









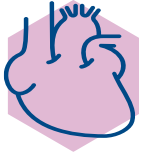


# Targeting the Complement Pathway with AAV-Based Gene Therapy for Geographic Atrophy

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4D Molecular Therapeutics

















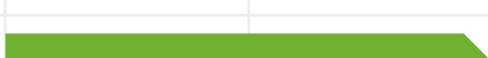






# 4D Molecular Therapeutics (4DMT)

BOLDLY INNOVATING TO UNLOCK THE POTENTIAL OF GENETIC MEDICINES FOR MILLIONS OF PATIENTS

<b>Company</b>	 Nasdaq <b>FDMT</b>	 Emeryville, CA	 ~140 Employees GMP Facilities R&D Headquarters
<b>Technology Platform</b>	Directed Evolution	~1 Billion AAV capsid sequences	Customized and evolved vectors Payload design and engineering GMP manufacturing
<b>Product Engine</b>	Vector Modularity 	Clinical-Stage Vectors in 3 Therapeutic Areas   	
<b>Clinical Development</b>		<b>5</b> Clinical Candidates for <b>7</b> Patient Populations	
<b>Strategy</b>		<b>Fully Integrated</b> Genetic Medicines Company	

# Pipeline: 5 Clinical-stage Product Candidates

3 THERAPEUTIC AREAS, INCLUDING RARE AND LARGE PATIENT POPULATIONS

VECTOR Delivery	PRODUCT CANDIDATE	INDICATION	EPIDEMIOLOGY (PREVALENCE)	RESEARCH CANDIDATE	IND-ENABLING	PHASE 1/2	PHASE 3	PRODUCT RIGHTS
<b>R100</b>								
<i>Intravitreal</i>								
OPHTHALMOLOGY								
	4D-I50	Wet AMD	~3M US/EUMM					 4DMT
		Diabetic Macular Edema	~1.2M US					 4DMT
	4D-I25	XLRP	~24K US/EUMM					 4DMT
	4D-I10	CHM	~13K US/EUMM					 4DMT
	4D-I75	Geographic Atrophy	~1M US					 4DMT
	Undisclosed	Rare Monogenic Disease	Undisclosed (Rare)					 astellas
<b>A101</b>								
<i>Aerosol</i>								
PULMONOLOGY								
	4D-710	CF Lung Disease (not modulator-amenable)	~6K US					 4DMT
		CF Lung Disease (modulator-amenable)	~34K US					 4DMT
	4D-725	A1AT Deficiency Lung Disease	~200K US/EUMM					 4DMT
<b>C102</b>								
<i>IV</i>								
CARDIOLOGY								
	4D-310	Fabry Disease Cardiomyopathy	~50-70K US/EUMM					 4DMT

# AAV-Based Gene Therapy for GA

## PRESENTATION SYNOPSIS

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- Addressing the challenges associated with efficient transgene delivery to the retina via intravitreal injection
- The role of the complement pathway and complement factor H (CFH) in the pathogenesis of geographic atrophy
- Therapeutic potential of short-form human CFH gene transfer via an evolved retinotropic AAV vector

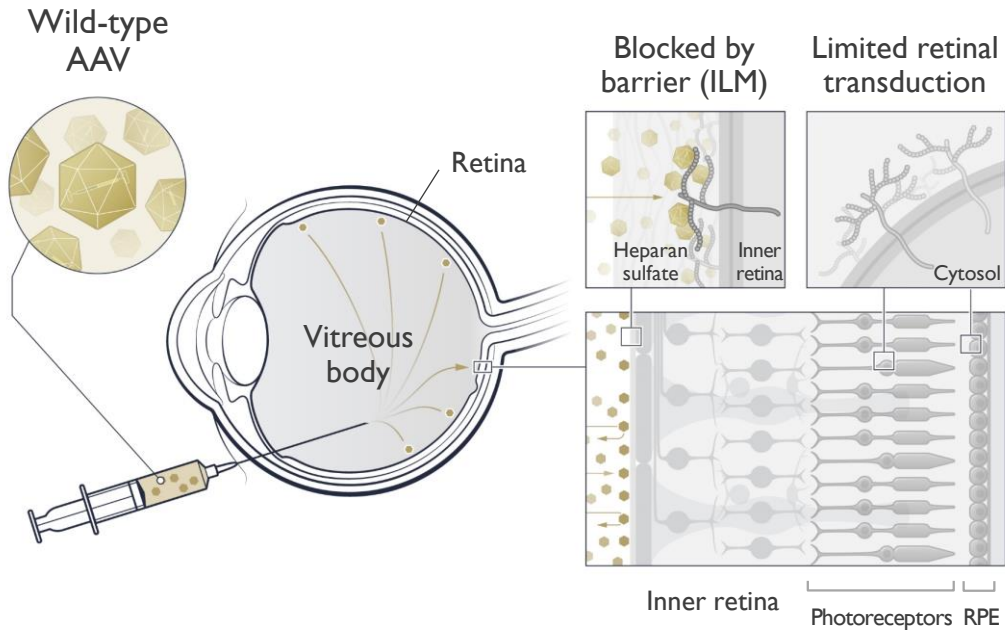
# Addressing the Challenges Associated with Retinal Transgene Delivery

