

# Reduced Ocular Inflammation and Improved GFP Expression in Rabbits with Controlled Release of AAV from Degradable Hydrogel Implants

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*\*Presenting Author*

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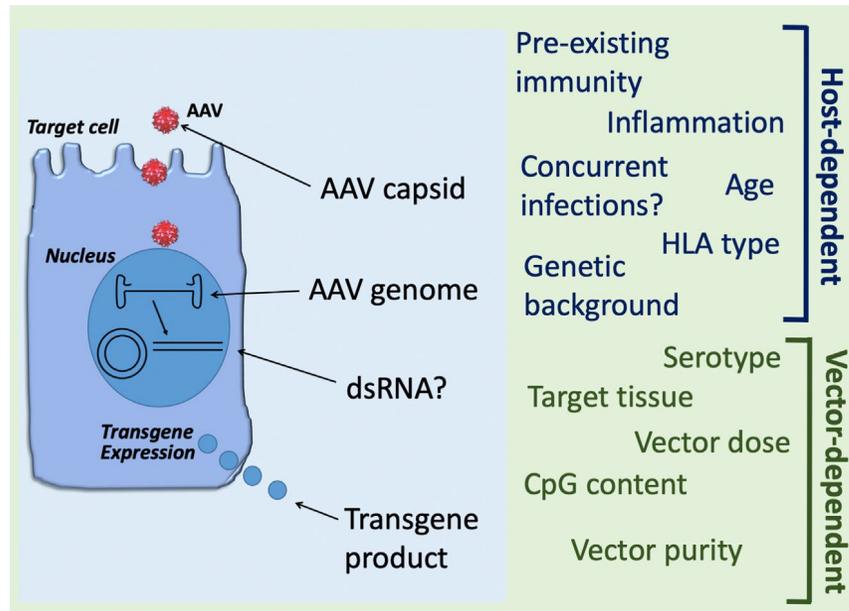
# Disclosures

- All authors are employees of Ocular Therapeutix, Inc.
- This study was funded by Ocular Therapeutix, Inc.
- The presentation discusses an investigational product and its efficacy and safety profile has not been established and it has not been approved by the FDA

# Background

## Ocular AAV Gene Therapy

*Dose-dependent inflammation is an obstacle to treatment efficacy and a multi-faceted strategy is often needed.*<sup>1-5</sup>

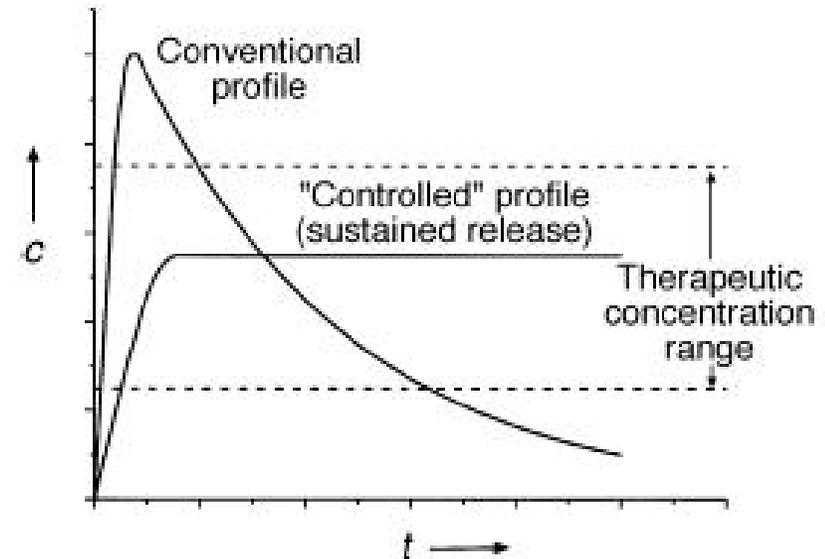


HLA, human leukocyte antigen

Figure used with permission from Verdera HC et al. *Mol Ther.* 2020;28(3):723-746.

## AAV Pharmacokinetics

*A sustained-release modality of AAVs, spreading a high total dose over multiple days, could result in reduced inflammation and improved efficacy outcomes*



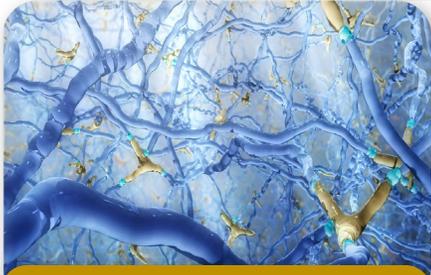
c, concentration; t, time

Figure used with permission from Santini JT Jr, et al. *Angew Chem Int Ed Engl.* 2000;39(14):2396-2407.

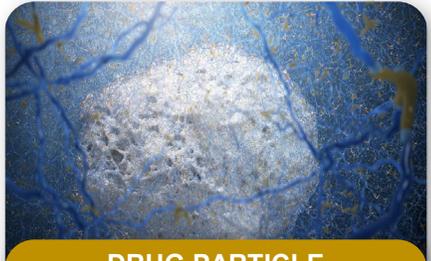
**References:** 1. Cukras C, et al. *Mol Ther.* 2018;26:2282-2294. 2. Chan YK et al. *Trans Vis Sci Tech.* 2021;10(4):3. 3. Bainbridge JWB et al. *N Engl J Med.* 2015; 372:1887-1897. 4. Dimopoulos IS et al. *Am J Ophthalmol.* 2018;193:130-142. 5. Verdera HC et al. *Mol Ther.* 2020;28(3):723-746.

**Abbreviation:** AAV, adeno-associated virus

# Ocular Therapeutix Hydrogel Platform for Drug Delivery



HYDROGEL MESHWORK



DRUG PARTICLE  
ENTRAPPED IN HYDROGEL



DRUG ELUTION FROM  
HYDROGEL FOLLOWING  
HYDRATION

## HYDROGEL PLATFORM ATTRIBUTES

### VERSATILE

- Steady state or tapered delivery
- Release in days or months
- Small molecules or large proteins
- Safety established in clinical and commercial products

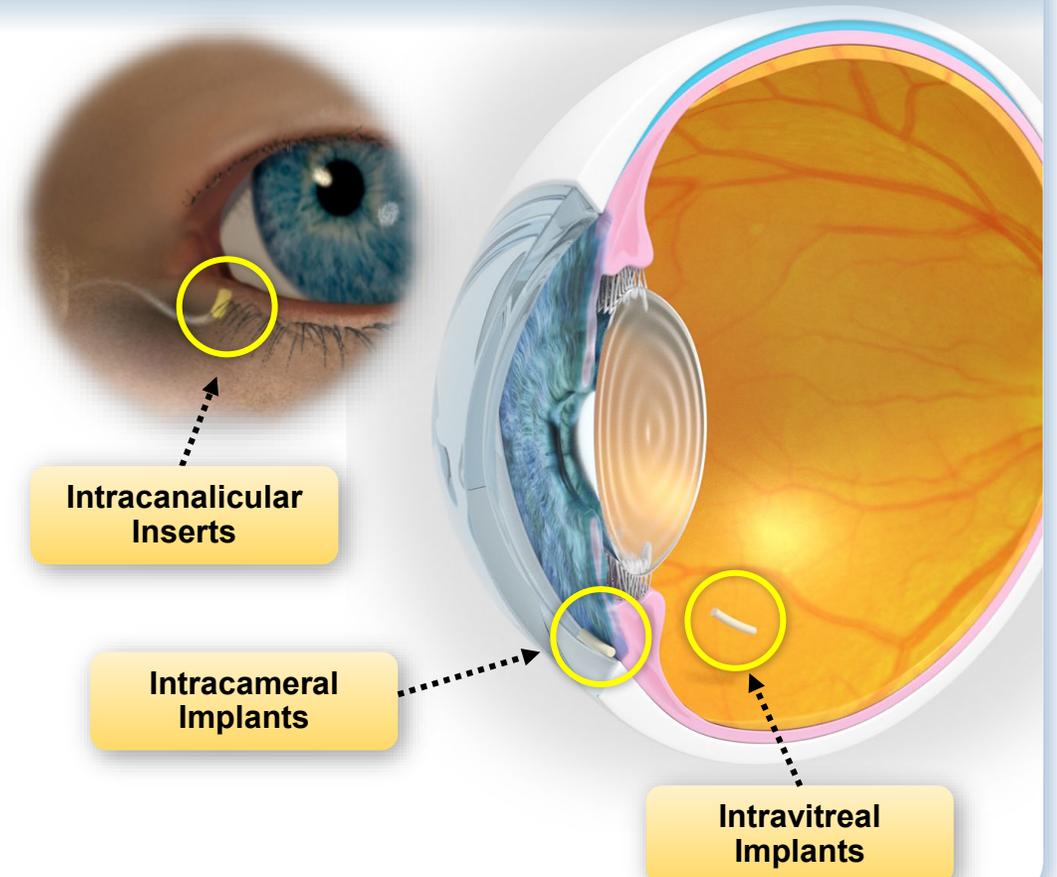
### BENEFICIAL

- 90% water, biocompatible
- Preservative-free
- Fully resorbs when drug is delivered

