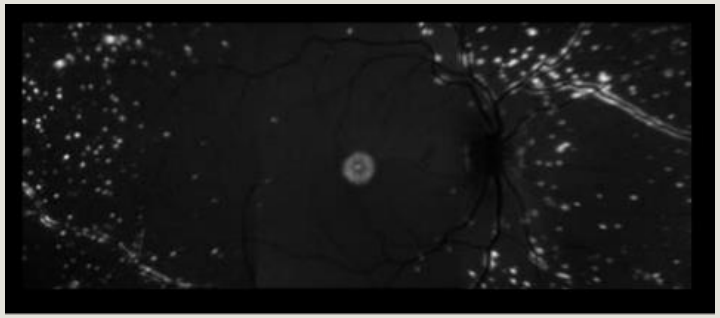
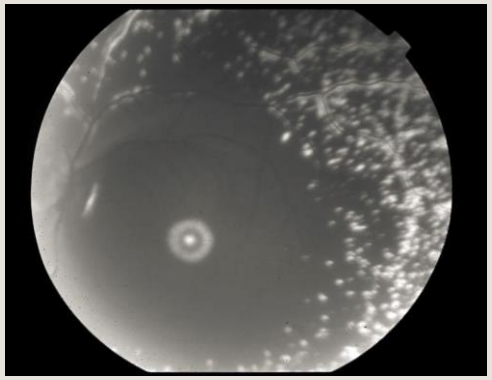
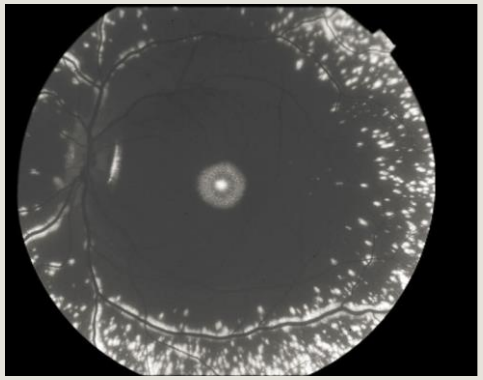
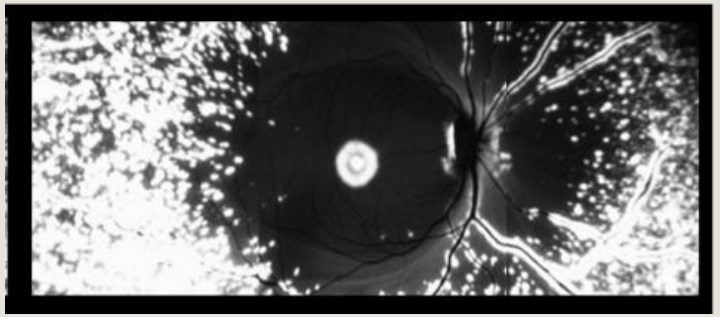
Rat retina



Improved Transduction in Nonhuman Primate

Fluorescence Fundus Images

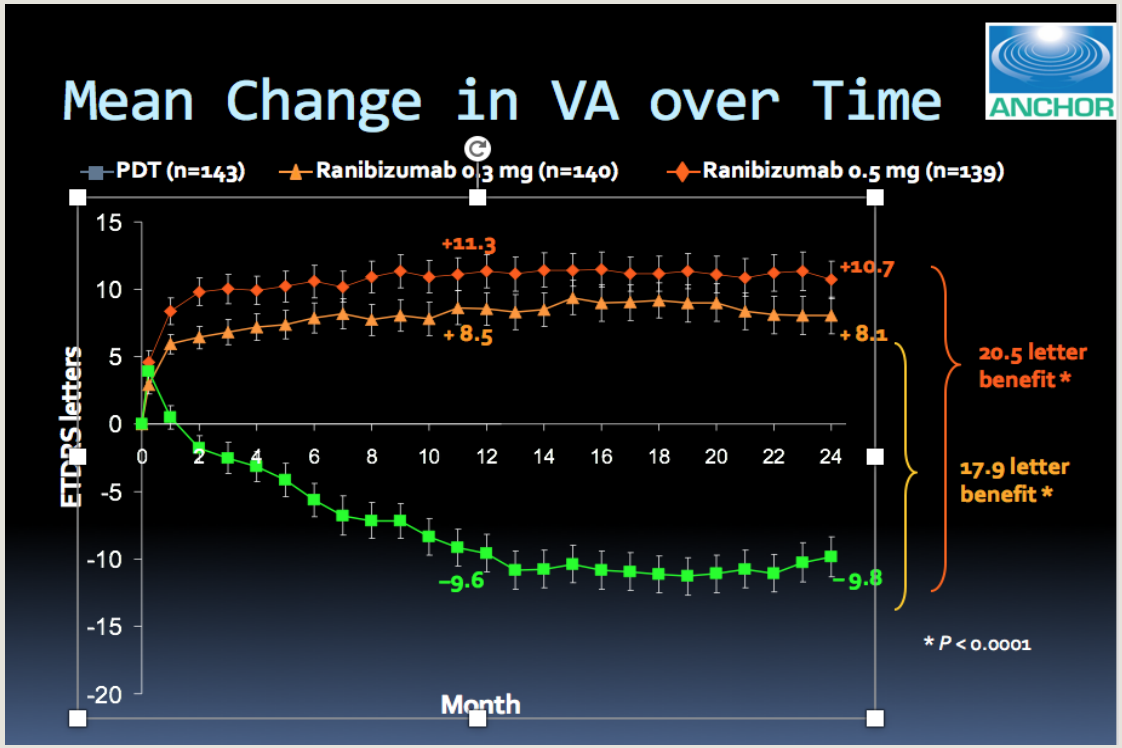
Heidelberg Spectralis Images

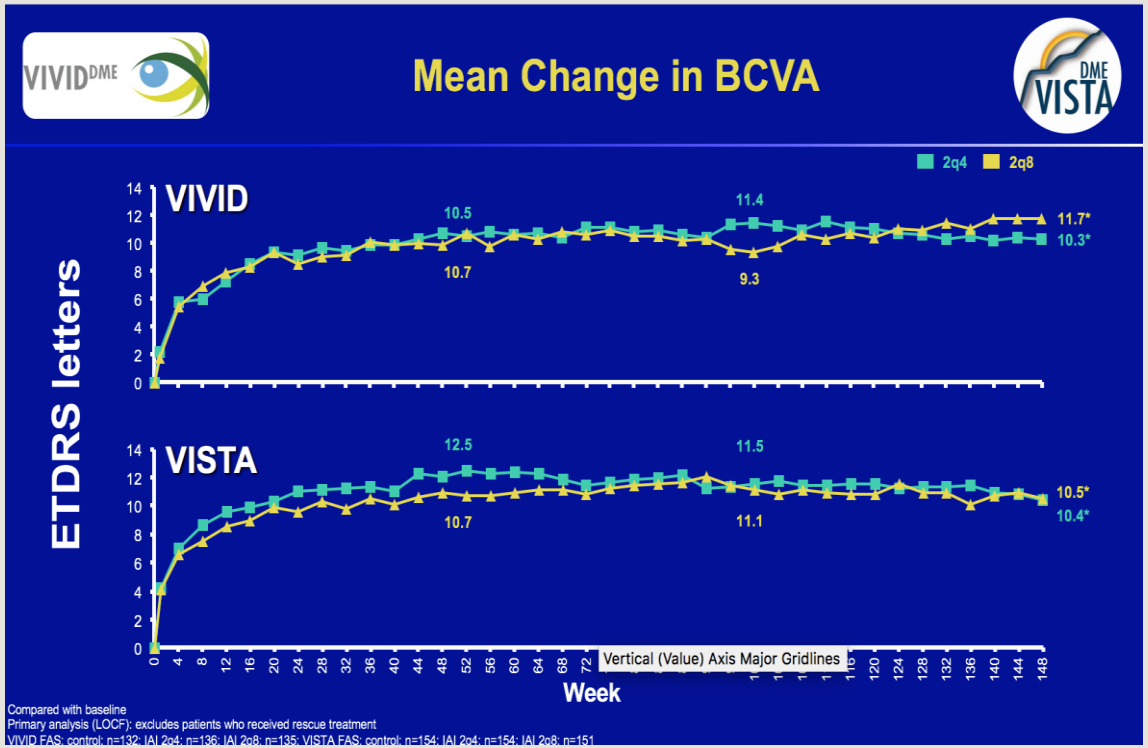
	4 wks	8 wks	12 wks	12 wks
AAV2.CMV-GFP				
7m8.CMV-GFP				

