Development of an Intravitreal (IVT) Gene Therapy for Geographic Atrophy (GA) by Overexpressing Complement Factor I (CFI) to Inhibit the Complement Cascade

Gustavo de Alencastro, Jie Ma, Anna Zaslavsky, Stephanie Lussier, Kelly Michiko Hanna, Kellie Schaefer-Swale, Alex Tai, Charu Kumar, Cameron K. Baker, Ali Aghajanirefah, Ruslan N. Grishanin, Brigit E. Riley
Contact: Gustavo de Alencastro - gdealencastro@adverum.com

Background and Product Vision

- Dry age-related macular degeneration (Dry AMD) with geographic atrophy (GA) is a highly prevalent disease, characterized by retinal pigment epithelium (RPE) and photoreceptor death leading to vision loss, which affects the quality of life in the aging population worldwide.
- Activation of components of the complement cascade have been associated with GA, and data from an approved product and clinical trials support that inhibition of the complement cascade can reduce GA lesion growth.
- Complement Factor I (CFI) is a rate-limiting enzyme in the complement cascade, naturally inhibiting the activity of proteins involved in complement overactivation. Variants in the CFI gene have been associated with an increased risk of developing Dry AMD. Thus, continuous overexpression of CFI in the ocular tissue has the potential to inhibit the complement cascade in the eye, halt GA lesion growth and preserve vision in patients with Dry AMD.
- Intravitreal (IVT)-delivery of adeno-associated viral (AAV) vectors engineered for broad retinal transduction and CFI expression have the potential to treat patients with Dry AMD. **The ability to administer AAV vectors to patients via IVT delivery, a routine in-office procedure, is ideal for highly prevalent ocular disorders such as Dry AMD.**

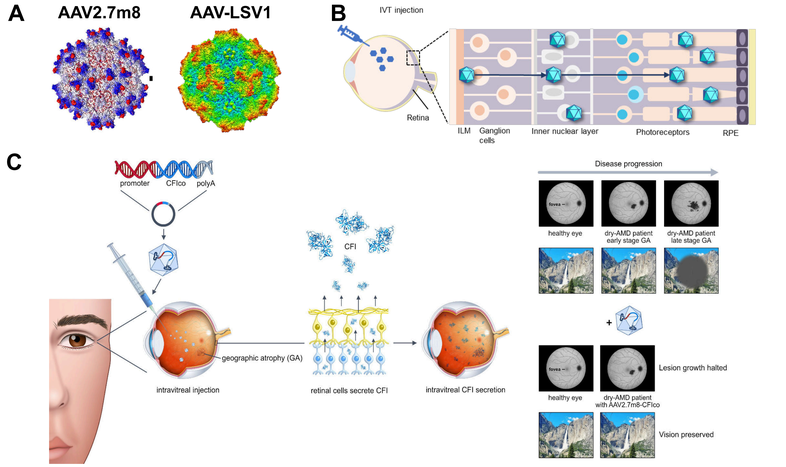

Figure 1. AAV-CFIco was packaged using Adverum's proprietary engineered AAV2.7m8 and AAV-LSV1 capsids for IVT delivery. **(A)** Cryogenic electron microscopy images of both retinotropic AAV chimeric capsids (AAV2.7m8 and AAV-LSV1) for IVT injection. **(B)** AAV2.7m8 was identified in screens across species from mouse, canine and non-human primate (NHP), and contains a peptide insertion. AAV-LSV1 was identified from a NHP screen and contains a loop swap variant. These capsid modifications allow the AAV vectors to bypass the inner limiting membrane (ILM) to efficiently transduce and deliver transgenes to target retinal cells. **(C)** Product vision for Dry AMD using the AAV-CFIco vector packaged into either AAV2.7m8 or AAV-LSV1. After IVT injection, the retinotropic AAV vector transduces retinal cells and secretes CFI in the extracellular space as well as in the vitreous cavity. Then, CFI inhibits the proteins overactivated in the complement cascade, halting the growth of the GA lesion before it covers the fovea of Dry AMD patients with early stage GA. Patients without any therapeutic intervention will eventually have their fovea covered by the GA lesion, leading to vision impairment. CFIco: codon-optimized human CFI sequence. RPE, retinal pigment epithelium.