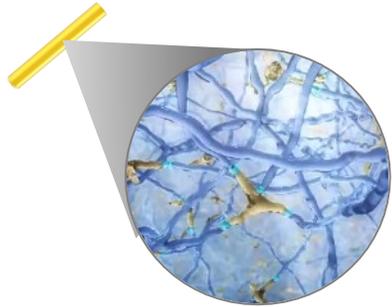
### PROPRIETARY

- Robust portfolio of patents
- Non-standard dosage forms
- Manufacturing trade secrets

## VERSATILE APPLICATIONS IN DIFFERENT AREAS OF OPHTHALMOLOGY



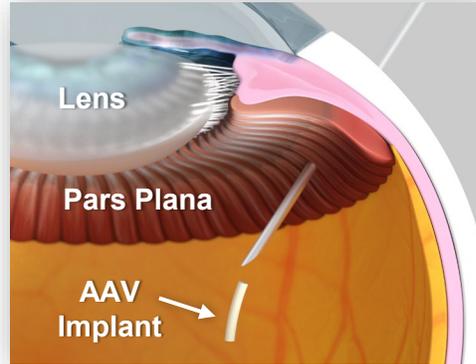
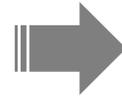
# AAV Intravitreal Implant



**Polyethylene Glycol Hydrogel**  
(Inactive Delivery Platform)

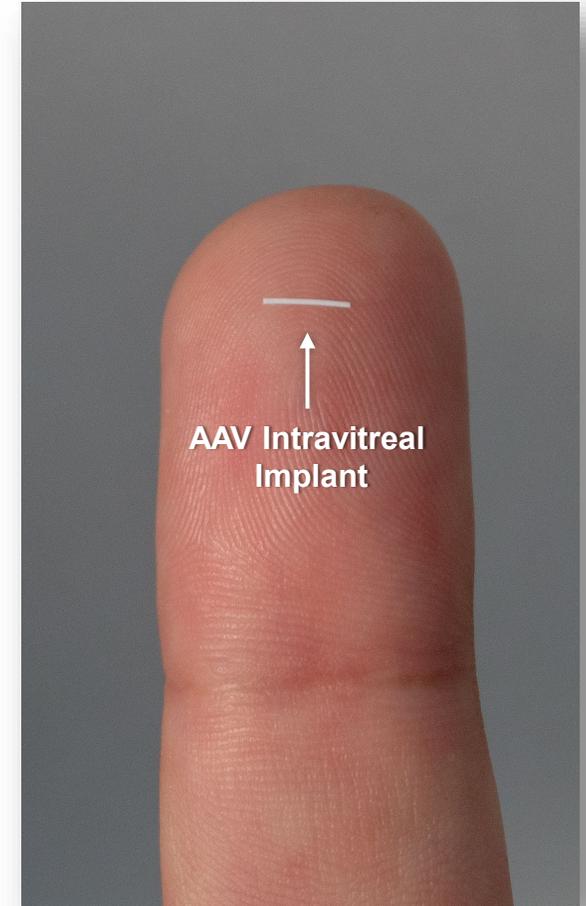


**Adeno-Associated Virus**  
(Gene Vector)

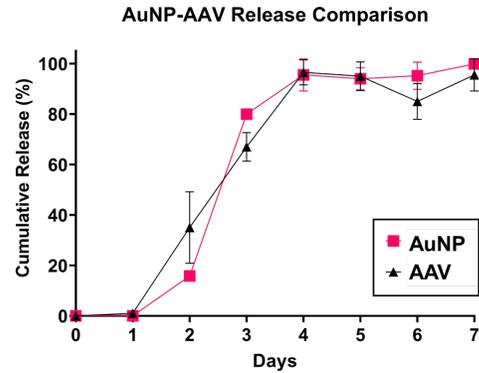
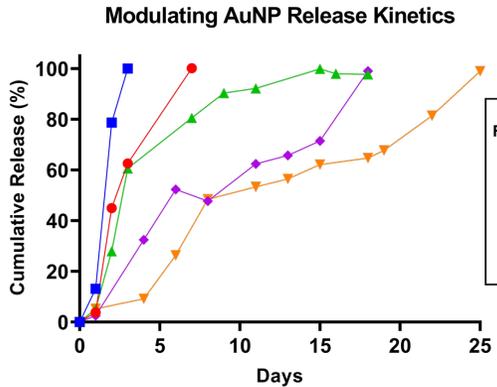


**AAV Intravitreal Implant**

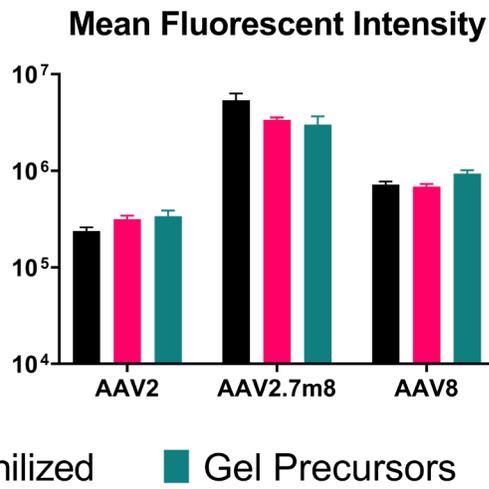
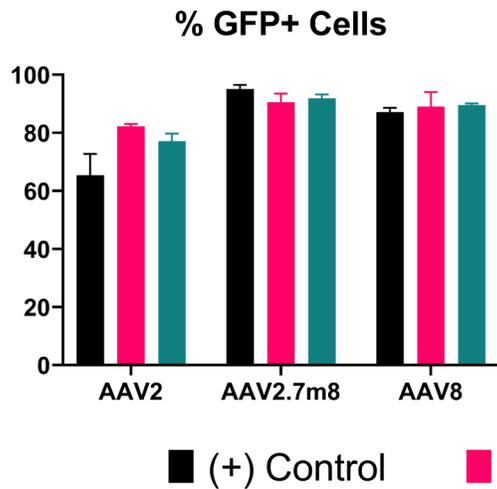
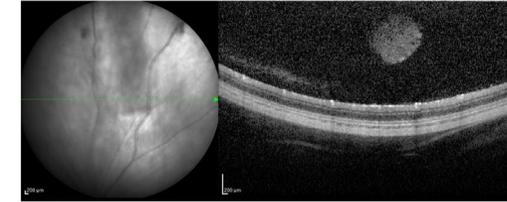
- Solid implant that can be placed next to tissue of interest and hydrates upon implantation
- Intravitreal injection offers an easier mode of delivery than subretinal injections
- AAV release is controlled via hydrogel degradation and mesh size opening



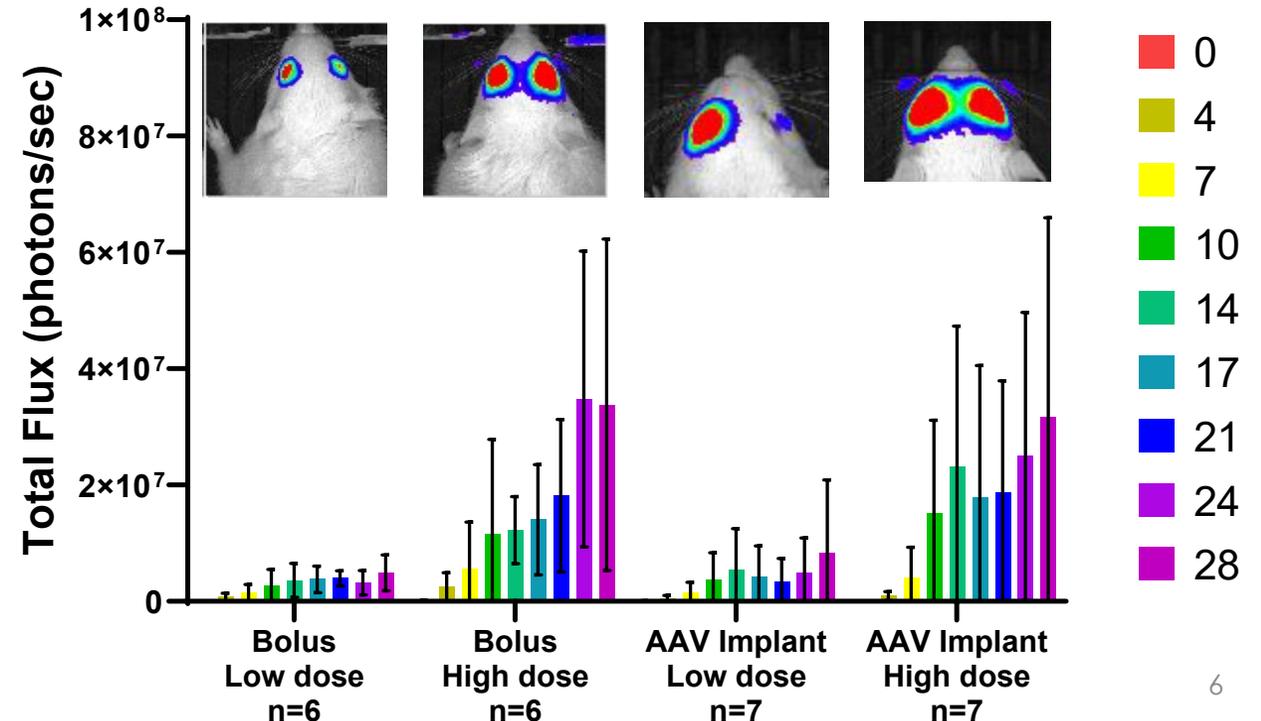
# Encapsulated AAVs Retain Infectivity In Vitro and In Vivo (Abstract# 286)



