Immunological response and durability of expression following sequential intravitreal administration of AAV2.7m8 gene therapy to the contralateral eye in non-human primates

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Purpose

- The adeno-associated viral (AAV) capsid AAV2.7m8 can transduce the retina following intravitreal (IVT) injection, which offers improved safety and convenience over subretinal injection, but introduces the vector into a less immune-privileged compartment.
- Adverum is developing ADVM-022, an intravitreally-delivered gene therapy candidate consisting of AAV2.7m8 encoding aflibercept, an approved standard of care to treat wet age-related macular degeneration (wAMD).
- Many patients develop wAMD bilaterally with variable time of onset.
- Sequential IVT gene therapy using AAV risks development of neutralizing antibodies (nAbs) following treatment of the first eye, which could impact efficiency of therapy in the second eye and potentially raise safety concerns.
- To assess this risk, we investigated the effect of prior exposure of AAV2.7m8-aflibercept (AAV2.7m8-afli) vector in one eye on the transduction efficacy and ocular tolerability of the same AAV vector in the contralateral eye of non-human primates (NHPs).

Study Design and Methods

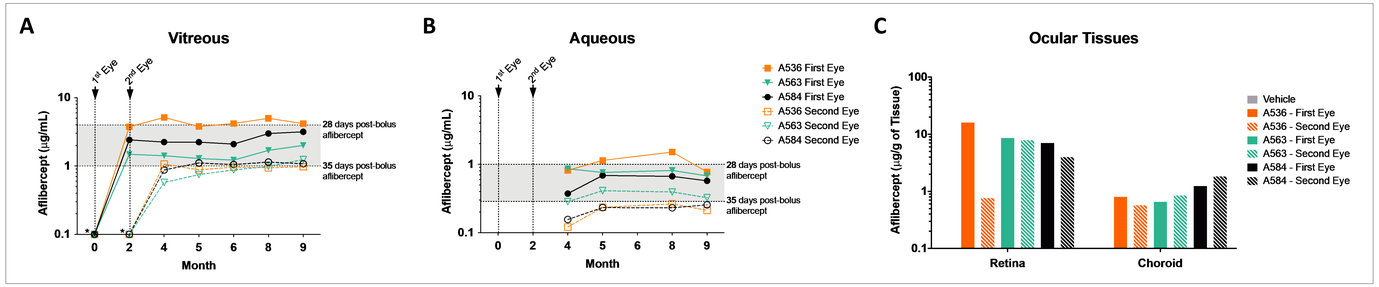
- Research-grade AAV2.7m8-aflibercept vector was produced in the baculovirus/SF9 cell system and purified by iodixanol gradient centrifugation.
- Male African green monkeys enrolled in the study were prescreened for the absence of pre-existing AAV2.7m8 neutralizing antibodies in serum using an *in vitro* transduction inhibition assay in HEK293T cells.
- NHPs were dosed IVT in one eye with $2x10^{12}$ vg AAV2.7m8-afli, and 2 months later the contralateral eye received an equal IVT dose of the same AAV vector. Vehicle controls were injected with formulation buffer in both eyes.

Group	Treatment	N	Dose/eye (vg)	Dosing schedule	Eye Treated	Follow up post-treatment
1	AAV2.7m8-afli	3	$2x10^{12}$	Day 0	OD	9 months
			$2x10^{12}$	Day 59	OS	7 months
2	Vehicle	1	0	Day 0	OU	9 months

- Aflibercept expression was monitored by ELISA in vitreous and aqueous fluids throughout the study duration up to 9 months, and in ocular tissue at study termination.
- Serum and vitreous nAbs and IgGs to AAV2.7m8 were assessed at various time points post dosing.
- Ocular tolerability was evaluated by ophthalmic examinations (ocular coherence tomography [OCT], fundus imaging, slit lamp, tonometry) throughout the study, and by histopathology of eye tissues at termination.

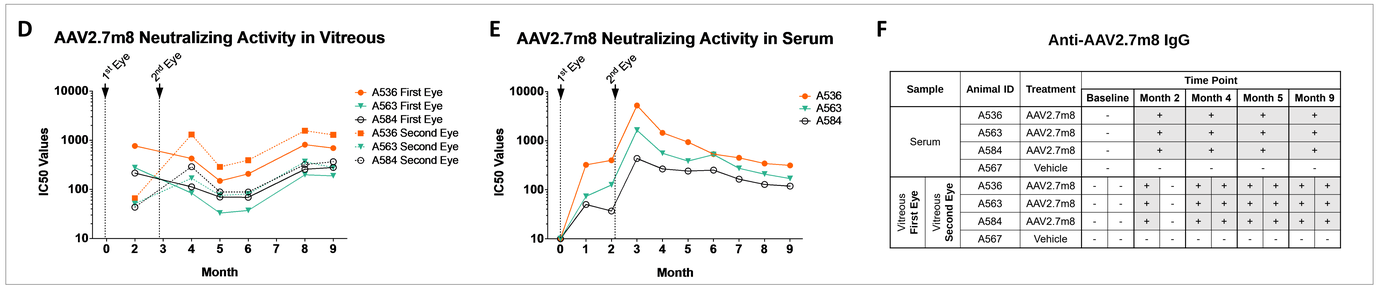
Results

Sustained aflibercept expression observed in ocular compartments following bilateral dosing with AAV2.7m8-afli, with meaningful levels detected in both eyes



