Preclinical Characterization of 4D-175, a Novel AAV-based Investigational Intravitreal Gene Therapy for Geographic Atrophy

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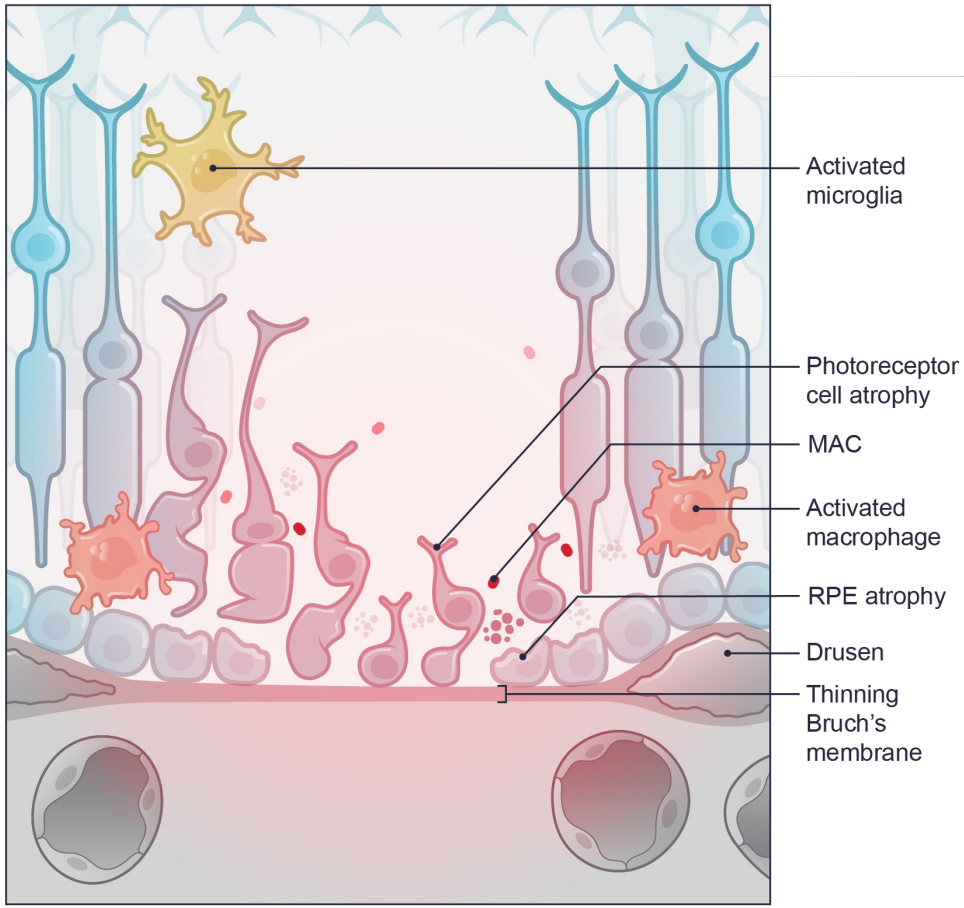
Geographic Atrophy

- Major cause of irreversible vision loss—affects an estimated 5 million individuals globally (1 million in the US)[1,2]
- Characterized by atrophic lesions caused by progressive degeneration of the RPE, photoreceptors, and choroid
- Current treatments reduce the rate of growth in GA lesions but require monthly or bimonthly intravitreal injections[3,4]
  - Increased risk of choroidal neovascularization[3,5]

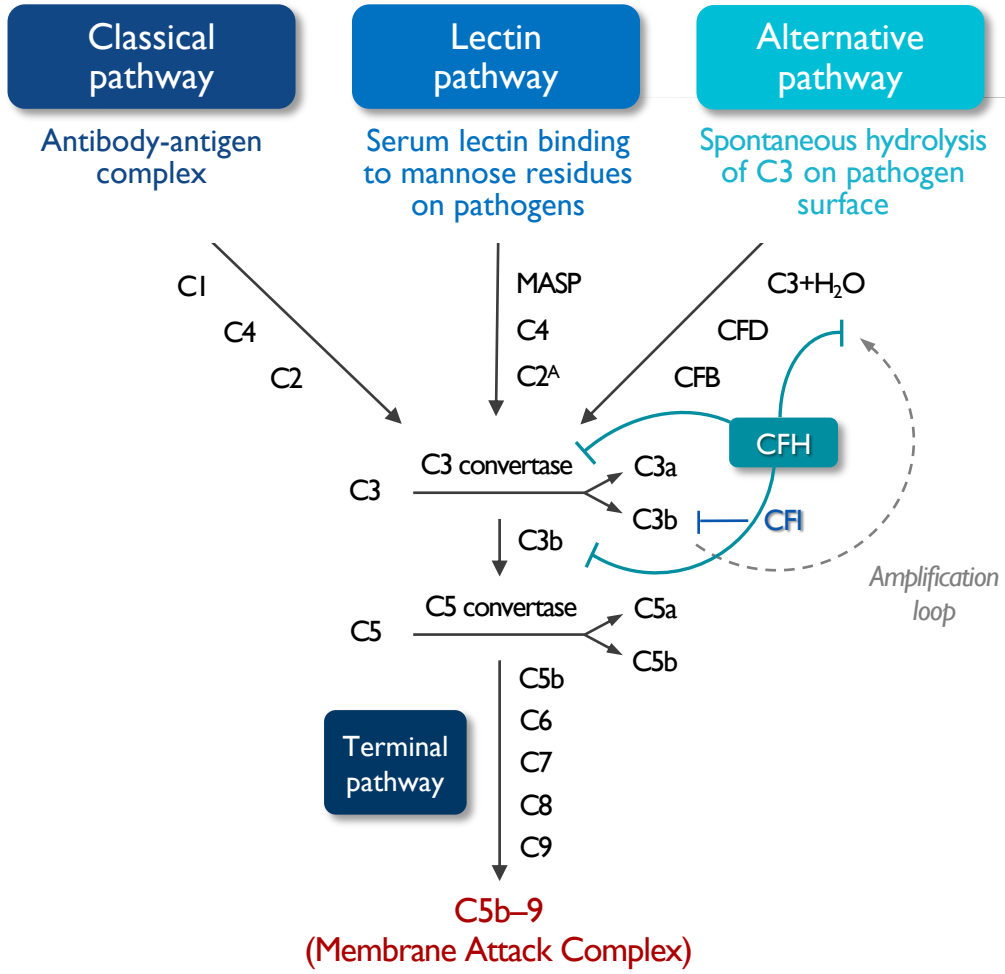


GA, geographic atrophy; MAC, membrane attack complex; RPE, retinal pigment epithelium.