Post-dose visualization of hydrogel implant via OCT



Ocular Luminescent Intensity



# Rabbit Proof-of-Concept

## Objective

- To evaluate ocular inflammation and GFP expression of **AAV2-CMV-eGFP** from bilateral intravitreal injections of AAV bolus (solution) or AAV implant

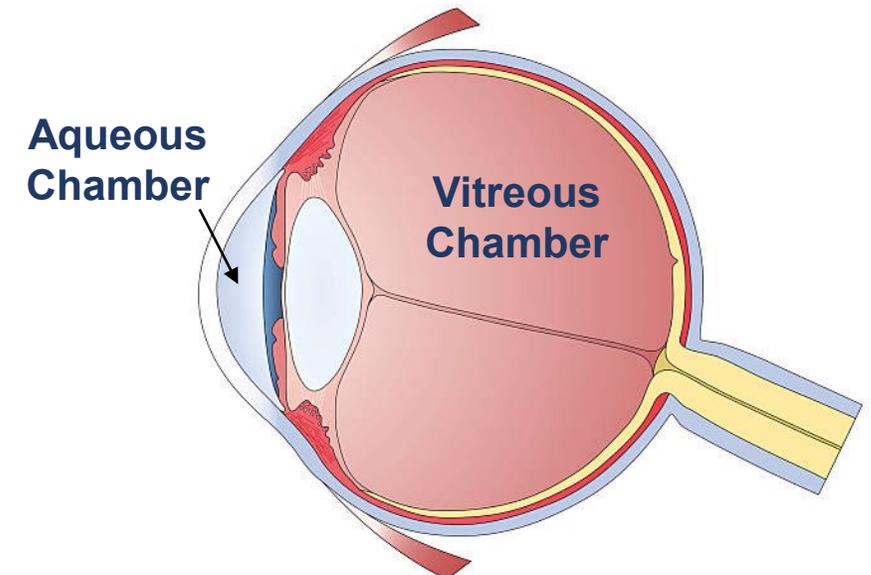
## Study Design

- Animals: New Zealand white rabbits were selected due to their larger globe size and high immunoreactivity
- Treatment: No anti-inflammatory treatment was given in any group

Group (n=3 animals per group)	Left Eye Treatment (OS)	Right Eye Treatment (OD)	Dose/eye	AAV Implant Release
1	Bolus vehicle	Implant vehicle	0	N/A
2	AAV Bolus (50µL)		1.2E+10 GC	N/A
3	AAV Implant		1.2E+10 GC	4 days

**Evaluations:** Eyes were assessed by ophthalmic exam, fundus autofluorescence imaging, and ocular biodistribution by qPCR through 8 weeks

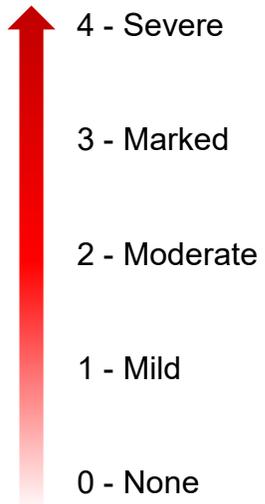
*Inflammation in the aqueous and vitreous chamber was graded by SPOT scoring system*



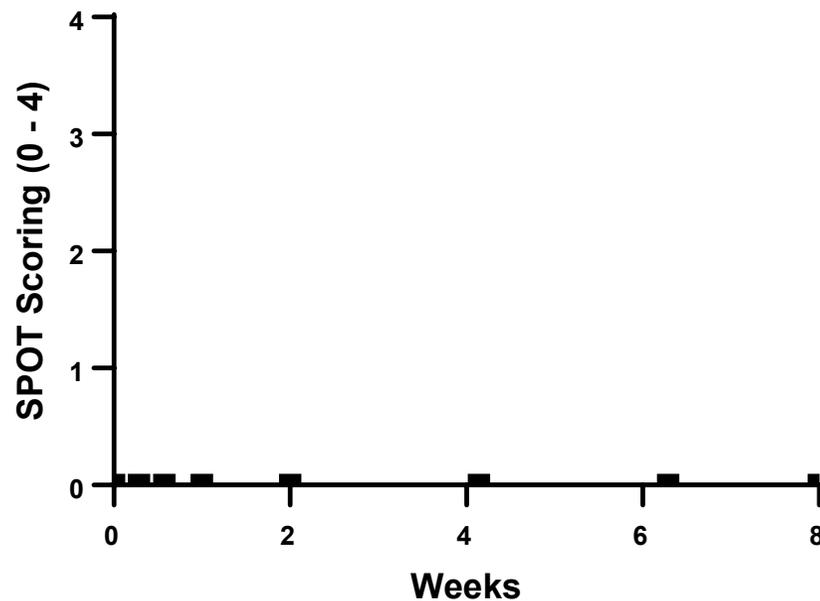
# Ocular Inflammation Scoring – Vehicle Test Articles

*Minimal inflammation observed from vehicle test articles*

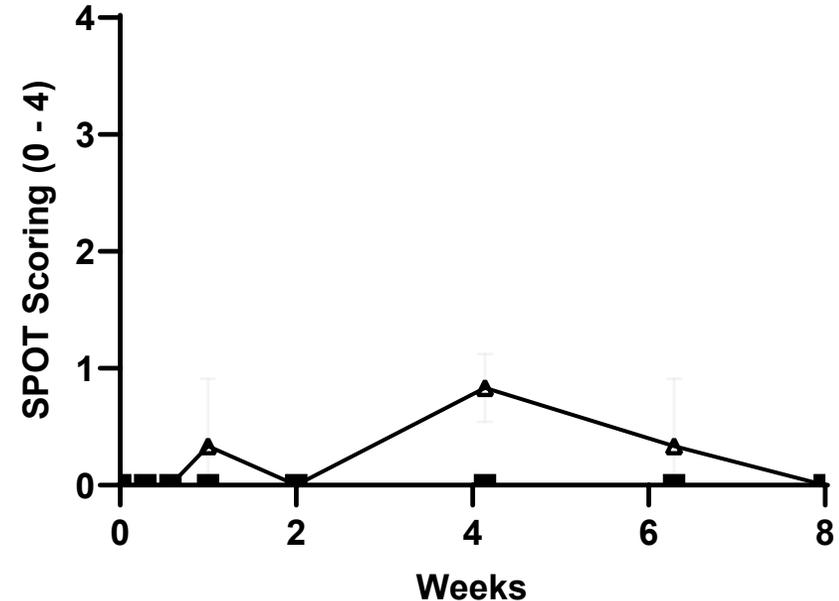
**SPOT Scoring for Ocular Inflammation**