Therapeutic Vector Evolution

# Intravitreal AAV-mediated Retinal Gene Therapy

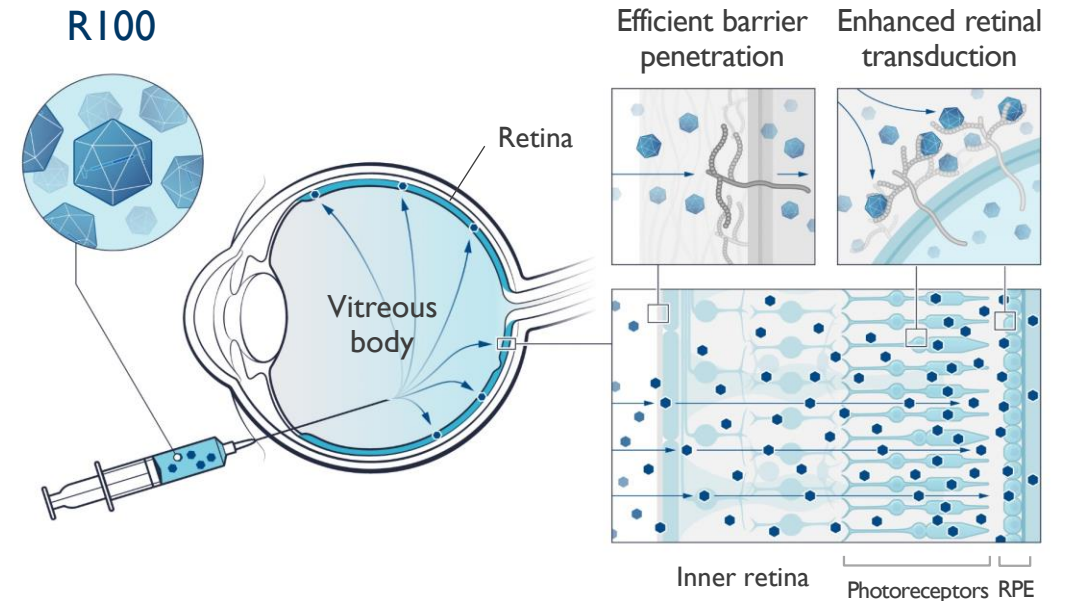
## RETINAL CELL TRANSDUCTION

### Wild-type AAV



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

### R100: Retinotropic AAV Vector

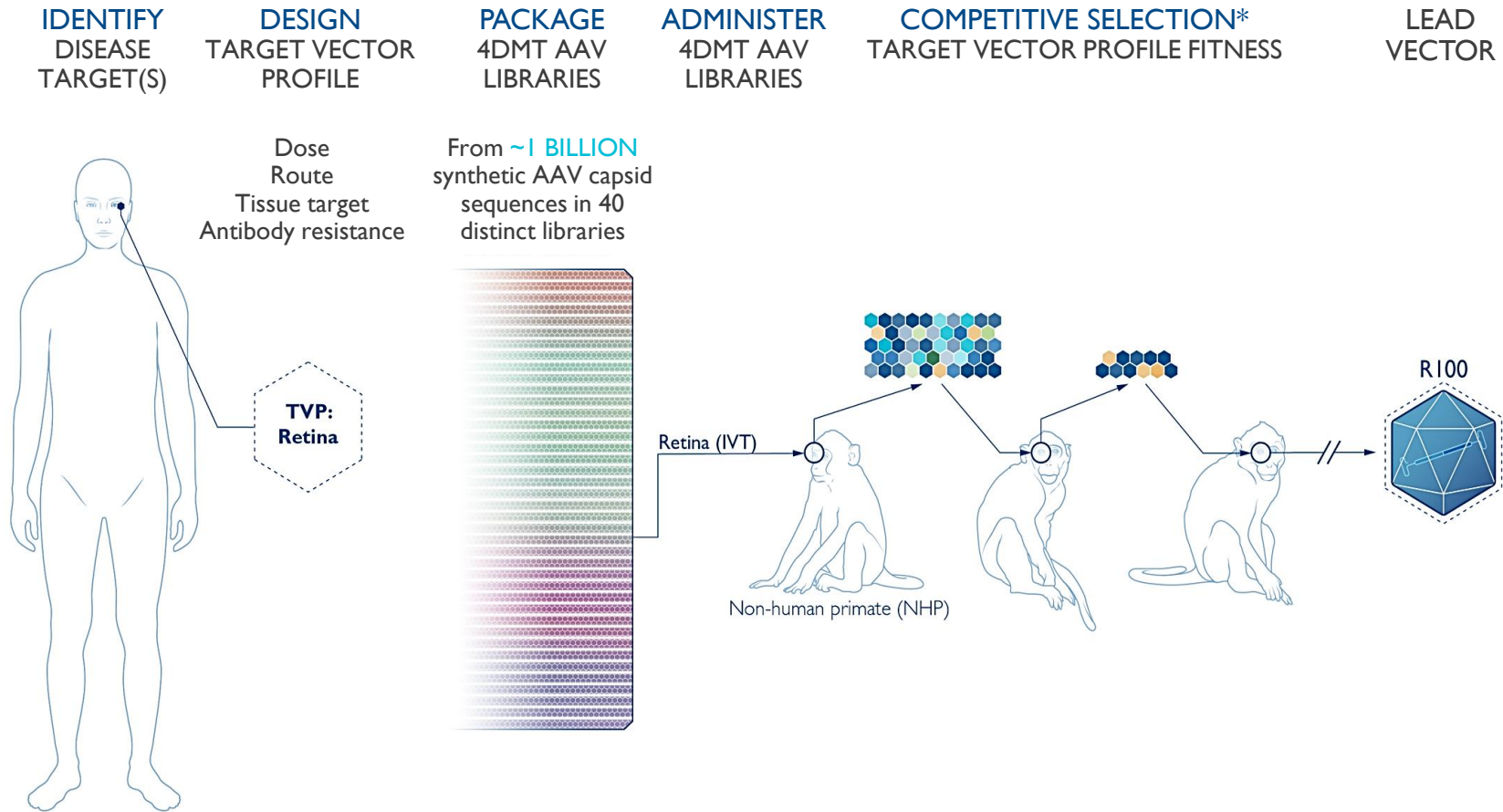


- Synthetic AAV capsid variant with enhanced capacity to penetrate vitreoretinal barriers

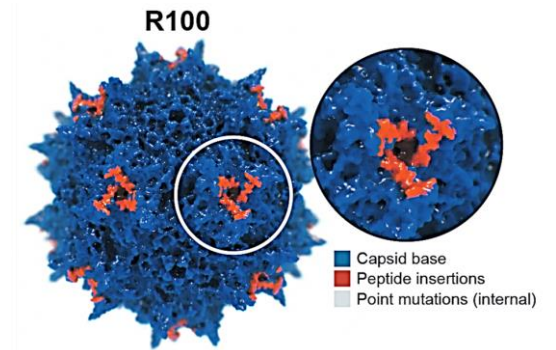
AAV, adeno-associated virus; ILM, inner limiting membrane; RPE, retinal pigment epithelium.

# Therapeutic Vector Evolution

## COMPETITIVE SELECTION FOR TARGET VECTOR PROFILE FITNESS



### R100 Retinotropic AAV Vector



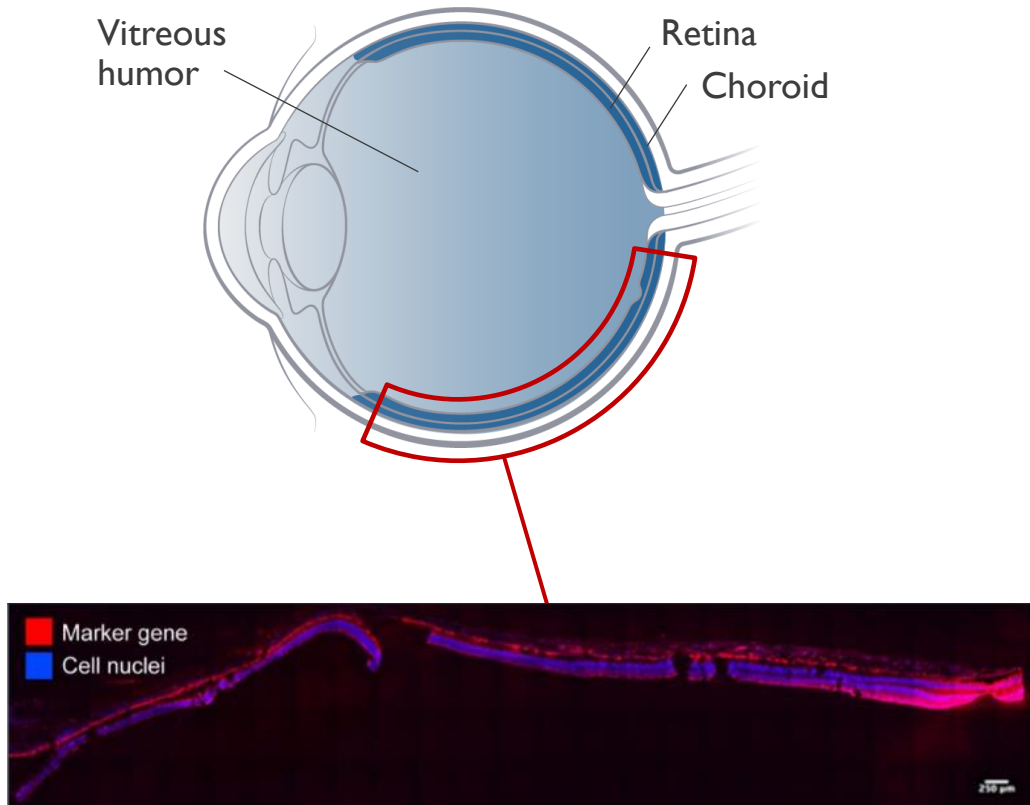
- Enhanced capacity to cross vitreoretinal barriers
- Transduction of all regions and layers of the retina
- Robust, widespread transgene expression following intravitreal administration

\*Capsid library placed under varying selective pressures. // Actual number of selection rounds varies by target. TVP, target vector profile.