Methods

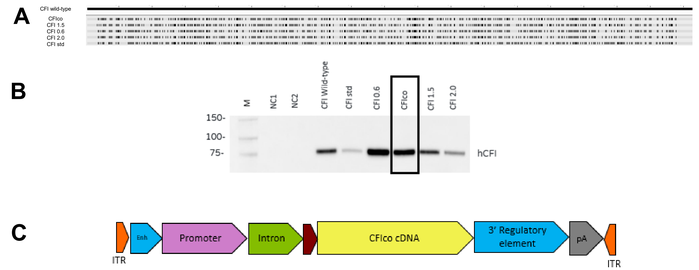

Figure 2. A codon-optimized human CFI sequence (CFIco), which has improved expression and CpG content over the wild-type sequence, was cloned into an AAV vector backbone and packaged using AAV2.7m8 and AAV-LSV1 capsids. **(A)** Five codon optimized human CFI cDNA sequences were generated and their nucleotide differences against the wild-type sequence are depicted by the black vertical lines. **(B)** Western blot analysis performed to quantify human CFI expression in cell culture media harvested from Ox293 cells after transfection with different versions of plasmids containing the different human CFI codon-optimized cDNAs. Two sequences had improved expression over the wild-type sequence (CFI 0.6 and CFIco). Cell culture media from untransfected cells were used as negative controls (NC1 and NC2). The values of the ladder bands (kDa) are indicated at the left of the gel image (M lane) **(C)** The human CFIco cDNA was inserted in a strong expression cassette flanked by AAV2 inverted terminal repeats (ITRs) (named AAV-CFIco). Enh: enhancer; pA: polyA.

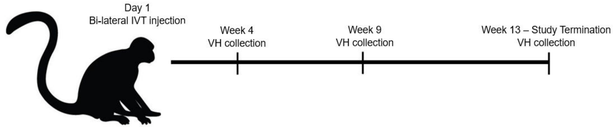

Figure 3. Overview of NHP study design. Scheme of the NHP study performed to evaluate human CFI expression in the vitreous humor after IVT injection of AAV-CFIco vector packaged into both the AAV2.7m8 and AAV-LSV1 serotypes. NHP subjects underwent bilateral administration of AAV or vehicle control. Animals were negative for neutralizing antibodies for AAV2.7m8 (Groups 2 and 3) and for AAV-LSV1 (Groups 4 and 5). Ocular Safety Assessments performed: Ophthalmic exams, Tonometry (intraocular pressure), Fundus and Optical Coherence Tomography (OCT), and Electroretinography (ERG). No steroids or other anti-inflammatory drugs were used at any point before or during the study, to unmask the extent of potential dose-related inflammatory response.

Table 1. Summary of NHP study design.

Group	Test Material	Serotype	Dose Level (vg/eye)	Human Equivalent Dose, HED (vg/eye)	Dose Volume (mL/eye)	Number of NHP subjects
1	Vehicle	N/A	0	0	0.05	3
2	AAV-CFIco	AAV2.7m8	3E10	6E10	0.05	3
3	AAV-CFIco	AAV2.7m8	1E11	2E11	0.05	3
4	AAV-CFIco	AAV-LSV1	3E10	6E10	0.05	3
5	AAV-CFIco	AAV-LSV1	1E11	2E11	0.05	3