**Aqueous Cells**



**Vitreous Haze**

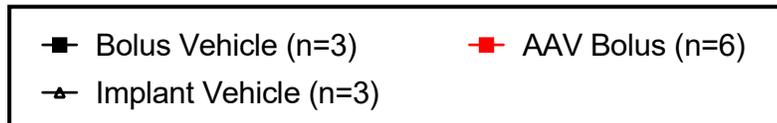
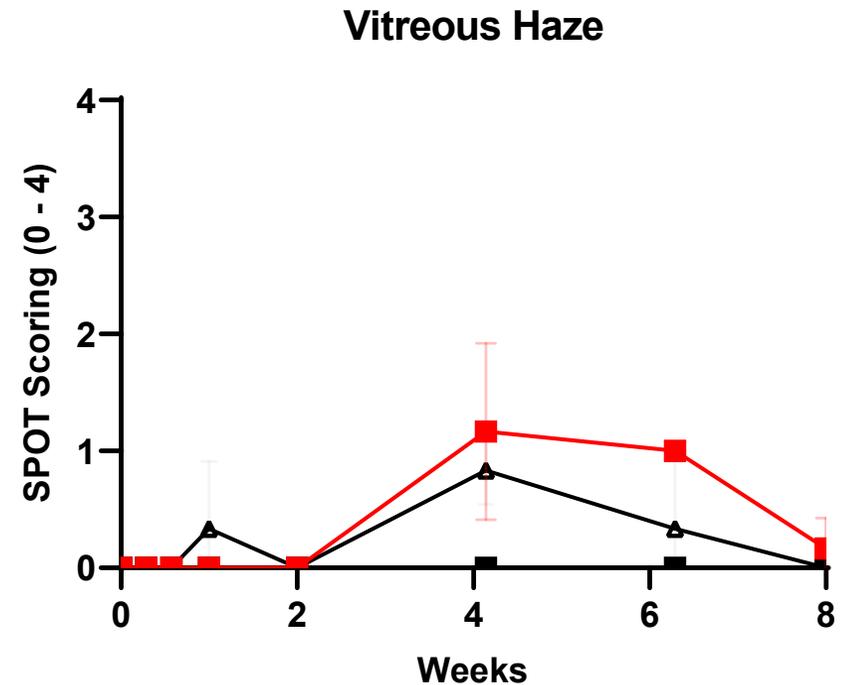
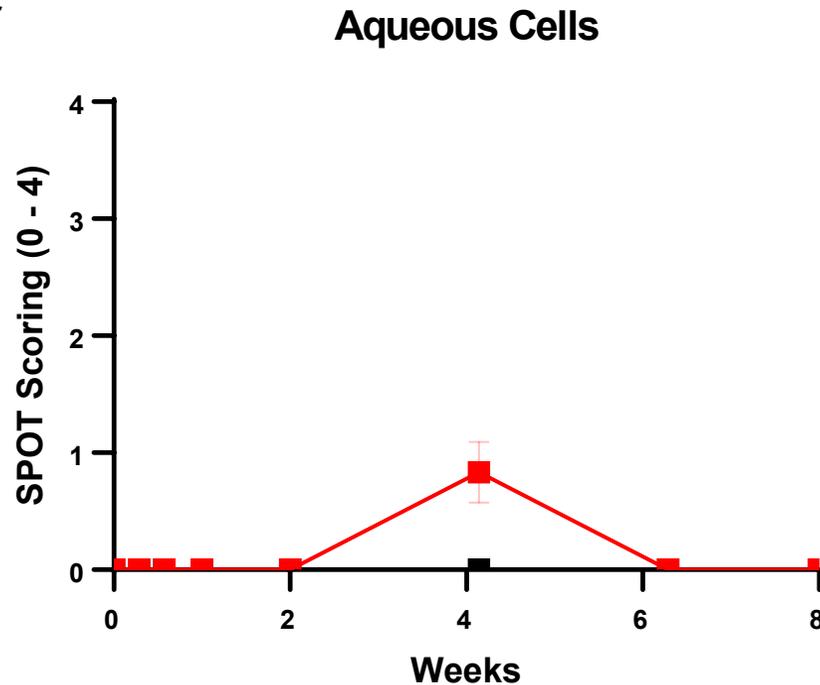
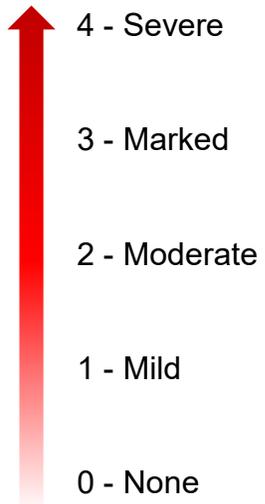


- Bolus Vehicle (n=3)
- ▲ Implant Vehicle (n=3)

# Ocular Inflammation Scoring – AAV Bolus

*AAV Bolus had mild inflammation that peaked at Week 4*

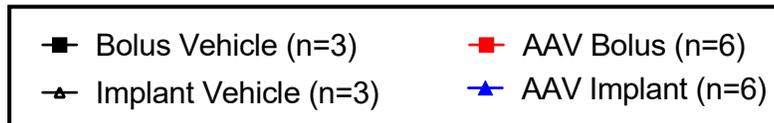
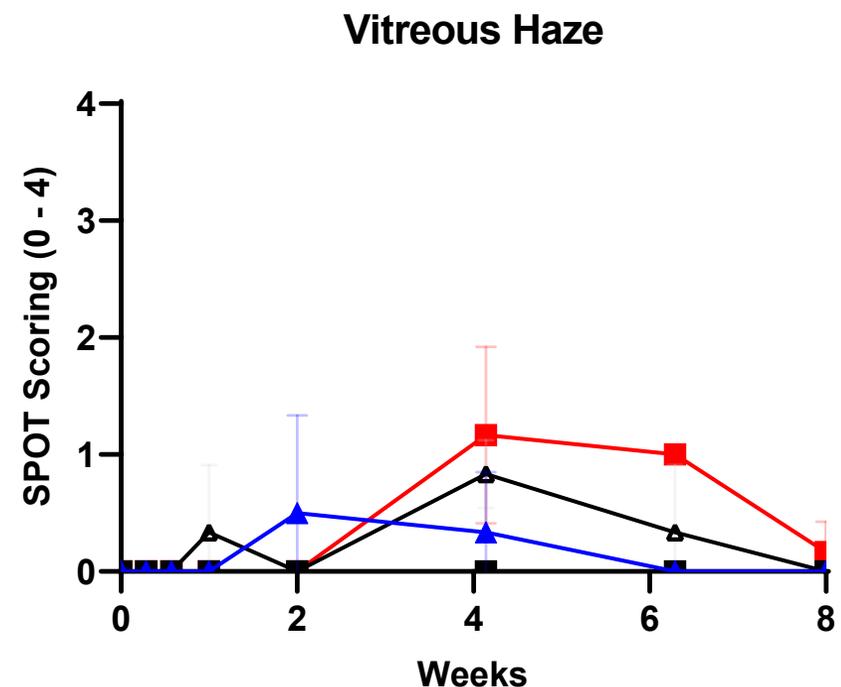
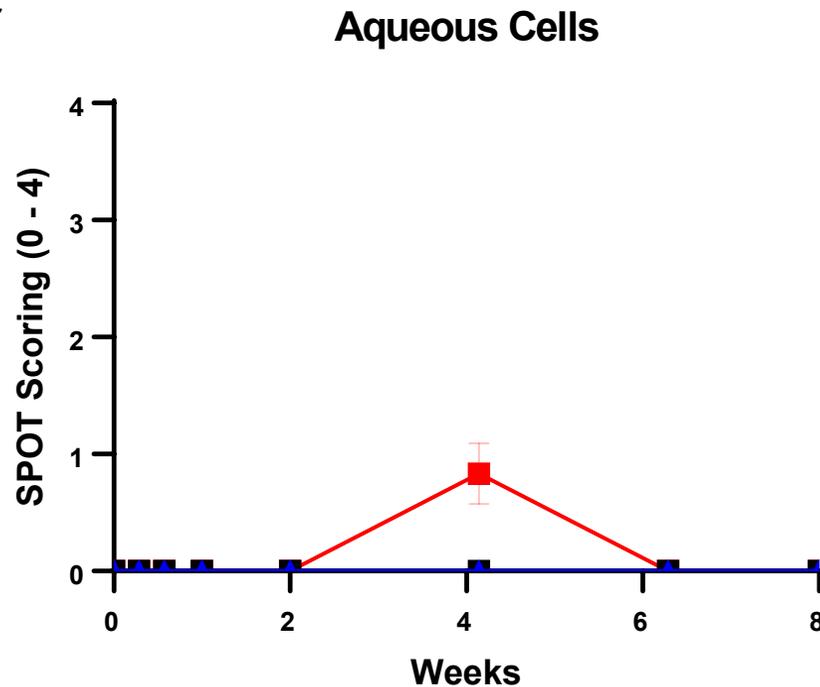
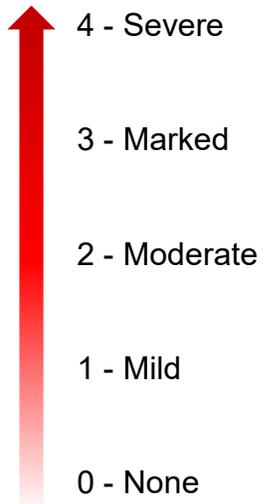
**SPOT Scoring for Ocular Inflammation**