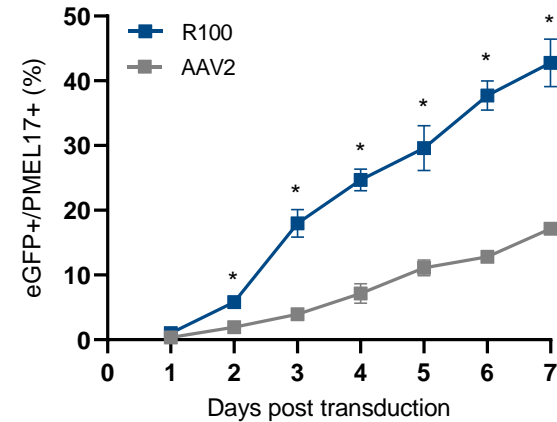
# R100: Evolved Retinotropic Intravitreal AAV Vector

## PROOF OF CONCEPT

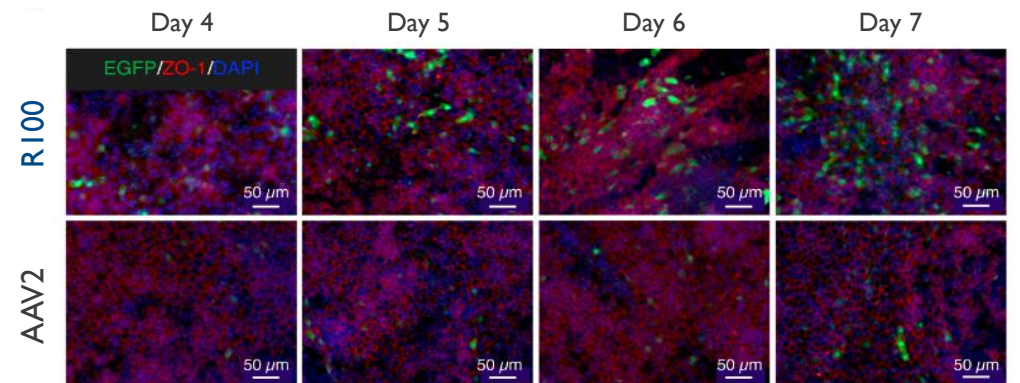
### NHP Retinal Transduction (IVT Delivery)



### Transduction of Human RPE Cells *In Vitro*



Superior transduction of human RPE cells *in vitro* compared to the wild-type AAV serotype AAV2



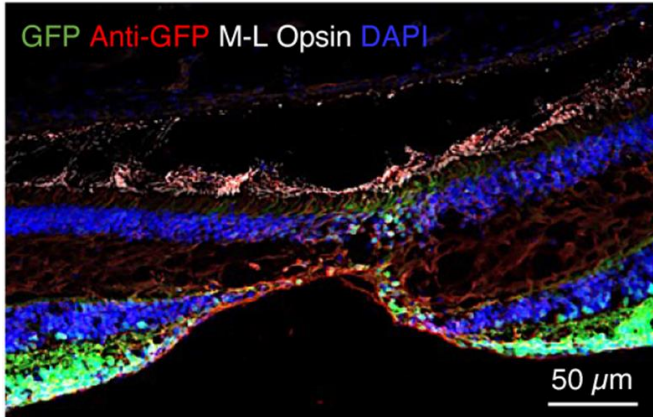
\*P < 0.05 (2-tailed t-test). EGFP, enhanced green fluorescent protein; IVT, intravitreal; NHP, nonhuman primate; RPE, retinal pigment epithelium.