1. Wong et al. *Lancet Glob Health* 2014;2:e106–16. 2. Freidman et al. *Arch Ophthalmol* 2004;122:564–72. 3. Syfovre [package insert]. Apellis Pharmaceuticals. 4. Izervay [package insert]. Iveric Bio, Inc. 5. Sivaprasad et al. *Eye* (Lond) 2023;37:402–7.

Complement Factor H (CFH)

- Key regulator of the complement system
  - Inhibits assembly of C3 and C5 convertases via competition with CFB for C3b binding[1]
  - Facilitates disassembly of convertases by displacing bound factor Bb[1]
  - Inactivates C3b by acting as a cofactor for CFI[1]
- Inactivation of alternative pathway on host cells localizes reaction to pathogens
- CFH dysfunction promotes assembly of C3 and C5 convertases and amplifies activation of the alternative complement pathway[2,3]



CFB, complement factor B; CFI, complement factor I.

1. Perkins et al. *Immunobiol* 2012;217:281–97. 2. Manuelian et al. *J Clin Invest* 2003;111:1181–90. 3. Prosser et al. *J Exp Med* 2007;204:2277–83.

Adapted from Khandhadia et al. *Immunobiology* 2012;217:127-46.

CFH Dysfunction in Geographic Atrophy

- Variants in the gene encoding CFH are strongly associated with the risk of GA[1,2]
  - Most common variant (Y402H) accounts for nearly 50% of the overall risk[2,3]
  - Rare variant (R1210C) is strongly associated with early onset GA[4]
- Murine models support a causal role for CFH dysfunction in retinal pathology[5]
  - CFH-deficient mice exhibit increased retinal C3 deposition and decreased visual acuity[2]; expression of human *CFH* rescues the phenotype[6]
  - Transgenic mice expressing human *CFH* Y402H variant develop AMD-like retinal pathology[7]
- Individuals with the Y402H variant have elevated levels of inflammatory markers in the choroid[8] and increased plasma concentrations of complement activation products[9]

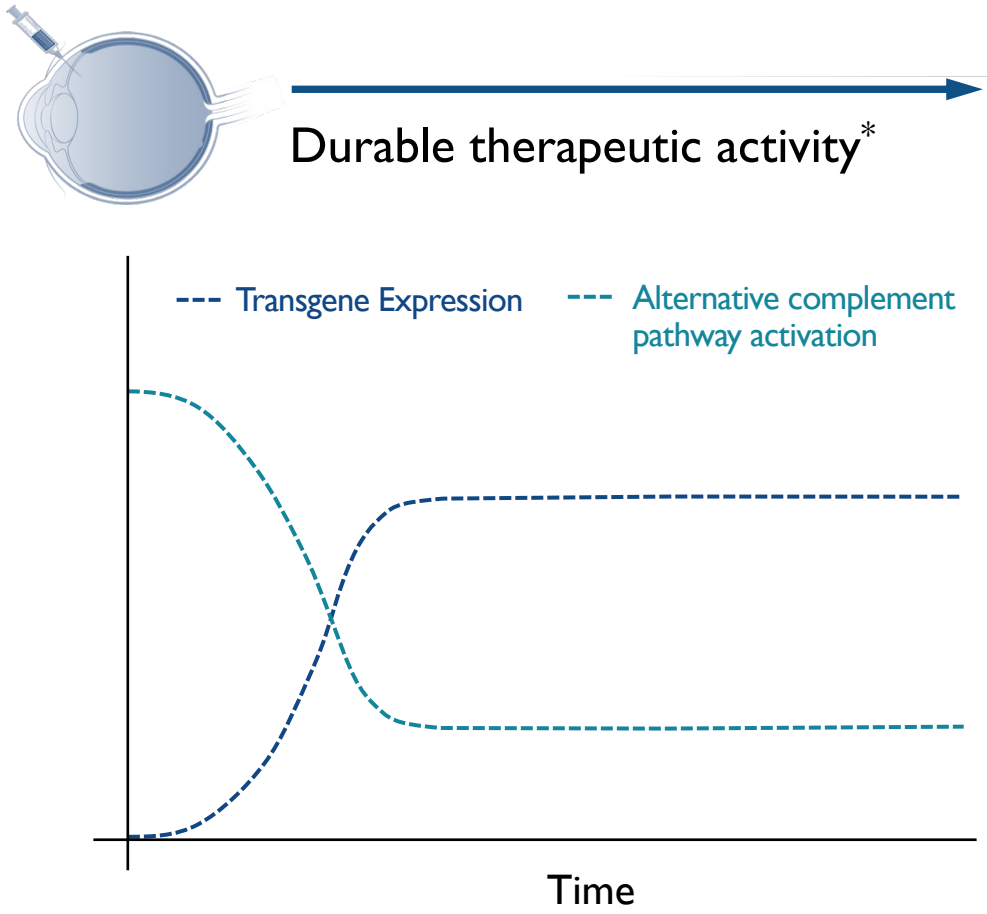
AMD, age-related macular degeneration; CFH, complement factor H; GA, geographic atrophy.

1. Mitchell et al. *Lancet* 2018:392:1147–59. 2. Klein et al. *Science* 2005;308:385-9. 3. Edwards et al. *Science* 2005;308:421-4. 4. Raychaudhuri et al. *Nat Genet* 2011;43:1232-6. 5. Ding et al. Adv Exp Med Bio 2014;801:213-19. 6. Coffey et al. *Proc Natl Acad Sci USA* 2007;104:16651-6. 7. Ding et al. *Am J Pathol* 2015;185:29-42. 8. Ufret-Vincenty et al. *Invest Ophthalmol Vis Sci* 2010;51:5878-87. 9. Smailhodzic et al. *Ophthalmology* 2012;119:339-46.

