AOPT | Association for Ocular Pharmacology and Therapeutics

14th Scientific Meeting

March 7th–10th, 2019

New Orleans

NEXT GENERATION OCULAR THERAPEUTICS

Association for Ocular Pharmacology and Therapeutics

www.aopt.org

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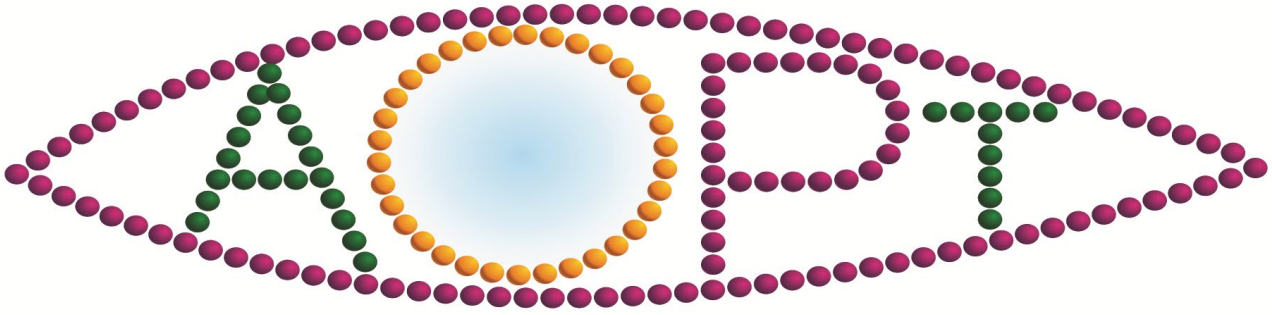

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Welcome Message

Welcome to New Orleans to take part in the 14th Scientific Meeting of the Association for Ocular Pharmacology and Therapeutics (AOPT). The topic of this year's conference is "Next Generation Ocular Therapeutics". Our Keynote Speaker is Casey Kopczynski, PhD, Chief Scientific Officer and Co-founder of Aerie Pharmaceuticals, Inc. who will present: "Between a ROCK and a Hardened Meshwork: The Discovery and Development of Rhopressa".

We are thankful to our hosts, who have helped put together an exciting meeting at a great venue, the Hotel Moteleone, in the heart of New Orleans. The meeting agenda is packed with exciting new information set in thirteen sessions including a session with young investigators presenting.

The Association for Ocular Pharmacology and Therapeutics is a global not-for-profit organization for scientists and individuals from all disciplines related to ocular pharmacology and its therapeutic applications. AOPT- has a diverse, multi-national membership composed of preclinical and clinical scientists, students, and healthcare professionals. Members are from academic institutions, pharma and biotech industries, device companies, clinics and private practice.

AOPT's mission is to serve as a global forum and network for the publication, dissemination and exchange of information and knowledge on treatments of eye diseases, from basic and clinical ocular pharmacology and therapeutics to related disciplines such as pharmacokinetics and dynamics, metabolism, translational research, safety, drug delivery, and pharmaceutics. The conference brings together those individuals at the forefront of scientific advancement related to ocular pharmacology and therapeutics. The program is rich in provocative presentations that we hope stimulate productive discussions and opportunities for networking and establishing potential collaborators.

What better place to come together to discuss science than in "The Big Easy".

Tom Yorio, PhD, FARVO

President, AOPT

AOPT Membership Information

There are four classes of membership in AOPT: Regular Members, Associate Members, Contributing Members, and Emeritus Members.

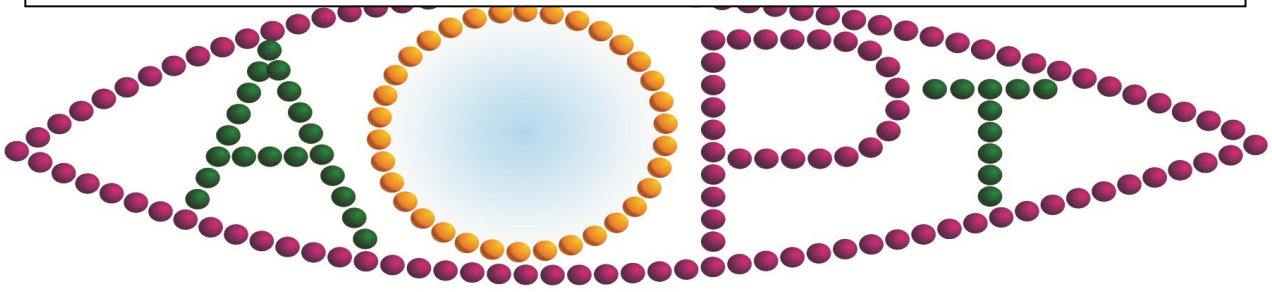
- **REGULAR MEMBERS**. The Regular Membership represent individuals demonstrating a genuine interest in or making significant contribution to ocular pharmacology and therapeutics. This may be evidenced by a) scientific publications; b) attendance at pharmacological, ophthalmological, optometric, or visual science meetings; c) direct involvement in research. A candidate for membership completes the online membership form and pays the appropriate membership dues. Membership is for two years. A subscription to the Journal of Ocular Pharmacology and Therapeutics is optional.

- **ASSOCIATE MEMBERS**. Associate Membership is for pre-doctoral and postdoctoral students. A candidate for this membership must have a pre-doctoral, or postdoctoral student status, and must complete the online membership form and pay the appropriate membership dues.

- **CONTRIBUTING MEMBERS**. Contributing Membership is restricted to corporations, associations, and individuals who support the objectives of AOPT but do not satisfy the requirements of Regular Membership or individuals elected to membership in any class who voluntarily choose to become Contributing Members. A candidate for contributing membership completes the online membership form and pays the appropriate membership dues.