- 5E+11 vg/eye

➢ GFP expression mediated by 7m8 comes on faster
➢ 7m8 vector mediates highest level of GFP expression across the retina and inside the fovea

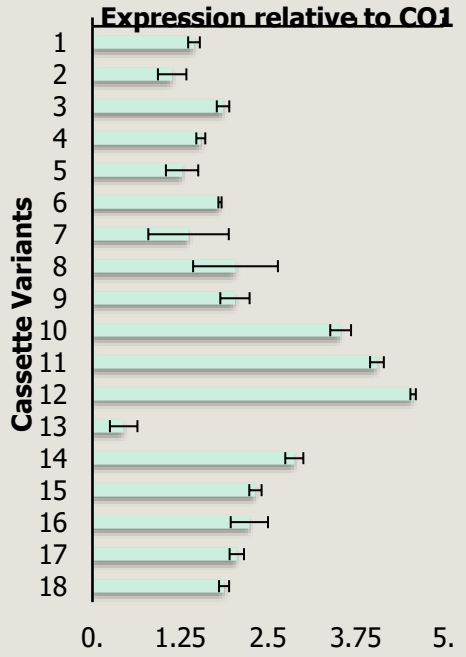
cDNA: sFLT vs Standard of Care Proteins



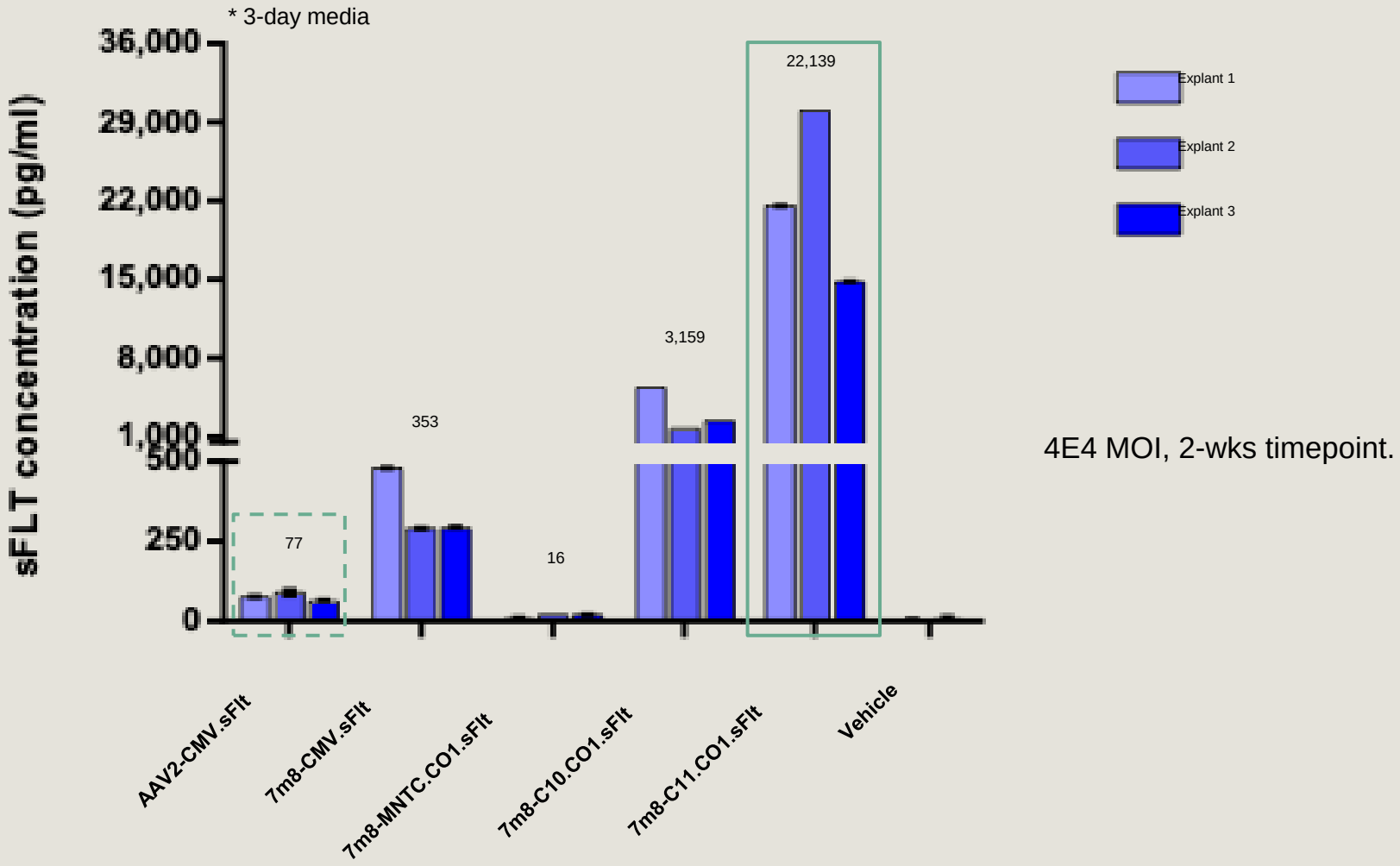


Codon optimization and assessment of regulatory elements

Variant	Enhancer	Promoter	Intron	5'UTR	cDNA	Enhancer	RNA export	polyA	Total size
1					CO1-cDNA				4220
2					CO1-cDNA				3575
3					CO1-cDNA				3465
4					CO1-cDNA				3665
5					CO1-cDNA				3630
6					CO1-cDNA				3660
7					CO1-cDNA				4360
8					CO1-cDNA				3890
9					CO1-cDNA				3355
10					CO1-cDNA				4080
11					CO1-cDNA				3625
12					CO1-cDNA				3740
13					CO1-cDNA				3660
14					CO1-cDNA				4180
15					CO1-cDNA				3995
16					CO1-cDNA				3815
17					CO1-cDNA				3620
18					CO1-cDNA				3220
CO1					CO1-cDNA				3355

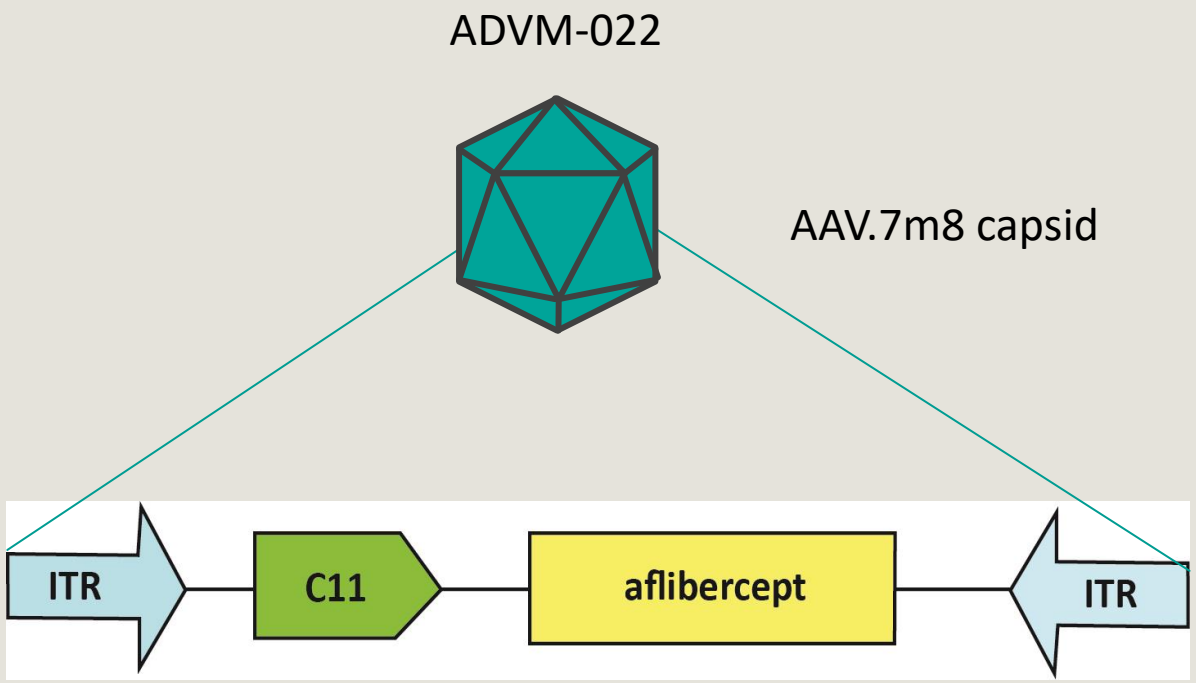


Evaluation of expression cassettes in pig retinal explants



4E4 MOI, 2-wks timepoint.