Gene Therapy for Geographic Atrophy

Therapeutic Rationale

- The retina is an opportune target for gene therapy
  - Small tissue volume
  - Relatively low dose requirements
  - Stable and non-dividing cell population
- Complement inhibition is a clinically validated therapeutic strategy in GA
  - Current therapies require monthly or bimonthly IVT injections
- Targeted delivery of therapeutic transgenes to the retina allows continuous steady state concentration
  - Potential for durable clinical benefit with single injection

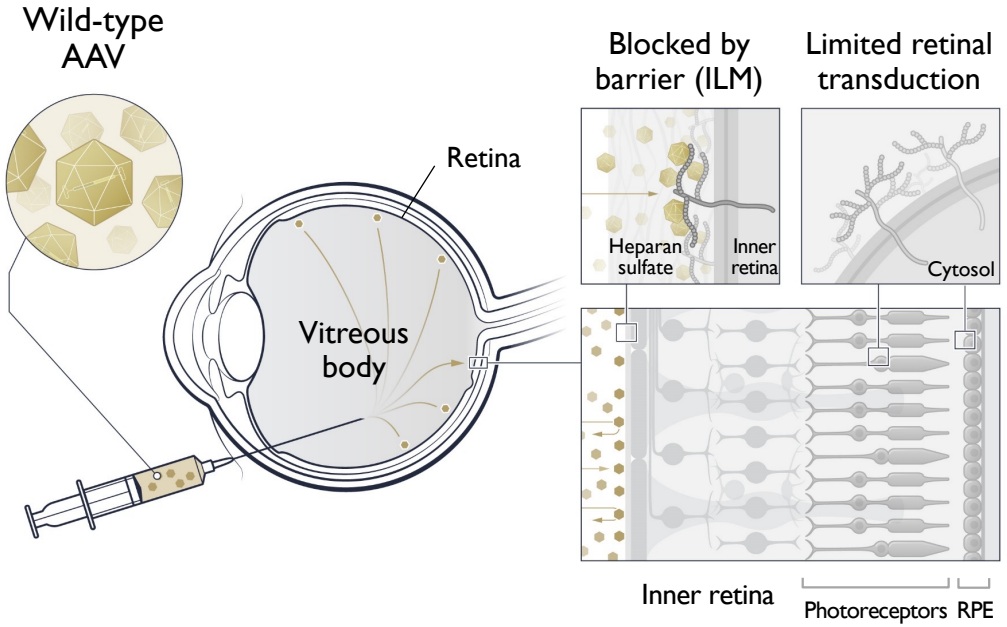


*Conceptual illustration.

Intravitreal AAV-mediated Retinal Gene Therapy

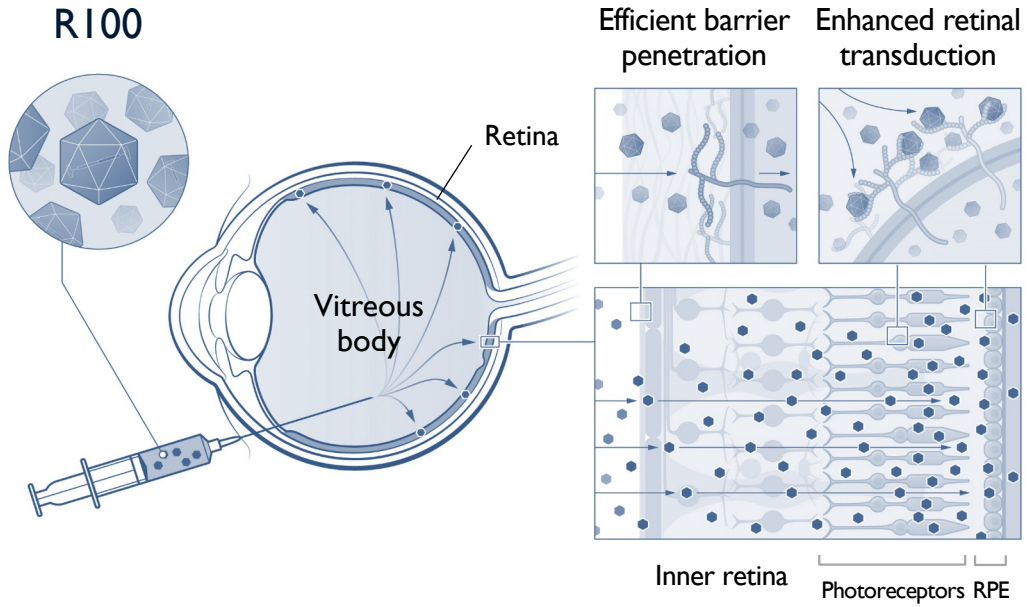
Retinal Cell Transduction

Wild-type AAV



- Conventional wild-type AAV vectors exhibit poor retinal cell transduction

R100: Retinotropic AAV Vector



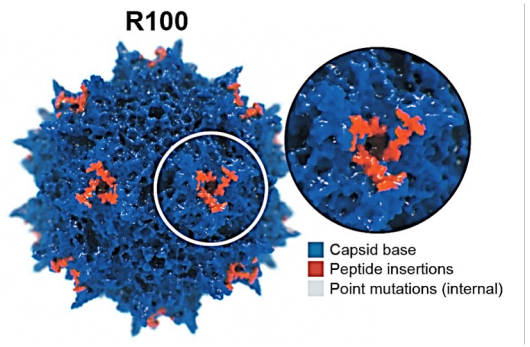
- Synthetic AAV capsid with enhanced capacity to penetrate vitreoretinal barriers

AAV, adeno-associated virus; ILM, inner limiting membrane; RPE, retinal pigment epithelium.