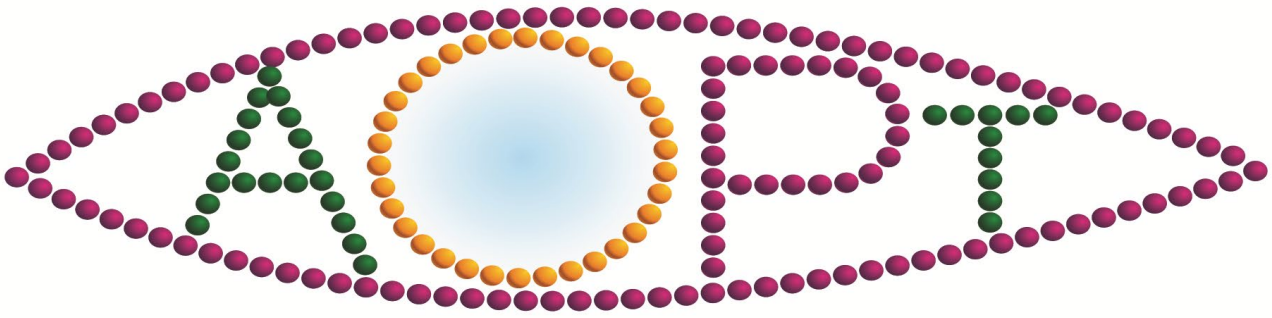
- **EMERITUS MEMBERS**. Any Regular Member may make a written request to the Treasurer that his/her membership be transferred to that of an Emeritus Member. The request is subject to approval of the membership committee. Emeritus Members have all the rights and privileges of Regular Members, except those of voting and holding elective office.

AOPT Official Journal: The Journal of Ocular Pharmacology and Therapeutics (JOPT), published by Mary Ann Liebert, Inc., publishers (140 Huguenot Street, 3rd Floor, New Rochelle, NY 10801), is the official Journal of AOPT. A substantially reduced subscription rate for this journal (electronic format) is an optional membership benefit, as indicated above.



AOPT Past Meetings

Meeting	Date	Location	Organizer
THIRTEENTH MEETING	FEB 16-19, 2017	FLORENCE, ITALY	Filippo Drago
TWELFTH MEETING	FEB 26 - MAR 1, 2015	CHARLESTON, SC	DAN STAMER
ELEVENTH MEETING	FEBRUARY 7-10, 2013	ALICANTE, SPAIN	JUANA GALLAR MARTINEZ
TENTH MEETING	FEBRUARY 17-20, 2011	FT. WORTH, TX	TOM YORIO / ABBOT CLARK
NINTH MEETING	FEBRUARY 18-21, 2009	SALZBURG, AUSTRIA	HERBERT REITSAMER
EIGHTH MEETING	FEBRUARY 9-11, 2007	SAN DIEGO, CA	JOHN LIU / ACHIM KRAUSS
SEVENTH MEETING	FEBRUARY 3-5, 2005	CATANIA, SICILY, ITALY	FILIPPO DRAGO
SIXTH MEETING	FEBRUARY 1-4, 2003	KONA, HI	PETER KADOR
FIFTH MEETING	NOVEMBER 2-5, 2000	BIRMINGHAM, AL	JIMMY BARTLETT
FOURTH MEETING	JANUARY 28-31, 1999	IRVINE, CA	ACHIM KRAUSS
THIRD MEETING	OCTOBER 22-24, 1997	BETHESDA, MD	PETER KADOR
SECOND MEETING	AUGUST 15-17, 1996	LOS ANGELES, CA	DAVID LEE
FIRST MEETING	JANUARY 26-29, 1995	NEW ORLEANS, LA	HERB KAUFMAN
OCULAR PHARMACOLOGY SYMPOSIUM	AUGUST 8-10, 1993	NOVI, MI	HITOSHI SHICHI



Your AOPT Board

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Dr. Thomas Yorio

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/President-Elect

Dr. Filippo Drago

Immediate-Past-President

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Dr. Ganesh Prasanna

Trustee

Dr. Cheryl Rowe-Rendleman

Trustee

Dr. Christine Wildsoet

Trustee

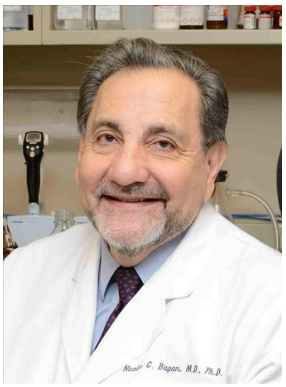
Dr. Heping Xu

Your Local Organizing Committee

[Dr. Shusheng Wang]

Organizer

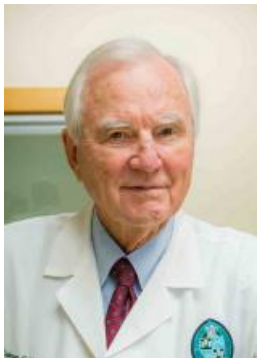
Tulane University



[Dr. Nicolas G. Bazan]

Co-organizer

Louisiana State University



[Dr. Delmar R. Caldwell]

Tulane University

[Dr. Jayne S. Weiss]

Louisiana State University

[Dr. Haydee E.P. Bazan]

Louisiana State University

[Dr. Maria Reinoso]

Louisiana State University

[Dr. Partha Bhattacharjee]

Xavier University

[Dr. Ash Jayagopal]

Roche

Travel Award Winners

National Eye Institute (NEI) Travel Awardees (21 in total)

Junior Faculty (4):

Dorota Stankowska, PhD, Research Assistant Professor, University of North Texas Health Science Center

Manas Biswal, PhD, Assistant Professor, University of South Florida

Shaoqing He, PhD, Research Assistant Professor, University of North Texas Health Science Center

Zhongjie (Kira) Fu, PhD, Instructor, Boston Children's Hospital, Harvard Medical School

Post-doctoral Fellows and Students (17):

Bo Yu, PhD, Postdoctoral Fellow, Tulane University

Fiona McDonnell, PhD, Postdoctoral Fellow, Duke University

Heather Schmitt, PhD, Postdoctoral Fellow, Duke University

Leona Ho, PhD, Postdoctoral Fellow, Duke University

Lin Cheng, PhD, Postdoctoral Fellow, University of Iowa Hospitals and Clinics

Mohamed M Ibrahim, PhD, Postdoctoral Fellow, University of Tennessee Health Science Center

Monika Lakk, PhD, Postdoctoral Fellow, University of Utah

Prabhavathi Maddineni, PhD, Postdoctoral Fellow, University of Tennessee Health Science Center