ADVM-022: AAV.7m8-aflibercept



Aflibercept expression cassette

C11=> strong ubiquitous expression cassette

AAV.7m8: Novel Capsid:

- AAV.7m8 Variant of AAV2
- Discovered by directed evolution (UC Berkeley)
- Exhibits robust tropism for retinal cells via intravitreal injection

Steve Ryan Laser Model

Laboratory Sciences

Subretinal Neovascularization

Natural History of an Experimental Model

Stephen J. Ryan, MD

• The disciform response is characterized by its many manifestations determined by subretinal neovascularization. Many advances have been made clinically and with histopathologic correlation, but a clear understanding of the pathogenesis of subretinal neovascularization has not yet been achieved at the basic level. The need to determine the pathogenesis and understand the rationale for therapy argues strongly for the development of a suitable experimental model of subretinal neovascularization. The macula is much more predisposed to the development of subretinal neovascularization than is the area nasal to the nerve head or the periphery. Spontaneous hemorrhages in experimentally induced subretinal neovascularization occurred, underscoring the clinical relevance of this model system. The delineation of the natural history in this report provides the basis for future morphologic correlation and subsequent manipulations of this model system.

(*Arch Ophthalmol* 1982;100:1804-1809)

The disciform response has long been recognized as a clinicopathologic entity common to many disease processes and characterized by subretinal neovascularization.[1] Clinical observations and histopathologic correlations have been made on some eyes,[2-7] but the precise pathogenesis of subretinal neovascularization and the role of many different factors in its development remain unclear. Thus, the development of an experimental model in which the pathogenesis of the disciform process can be elucidated remains an important goal.[8-10]

Argon laser photocoagulation has been reported to induce subretinal neovascularization clinically.[11,12] Thus, the laser has been used to cause subretinal neovascularization in a reproducible manner in rhesus monkey eyes.[13] The rhesus monkey (*Macaca mulatta*) was chosen for these studies because its distinct retinal and choroidal circulation and macular anatomy are similar to those of man. The morphology, including histology and ultrastructure, has been well described previously, since this animal has been extensively studied in vision research.[14,15]

This report details the results of a natural history study of subretinal neovascularization in the rhesus monkey model.

MATERIALS AND METHODS

Preoperatively, fundus photographs and fluorescein angiograms were obtained on all 44 adult rhesus monkeys in these studies. Animals who had drusen or other abnormalities of the retina, including the macula, were excluded from the study.

For laser treatment, a combination of ketamine hydrochloride (10 mg/kg), phenothiazine (1.5 mg/kg), and atropine sulfate (.05 mg/kg) was administered intramuscularly as a sedative. This treatment was followed by anesthesia with intravenous pentobarbital sodium (5 mg/kg). Topical 10% phenylephrine hydrochloride and 1% tropicamide produced pupillary dilation.

The radiation system used (Coherent Radiation System-900 Argon Laser) had a principal output at 5,145 (green) and 4,880 (blue) A and was used through a slitlamp (Zeiss) and a Goldmann fundus contact lens.

Since the experimental goal was to produce rather than obliterate neovascularization, the exact opposites of the clinical or therapeutic criteria for laser photocoagulation were used: a small spot size (100 μm) of high intensity (600 to 900 mW, usually in the vicinity of 700 mW) and short duration (0.1 s).

The laser burn, or spot, was applied in the manner of a grid (Fig 1). Eight spots were applied in the macular region. Laser burns were applied at different distances from the center of the fovea, as detailed in the diagram. The fovea itself was never treated in both eyes of the same animal.

The same grid pattern was applied nasal to the optic nerve head in some instances (Fig 1). The center of these eight points was located approximately the same distance nasal to the optic nerve head as the fovea was temporal to the disc.

The periphery was treated in the following manner: Eight spots were placed in a rectangular pattern. The corners were located directly over the areas drained by the vortex veins, with the remaining four spots put on the imaginary lines connecting the four corner laser burns, one to a side (Fig 2). One spot was put directly above the disc, one was put directly below the disc, and the other two were put on a line running through the disc and macula.

Most animals had treatment applied to more than one area so that an animal served as its own control subject. Some of the animals had laser burns applied in all three areas at
nasal to the
ery), whereas
two areas at
and periphery
applied in one
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total of 779 la

Each eye wa
laser treatme
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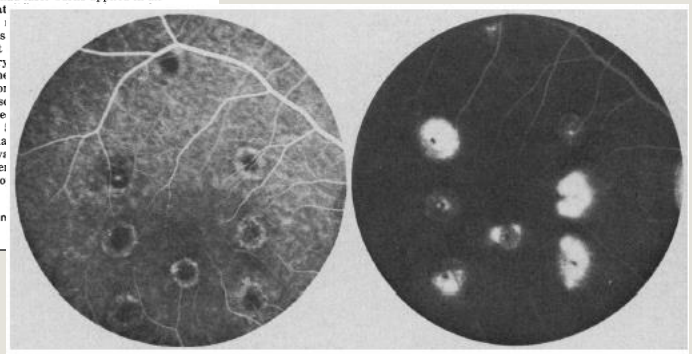
Accepted for publication Oct 19, 1981.

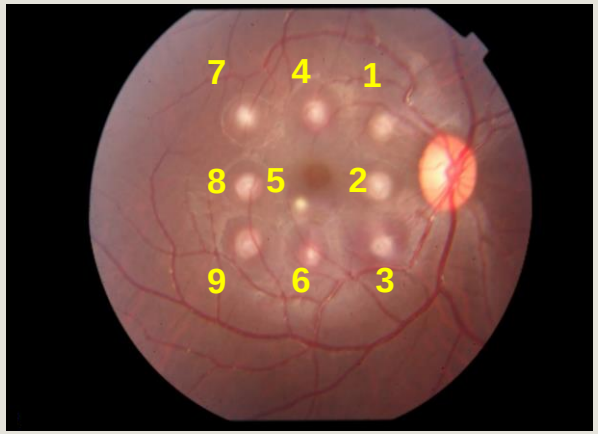
From the Department of Ophthalmology, University of Southern California and the Estelle Doheny Eye Foundation, Los Angeles.

Reprint requests to University of Southern California, Estelle Doheny Eye Foundation, 1355 San Pablo St, Los Angeles, CA 90033 (Dr Ryan).



Drug	**Did it work in Non-Human Primates?**	**Did it work in Rodents?**
Ranibizumab	Yes in laser induced CNV (Krzystolik 2002, Husain 2005)	**Mixed.** Yes in mice expressing human VEGF (Miki 2009); No in rats with laser induced CNV (Lu 2009)
Bevacizumab	Yes in laser induced CNV (Lichtlen 2010, Goody 2011)	**Mixed.** Yes when administered IVT in laser induced CNV in mice (Hollanders 2014, Davis 2012); No in rats with laser induced CNV (Lu 2009); No when administered intraperitoneally in laser induced CNV in mice (Yu 2008); Yes in mice expressing human VEGF (Miki 2009); Yes in IVT VEGF induced CNV in rabbits (Ameri 2007)
Aflibercept	Yes in laser induced CNV (Nork 2011)	**Yes.** Yes in laser induced CNV in mice (Saishin 2003); Yes in subretinal CNV in mice expressing VEGF (Saishin 2003); Yes when administered subcutaneously in subretinal matrigel induced CNV in rats (Cao 2010); Yes when administered intraperitoneally in injury induced inflammatory NV in mice expressing VEGF (Cursiefen 2010)
Pegaptanib	No data	**Mixed.** No in rats with laser induced CNV (Lu 2009); Yes in rat model of proliferative retinopathy (hypoxia induced) (Ishida 2003). Prevents leukostasis and BRB breakdown in diabetic rats (Ishida 2003); prevents vascular permeability in guinea pigs (Eyetech 2002), prevents VEGF induced corneal angiogenesis in rats (Eyetech 2002), prevents NV in retinopathy of maturity in mice (oxygen induced) (Eyetech 2002)

Laser-induced CNV model in nonhuman primates- Rx-Gen (Matt Lawrence)