# Ocular Inflammation Scoring – AAV Implant

*AAV Implant exhibited trace inflammation at Week 4*

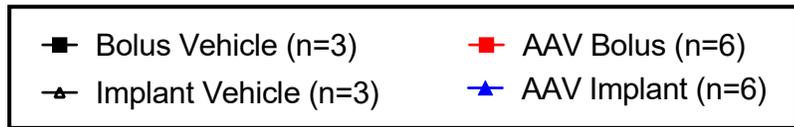
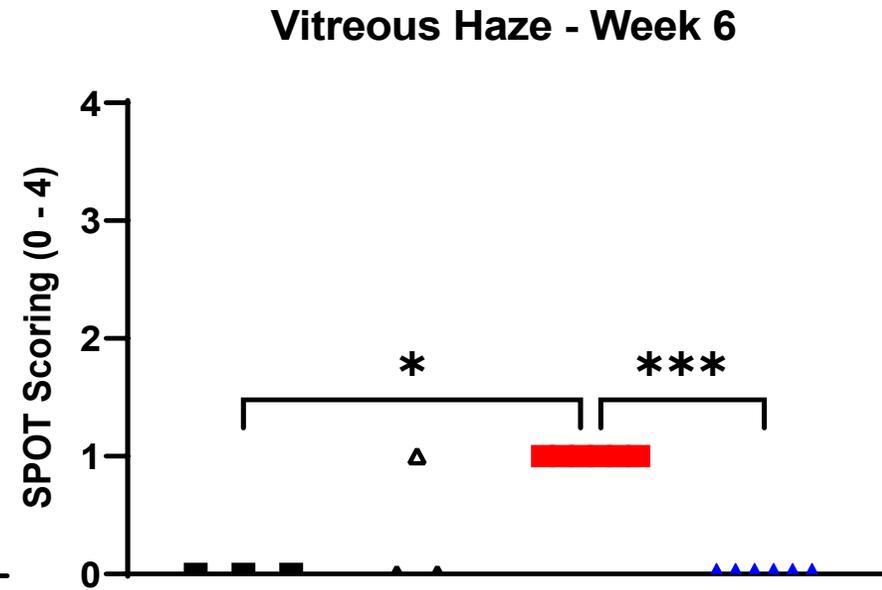
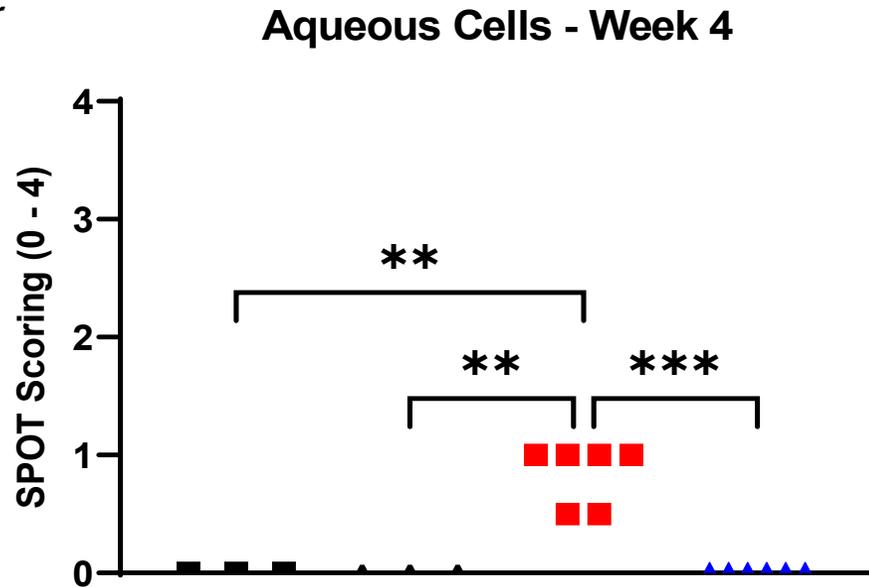
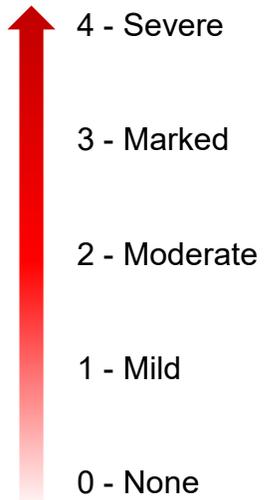
**SPOT Scoring for Ocular Inflammation**



# Ocular Inflammation Scoring – AAV Implant

*Greater inflammation in AAV Bolus compared to AAV Implant*

**SPOT Scoring for Ocular Inflammation**

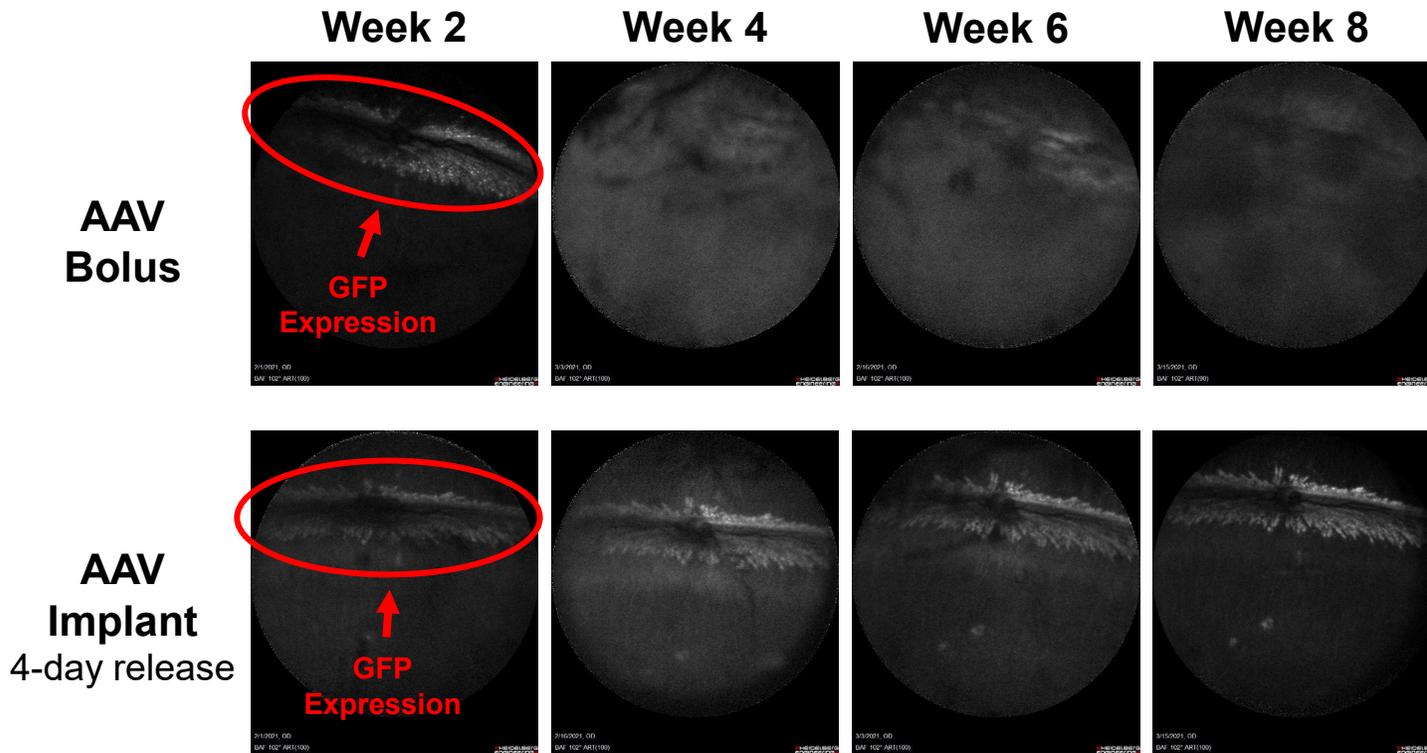


**Dunn's multiple comparisons**  
 \*: <0.05  
 \*\*: <0.01  
 \*\*\*: <0.001

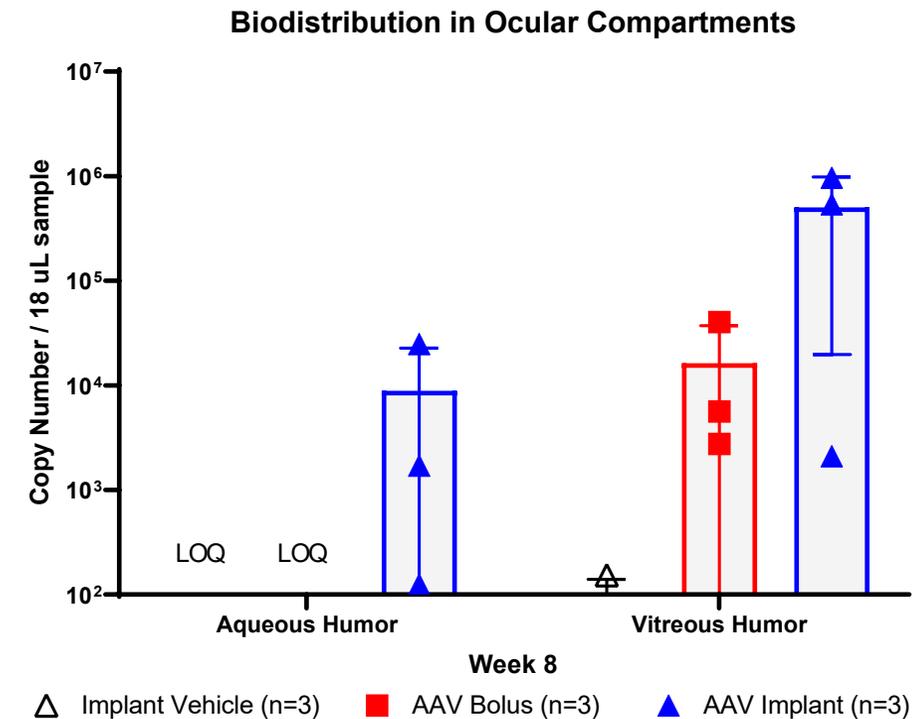
# Sustained GFP Expression Following Injection with AAV Implants

- AAV Bolus eyes experienced loss in GFP expression after 4 weeks
- AAV Implant eyes retained GFP expression over 8 weeks

*Elevated copy numbers in ocular chambers with AAV Implant*



*Representative fundus autofluorescence images*



# Rabbit Proof-of-Concept: Release Kinetics

## Objective

- To evaluate a higher dose of **AAV2.7m8-CMV-eGFP** from bilateral intravitreal injections of AAV Bolus (solution) or AAV Implant

## Study Design

- Animals: New Zealand white rabbits
- Treatment: Two different AAV Implant release kinetics were administered to assess a dose-rate response