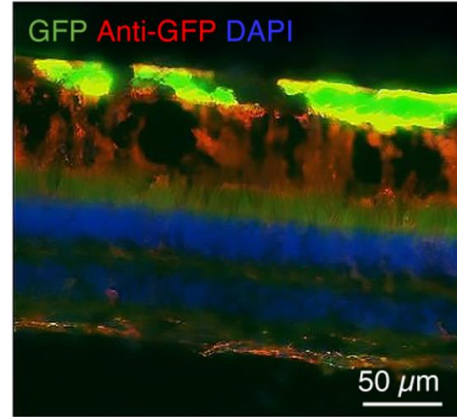
# R100-mediated Transgene Expression

ROBUST PAN-RETINAL EXPRESSION FOLLOWING IVT ADMINISTRATION IN NONHUMAN PRIMATES

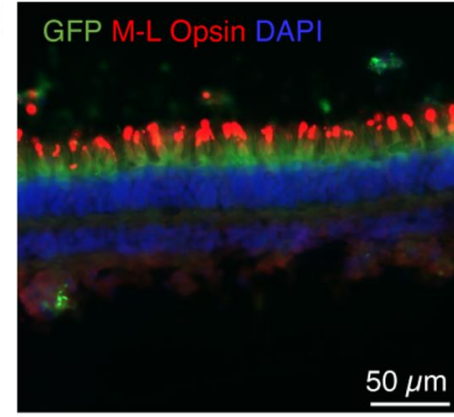
Central Retina



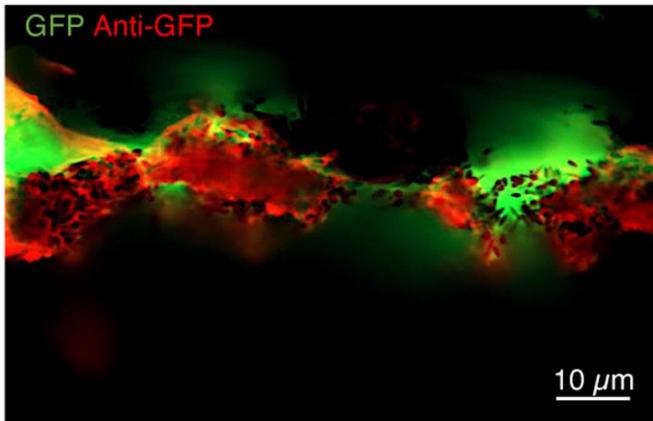
Peripheral PR



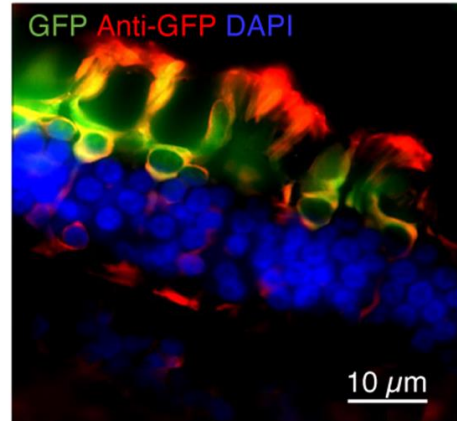
Peripheral PR



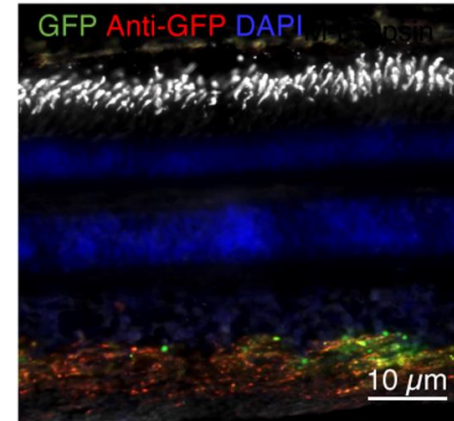
Retinal Pigment Epithelium



Photoreceptors



Retinal Ganglion Cells

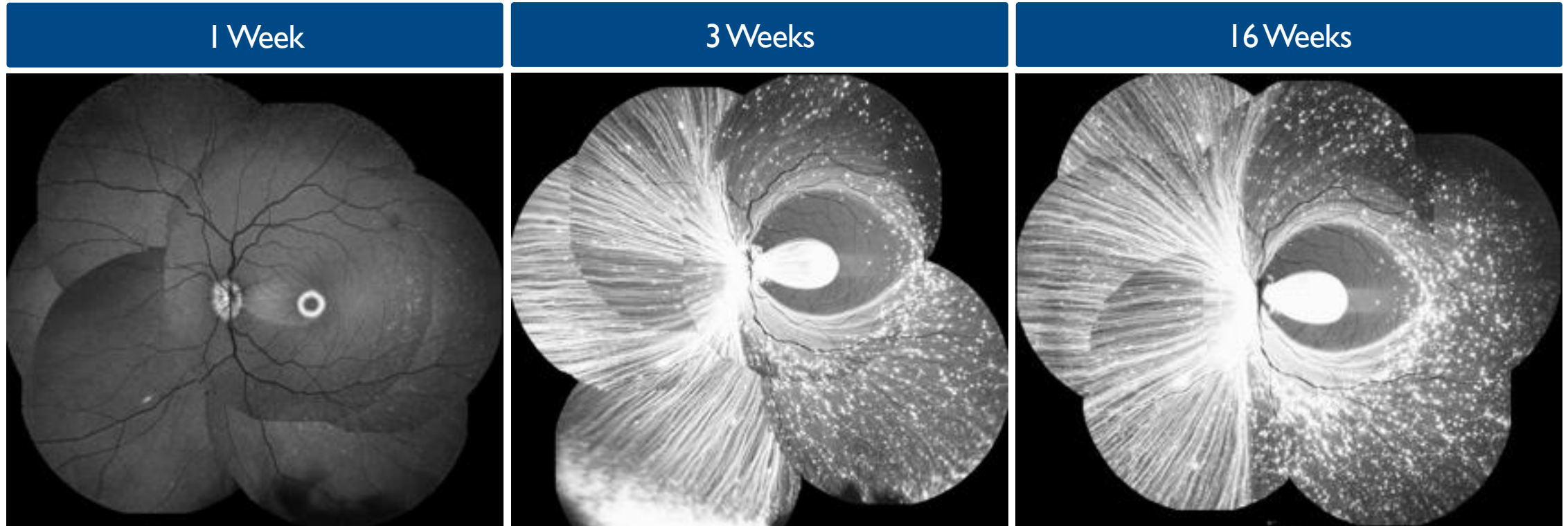


- NHP retina 3 weeks after IVT administration of R100.CAG-EGFP ( $1 \times 10^{12}$  vg/eye)
- R100 demonstrated robust transduction of multiple cell types and multiple cell layers in both the central and peripheral retina

GFP, green fluorescent protein; PR, photoreceptors.

# R100 Intravitreal Retinotropic AAV Vector

IN-LIFE IMAGING IN NONHUMAN PRIMATES FOLLOWING IVT ADMINISTRATION OF R100.CAG-EGFP\*



- Durable and widespread retina transduction observed across all regions of the eye

\* $1 \times 10^{12}$  vg/eye. EGFP, enhanced green fluorescent protein; IVT, intravitreal.

# R100 Biodistribution and Safety


## GLP TOXICOLOGY AND BIODISTRIBUTION STUDIES—NONHUMAN PRIMATES

Species	4D-110		4D-125	4D-150
	NHP	NHP	NHP	NHP*
Eyes dosed, N	27 (unilateral)	34 (bilateral)	30 (unilateral)	42 (bilateral)
Route of administration	IVT	IVT	IVT	IVT
Highest dose to date	1E12 vg/eye	1E12 vg/eye	1E12 vg/eye	1E12 vg/eye
Clinical evaluation	No adverse findings <sup>†</sup>	No adverse findings <sup>†</sup>	No adverse findings <sup>†</sup>	No adverse findings <sup>†</sup>
Clinical pathology	No adverse findings	No adverse findings	No adverse findings	No adverse findings
Hematology	No adverse findings	No adverse findings	No adverse findings	No adverse findings
Hematocrit	No adverse findings	No adverse findings	No adverse findings	No adverse findings
Clinical chemistry	No adverse findings	No adverse findings	No adverse findings	No adverse findings
Liver enzymes (ALT/AST)	No adverse findings	No adverse findings	No adverse findings	No adverse findings
Gross pathology	No adverse findings	No adverse findings	No adverse findings	No adverse findings
Histopathology	No adverse findings	No adverse findings	No adverse findings	No adverse findings
Cellular immune response <sup>‡</sup>	Anti-capsid: negative Transgene: negative	Anti-capsid: negative Transgene: negative	Anti-capsid: negative Transgene: negative	Anti-capsid: positive Transgene: positive

\*African green monkey. <sup>†</sup>Transient, steroid-responsive uveitis. <sup>‡</sup>Enzyme-linked immunosorbant assay. IVT, intravitreal; NHP, nonhuman primate.

# Vector Modularity

BROAD APPLICATION ACROSS MULTIPLE RETINAL DISEASES



R100

Candidate	Transgene	Promotor	Target Indication	Patients Treated*
4D-150	Aflibercept VEGF-C miRNA	Ubiquitous	Wet AMD, DME	>60
4D-110	<i>CHM</i>	Ubiquitous	Choroideremia	13
4D-125	<i>RPGR</i>	Photoreceptor-specific	X-linked retinitis pigmentosa	15
4D-175	<i>sCFH</i>	Ubiquitous	Geographic atrophy	NA

- Therapeutic vector profile supports modular design of retinal gene therapy candidates

\*Phase 1/2 clinical trials. AMD, age-related macular degeneration; DME, diabetic macular edema; RPGR, *retinitis pigmentosa GTPase regulator*; sCFH, short-form complement factor H.

# Geographic Atrophy

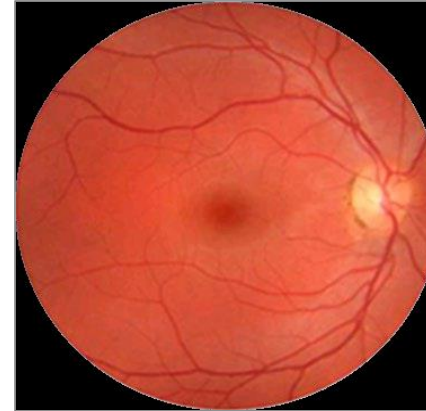
Therapeutic Rationale for Targeting the  
Complement System

# Geographic Atrophy (GA)

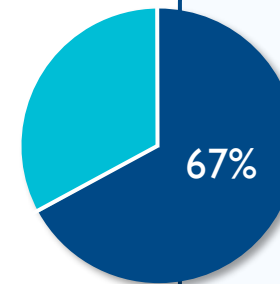
## OVERVIEW

- Advanced form of age-related macular degeneration that leads to irreversible vision loss
- Affects an estimated 5 million individuals globally (1 million in the US)<sup>1,2</sup>
- Characterized by atrophic lesions in the outer retina caused by degeneration of the retinal pigment epithelium (RPE), photoreceptors, and choriocapillaris<sup>3</sup>
- Clinical course: progressive loss of central vision and light sensitivity