Sri Konda, B. M., Postdoctoral Fellow, University of Wisconsin

Madhoosudan Patil, PhD, Postdoctoral Fellow, University of Colorado Anschutz Medical Campus

Yanfei Wang, PhD, Postdoctoral Fellow, Harvard Medical School

Amanda Roberts, M.A., graduate student, University of North Texas Health Science Center

Daniel Grigsby, B.S., Predoctoral Fellow, Duke University

Jackson Baumann, B.S., Graduate Student, University of Utah

Jerome Cole II, Undergraduate Student, University of Tennessee Health Science Center

Navita Lopez, M.S., Graduate Student, University of North Texas Health Science

Center

Renuka Chaphalkar, M.S., Predoctoral Fellow, University of North Texas Health Science Center

BrightFocus Foundation Travel Awardees (8 in total)

Post-doctoral Fellows and Students:

Mayur Choudhary, PhD, Postdoctoral Fellow, Duke University

Syed Zaidi, PhD, Postdoctoral Fellow, Medical University of South Carolina

Bomina Park, Graduate student, Indiana University School of Medicine

Pinkal Patel, M.S., Graduate Student, University of North Texas Health Science Center

Natalia Hudson, PhD, Postdoctoral Fellow, Trinity College

Shuo Huang, PhD, Postdoctoral Fellow, Boston Children's Hospital, Harvard Medical School

Yao Tong, M.S., Predoctoral fellow, Tulane University

Sai Vuda, undergraduate student, Case Western Reserve University

Cooper Vision Travel Awardees (6 in total)

Junior Faculty:

Ariadna Diaz-Tahoces, PhD, independent researcher, Instituto de Neurociencias de AlicanteDoreen Schmidl, PhD, junior faculty, Medical University of Vienna

Post-doctoral Fellows and Students:

Giulia Malaguarnera, PhD, Postdoctoral Fellow, University of Catania

Anita Ghosh, B.S., predoctoral fellow, Loyola University Chicago

Manuel Chacon, B.S., predoctoral fellow, Instituto Universitario Fernández-Vega

Kameron Kilchrist, M.S., predoctoral fellow, Vanderbilt University

Foundation Fighting Blindness Travel Awardees (3 in total)

Junior Faculty:

Qingguo Xu, PhD, Assistant Professor, Virginia Commonwealth University

Post-doctoral Fellows and Students:

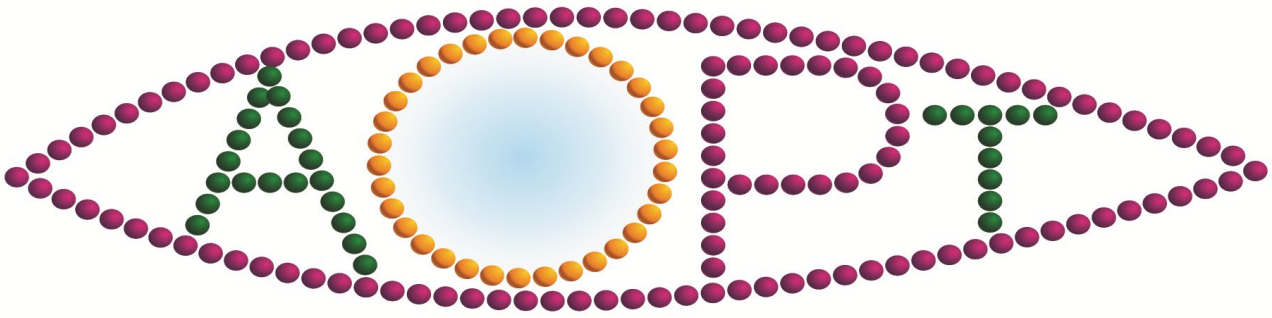
Chiara Platania, PhD, Postdoctoral Fellow, University of Catania

Jarel Gandhi, PhD, Postdoctoral Fellow, Mayo Clinic

Omar Consulting Group Minority Travel Awardees (2 in total)

Felix Yemanyi, graduate student, University of Houston

Josiah Sherman, undergraduate student, Xavier University of Louisiana



General Information

On-site Registration Desk (Iberville Ballroom)

Thursday, March 7th, 15:00 – 19:00

Friday, March 8th, 07:30 – 12:30

Late registrants can obtain their badges from on call assistant.

Name Badges

All participants must wear their name badges throughout the meeting. Name badges allow admission to all sessions, breaks, lunches, receptions and the banquet.

Welcome Reception

The Welcome reception will be held on Thursday March 7, 2019 from 18:50 to 20:00 in Iberville Ballroom of the Hotel Monteleone.

AOPT Business Meeting

The AOPT Business Meeting will be held on Friday, March 8 from 16:20-17:20. All AOPT members are encouraged to attend.

AOPT Banquet

The AOPT Banquet, open to all registered participants, will be held Saturday, March 9 from 19:00 to 21:30 in Royal room. Travel awards will be presented during the banquet.

Clothing

Clothing is business casual for all occasions.

Internet Access

Free WIFI connection is provided in rooms of all registered participants and in hotel lobby.

Liability and Personal Insurance

The AOPT 2019 Organizers cannot accept liability for personal accidents or loss of or damage to private property of participants and accompanying persons. Participants are recommended to take out their own personal travel and health insurance for their trip to New Orleans.

Safety and Security

We kindly request that you do not leave bags, suitcases or backpacks unattended at any time during the meeting.

Information for Presenters

Language

The official language of the AOPT 2019 meeting is English.

Oral Presentations

Presenters using a Powerpoint presentation should bring it on memory stick (USB), and load it in the designated computer between 7:30-8:00 am (morning sessions) or during the lunch break (afternoon sessions). Presenters combining Powerpoint and video films are requested to double-check their presentations before the session begins to be sure they work properly. If you are a Macintosh user, please convert you file to Powerpoint to be used on the PC. Alternatively, MAC users can bring their own computer and a VGA adaptor.