R100: Targeted and Evolved AAV Vector

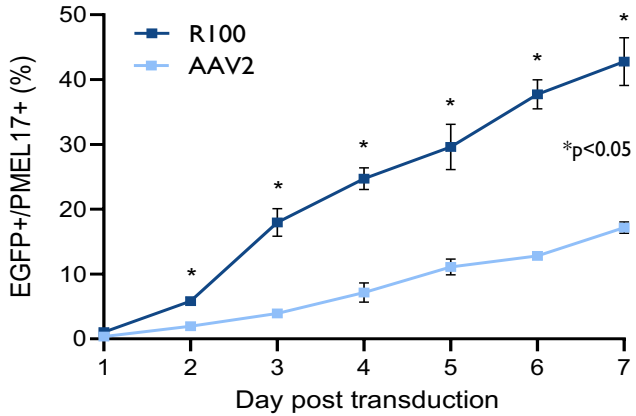
Extensive Characterization in Preclinical and Clinical Studies

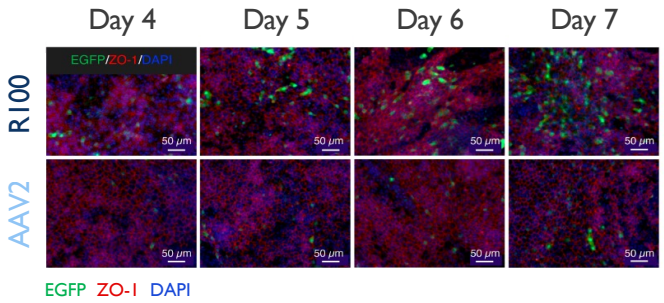
R100
Retinotropic AAV Vector



- Enhanced capacity to cross vitreoretinal barriers
- Transduction of all regions and layers of the retina
- Robust transgene expression following IVT administration

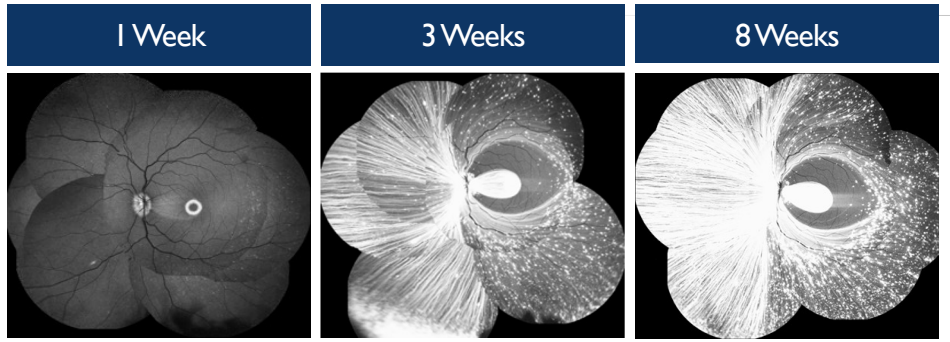
Human RPE Cell Transduction





- Superior transduction of human RPE cells compared to AAV2 *in vitro*

Retinal Transduction in NHPs



Intravitreal administration of R100.CAG-EGFP $1x10^{12}$ vg/eye.

Clinical Validation

Vector	Product	Target Indication	N
R100	**4D-150** *AFLB, miR-(VEGFC)*	Wet AMD, DME	130
	4D-110 *CHM*	Choroideremia	13
	4D-125 *RPRG*	XLRP	15

4 Phase 1/2 clinical trials (N=158).

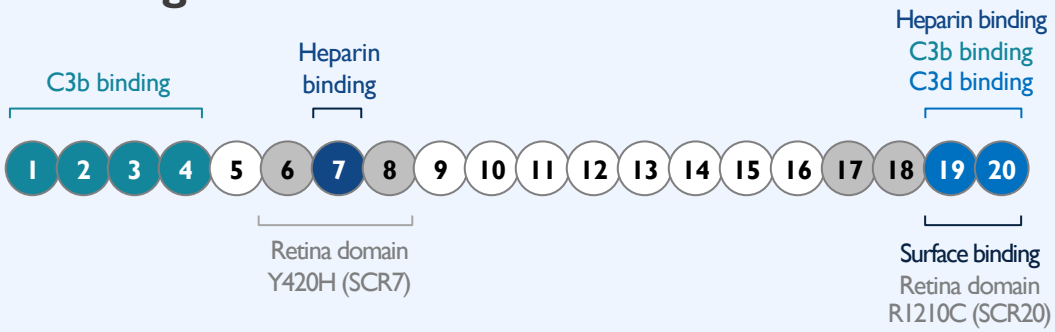
EGFP, enhanced green fluorescent protein; NHP, nonhuman primates; RPE, retinal pigment epithelium; XLRP, X-linked retinitis pigmentosa.

4D-175 Transgene Design and Function

Short-form Complement Factor H (*sCFH*)

Transgene Design

Full-length Human CFH[1]



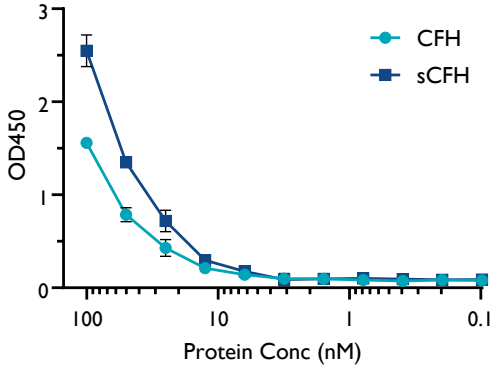
Short-form CFH (sCFH)

- Reduced size of the sCFH protein predicted to result in increased penetration of the RPE and choroid[2,3]

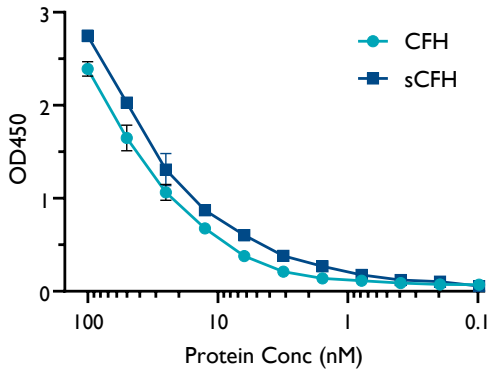
1. de Córdoba SR, de Jorge EG. *Clin Exp Immunol* 2008;151:1–13. 2. Moore et al. *IOVS* 2001;42:2970-5.
3. Bok et al. *IOVS* 1985;26:1659-94.