Normal



Geographic Atrophy



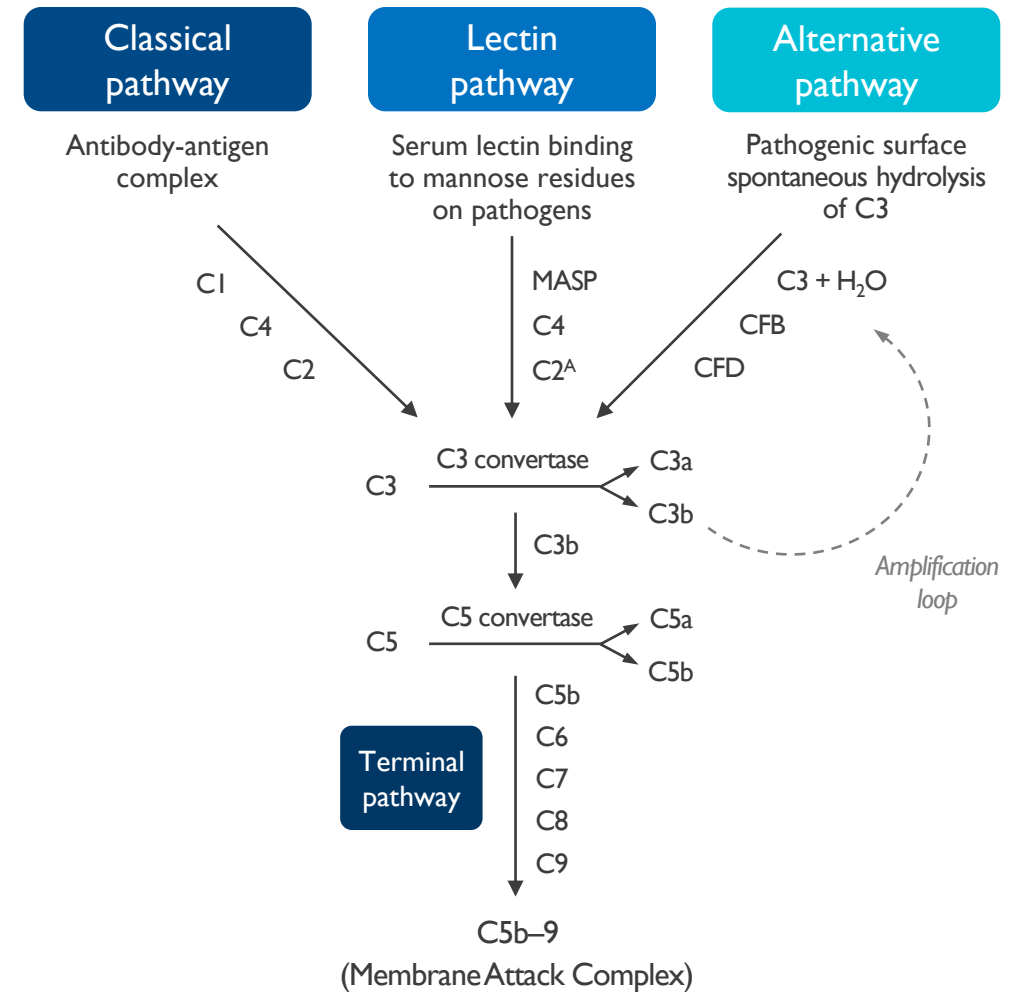
Approximately 2/3 of patients with GA will become ineligible to drive<sup>4</sup>

Median time to vision loss below the standard for eligibility to drive: 1.6 years<sup>4</sup>

1. Wong et al. *Lancet Glob Health* 2014;2:e106–16. 2. Freidman et al. *Arch Ophthalmol* 2004;122:564–72. 3. Holtz et al. *Ophthalmology* 2014;121:1079–1091. 4. Chakravarthy et al. *Ophthalmology* 2018;125:842–9.

# Complement Inhibition is a Validated Therapeutic Approach in GA

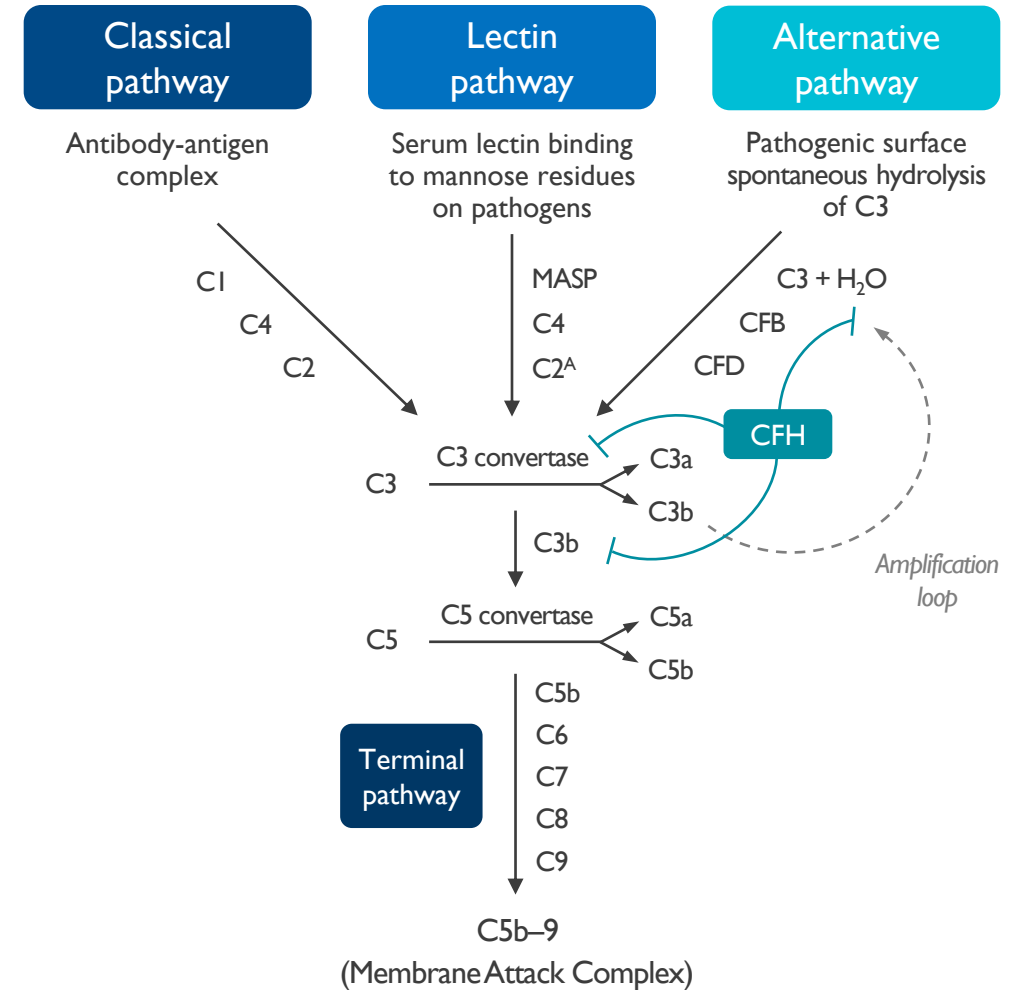
- Complement-mediated inflammation is recognized as a main contributor to the pathogenesis of GA
- Hyperactivation of the complement system leads to chronic inflammation and RPE damage<sup>1</sup>
- Biochemical analysis of drusen identified the presence of multiple complement proteins<sup>2</sup>
- Clinical trials have demonstrated that complement inhibition attenuates the growth of GA lesions<sup>3-5</sup>



1. Lachmann PJ. *Adv Immunol* 2009;104:115-49. 2. Anderson DH et al *Prog Retin Eye Res* 2010;29:95-112.  
3. Liao D et al. *Ophthalmology* 2020;127:186-95. 4. Jaffe GJ, et al. *Ophthalmology* 2021;128:576-86.  
5. Wykoff C. Presented at American Academy of Ophthalmology, November 21, 2021.

