Doctoral Dissertation Defense Announcement

The Development of a Prostaglandin-Based rAAV Gene Therapy for the Treatment of Glaucoma

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Committee in Charge:
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Iris Kassem, PhD
Matthew Veldman, PhD
Robert Nickells, PhD

Date: Friday, December 1, 2023
Time: 1:00 PM (CST)
Defense Location: Kerrigan Auditorium
Zoom: Zoom Link Provided Upon Request
Graduate Studies:
Biochemistry of the Cell
Intro to Biomedical Research
Techniques in Molecular & Cell Biology
Molecular and Cellular Biology
Mechanism of Cellular Signaling
Classical and Molecular Genetics
Ethics and Integrity in Science
Cellular and Molecular Neurobiology
Research Ethics Discussion Series
Introduction to Biostatistics
Advanced Cell Biology
The Biology of Vision
Developing Soft Skills
Career Development in Biomedical Science
Reading and Research
Doctoral Dissertation
Abstract

Glaucoma affects approximately 80 million people worldwide and as such is a leading cause of blindness. Glaucoma is characterized by an imbalance between aqueous humor production and drainage. Consequently, this insufficient drainage causes increases in intraocular pressure (IOP), causing undue stress upon the retina and the retinal ganglion cells (RGCs), ultimately leading to degeneration and loss of vision. While the most commonly prescribed treatment regimens such as topically applied prostaglandin analogue eye drops are quite effective, lowering IOP by approximately 25-35%, they exhibit several drawbacks. One of which is extremely poor patient compliance. The research enclosed focuses on the development of a singly administered adeno-associated vector (rAAV) gene therapy to vectorize the effects of prostaglandin eye drops, lowering IOP long term without the need for daily administrated drops. This would represent a paradigm shift in the treatment options for patients experiencing difficulties with drop administration and regimens.

Firstly, we characterized the efficacy and tolerability of a prostaglandin-based transgene when delivered intracamerally in the normotensive brown Norway (BN) rat delivered at three different dosages of rAAV vector. We determined that IOP can be reduced at near or exceeding clinical relevance in low and high dose treated eyes which can be partially reversed when administering a ligand specific to our off-type riboswitch causing transgene transcript cleavage. Some adverse events such as pigment dispersion were noticed mainly in high dose treated groups, however the treatment was mostly well-tolerated.

Secondly, we tested three rAAV serotypes for tropism and tolerability in the African green monkey (AGM) anterior chamber. As large animal models are necessary to determine translatability to the clinic, we partnered with a contract research organization, RxGen, with an available colony of AGMs. We tested
three different capsid serotypes, namely rAAV2/2[MAX], rAAV2/6, and rAAV2/9 for their tolerability and tropism when intracamerally injected. While we found transient phases of inflammation, we did not find any major adverse events during the study.

Thirdly, we investigated the tropism of several rAAV serotypes and capsid modifications in the C57BL/6J mouse. As we found pigment dispersion in the BN rats, we aimed to evaluate all capsid serotypes available in our lab to find a candidate that does not transduce iris tissue. We tested 9 serotypes, namely rAAV2/1, 2/2, 2/2[7m8], 2/2[MAX], 2/5, 2/6, 2/7, 2/8, and 2/9 and evaluated transduction and biodistribution profiles of each, in vivo and histologically. We found rAAV2/5 to primarily target the corneal endothelium, which was subsequently used to package the therapeutic transgene for further study.

Finally, we evaluated the efficacy of the prostaglandin-based transgene when intracamerally injected into glaucomatous DBA/2J mice. From our previous aim, we packed the transgene into a rAAV2/5 capsid and intracamerally injected DBA/2J mice at a low and high dose, which exhibited significant clinical levels of reduction in IOP, and partial preservation of retinal ganglion cells. Validation of IOP reduction in glaucomatous animals represents significant progress in the development of an enduring treatment for glaucoma in which patients will no longer be required to administer medication daily.