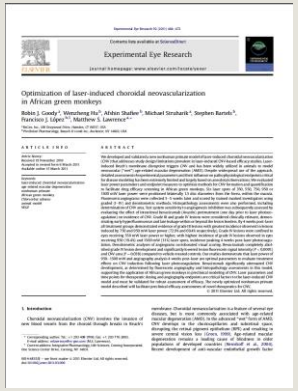


9 laser spots over the macula (1 next to fovea)

Lesions with various degrees of neovascularization and leakage

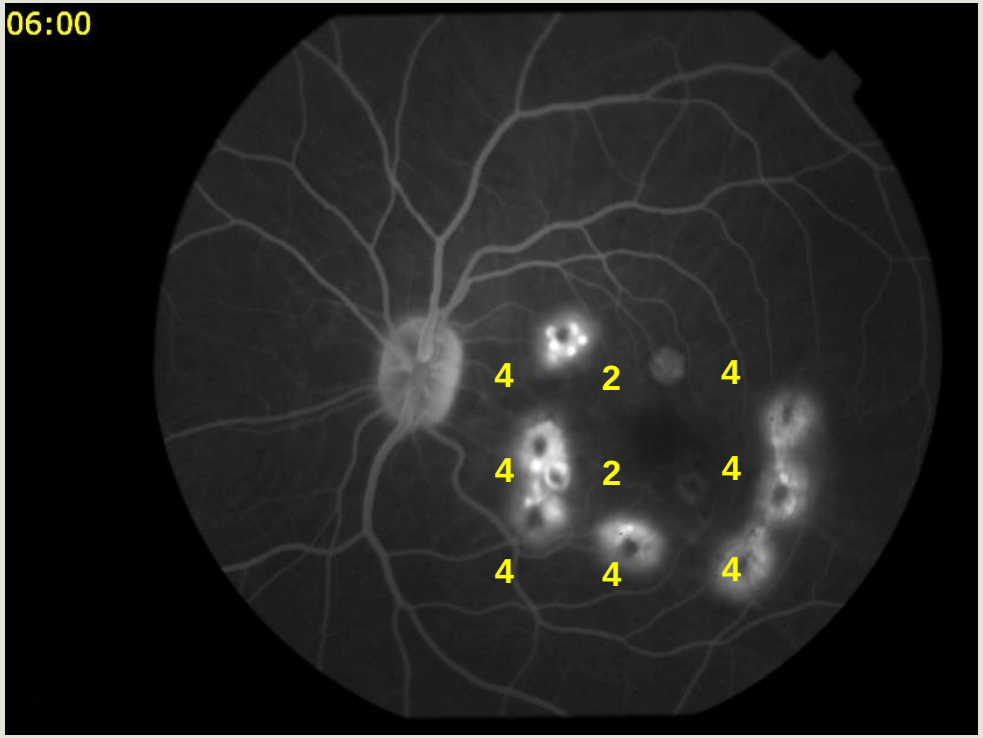
Lesions scored by fluorescein angiography and graded (I-IV) according to onset and persistence of fluorescence over time

Only grade IV lesions considered as representative of CNV


Experimental Eye Research

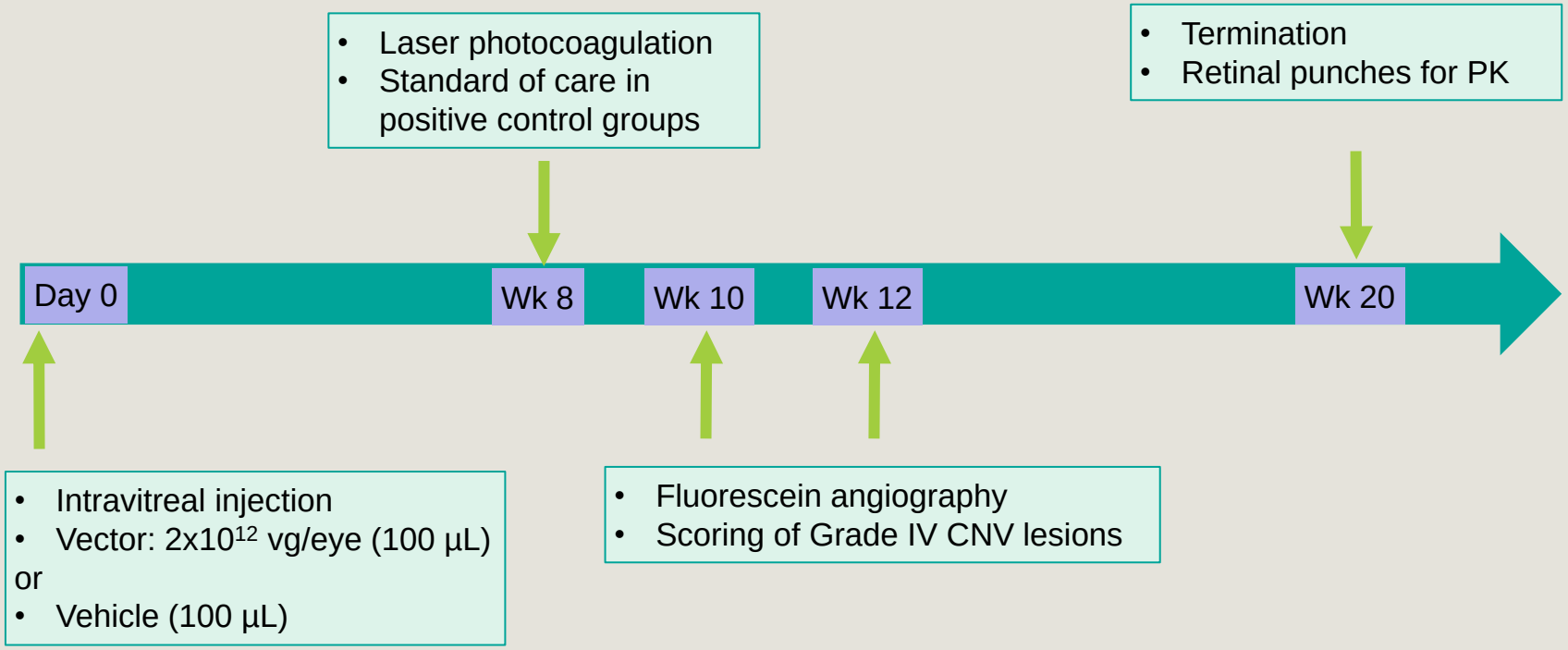
Optimization of laser-induced choroidal neovascularization in African green monkeys