# Complement Factor H (CFH)

- A key regulator of the complement system
  - Inhibits assembly of C3 and C5 convertase enzymes via competition with factor B for C3b binding<sup>1</sup>
  - Facilitates disassembly of the convertases by displacing bound factor Bb<sup>1</sup>
  - Inactivates C3b by acting as a cofactor for complement factor I (CFI)<sup>1</sup>
- Inactivation of alternative pathway on host cells localizes reaction to pathogens
- CFH dysfunction decreases inactivation of the complement cascade and amplifies activation of the alternative complement pathway<sup>2,3</sup>



1. Perkins et al. *Immunobiol* 2012;217:281–297. 2. Manuelian et al. *J Clin Invest* 2003;111:1181-90. 3. Prosser et al. *J Exp Med* 2007;204:2277-83.



# Rationale for *CFH* Gene Transfer in GA

## SUPPORTED BY MULTIPLE LINES OF EVIDENCE

- Variants in the gene encoding *CFH* are strongly associated with the risk of GA<sup>1,2</sup>
  - Most common *CFH* variant (Y402H) accounts for nearly 50% of the overall risk<sup>2,3</sup>
  - Rare *CFH* variant (R1220C) is strongly associated with early onset GA<sup>4</sup>
- Studies in murine models support a causal role for *CFH* dysfunction in retinal pathology<sup>5</sup>
  - *CFH*-deficient mice exhibit increased retinal C3 deposition and decreased visual acuity<sup>2</sup>; expression of human *CFH* rescues the phenotype<sup>6</sup>
  - Transgenic mice expressing the human *CFH* Y402H variant develop AMD-like retinal pathology<sup>7</sup>
- Individuals with AMD carrying the *CFH* Y402H variant have elevated levels of inflammatory markers in the choroid<sup>8</sup> and increased plasma levels of complement activation products<sup>9</sup>

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.

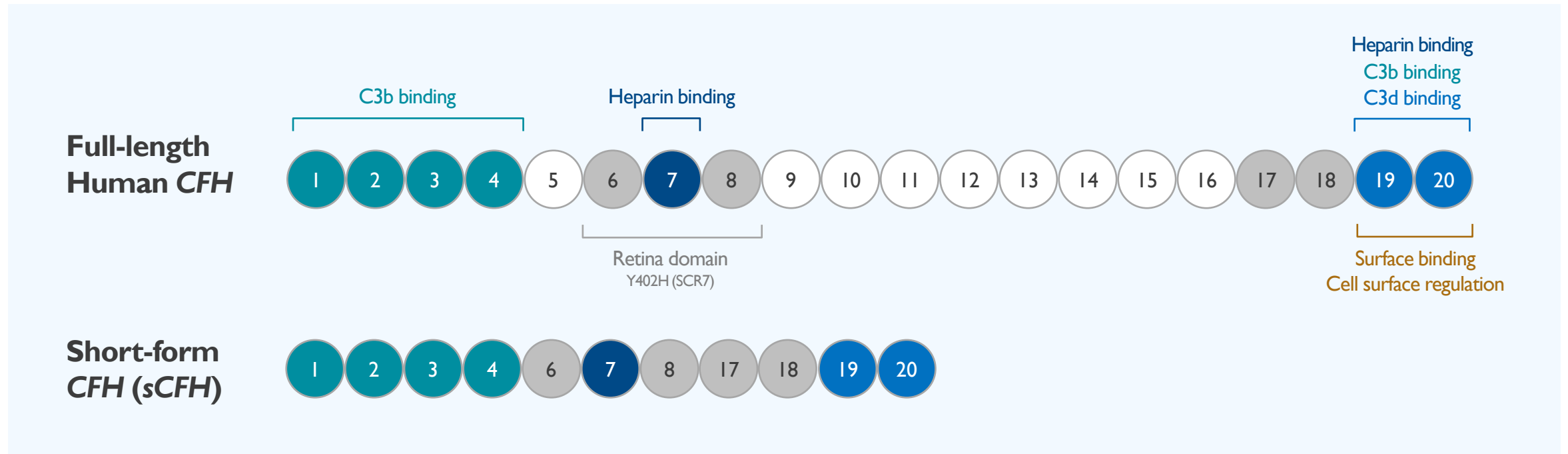
# 4D-175

Investigational Intravitreal Gene Therapy for  
Geographic Atrophy

# 4D-I75 Transgene Selection

## SHORT-FORM COMPLEMENT FACTOR H (sCFH)

- Full-length human *CFH* gene and promoter exceed the payload capacity of the AAV capsid
- To reduce payload size, 4D-I75 employs an engineered short-form *CFH* transgene

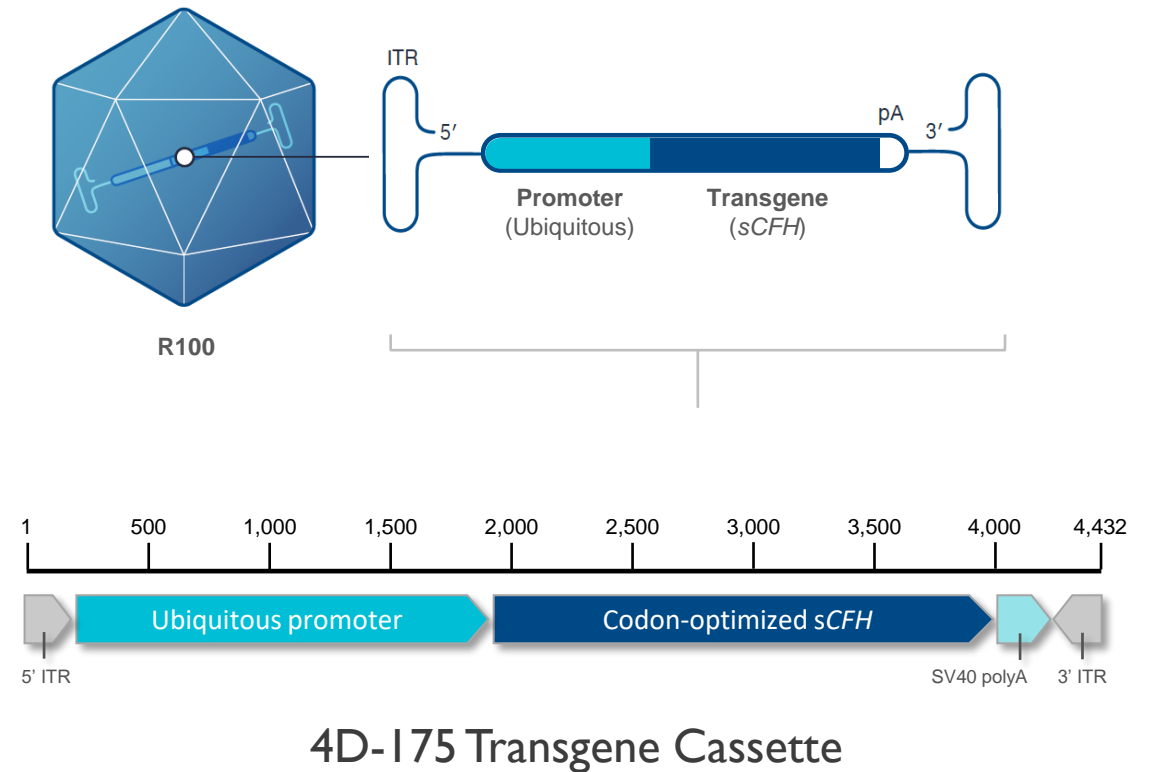


Circles represent short consensus repeats (SCRs). de Córdoba SR, de Jorge EG. *Clin Exp Immunol* 2008;151:1-13.