Pharmacological Activity

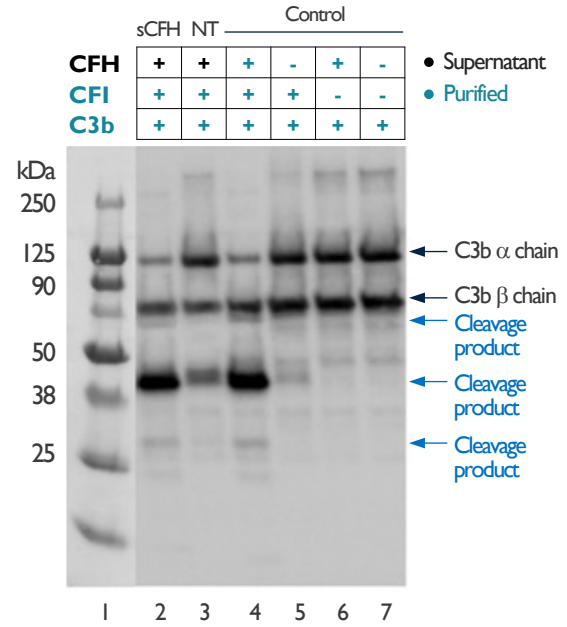
Heparin Binding



hC3b Binding



C3b Cleavage

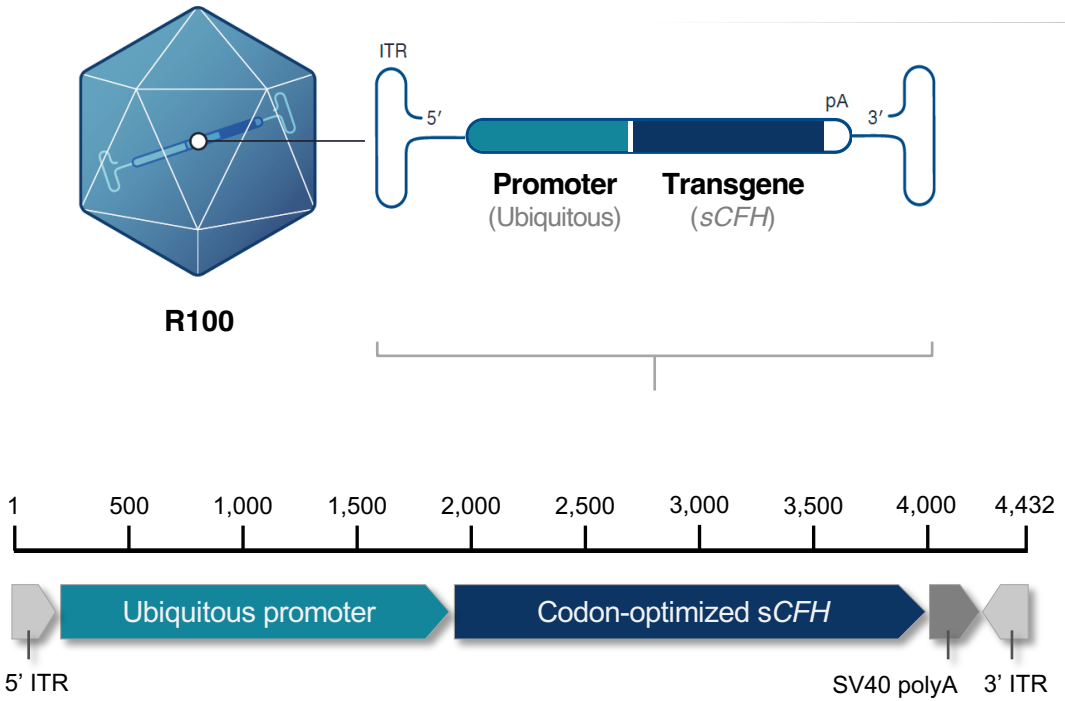


sCFH exhibits proper heparin and C3b binding and **inhibits complement activity *in vitro***

4D-175 Design

Intravitreal Gene Therapy for Geographic Atrophy

- Clinically validated retinotropic AAV vector (R100)
- Codon-optimized sequence encoding a shortened form of human complement factor H (sCFH)
- Ubiquitous promotor to drive transgene expression
- Therapeutic objective: Restore normal complement regulation in the retina through durable expression of CFH



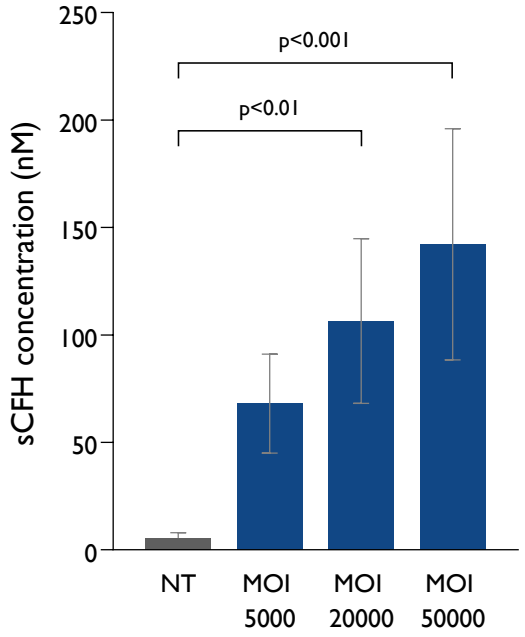
4D-175 Transgene Cassette

AAV, adeno-associated virus; CFH, complement factor H; ITR, inverted terminal repeat; pA, poly adenylation tail.