Poster Presentations

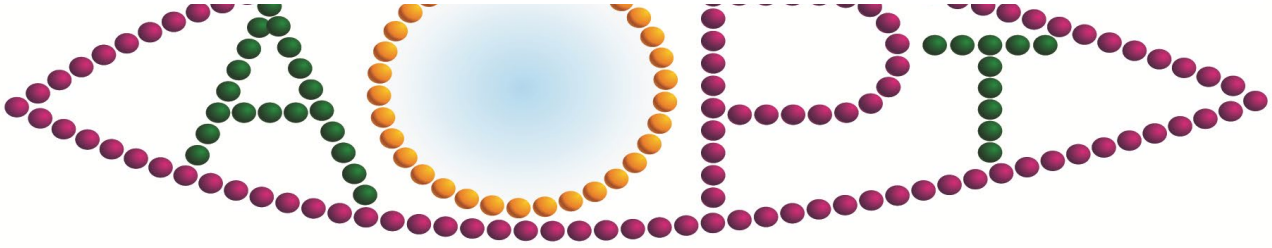
Posters must be mounted by 18:50 on Thursday, March 7 and remain on display until the end of the day (17:00) on Saturday, March 8. Poster presenters are required to stand by their posters for informal discussions during the designated poster session on Friday, March 8 (17:30-19:00) and requested to check on their poster during the coffee and lunch breaks. Posters left up on Sunday will removed and discarded. AOPT 2019 is not responsible for poster materials left at meeting's end.

Disclosures

All Commercial Relationships must be indicated on a slide of the presentation and on the posters, even if they were not indicated at abstract submission.

Recording Policy

Recording (photographing, audiotaping, or videotaping) of any presentation or poster is PROHIBITED, except by AOPT agents for official purposes or by authors who want to photograph their own poster presentations.



Hotel Amenities

Hotel Monteleone
NEW ORLEANS
The French Quarter's Most Celebrated Hotel

HISTORIC LUXURY HOTEL IN NEW ORLEANS

Hotel Address: 214 Royal St, New Orleans, LA 70130

Phone: (504) 523-3341

Driving Directions

From The East (Mississippi, Alabama, Etc.)

Head I-10 West toward Baton Rouge. Exit Orleans Ave/Vieux Carre (235A). Proceed down ramp and turn left at the light onto Orleans Ave. Continue on Orleans Ave under Interstate. Orleans Ave becomes Basin Street. Continue on Orleans/Basin for .6 miles. Turn left on Conti Street. Continue six blocks (.5 miles), turn right on Decatur Street, then right on Bienville Street. Turn left on Royal Street and a valet attendant at the Front Entrance of hotel will be waiting for your arrival.

From The West (Airport, Baton Rouge, Texas, Etc.)

Head I-10 East toward New Orleans Business District and Slidell. Exit Superdome/Poydras Street (Exit 234B). Proceed down ramp and continue straight for eight blocks, staying in left lane. Turn left onto Camp Street and continue four blocks to Canal Street (a big, two-way boulevard). Cross over Canal Street and continue onto Chartres Street (in the French Quarter). Go two blocks on Chartres Street and turn left on Bienville Street. Turn left on Royal Street and a valet attendant at the Front Entrance of hotel will be waiting for your arrival.

Dinning and Bar

Experience the best in New Orleans dining with our Criollo Restaurant, or visit the historic Carousel Bar & Lounge, which was voted one of the top 20 bars in the world by Vogue Australia.

Parking

Hotel Monteleone owns and operates two convenient, 24-hour valet parking facilities for our guests. One is located on the property, and the other is off-site, one block from the hotel.

Either way, when you come to stay, simply take Chartres Street into the French Quarter, then take a left on Bienville Street. Turn on Royal Street and our valet will be at the front of the hotel ready to take your car.

The rates are **discounted $35 plus tax** for regular-size vehicles for AOPT attendees, per day. Rates are subject to change without notice.

Business Center (Available 24/7 using room key)

Whether you are traveling for business or pleasure, our business center has everything you need, conveniently located on the 2nd floor. We offer free Wi-Fi, computers, and printers for your convenience. We also offer complimentary boarding pass printing.

Fitness Center (24/7 State-of-the-Art Facility)

Our rooftop Fitness Center offers panoramic views of both the Mississippi River and the French Quarter. Our cutting-edge exercise facility is on-premises and available 24/7 with room key access. Our New Orleans luxury hotel makes it easy for our visitors to keep fit and relieve stress during their stay.

Visitors can work out on the Techno-Gym, state-of-the-art, equipment catering to a variety of fitness needs. The 24-hour Fitness Center is provided for the exclusive use of hotel guests, including full cardio machines and gym equipment. Chilled water and complimentary fruit provide a source of hydration and a nutritious snack.

Spa Aria: Comforting, Rejuvenating, Unforgettable – A Breath Of Fresh Air

A cozy retreat tucked within our exquisite New Orleans luxury hotel, Spa Aria transcends relaxation. The spa envelops you like the warmth of a summer evening in New Orleans. From the soft glow of candles to the infusion of fragrance, Spa Aria transports you to a place of repose.

Whether it's your head, your toes, or your entire body that needs soothing, our gifted staff will guide each guest through a satisfying journey. Using intuitive hands and world-renowned products from Pevonia, our pampering is certain to ease the most exhausted souls.

Yoga at Hotel Monteleone

Enjoy complimentary yoga in partnership with lululemon on the Hotel Monteleone pool deck. This gentle vinyasa-style class will leave you refreshed and ready to take on the day. Please reserve your spot with the concierge. Mats will be provided.

For your workout gear needs, lululemon is located in Canal Place at 333 Canal Street, a short five-minute walk from the hotel.

Tuesday & Thursday from 7:00-8:00 AM, Saturday from 8:00-9:00 AM

Pressreader

When you stay with us, enjoy complimentary access to thousands of newspapers and magazines. Connect to our Wi-Fi and download the free PressReader app to start reading.

Pet Amenities

Our New Orleans luxury hotel amenities aren't just for humans — Hotel Monteleone is pleased to offer pet-friendly hotel packages to pamper your favorite four-legged friends, too. This pet-friendly hotel is one of the few New Orleans luxury hotels in Louisiana that offers services for pets.

"Monte's Pet Package" includes a mat with water and food bowls, a bag of

treats, and a pet-friendly brochure filled with places in the city where your pets are welcome. Upon check-in, guests who have a pet must sign a pet application that outlines specific rules and fees that are charged (a $100 non-refundable cleaning fee and a $25 per night "pet rate").