Representative fundus fluorescein angiogram



Vehicle_IVT
70 d post treatment
14 d post lesion

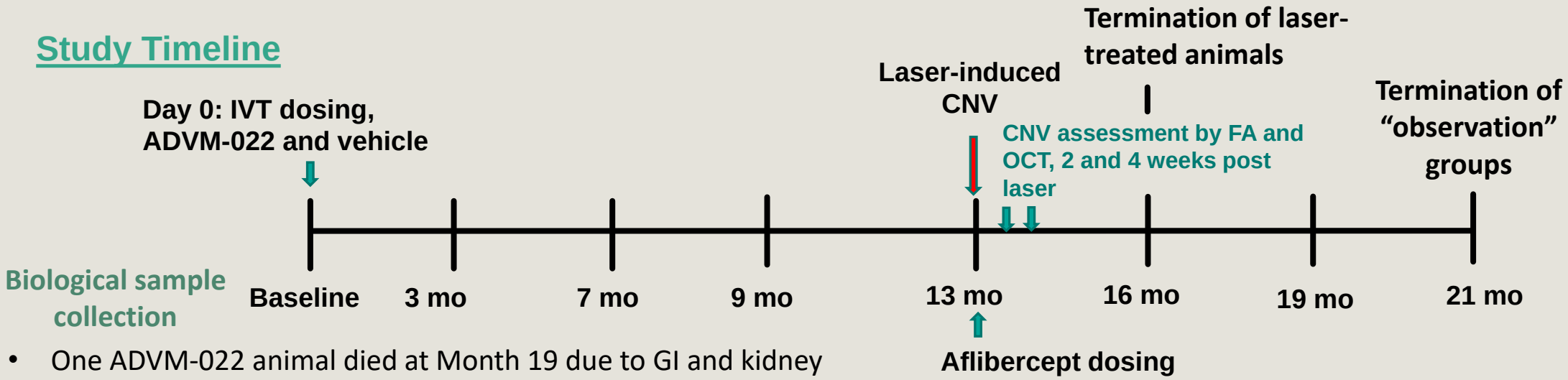
Experimental Design



Long-term Efficacy Study Design

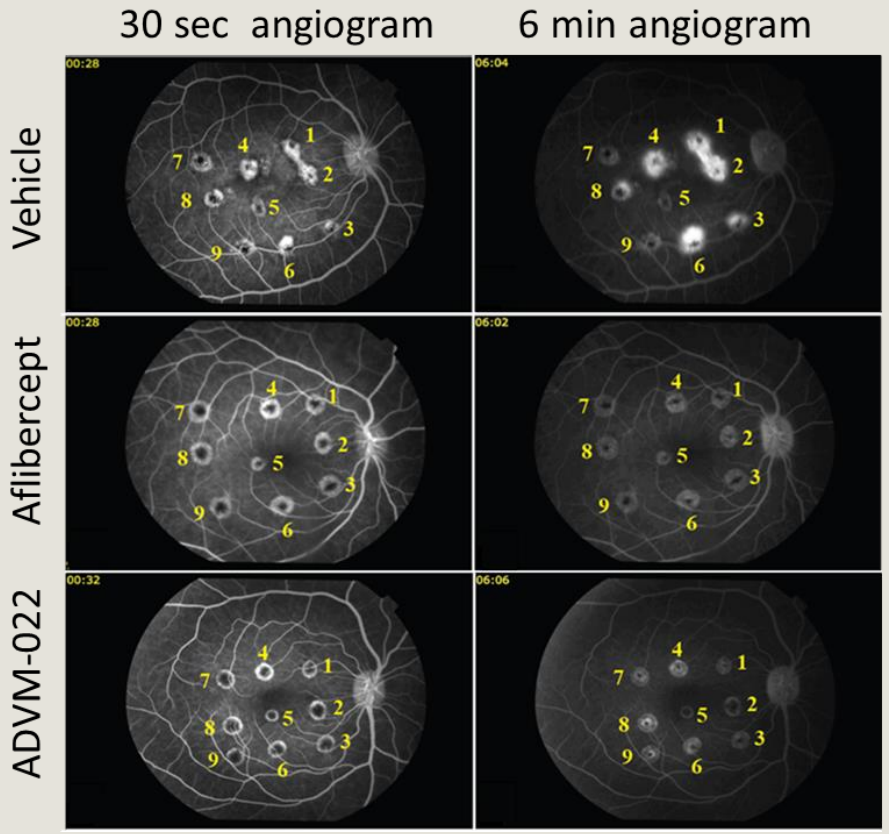
Group	Treatment	N	Dose (IVT; OU)	Treatment delivery	Laser (OU) relative to Day 0
1a	ADVM-022	2M/2F	$2x10^{12}$ vg/50µL	Day 0	13 month
1b		2M/1F	$2x10^{12}$ vg/50µL	Day 0	Not lasered
2a	Vehicle	2M/2F	50µL	Day 0	13 months
2b		2M/1F	50µL	Day 0	Not lasered
3	Aflibercept	2M/2F	1.2 mg/30µL	Day of laser	13 months

Study Timeline

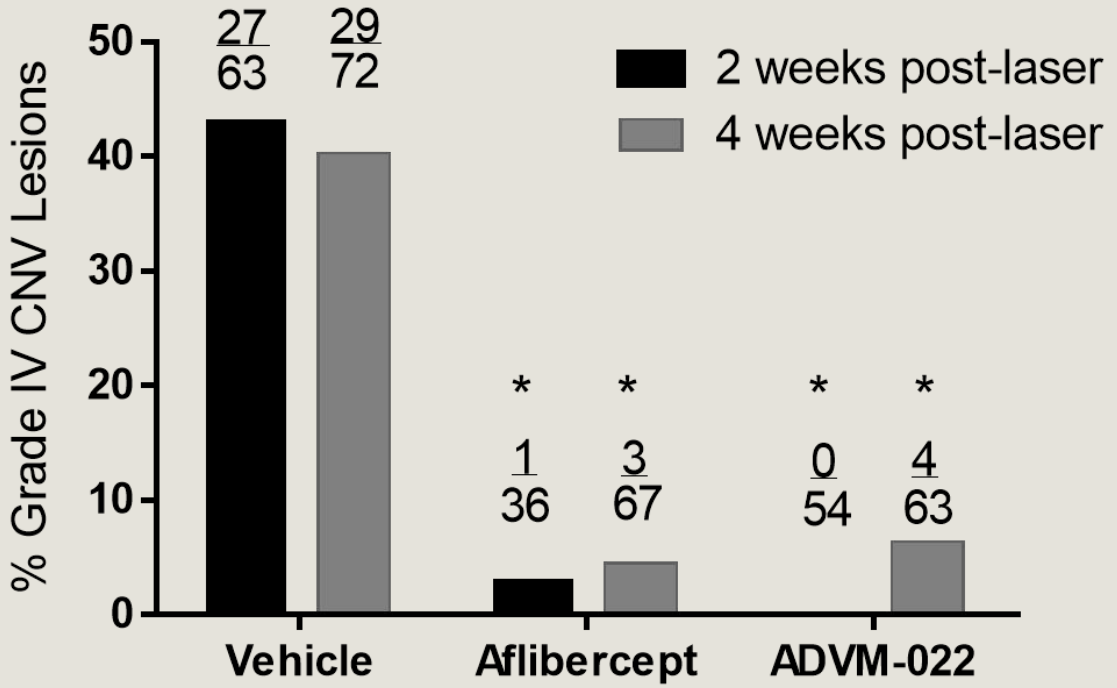


- One ADVM-022 animal died at Month 19 due to GI and kidney conditions unrelated to test article

Single dose IVT ADVM-022 Significantly Reduces the Incidence of Grade IV Lesions When Administered 13 Months Prior to Laser-induced CNV