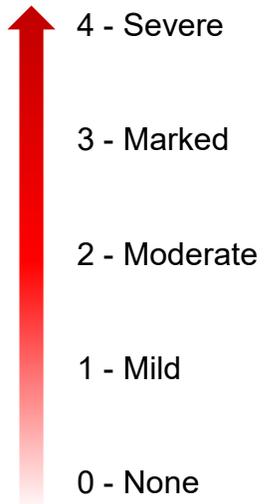
Group (n=3 animals per group)	Left Eye Treatment (OS)	Right Eye Treatment (OD)	Dose/eye	AAV Implant Release
1	Bolus vehicle	Implant vehicle	0	N/A
2	AAV Bolus		3.6E+10 GC	N/A
3	AAV Implant (Fast Release)		3.6E+10 GC	4 days
4	AAV Implant (Medium Release)		3.6E+10 GC	2 weeks

- Evaluations: All eyes were examined by ophthalmic exam and fundus autofluorescence imaging through 13 weeks

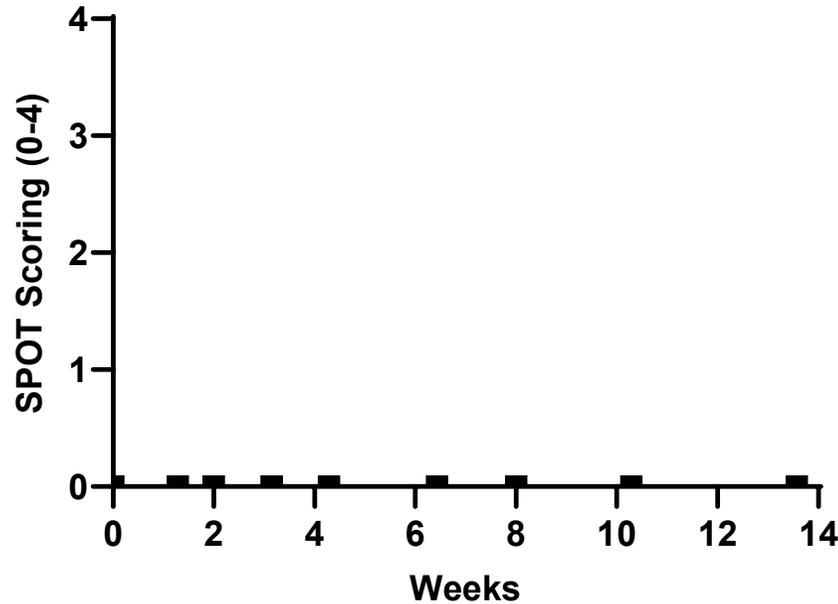
# Ocular Inflammation Scoring – Vehicle Test Articles

*No inflammation from vehicle test articles*

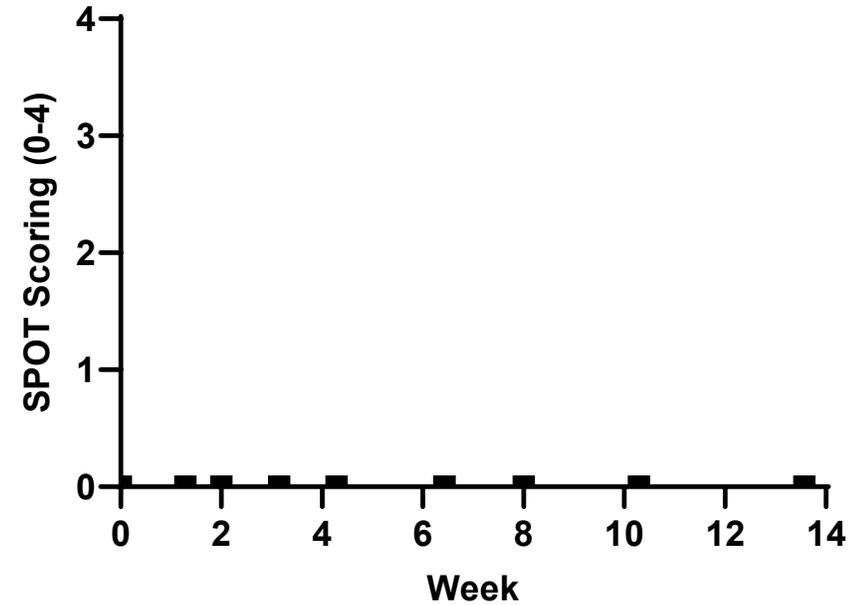
**SPOT Scoring for Ocular Inflammation**



**Aqueous Cells**



**Vitreous Haze**



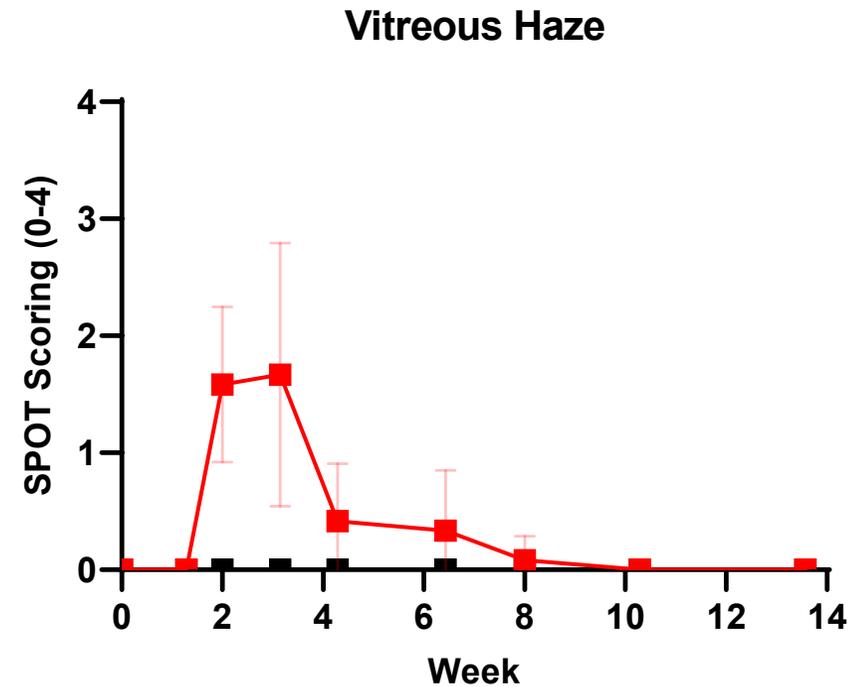
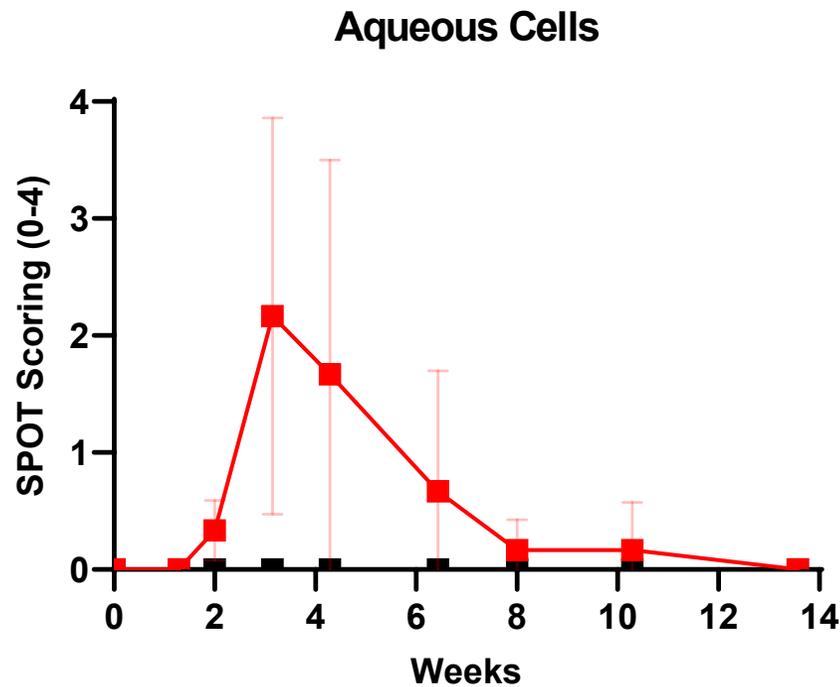
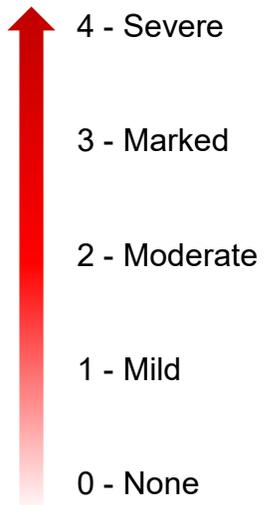
■ Bolus Vehicle (n=3)

▲ Implant Vehicle (n=3)