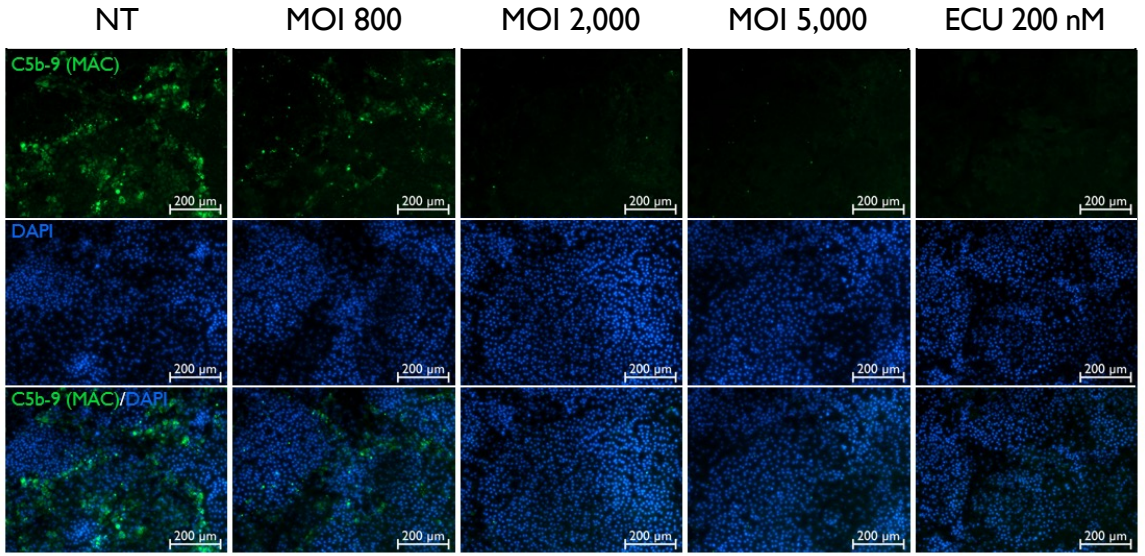
4D-175 Preclinical Characterization

Robust Transgene Expression and Functional Activity in Human Retinal Cells *In Vitro*

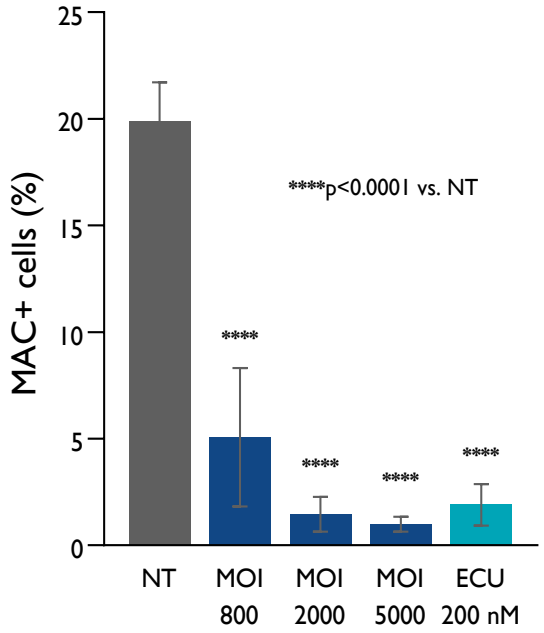
Transgene Expression*



MAC (C5b–9) Formation in iPSC-derived RPE Cells†



C5b-9 (MAC): membrane attack complex
DAPI: 4′,6-diamidino-2-phenylindole



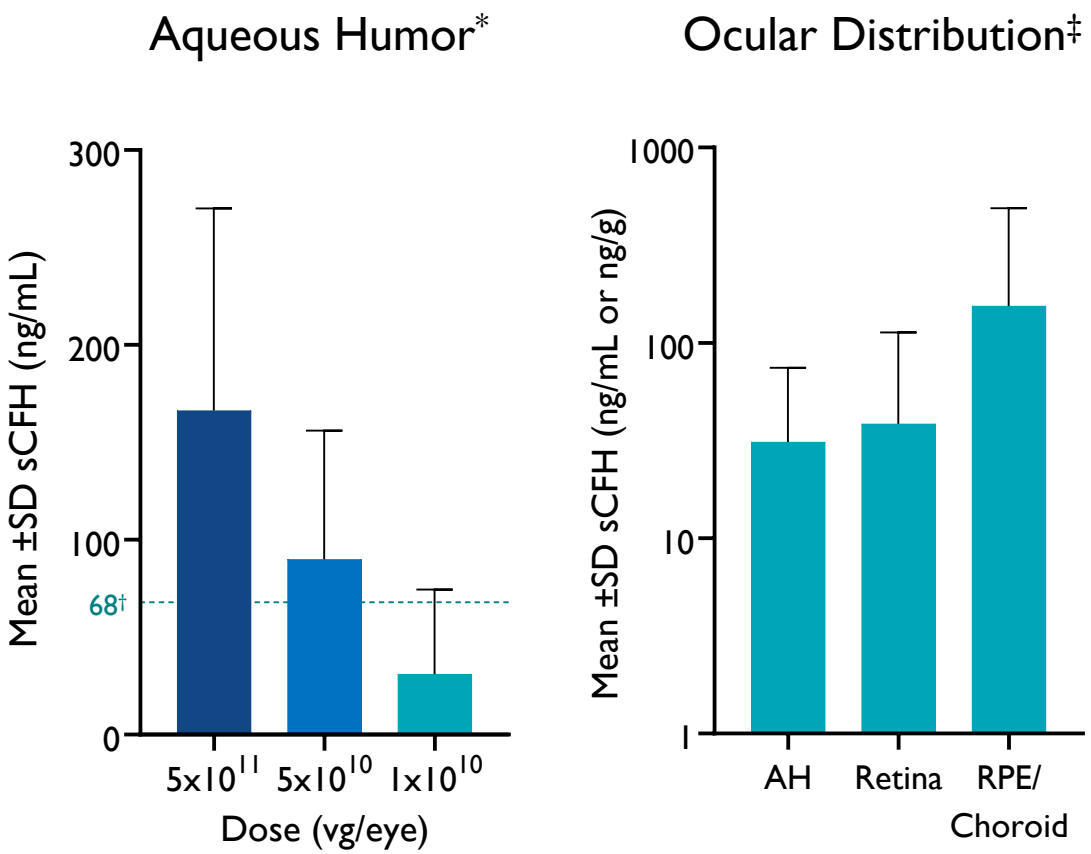
Dose-dependent transgene expression and inhibition of alternative complement pathway in human RPE cells

*iPSC-derived RPE cells (assessed by enzyme-linked immunosorbent assay). †Assessed by immunocytochemistry and flow cytometry; alternative complement pathway activated by addition of serum (1%) and zymosan (0.5 mg/mL serum) to culture medium. ECU, eculizumab (anti-C5 antibody; positive control); iPSC, induced pluripotent stem cells; MAC, membrane attack complex; MOI, multiplicity of infection; NT, non-transduced; RPE, retinal pigment epithelium.

