Controlled Release of Adeno-Associated Viruses (AAVs) Using Hydrogel Implants Improve GFP Expression and Reduce Anti-Drug Antibody (ADA) Titers and Inflammation in Rabbits

Meryem Pehlivaner, PhD[1]; Steven Lu, PhD[1]; Nelson Bello[1]; Rabia Gurses-Ozden, MD[1]; Rami El Hayek, PhD[1]; Charles D. Blizzard[1]; Peter K. Jarrett, PhD[1]

Affiliations: [1]Ocular Therapeutix, Bedford, MA, USA

PURPOSE

- Strategies to reduce inflammation after ocular gene therapy are often needed for improving treatment efficacy.
  - Spreading a high dose of adeno-associated virus (AAV) over multiple days using a sustained-release modality could reduce inflammation and improve treatment outcomes
- The solid AAV hydrogel implant can be placed near the target tissue and may improve the ease of vector-delivery methods
  - The Ocular Therapeutix hydrogel platform is 90% water, biocompatible, preservative-free, and fully resorbs when the drug is delivered
  - Previous studies suggest the use of a hydrogel platform for controlled delivery of AAVs in ocular gene therapy is feasible[1]

- This study tested the hypothesis that modulating the pharmacokinetics of AAV delivery via a degradable hydrogel implant can reduce the risk of developing severe ocular inflammation leading to improved transgene expression, and lower systemic exposure to AAVs and reduce ADA titers

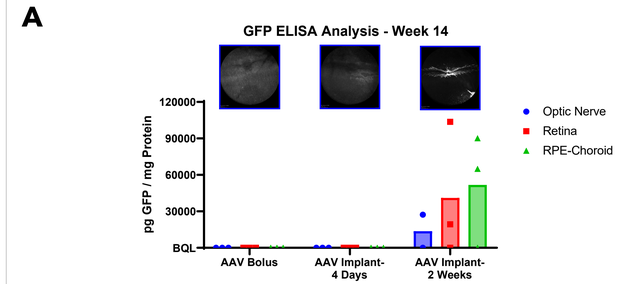
METHODS

- AAV2.7m8-CMV-GFP loaded hydrogel implants at a dose of 3.6E+10 GC/implant were formulated to release AAVs over 4 (Fast Release) or 14 days (Medium Release)
- A single implant was injected intravitreally in both eyes of New Zealand White rabbits
- Bilateral injections of a bolus 50µL AAV solution at the same dose were performed as a positive control
- The impact of sustained AAV delivery on inflammation and green fluorescent protein (GFP) expression was investigated through ocular examinations, fundus autofluorescence (FAF) imaging, immunohistochemistry (IHC) and enzyme-linked immunoassay (ELISA) quantification in ocular tissues (n=3 rabbits/6 eyes per group). Vector genomes in plasma were quantified by quantitative polymerase chain reaction and ADA titers in serum were determined by ELISA

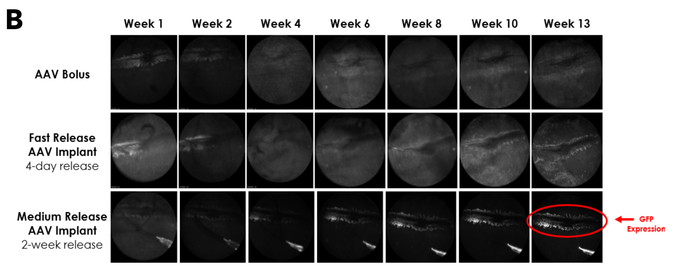
Group (n=3 animals/group)	Treatment (OU)	AAV Implant Release	Dose/eye
1	AAV Bolus	N/A	3.6E10 GC
2	Fast Release Implant	4 Days	3.6E10 GC
3	Medium Release Implant	2 Weeks	3.6E10 GC

RESULTS

- **FAF imaging and protein quantification demonstrated greatest GFP expression in the medium release implant group**