# Ocular Inflammation Scoring – AAV Bolus

*AAV Bolus had moderate inflammation that peaked at Week 3*

**SPOT Scoring for Ocular Inflammation**

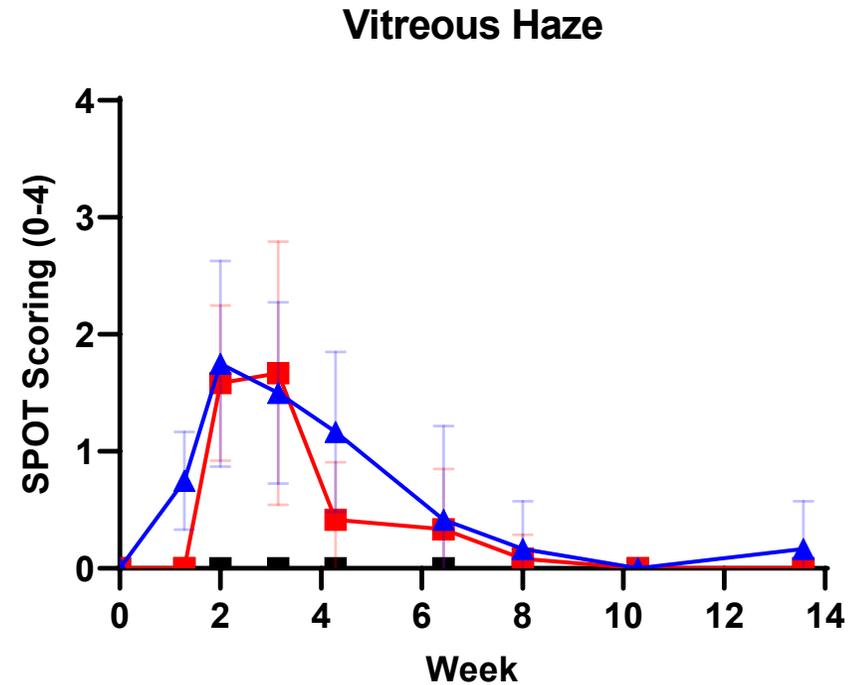
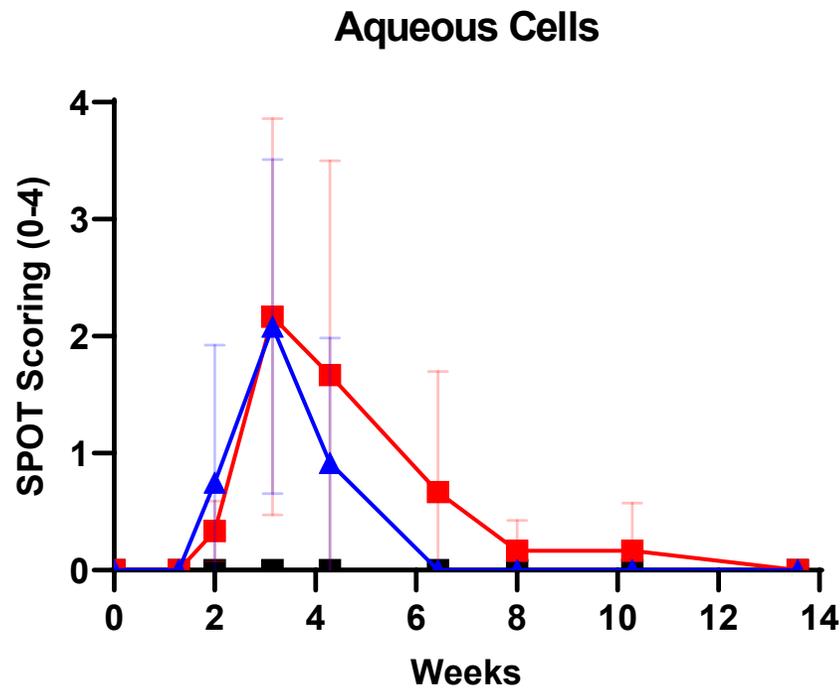
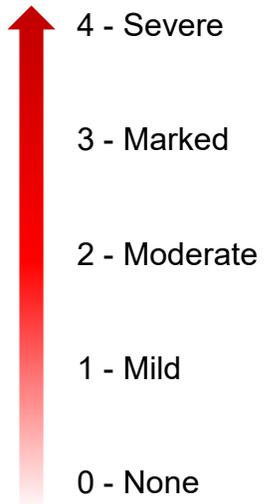


■ Bolus Vehicle (n=3)    ■ AAV Bolus (n=6)  
▲ Implant Vehicle (n=3)

# Ocular Inflammation Scoring – Fast Release AAV Implant

*4-day release AAV implant exhibited similar inflammation to AAV Bolus*

**SPOT Scoring for Ocular Inflammation**

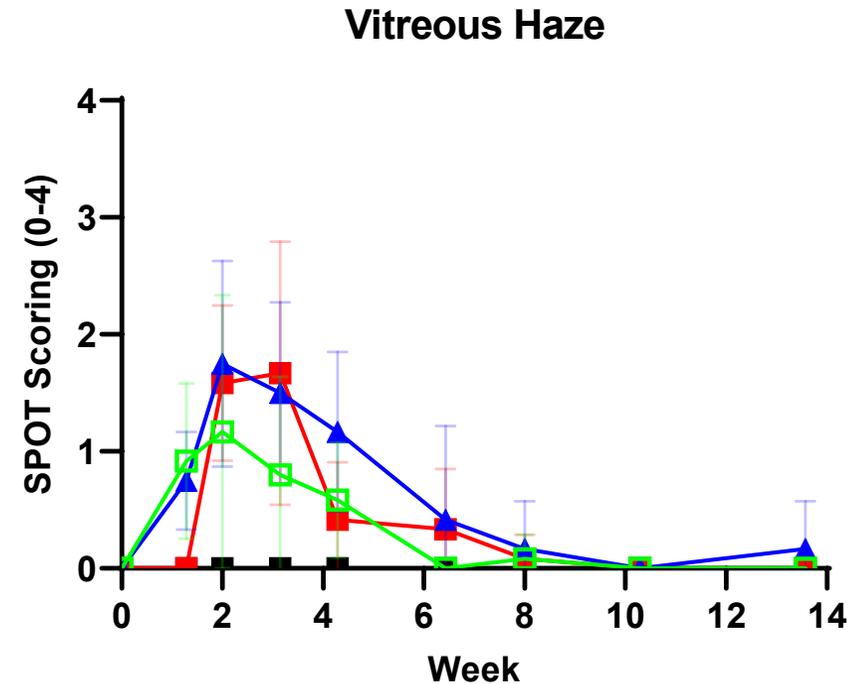
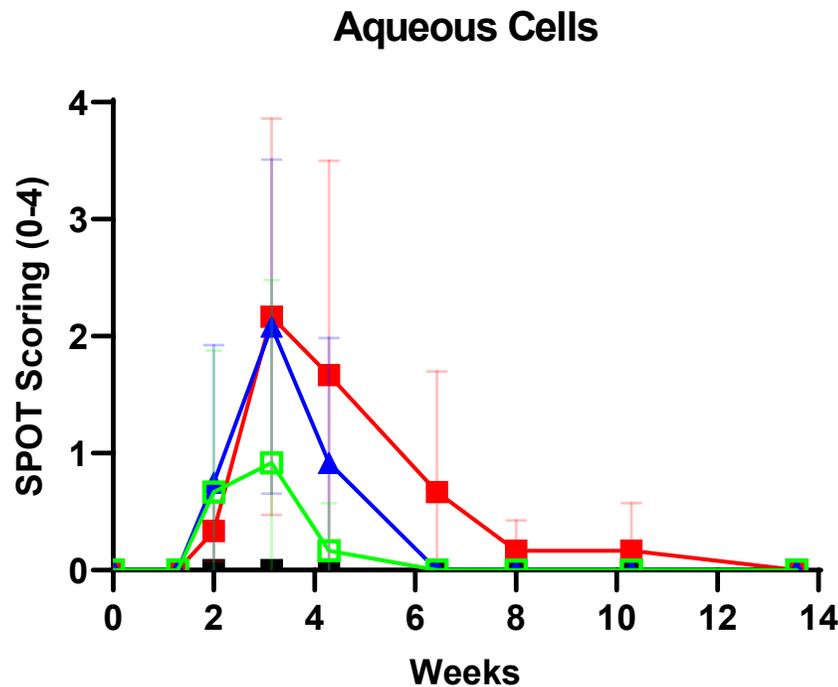
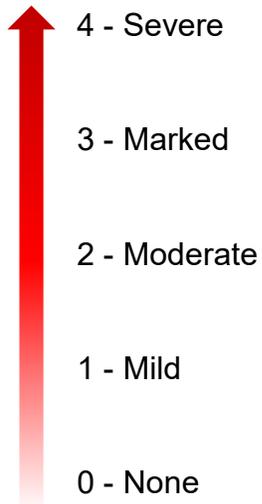


- Bolus Vehicle (n=3)
- AAV Bolus (n=6)
- ▲ Implant Vehicle (n=3)
- ▲ AAV Implant - 4 Days (n=6)