4D-175 Preclinical Characterization

NHP Ocular Pharmacodynamics and Tolerability

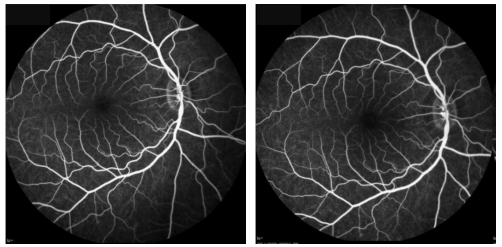
4D-175 Ocular Biodistribution



4D-175 Safety and Tolerability

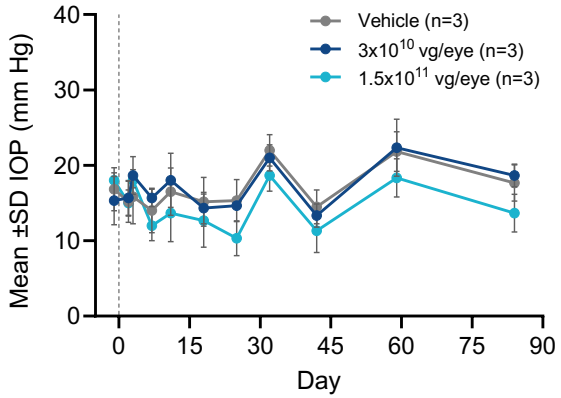
Fluorescein Angiography

NHP 2502 ($1.5x10^{11}$ vg/eye)



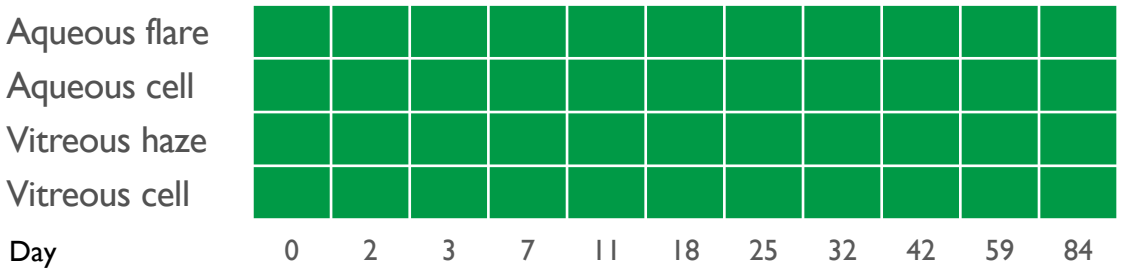
Baseline Week 12

Intraocular Pressure (IOP)



Ophthalmic Examination

Highest Reported Score¶



*Day 15 following IVT administration of 4D-175. †Target mean AH CFH concentration [1]. ‡$1x10^{10}$ vg/eye; tissue concentrations assessed at necropsy. ¶Uveitis score ($3x10^{10}$ and $1.5x10^{11}$ vg/eye; n=3 animals per group). 1. Altay et al. *Eye* 2019;33:1859–64.

Conclusions

- *In vitro* experiments demonstrated that the *sCFH* transgene-derived protein exhibits functional activity consistent with wild type full-length CFH
  - Proper heparin and C3b binding
  - Appropriate C3b cleavage and corresponding inhibition of alternative complement pathway activity
- Transduction of human RPE cells with 4D-175 led to dose-dependent transgene expression and inhibition of MAC formation
  - 75–95% reduction in complement-dependent MAC deposition
- IVT administration of 4D-175 ($1x10^{10}$ to $5x10^{11}$ vg/eye) to NHPs was safe and well tolerated and resulted in robust transgene expression in the retina and RPE/choroid
- 4D-175 IND filing anticipated in 1H 2024