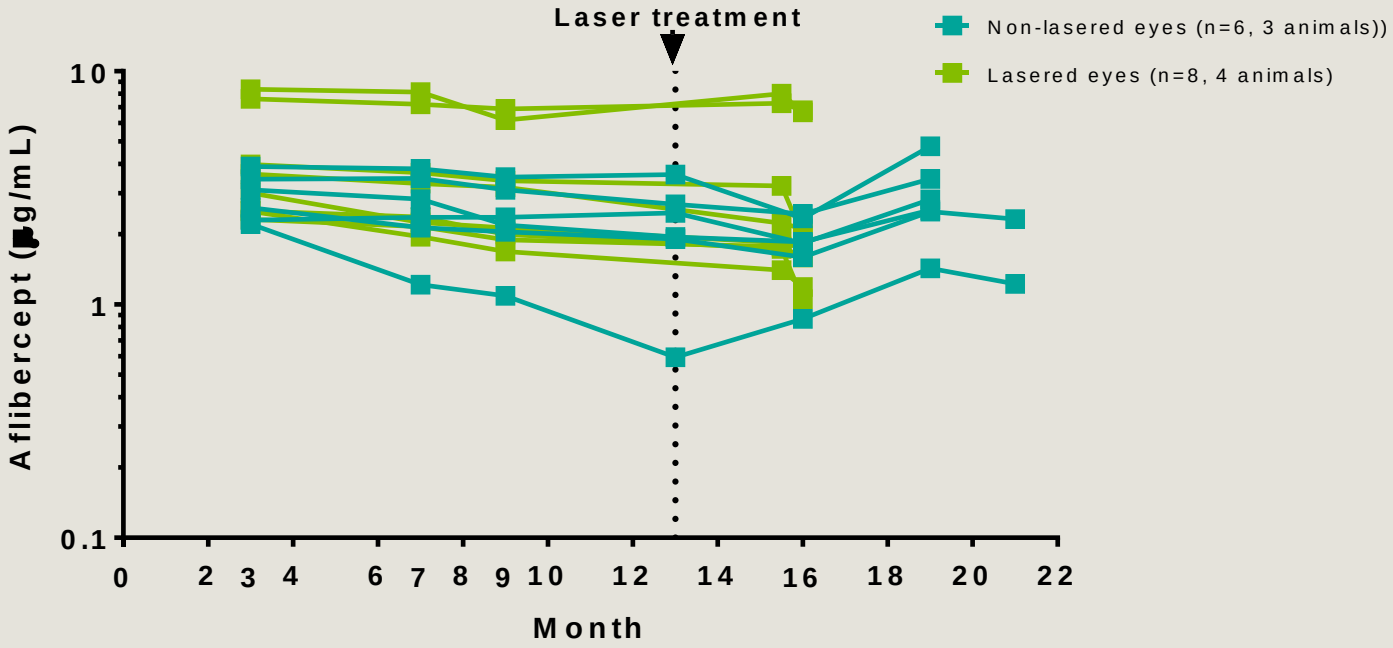
Representative Fluorescein Angiography



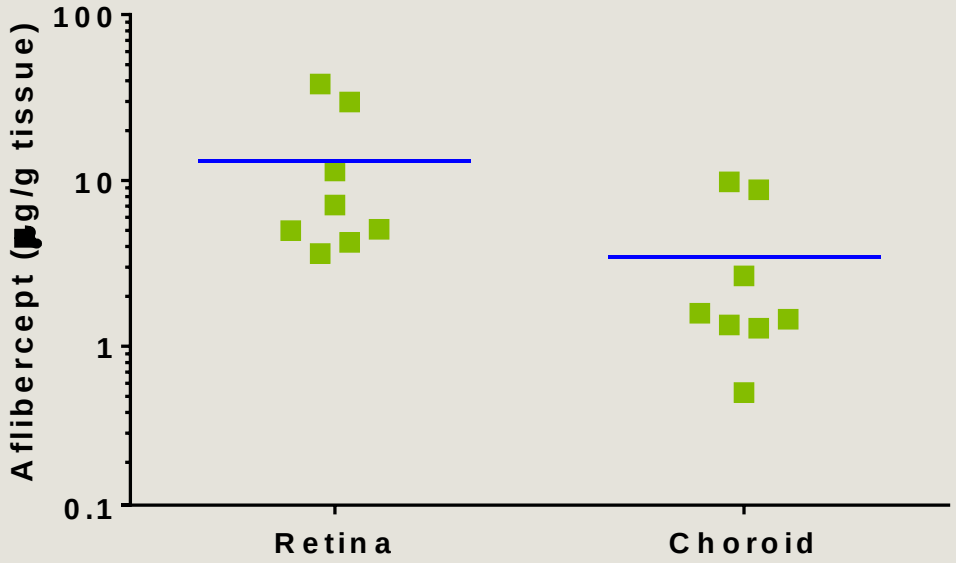
* P < 0.0001

ADVM-022 Delivered Intravitreally Results in the Long-Term Sustained High Levels of Aflibercept Expression in the Eye

Longitudinally assessed expression in vitreous humor up to 21 month after ADVM-022 dose (7 animals)



Aflibercept levels in retina and choroid 16 months post ADVM-022 delivery (n=8 eyes, laser-treated group)



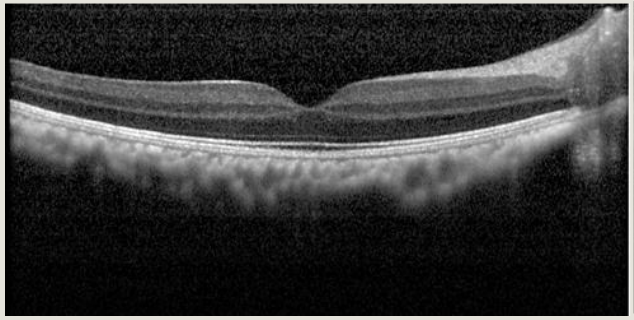
Long-term Expression of Aflibercept in Retina From ADVM-022 Does Not Affect Retinal Morphology

Vehicle control

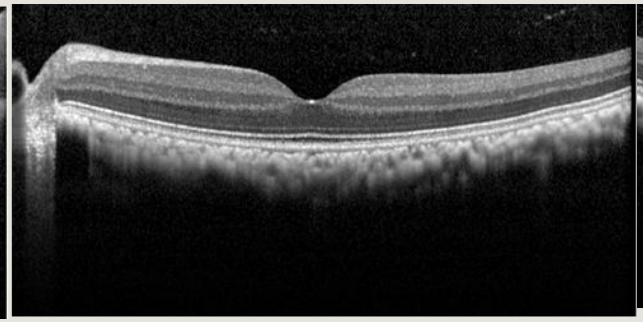
ADVM-022

Baseline

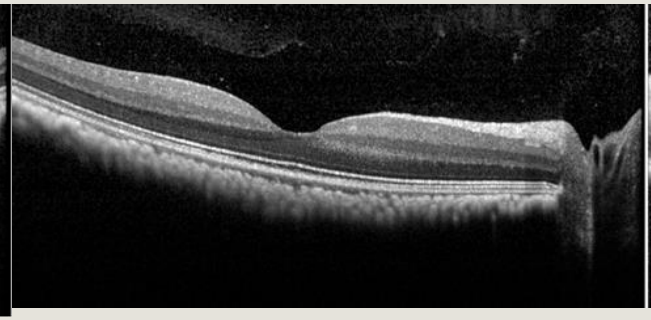
OD



OS



OD



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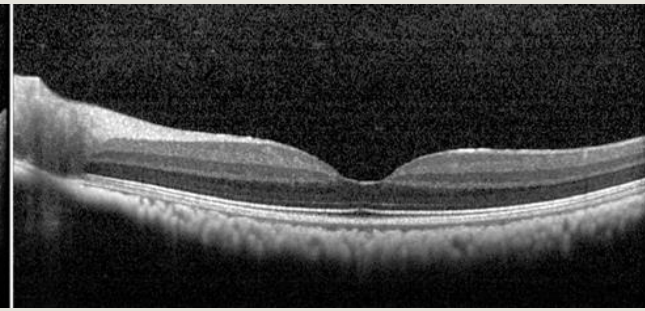
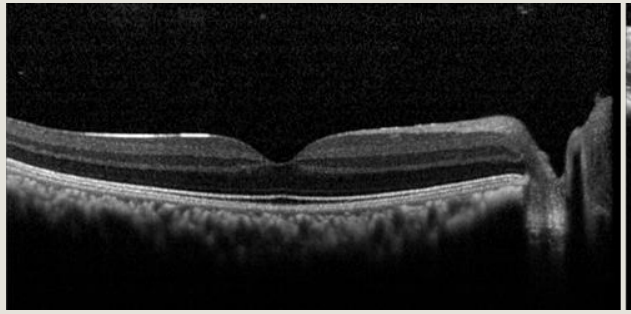


Month 21 post-dose

OD



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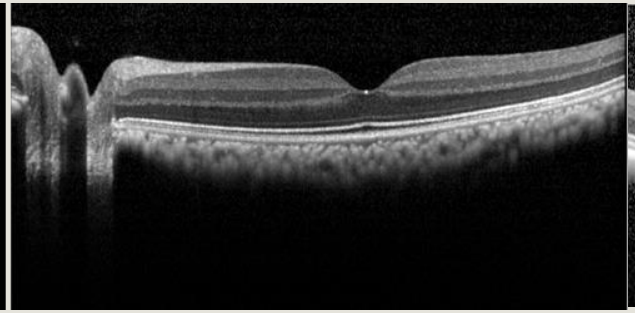

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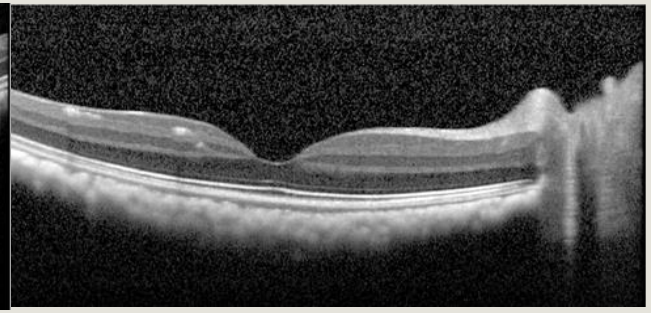