WiFi Service

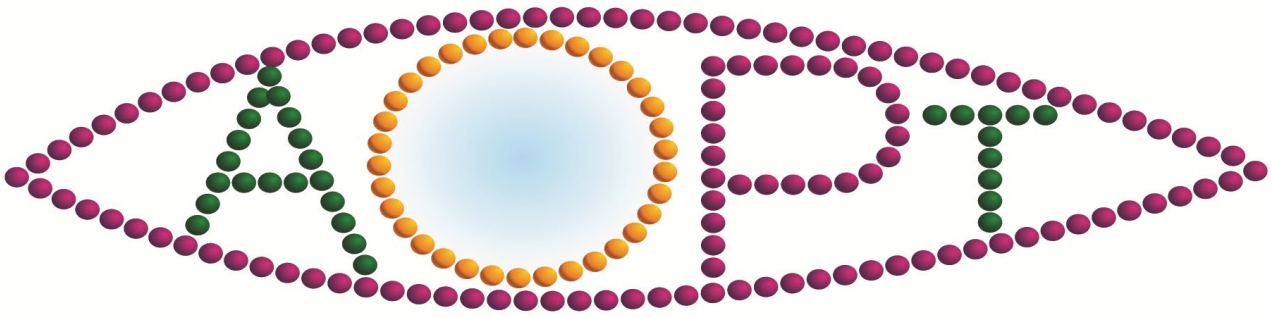
We provide complimentary Wi-Fi up to 2M, then it's $5.95 – Ultimate up to 20M in guest rooms.

Wheelchair Access

Hotel Monteleone has full wheelchair access via the Bienville Street entrance. We also have Traditional King and Traditional Double Double rooms with wheelchair access. Some of these rooms offer roll-in showers. These rooms are available on request. Shower chairs are also available upon request.

Childcare Service

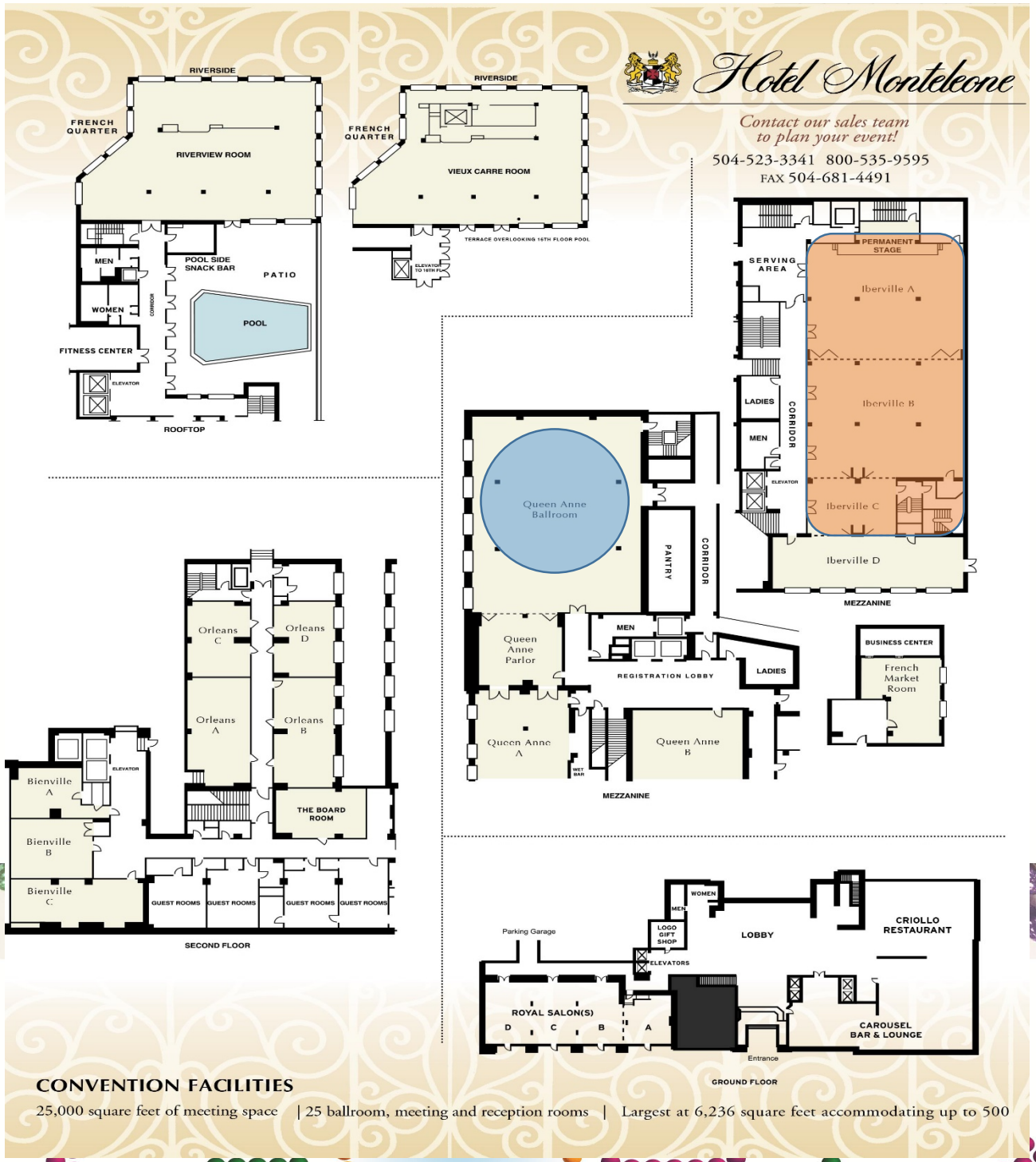
Certified child care service is provided for single parents who wish to attend the meeting (https://child-care-preschool.brighthorizons.com/la/neworleans/kidopolis). See their website for rate.