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4D Molecular Therapeutics

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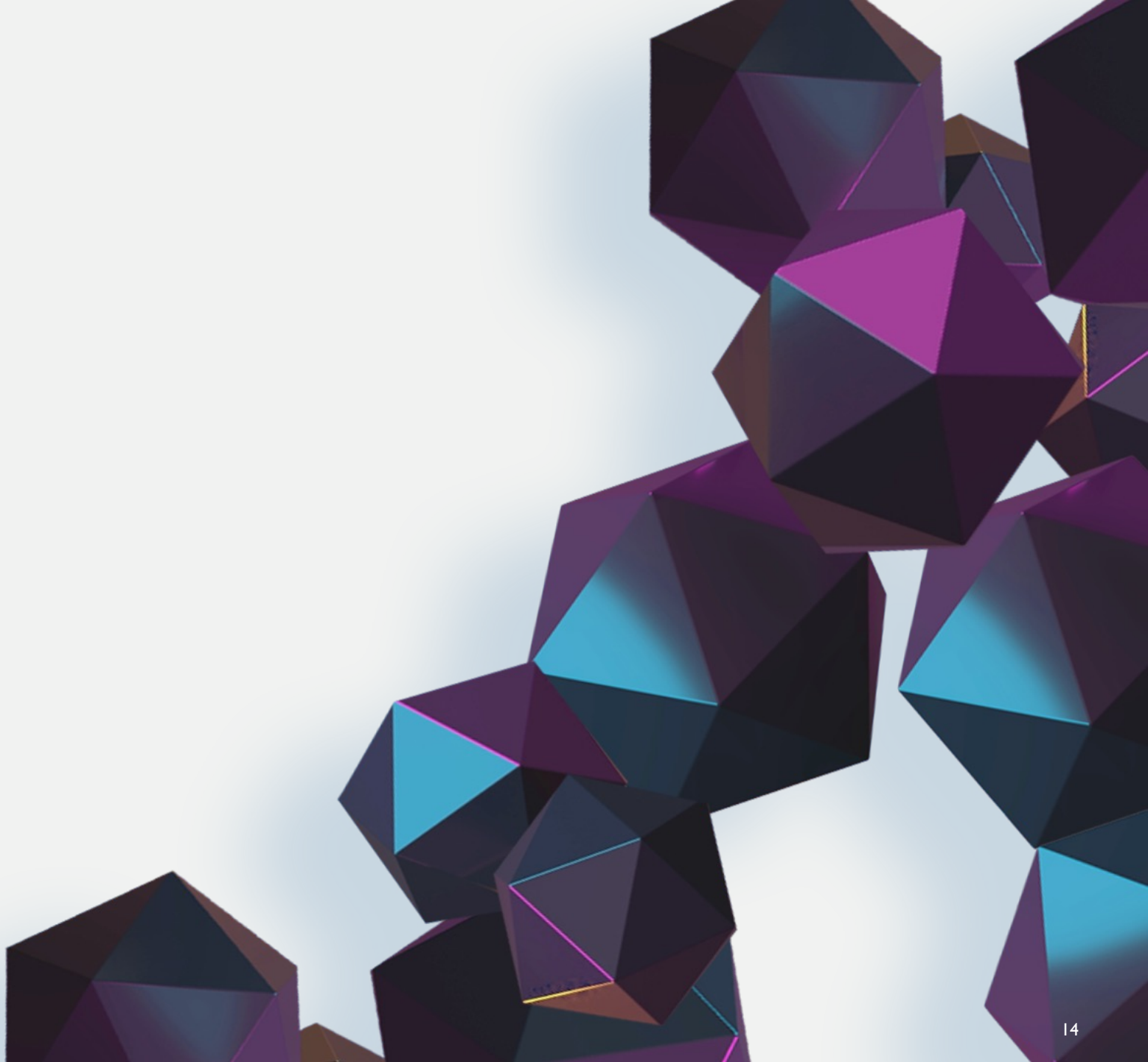
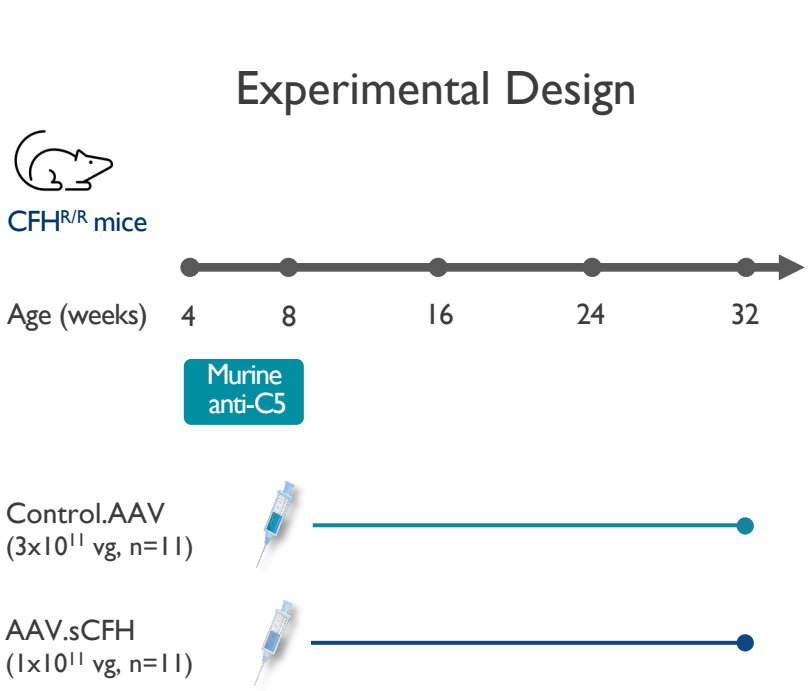
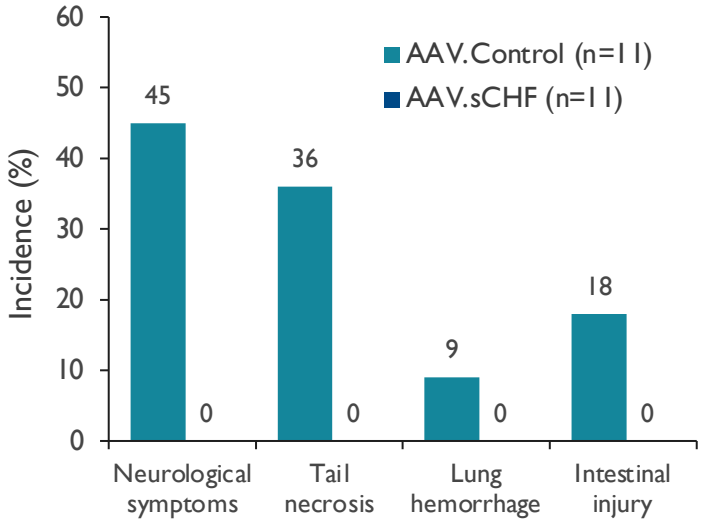
Back-up

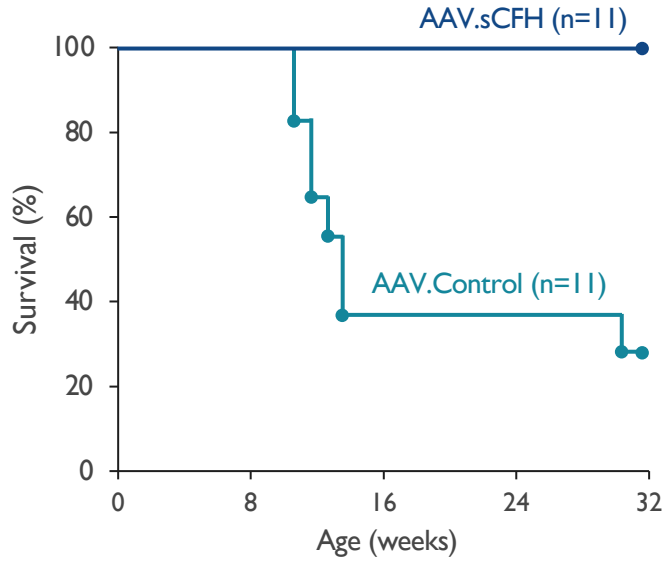


Confirmed Pharmacological Function of *sCFH In Vivo*

Phenotypic Correction in Mouse Model of aHUS







- sCFH prevented aHUS phenotypes and extended survival compared to controls in a mouse disease model

aHUS, atypical hemolytic uremic syndrome; sCFH, short-form complement factor H transgene.