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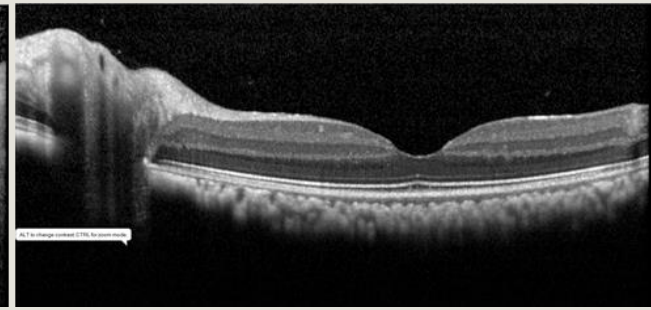
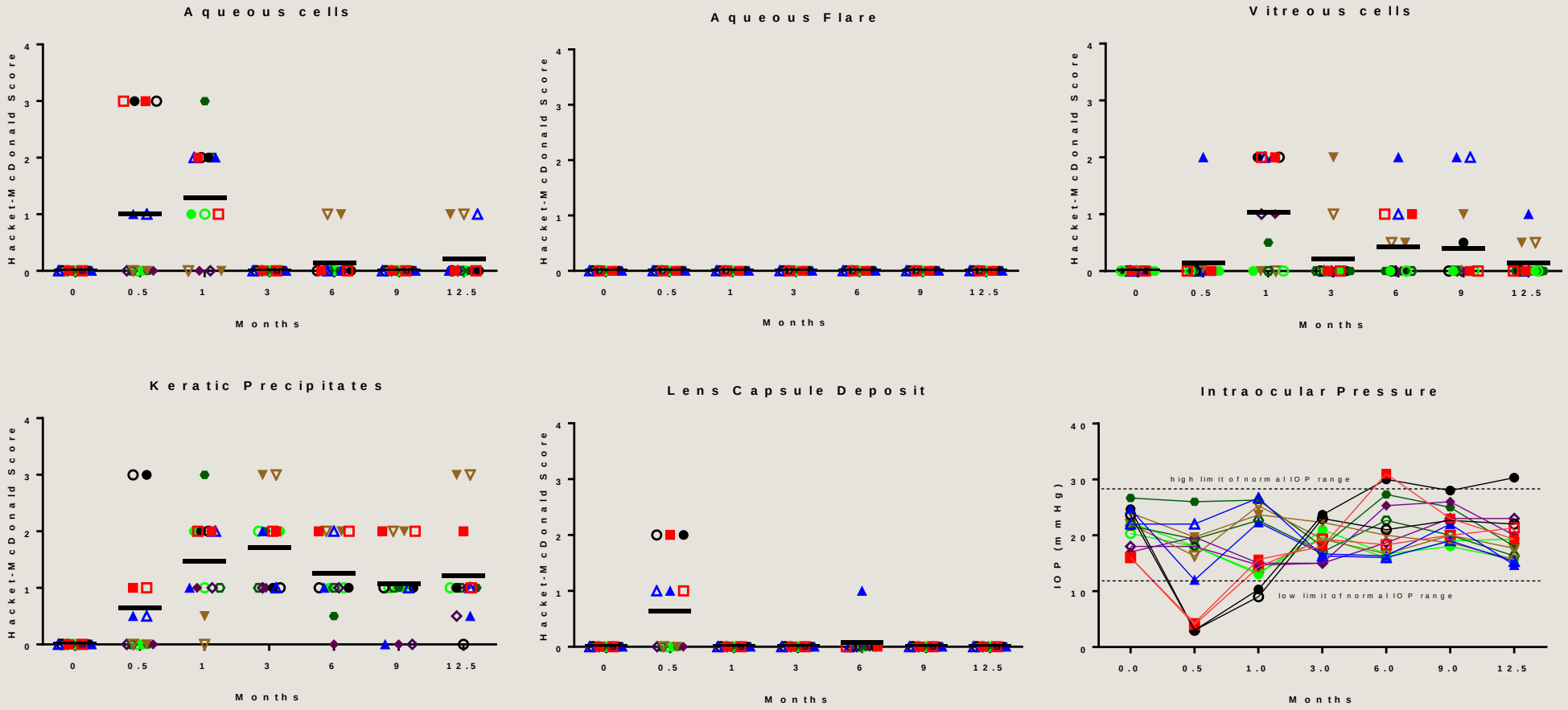


12.5-month Long-term Study Indicates Self-limiting Mild-to-Moderate Inflammatory Response in ADVM-022 ($2X10^{12}$ vg/eye) Injected Eyes

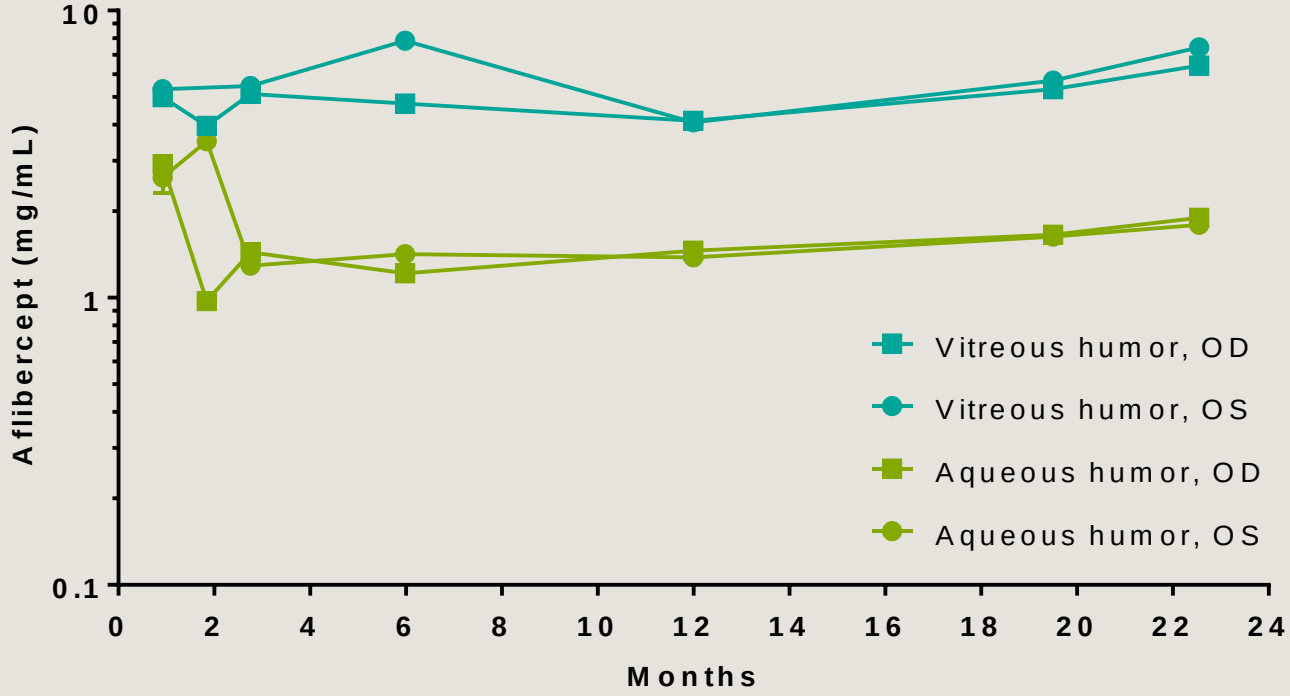


n=14 eyes.

Horizontal bars represent mean value

ADVM-022 Dosed Intravitreally Results in the Long-Term Sustained High Levels of Aflibercept Retina Expression

Aflibercept levels in vitreous and aqueous humors up to 22.5 month after ADVM-022 dose



PK Study Comparing ADVM-022 to Bolus Aflibercept Recombinant Protein

IVT administration of either ADVM-022 or aflibercept to NHPs

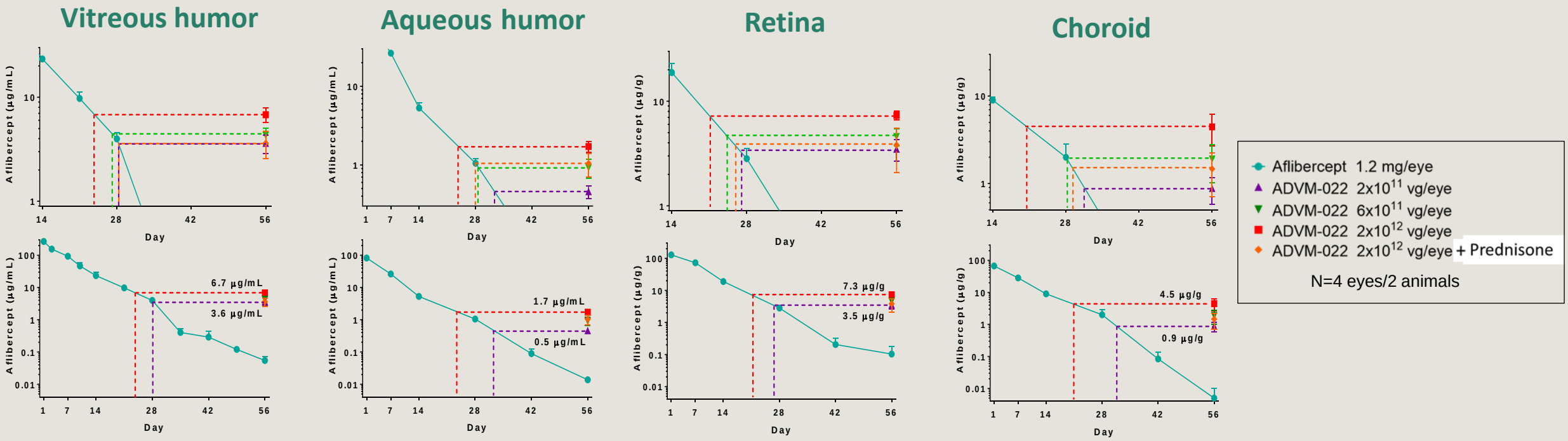
Group	Treatment	N	Dose (IVT, OU)	Administration	Sample collection										
					Day 1	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
1a	**Aflibercept**	2(M/F)	1.2 mg	Day 0	■										
1b	**Aflibercept**	2(M/F)	1.2 mg	Day 0		■	■								
1c	**Aflibercept**	2(M/F)	1.2 mg	Day 0				■	■						
1d	**Aflibercept**	2(M/F)	1.2 mg	Day 0						■	■				
1e	**Aflibercept**	2(M/F)	1.2 mg	Day 0								■	■		
1f	**Aflibercept**	2(M/F)	1.2 mg	Day 0										■	■
2	**ADVM-022**	2(M/F)	2 x 10^{11} vg	Day 0											■
3	**ADVM-022**	2(M/F)	6 x 10^{11} vg	Day 0											■
4	**ADVM-022**	2(M/F)	2 x 10^{12} vg	Day 0											■
5	**ADVM-022***	2(M/F)	2 x 10^{12} vg	Day 0											■