# 4D-I75 Product Design

## INTRAVITREAL GENE THERAPY FOR GEOGRAPHIC ATROPHY

- Intravitreal AAV-based gene therapy
- Clinically validated retinotropic R100 AAV capsid variant
- Codon-optimized sequence encoding a shortened form of human complement factor H (sCFH) under the control of a ubiquitous promoter
- Therapeutic objective: Restore normal complement regulation in the retina through durable expression of CFH

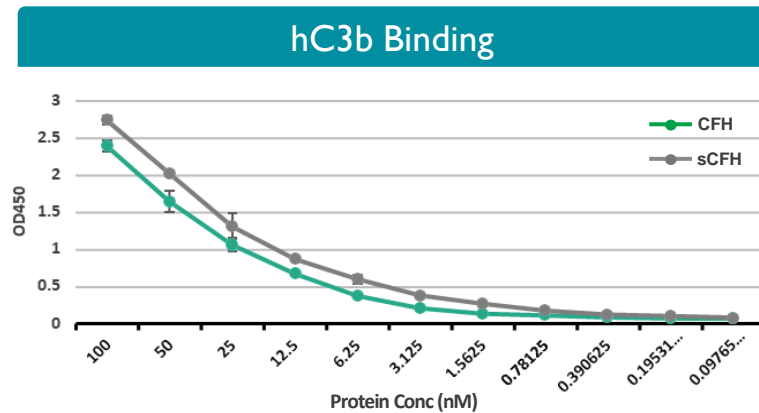
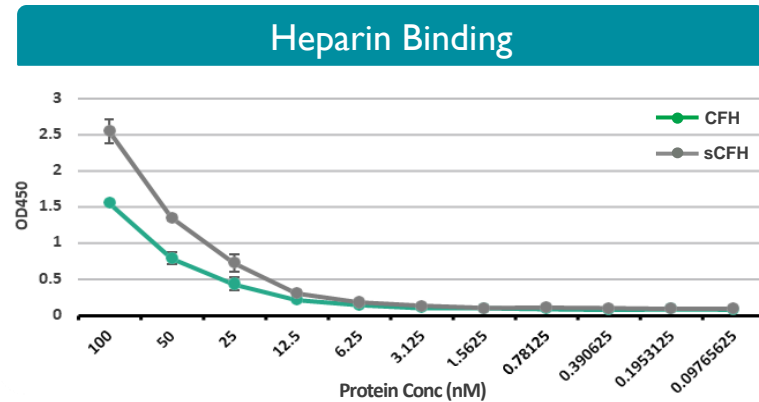


CFH, complement factor H; ITR, inverted terminal repeat; pA, poly adenylation tail.

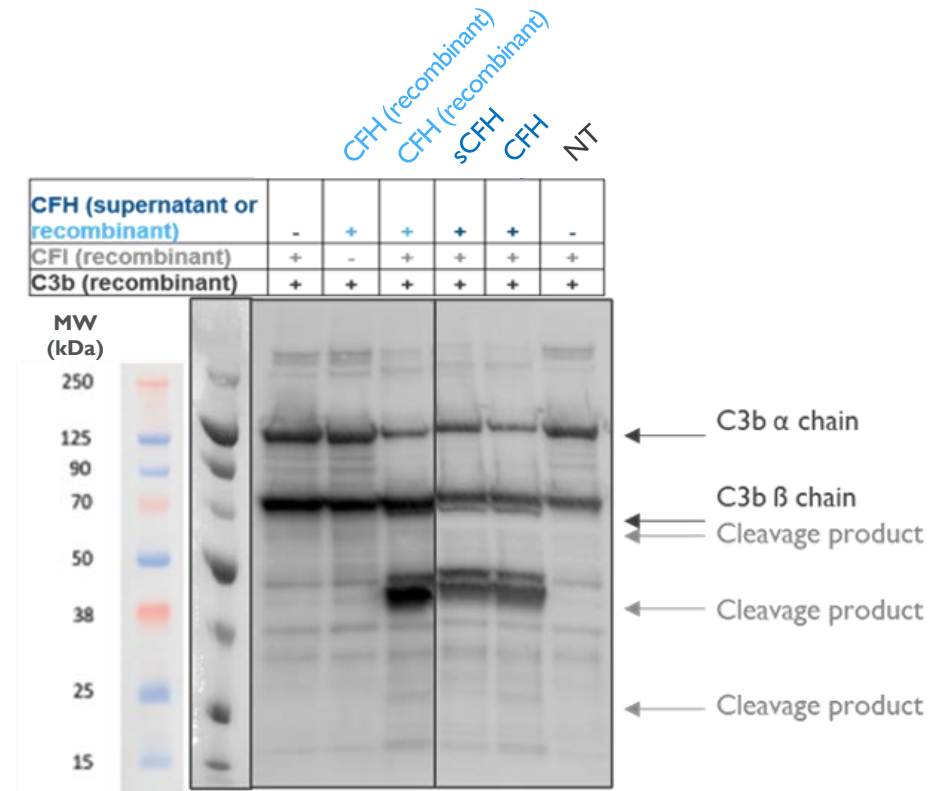
# 4D-175 for the Treatment of GA

## CONFIRMED PHARMACOLOGICAL FUNCTION OF sCFH PROTEIN

### 4D-175 sCFH Binds Heparin and C3b



### 4D-175 sCFH Cleaves C3b Appropriately



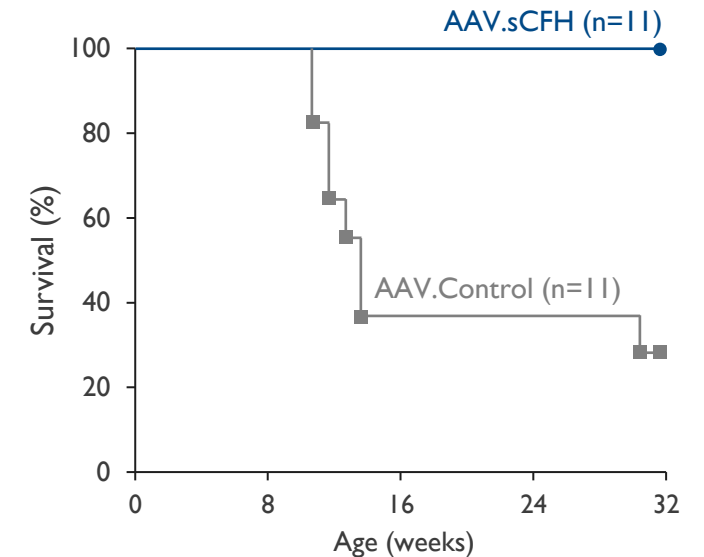
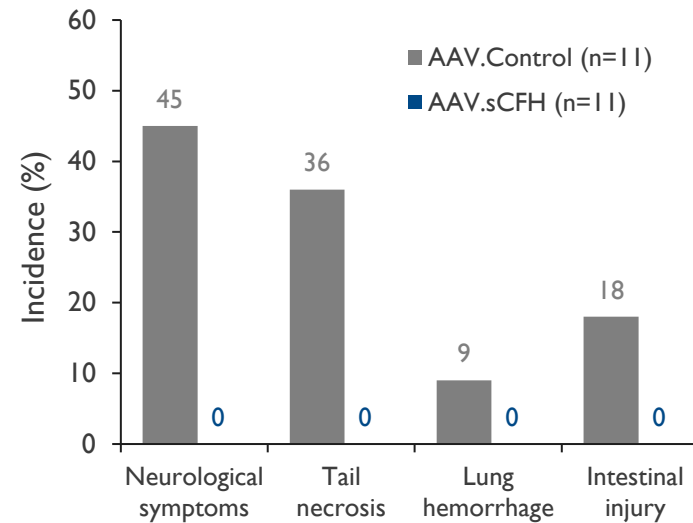
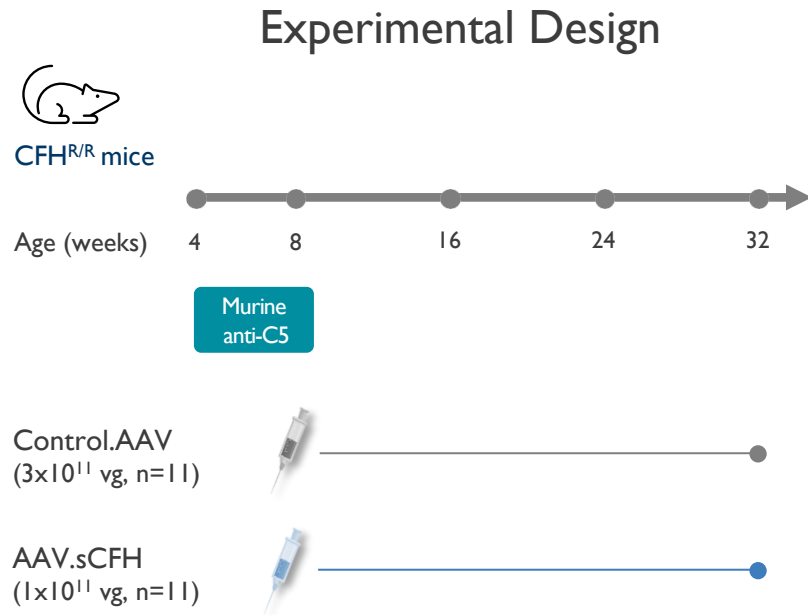
- The C3b alpha chain is degraded into iC3b in the presence of CFH + CFI
- iC3b is detected as a reduction of the alpha chain (116 kDa) and appearance of 2 iC3b breakdown bands (68 and 43 kDa)