Results

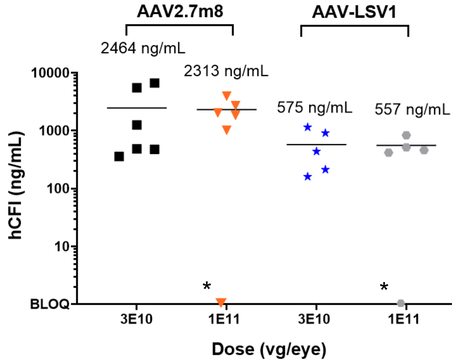

Figure 4. Mean peak human CFI (hCFI) values exceeded 500 ng/mL after a dose of 3E10 vg/eye with both AAV2.7m8 and AAV-LSV1 vectors in vitreous humor. NHP subjects were treated with a dose of 3E10 (black and blue symbols) or 1E11 (orange and gray symbols) vg/eye. The peak human CFI value detected in vitreous humors for each eye of the NHP subjects is represented here. Black horizonal bars denote group mean and asterisks (*) denote outlier samples which are hypothesized to be the result of anti-drug antibodies (NHP subjects 7 and 15). NHP subject 5 was euthanized on Week 12, which was unrelated to test article. A subset of vitreous humor samples were not collected at Week 4 but were collected at later timepoints. This was due to initial uncertainty of the difficulty of sample collection. Vitreous humor from NHP subject 5 was collected at Week 12.

Table 2. AAV-CFIco packaged into both AAV2.7m8 and AAV-LSV1 capsids were well tolerated as depicted in the heat map of ocular scores (vitreous cells) below.

Group/Serotype/ Dose	NHP subject	Baseline	D12	D27	D37	D52	D79	Color Codes
1 Vehicle	1 OD	0	0	0	0	0	0	4
	1 OS	0	0	0	0	0	0	3
	2 OD	0	0	0	0	0	0	2
	2 OS	0	0	0	0	0	0	1
	3 OD	0	0	1	0	0	0	0.5
	3 OS	0	0	1	0	0	0	0
2 AAV2.7m8 3E10 vg/eye	4 OD	0	0	0	0	0	0	
	4 OS	0	0	0	0	0	0	
	5 OD	0	0	0	0	0	0	
	5 OS	0	0	0	0	0	1	
	6 OD	0	0	1	0	0	0	
	6 OS	0	0	1	0	0	0	
3 AAV2.7m8 1E11 vg/eye	7 OD	0	0	1	1	1	1	
	7 OS	0	0	0	1	1	2	
	8 OD	0	0	0	2	1	1	
	8 OS	0	0	0	0	0	1	
	9 OD	0	0	1	1	1	1	
	9 OS	0	0	1	1	1	1	
4 AAV-LSV1 3E10 vg/eye	10 OD	0	0	0	0	0	0	
	10 OS	0	0	0	0	0	0	
	11 OD	0	0	1	1	0	0	
	11 OS	0	0	1	1	0	0	
	12 OD	0	0	0	0	0	0	
	12 OS	0	0	1	0	0	0	
5 AAV-LSV1 1E11 vg/eye	13 OD	0	0	0	0	0	0	
	13 OS	0	0	0	0	0	0	
	14 OD	0	0	0	0	0	0	
	14 OS	0	0	0	0	0	0	
	15 OD	0	0	0	0	0	1	
	15 OS	0	0	0	0	1	1	

Summary of AAV2.7m8-CFIco and AAV-LSV1-CFIco Tolerability

- Administration of both AAV test articles in NHP subjects was well tolerated.
- No adverse systemic clinical signs were observed.
- In life observations were dose-dependent and limited to non-adverse slight to mild dose-dependent ocular inflammation characterized by pigment and cells in the vitreous humor.

Conclusions

- AAV-CFIco packaged into both AAV2.7m8 and AAV-LSV1 capsids provided robust levels of intraocular human CFI.
- IVT administration of AAV-CFIco with both AAV2.7m8 and AAV-LSV1 capsids was well tolerated by NHP subjects.
- Based on our experience and nonclinical/clinical translatability of both the AAV cassette and capsid, we anticipate potential best in class AAV product for CFI expression.
- The ability to administer AAV vectors via IVT delivery, a routine in-office procedure, is ideal for highly prevalent ocular disorders such as Dry AMD.

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

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