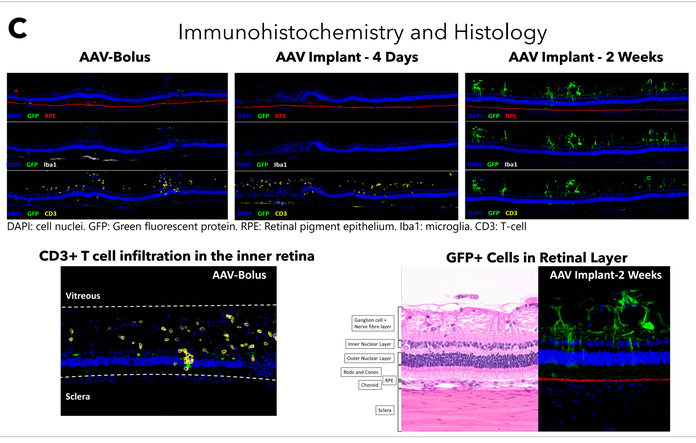


Quantification of GFP in rabbit ocular tissues via ELISA at Week 14 and representative Week 13 FAF images



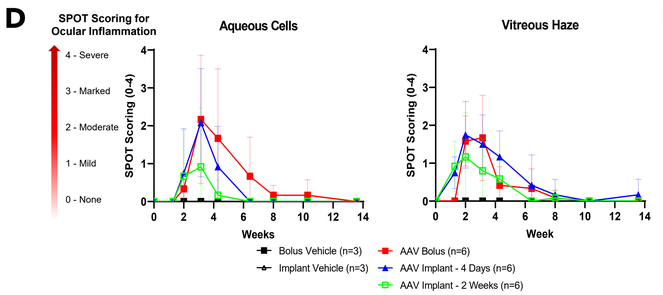
FAF images through Week 13

- **AAV Bolus had signs of local inflammatory response- Infiltration with T cells and macrophages**

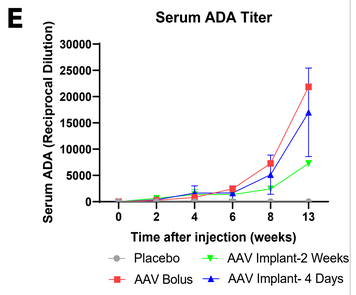


DAPI: cell nuclei. GFP: Green fluorescent protein. RPE: Retinal pigment epithelium. Iba1: microglia. CD3: T-cell

- **Medium release group resulted in lower peak inflammation at Week 3 and lower ADA titer levels at Week 13**

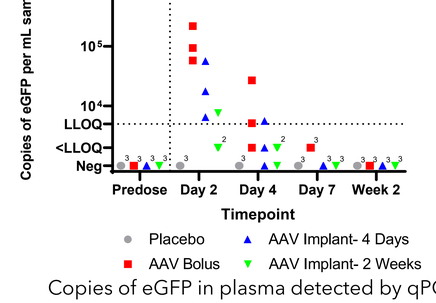


Ocular inflammation measured by SPOT scoring

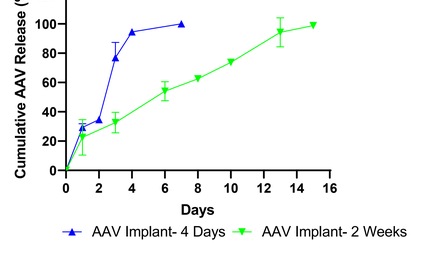


Effect of controlled release on ADA titer

- **Controlled release of AAV can lower systemic levels as evidenced by lower copy numbers from the AAV implant groups in plasma**



Copies of eGFP in plasma detected by qPCR



In vitro release profiles of AAV implants

CONCLUSIONS

- **Our data demonstrates that modulating AAV pharmacokinetics using hydrogel implants for sustained delivery can impact ocular inflammation, adaptive immune response and transgene protein expression**
- **The controlled release of AAVs from degradable hydrogel implants showed decreased inflammation and increased in vivo transduction compared to a single bolus administration without modifying the AAV construct**
- **Sustained-release AAV implants may provide a promising platform to improve safety and efficacy for ocular gene therapy**

Presentation Disclosures: This poster discusses an investigational product; its efficacy and safety profile has not been established and it has not been approved by the U.S. Food and Drug Administration (FDA). **Funding**: These studies were funded by Ocular Therapeutix **Financial Disclosures**: All authors are employees of Ocular Therapeutix.

References: **1.** Lu S, et al. Development of Hydrogel Implants for the Sustained Delivery of Adeno-Associated Viruses in Ocular Gene Therapy. Presented at The American Society of Gene and Cell Therapy. May 16-19, 2022; Washington, DC.

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