Data on file.

# 4D-175 for the Treatment of GA

## CONFIRMED PHARMACOLOGICAL FUNCTION OF sCFH *IN VIVO*

### Phenotypic Correction in Mouse Model of aHUS



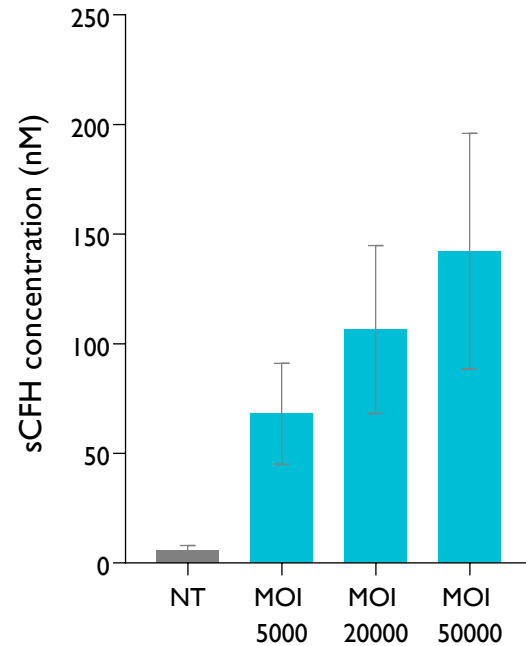
- sCFH prevented aHUS phenotypes and extended survival compared to controls in a mouse disease model

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

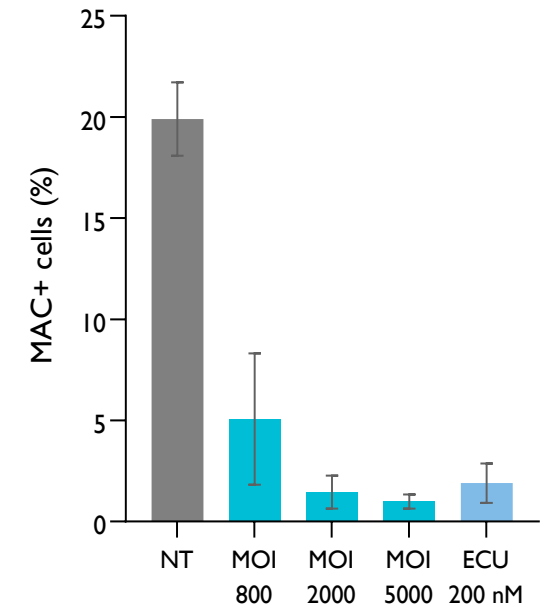
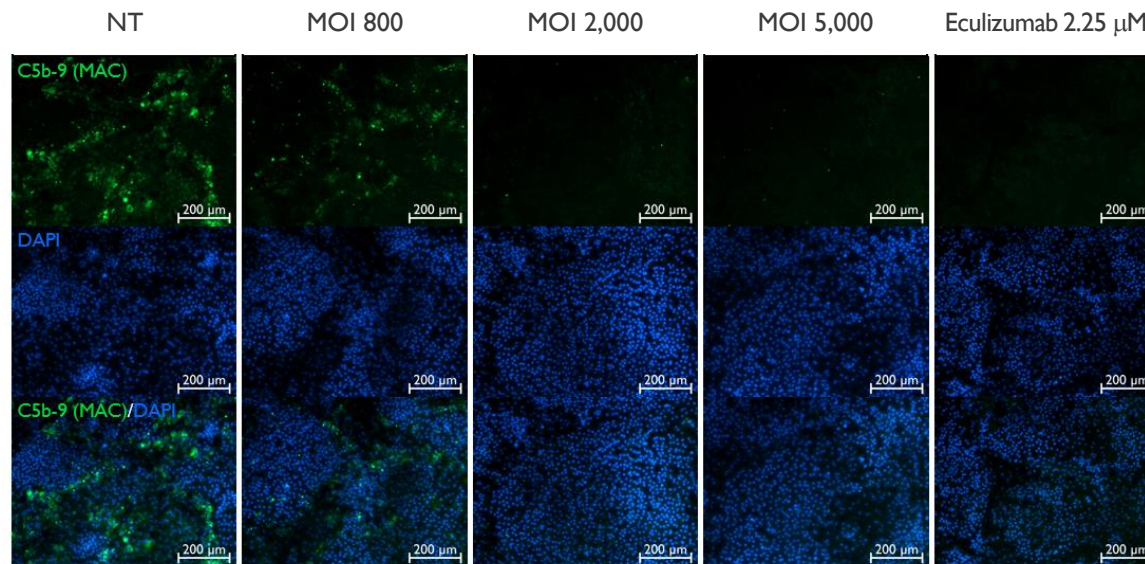
# 4D-175 for the Treatment of GA

PRELIMINARY *IN VITRO* DATA SUPPORT TRANSGENE EXPRESSION AND ACTIVITY

## Transgene Expression\*



## MAC Formation in iPSC-derived RPE Cells†



- Robust 4D-175 transgene expression and prevention of membrane attack complex (MAC) formation *in vitro*

\*iPSC-derived RPE cells. iPSC, induced pluripotent stem cells; MOI, multiplicity of infection; RPE, retinal pigment epithelium. †Assessed by immunocytochemistry (left panel) and flow cytometry (right panel).

# Summary

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- R100: Synthetic retinotropic AAV vector
  - Enhanced capacity to penetrate vitreoretinal barriers following IVT administration
  - Widespread transduction of retina cells allows efficient transgene delivery at low doses
  - Clinically validated: 4 clinical trials evaluating 3 separate investigational retinal gene therapies
- 4D-175: Investigational IVT gene therapy for GA
  - R100 vector carrying a codon-optimized sequence encoding sCFH
  - Robust transgene expression and complement inhibition in RPE cells *in vitro*
  - Confirmed pharmacological function of sCFH in *in vivo* models of complement-mediated diseases
- IND filing anticipated in 1H 2024





**THANK YOU**

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