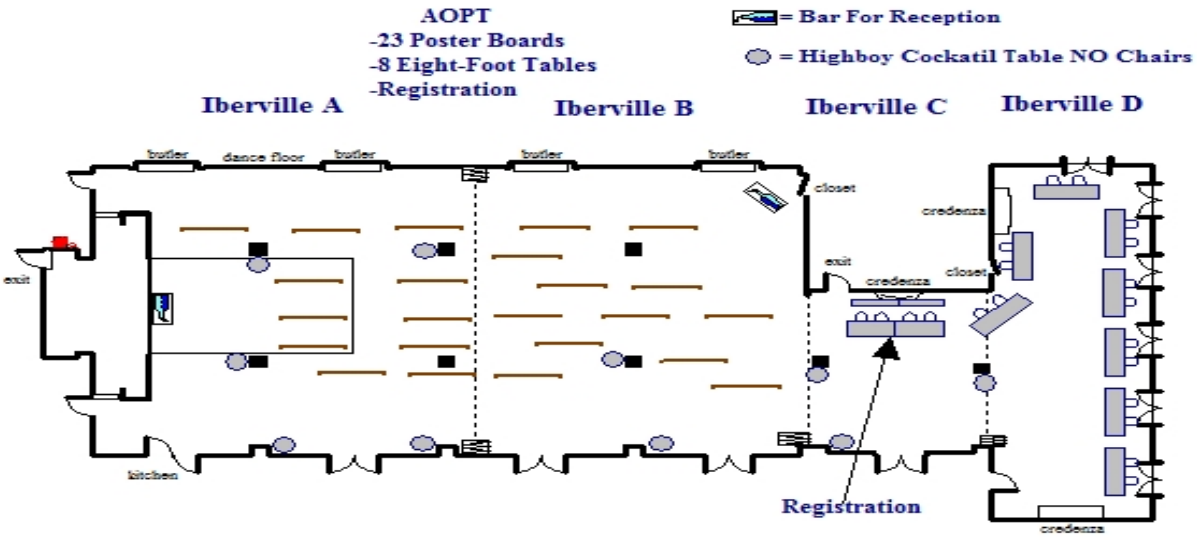
Hotel Floor Plan

Queen Anne Ballroom: Platform Session

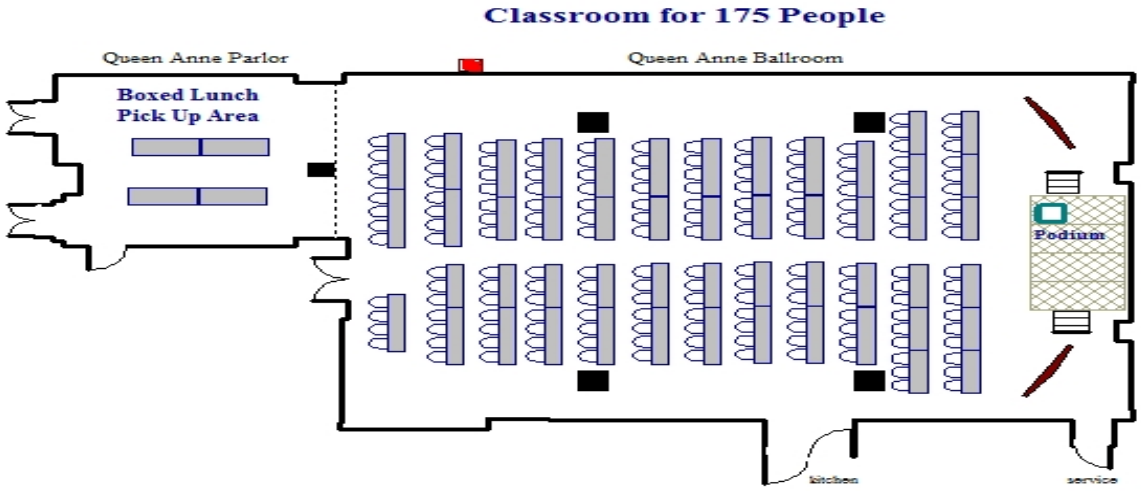
Iberville Ballroom: Poster Session, Registration, Reception



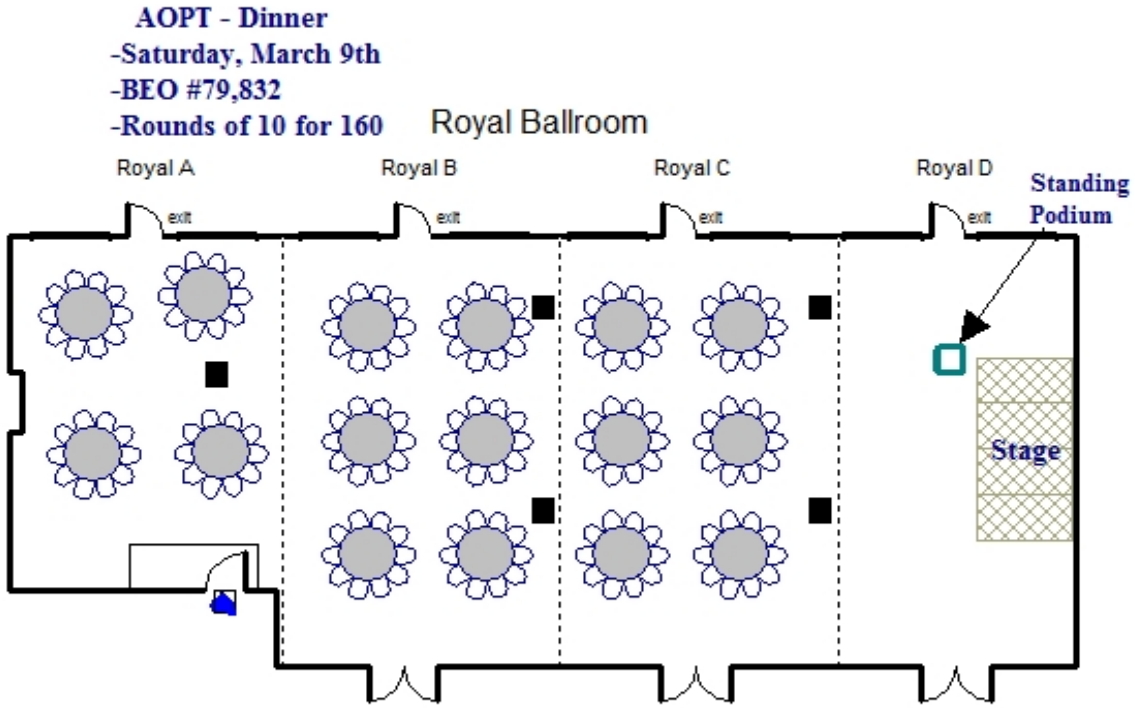
Iberville Ballroom: Registration, Reception, Poster Session