Aflibercept expression levels were measured in **(A)** vitreous and **(B)** aqueous humor samples collected throughout the study, as well as from **(C)** retina and choroid tissue collected 9 and 7 months after IVT injection to first and second eyes, respectively. Arrows with dotted vertical lines indicate time of injection. Asterisk (*) indicates values below the lower limit of quantification of the ELISA assay (<0.016 µg/ml). In A and B: shaded boxed area indicates time interval at which similar protein levels are detected after a single 1.2 mg/eye bolus IVT injection aflibercept in NHP eyes.

Antibody-mediated immune response following bilateral administration of AAV2.7m8-afli

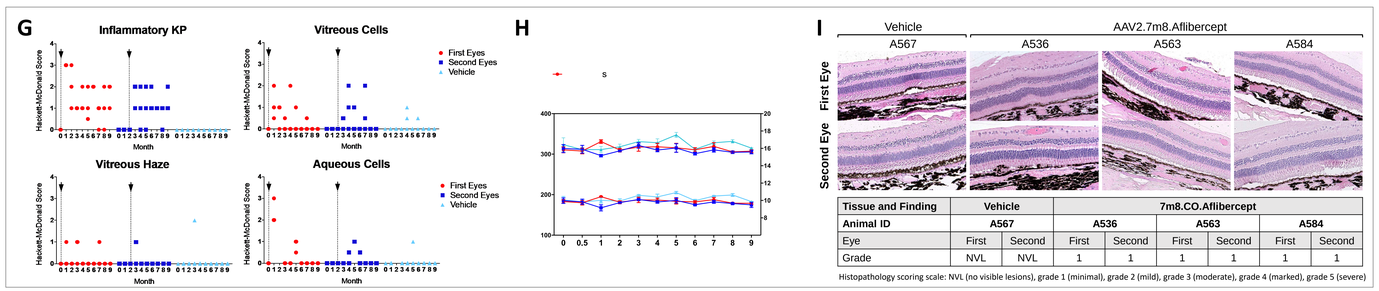


Sample		Animal ID	Treatment	Time Point				
				Baseline	Month 2	Month 4	Month 5	Month 9
Serum		A536	AAV2.7m8	-	+	+	+	+
		A563	AAV2.7m8	-	+	+	+	+
		A584	AAV2.7m8	-	+	+	+	+
		A567	Vehicle	-	-	-	-	-
Vitreous First Eye	Vitreous Second Eye	A536	AAV2.7m8	- -	+ -	+ +	+ +	+ +
		A563	AAV2.7m8	- -	+ -	+ +	+ +	+ +
		A584	AAV2.7m8	- -	+ -	+ +	+ +	+ +
		A567	Vehicle	- -	- -	- -	- -	- -

Presence of anti-AAV2.7m8 antibodies in the **(D)** vitreous humor and **(E)** serum of 3 NHPs throughout sequential IVT dosing as determined by *in vitro* transduction inhibition in HEK293T cells. Arrows with dotted vertical lines indicate time of injection. **(F)** Presence of anti-AAV2.7m8 immunoglobulin (IgG) in serum and vitreous samples at various timepoints throughout the study.

No apparent T-cell-mediated response was detected in PBMCs collected at the time of sacrifice (data not shown).

Sequential AAV2.7m8-afli IVT injections were well-tolerated as assessed by ophthalmic examinations and histopathology



Tissue and Finding	Vehicle		7m8.CO.Aflibercept					
Animal ID	A567		A536		A563		A584	
Eye	First	Second	First	Second	First	Second	First	Second
Grade	NVL	NVL	1	1	1	1	1	1

Histopathology scoring scale: NVL (no visible lesions), grade 1 (minimal), grade 2 (mild), grade 3 (moderate), grade 4 (marked), grade 5 (severe)

(G) Ocular health parameters scored by the Hackett-McDonald irritation and inflammation scoring system. No aqueous flare was detected. Arrows with dotted vertical lines indicate time of injection. **(H)** Average retinal thickness and volume as assessed by OCT. Mean values (n=3) ± SEM. **(I)** H&E staining and pathology assessment of representative retinal tissue sections. Test article-related findings were limited to minimal perivascular infiltrates of mononuclear cells.

Conclusions

- Our data demonstrate that development of immunity following IVT administration of AAV2.7m8 capsid in one eye does not completely block transduction following sequential dosing in the contralateral eye in NHPs.
- Even in the presence of neutralizing antibodies, staggered IVT dosing to both eyes with AAV2.7m8-aflibercept does not exacerbate inflammatory response with histological observations limited to minimal perivascular infiltrates and mild inflammation.
- These data suggest that patients with pre-existing neutralizing antibodies can be safely enrolled in clinical studies and that it is possible to use the same vector to treat bilateral disease with variable time of onset.