■ Terminal collection of aqueous and vitreous humor, retina, and choroid

■ Additional in-life vitreous humor collection days

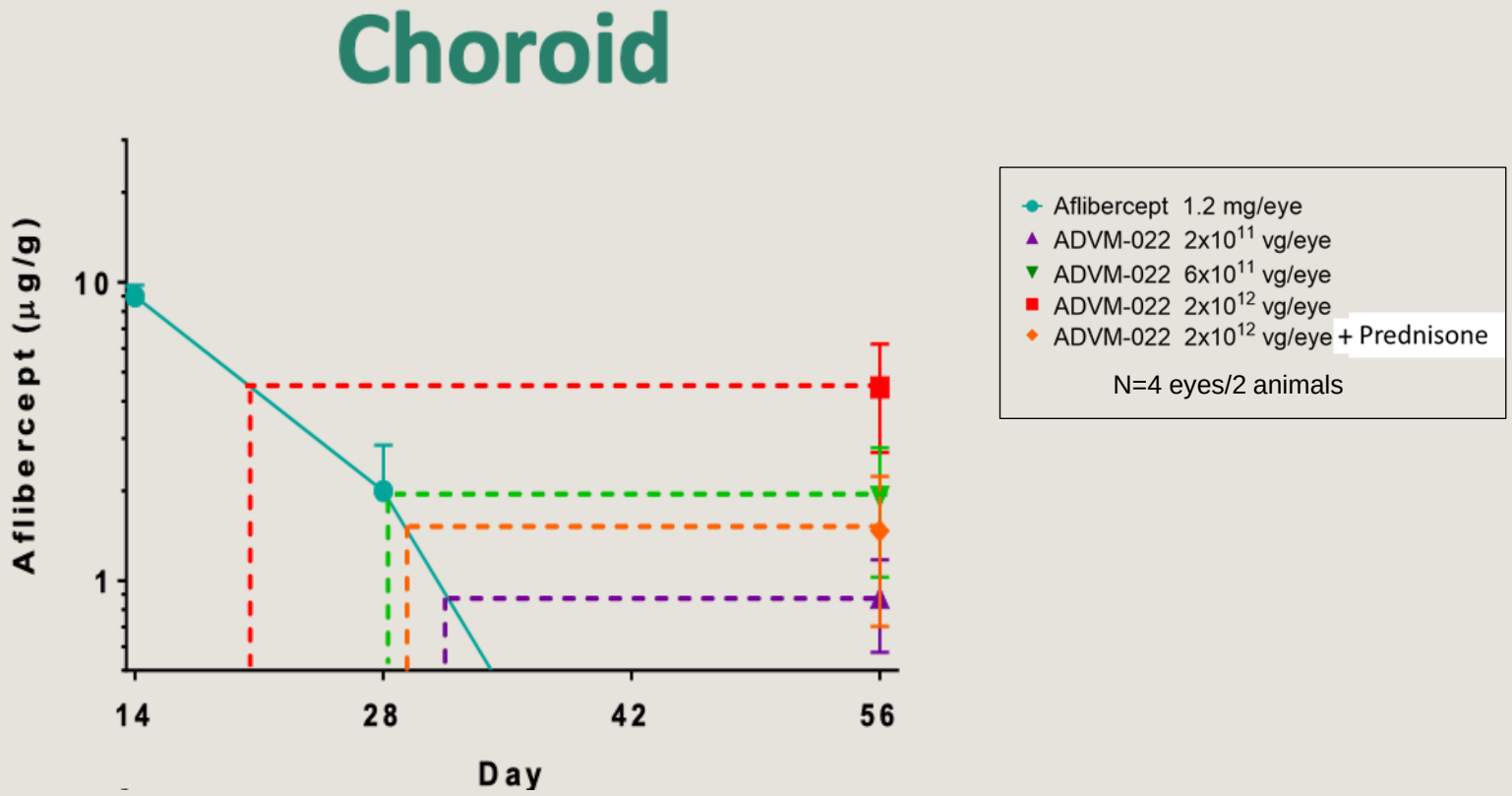
* Oral treatment with Prednisone

Dose-ranging Pharmacokinetics of IVT ADVM-022 in NHP



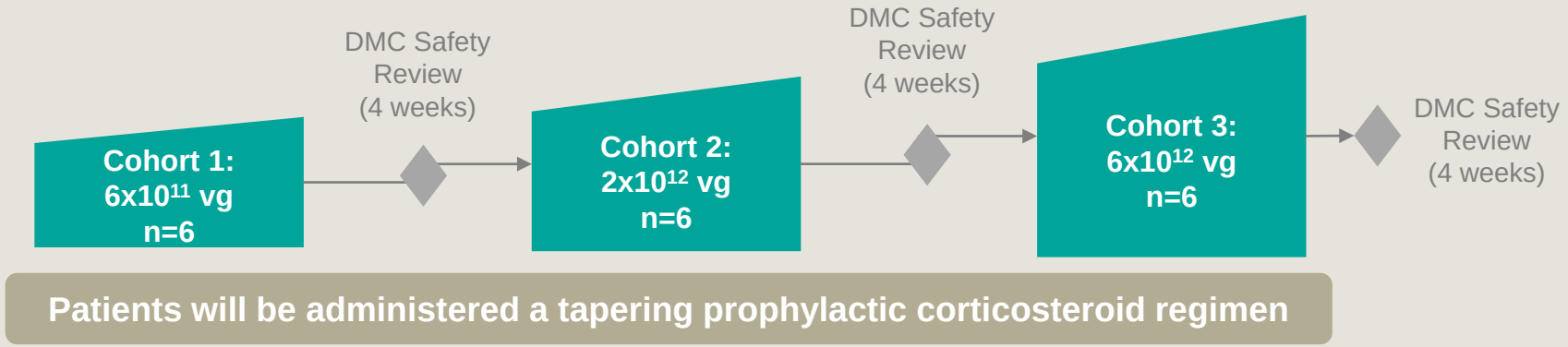
- **IVT ADVM-022 provides ocular expression of aflibercept in all compartments including retina and choroid where CNV occurs**
  - **Levels comparable to aflibercept-injected eyes 21-31 days post-dose**

Dose-ranging Pharmacokinetics of IVT ADVM-022 in NHP



- **IVT ADVM-022 provides levels comparable to aflibercept-injected eyes 21-31 days post-dose**

ADVM-022 OPTIC Phase 1 Trial Design - Initiated 4Q18*



Patients will be administered a tapering prophylactic corticosteroid regimen

***First patient dosed mid November, 2018**

OCT = Optical Coherence Tomography

KEY POINTS

- Non Human Primate Models Demonstrate:
  - Robust expression of aflibercept sustained 22.5 months after single IVT injection
  - ADVM-022 delivered aflibercept is sustained at levels similar to the aflibercept recombinant protein-injected eyes 21-31 days post-dose.

- Human Phase 1 Trial ADVM-022 – OPTIC
  - IND Active – August 2018
  - Granted Fast Track – September 2018
  - First patient dosed November 2018 – Preliminary Data expected 1Q20.