Queen Anne Ballroom: Platform Session



Royal Ballroom: AOPT BANQUET (MARCH 9, 2019)



Your Local Program Committee

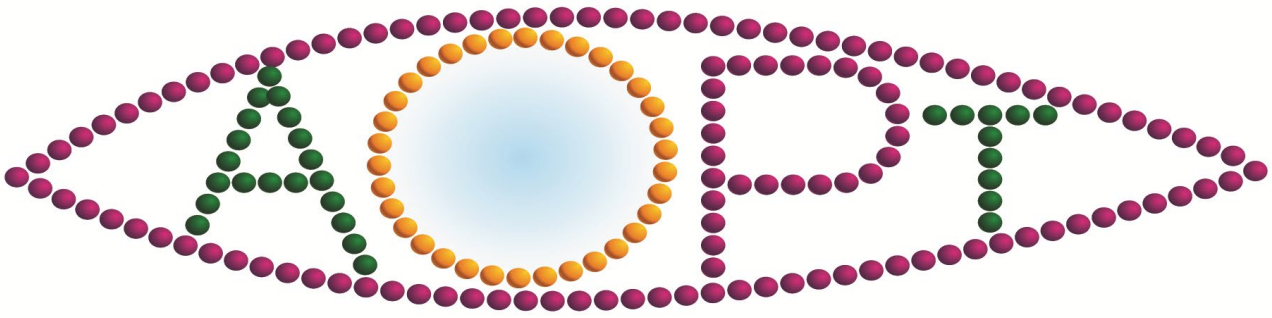
On behalf of the local Program Committee, I am pleased to welcome you to New Orleans, LA, for the 14th Congress on Ocular Pharmacology and Therapeutics. It is really homecoming for AOPT since the first official AOPT meeting took place in New Orleans in 1995. Our Program this year contains 67 platform presentations and a poster session with 43 presentations. We were fortunate to receive support from the **National Eye Institute**, **BrightFocus Foundation, CooperVision, Foundation Fighting Blindness, Regeneron, Roche** and **Omar Consulting Group** to provide 40 travel awards to students (both graduate students and undergraduate students), postdoctoral fellows, and junior faculty from all over the world. The travel award applicants were exceptional, and we are very grateful to our Award Committee (Carol Toris, Catherine Bowes Rickman, Christine Wildsoet, Malinda Fitzgerald, and Shusheng Wang) for tackling the selection of the finalists. We are thankful for the AOPT board members and session moderators for identifying exceptional speakers for the conference. Everything is lining up for an informative, interactive, and productive three and half days of cutting edge science covering all aspects of ocular pharmacology and therapeutics.

Shusheng Wang

Local Program Committee Chair

Nicolas G. Bazan

Local Program Committee Co-chair



Program At-A-Glance

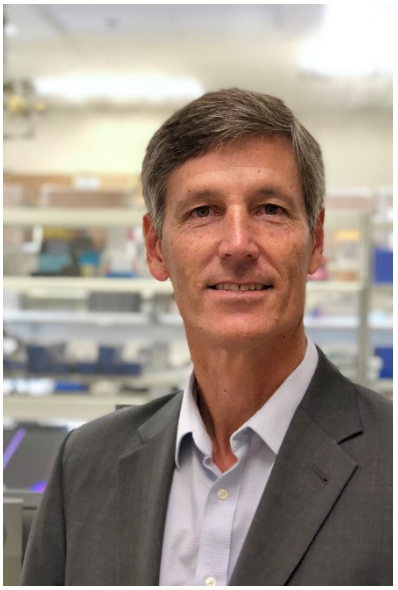
Thursday, March 7, 2019	
15:00-16:30	AOPT Board Meeting
15:00-17:15	Registration
17:15-17:20	Opening Remarks
17:20-18:50	Session 1
18:50-20:00	Welcome Reception

Friday, March 8, 2019	
8:30-10:00	Session 2
10:00-10:20	Break & Exhibits
10:20-11:50	Session 3
11:50: 13:00	Lunch-n-Learn
13:00-14:30	Session 4
14:30-14:50	Break & Exhibits
14:50-16:20	Session 5 (Young Investigator)
16:20-17:20	AOPT general business meeting
17:30-19:00	Poster Session

Saturday, March 9, 2019	
8:30-10:00	Session 6
10:00-10:20	Break & Exhibits
10:20-11:50	Session 7
11:50: 13:00	Lunch-n-Learn
13:00-14:30	Session 8
14:30-14:50	Break & Exhibits
14:50-16:20	Session 9
17:00-18:20	Keynote Address
19:00-21:30	Banquet Dinner

Sunday, March 10, 2019	
8:30-10:00	Session 10
10:00-10:20	Break & Exhibits
10:20-11:50	Session 11
11:50: 13:00	lunch on your own
13:00-14:30	Session 12
14:30-14:50	Break & Exhibits
14:50-16:20	Session 13
16:20-16:40	Closing Remarks

Keynote Speaker



Casey Kopczynski, PhD, Chief Scientific Officer and Co-founder

Aerie Pharmaceuticals, Inc.

Dr. Kopczynski has served as Aerie's Chief Scientific Officer since co-founding the company in 2005. Aerie Pharmaceuticals is an ophthalmic pharmaceutical company focused on the discovery, development, and commercialization of first-in-class therapies for the treatment of patients with glaucoma, retinal diseases and other diseases of the eye.

As the CSO at Aerie, Dr. Kopczynski

- Provided strategic leadership of R&D programs comprised of technical, regulatory, quality, clinical, legal, financial and commercial functions.
- Built and led team that discovered and developed netarsudil, a new class of glaucoma drug that received FDA marketing approval in 2017.
- Developed a highly efficient and productive R&D model that relies upon a multidisciplinary team of in-house scientists to drive innovation and utilizes high quality outsourcing partners for routine, resource-intensive activities. Expanded R&D pipeline to include products for age-related macular degeneration, diabetic retinopathy, and glaucoma neuroprotection.
- Is responsible for evaluating potential in-licensing or merger/acquisition opportunities. Lead technical evaluations and due diligence efforts up to and including final decision.