Together these findings can be utilized to develop future gene therapy-based treatments aimed at reducing patient burden and eliminating compliance and adherence issues of current treatments available for glaucoma. Our results demonstrate a dosage dependent decrease in IOP which can facilitate dosage adjustments based on severity of the disease, while also including a fail-safe wherein transcript expression can be halted based on riboswitch activity.
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**Education**

**Medical College of Wisconsin** - Milwaukee, WI  
PhD. Candidate (Expected December 2023)  
Cell Biology, Neurobiology, and Anatomy  
August 2017 - Present

**Marian University** - Fond du Lac, WI  
Bachelor of Science  
Biology & Chemistry  
Magna Cum Laude  
May 2016

**Research Experience**

**Doctoral Research**  
PhD Candidate  
August 2017 - Present

- Thesis work: Development of an rAAV Gene Therapy for the Treatment of Glaucoma  
- Characterizing most effective capsid serotypes in transduction of the anterior chamber  
- Development of a novel prostaglandin-based rAAV therapy for reduction of intraocular pressure  
- Mentored 9 graduate students during rotations and upon joining the ocular gene therapy lab, 1 medical student for a period of 1 year, and trained 3 research technologists within the laboratory.

**Department of Ophthalmology, Medical College of Wisconsin** - Milwaukee, WI  
Research Technologist 1  
July 2016 - 2017

- Improvement of Photoreceptor Targeting via Intravitreal Delivery in Mouse and Human Retina Using Combinatory rAAV2 Capsid Mutant Vectors  
- Research projects involving primary tissue culture, molecular cloning, and virus production  
- Assemble relevant protocols and standard operating procedures  
- Manage and maintain lab supplies and equipment

**Undergraduate research**

**Senior Thesis Research Project**

- Cytokinetic Midbody Identification and Structural Changes in Danio rerio Embryos  
- Studied the structure, location, and composition of cytokinetic midbodies in Danio rerio embryos using immunofluorescent microscopy.

**Leadership**

Milwaukee Public STEM Fair, 2017: Exhibited science experiments to K-12 students  
Milwaukee Public STEM Fair MCW Student Coordinator, 2018

**Professional Training**

Ocuscience Ophthalmic Imaging and Electoretinography in Animals: Investigating Retinal Structure and Function Lecture and Wet Lab, 2021
Publications


Presentations

2017 ARVO Abstract #2689920: Intravitreal transduction profile of recombinant adeno-associated virus in murine and human retina - Poster Format, Designated hot topic which is given to 3% of abstracts submitted to ARVO

2019 ARVO Abstract # 3146923: Biodistribution and tolerability of rAAV vectors in the anterior chamber for the treatment of primary open angle glaucoma - Poster Format

2021 ARVO Abstract #3543891: Tolerability and efficacy of an rAAV-mediated gene therapy for the treatment of primary angle glaucoma in brown Norway rats- Paper Format , Virtual

2021 Graduate Student Association Research Symposium: Tolerability and efficacy of an rAAV-mediated gene therapy for the treatment of primary open angle glaucoma in brown Norway rats – Poster Format, Best Poster Award

2021 Graduate School & Office of Postdoctoral Education Research Poster Session; Tolerability and efficacy of an rAAV-mediated gene therapy for the treatment of primary open angle glaucoma- Poster Format

2022 ARVO Abstract #3711446: Safety and efficacy or recombinant adeno-associated virus (rAAV) mediated transduction in the anterior chamber of the African Green Monkey (Chlorocebus sabaeus) - Poster Format

2022 ASGCT Abstract #1328: Evaluation of rAAV Serotypes in the Murine Anterior Chamber for the Treatment of Glaucoma- Poster Format, Outstanding Poster Award

2023 ARVO Abstract #3880597: Effects of a Prostaglandin-based rAAV gene therapy for the treatment of glaucoma in DBA/2J mice- Paper Format

Professional Affiliations

Scientific Memberships

- American Society for Gene and Cell Therapy (ASGCT) Member-in-Training, 2022
- McPherson Eye Research Institute Member, 2021-2023

Honors & Awards

McPherson Eye Research Institute Distinguished Paper Award- Prostaglandin-based rAAV-mediated glaucoma gene therapy in Brown Norway rats, 2023
American Society for Gene and Cell Therapy Outstanding Poster Award, 2022
Graduate School & Postdoctoral Education Research Poster Session: Best Poster Award, 2021
Marian University Biology Academic Achievement Award, Recipient, 2016
Marian University John May Biology Research Award, Recipient, 2016