# Ocular Inflammation Scoring – Medium Release AAV Implant

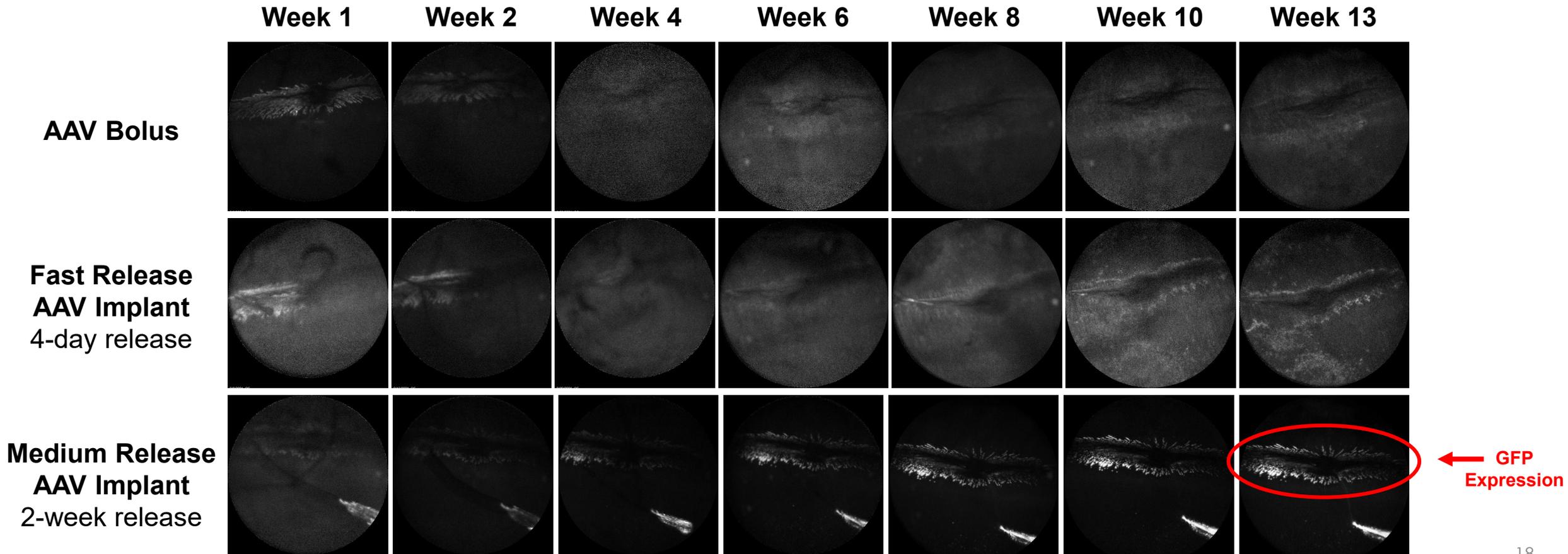
*2-week release AAV implants had lower (mild) inflammation than faster release groups*

**SPOT Scoring for Ocular Inflammation**

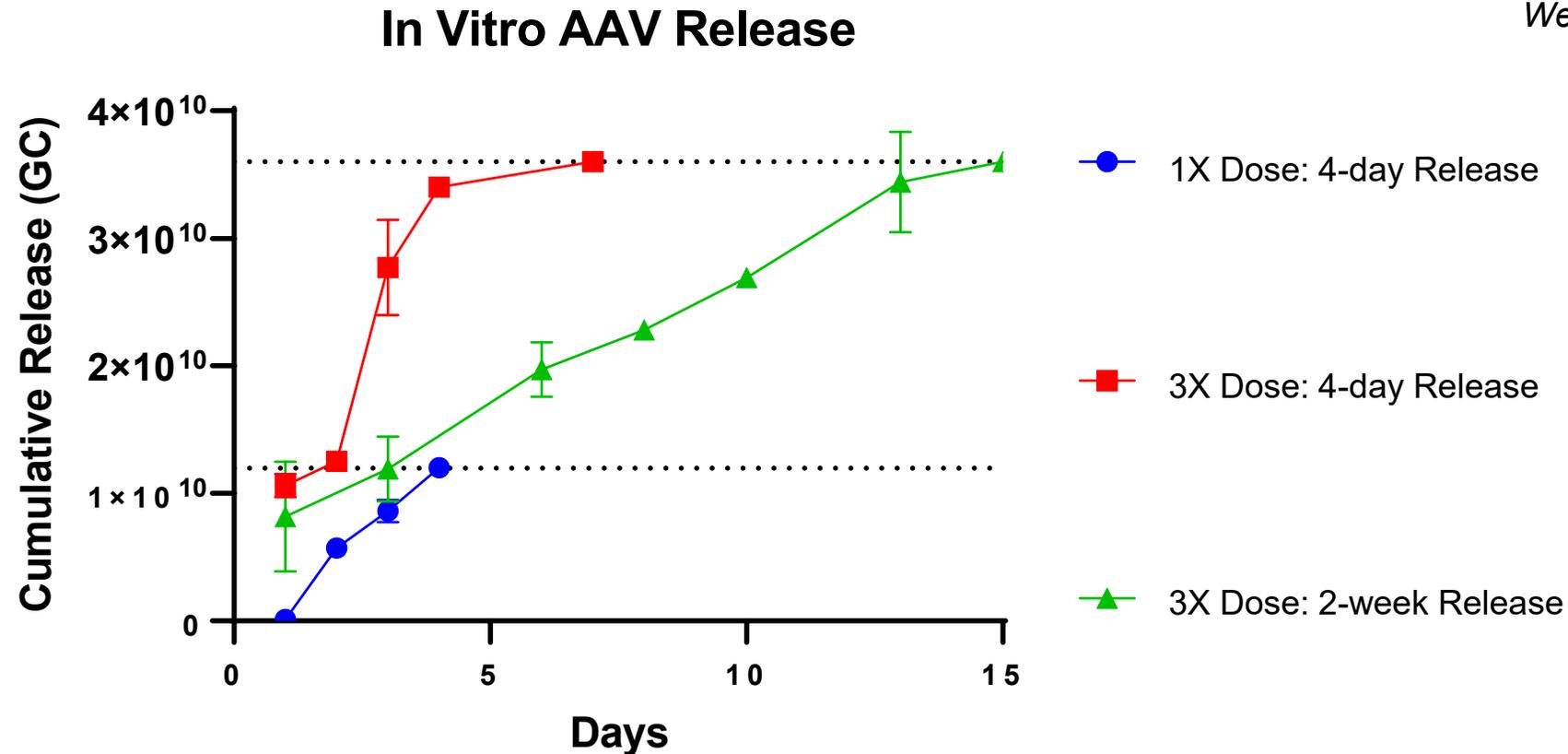


# Stable Expression of GFP from Medium Release AAV Implant

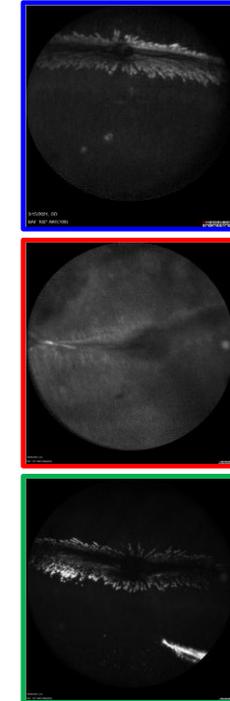
- *AAV Bolus and fast release (4-day) AAV Implant experienced loss in GFP expression after 2 weeks*
- *Medium release (2-week) AAV Implant eyes retained GFP expression over 13 weeks*



# Potential AAV Dose-Rate Response on GFP Expression



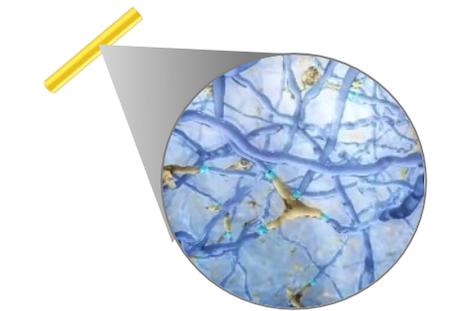
Week 8 GFP Expression



- *Estimated per day release is greatest in 3X Dose, 4-day release formulation*
- *At higher doses, a longer release time frame may be needed to maintain GFP expression*

# Conclusions

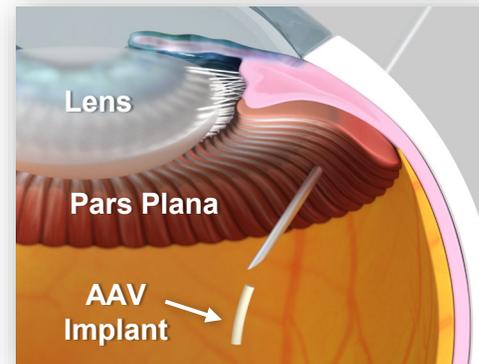
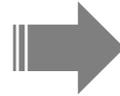
- AAV is compatible with hydrogel platform, retains infectivity in vitro and in vivo
- **Modulating AAV pharmacokinetics can influence ocular inflammation and transgene expression**
- Hydrogel platform for sustained-release of AAVs provides an avenue to reduce inflammation without modifying the AAV construct
- Sustained-release AAV implants are a promising therapeutic benefit for ocular gene therapy



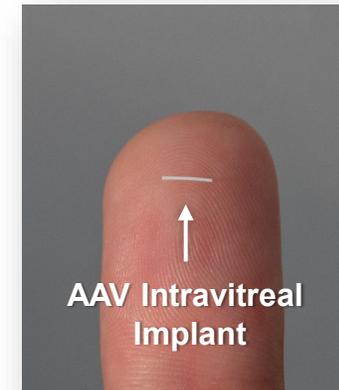
**Polyethylene Glycol Hydrogel**  
(Inactive Delivery Platform)



**Adeno-Associated Virus**  
(Gene Vector)



**AAV Intravitreal Implant**



**AAV Intravitreal Implant**