Prior to Aerie, he was Vice President of Research at Ercole Biotech, Inc., a company developing gene-based drugs for the treatment of cancer, inflammation and orphan genetic diseases, and Managing Partner at Biotech Initiative, LLC, a consulting practice dedicated to emerging biotech companies.

Education

B.A. in Biology, Washington University, St Louis, MO

Ph.D. in Molecular, Cellular and Developmental Biology, Indiana University

Jane Coffin Childs Postdoctoral Research Fellow, University of California, Berkeley

Professional Experience

Director of Research, Exelixis, Inc

Founding Partner, Biotech Initiative, LLC

Vice President of Research, Ercole Biotech, Inc

Chief Scientific Officer and Co-founder, Aerie Pharmaceuticals

Platform Sessions

Thursday, March 7

Welcome and Opening Remarks (17:15-17:20)

Session 1: NOLA Ophthalmic Research (17:20-18:50)

Session Moderators:

Nicolas G. Bazan, MD, PhD, Professor, Director, Neuroscience Center of Excellence, School of Medicine, Louisiana State University

Partha Bhattacharjee, D.V.M., PhD, Associate Professor, Department of Biology, Xavier University of Louisiana

Session Speakers:

Partha Bhattacharjee, D.V.M., PhD, Associate Professor, Department of Biology, Xavier University of Louisiana

Title of Talk: "LRP-1 targeted retinal neuroprotection in diabetic db/db mice"

Katelyn Robillard, Neuroscience Center of Excellence, School of Medicine, Louisiana State University

Title of Talk: "Antisense and Gene Therapy Rescues Hearing, Balance and Vision in Usher syndrome"

William Gordon, PhD, EENT Professor of Neuroscience and Ophthalmology, Neuroscience Center of Excellence, School of Medicine, Louisiana State University

Title of Talk: "Molecular organization of lipids in the human macula and retinal periphery"

Minghao Jin, PhD, Associate Professor, Neuroscience Center of Excellence, School of Medicine, Louisiana State University

Title of Talk: "Mechanisms by which ciliary neurotrophic factor (CNTF) protects rods and cones"

Nicolas G. Bazan, MD, PhD, Professor, Director, Neuroscience Center of Excellence, School of Medicine, Louisiana State University

Title of Talk: "Neuroprotection by novel lipid mediators: significance in retinal degenerations."

Friday, March 8

Session 2: Therapeutic Modalities in Ophthalmology (8:30-10:00)

Session Moderators:

Ashwath Jayagopal, PhD, MBA, Section Head, Ophthalmology Discovery and Biomarkers, Roche

Daniel Stamer, PhD, Professor, Ophthalmology and Biomedical Engineering, Duke Eye Center, Duke University, Durham NC USA

Session Speakers:

Andras Komaromy, DrMedVet, PhD, College of Veterinary Medicine, Michigan State University

Title of Talk: "AAV-mediated gene therapy for long-term effective intraocular pressure (IOP) control in a canine open-angle glaucoma (OAG) model"

Mike Robinson, MD, VP & Therapeutic Area Head, Allergan

Title of Talk: "Bimatoprost Sustained-Release Implants for Glaucoma Therapy"

Jian-xing Ma, MD, PhD, Laureate Professor and Chairman, Department of Physiology, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104, United States

Title of Talk: "Activation of PPARa, a Potential therapeutic strategy for Age-Related Macular Degeneration"

Dan Chung, DO, MA, Clinical Ophthalmic Lead, Spark Pharmaceuticals

Title of Talk: "Development of Luxturna™ (voretigene neparvovec-rzyl): Gene Therapy for *RPE65* Biallelic Mutation Associated Inherited Retinal Disease"

Kameron V. Kilchrist, Vanderbilt University

Title of Talk: "Small molecule ligand targeting of locked nucleic acids to enable corneal delivery"

Break and Exhibits (10:00-10:20)

Session 3: Inflammation in Retinal Degenerative Diseases: Immune Therapy (10:20-11:50)

Session Moderators:

Heping Xu, MD, PhD, School of Medicine, Denitstry and Biomedical Sciences, Queens's University Belfast, UK

Florian Sennlaub, MD, PhD, Team Leader, Institute of Vision, France

Session Speakers:

Florian Sennlaub, MD, PhD, Team Leader, Institute of Vision, France

Title of Talk: "HTRA1 inactivates thrombospondin-1 mediated subretinal immune-suppression"

Dongfeng Chen, MD, PhD, Associate Professor, Schepens Eye Research Institute of Massachusetts Eye and Ear

Title of Talk: "An immune target for neuroprotection in glaucoma"

Patrice E. Fort, PhD, Assistant Professor, Ophthalmology and Visual Sciences, Molecular & Integrative Physiology, Kellogg Eye Centre

Title of Talk: "Role and regulation of the innate inflammatory system in diabetic retinopathy"

Xavier Guillonneau, PhD, Group leader, Institut de la Vision, France.

Title of Talk: "Monocyte-Derived Macrophages in Diabetic Retinopathy"

Heping Xu, MD, PhD, School of Medicine, Denitstry and Biomedical Sciences, Queens's University Belfast, UK

Title of Talk: "Immune suppression as an alternative approach to control retinal angiogenesis"

Lunch & Symposium (11:50-13:00)

Maximizing collaboration between academia and industry

Panelists academia: Tom Yorio, Carol Toris, Iok-Hou Pang

Panelists industry: Achim Krauss, Ashwath Jayagopal

Session 4: Gene Therapy Approaches in Treating Eye Diseases (13:00-14:30)

Session Moderators:

Alfred Lewin, PhD, Professor, Department of Molecular Genetics & Microbiology, College of Medicine, University of Florida

Stephen Tsang, MD, PhD, Department of Pathology and Cell Biology, Columbia University

Jijing Pang, MD, PhD, Department of Ophthalmology Research, College of Medicine, University of Florida

Session Speakers:

Stephen Tsang, MD, PhD, Department of Pathology and Cell Biology, Columbia University

Title of the talk: "Precision Genome Surgery for Imprecision Medicine"

William A. Beltran, DVM, PhD, School of Veterinary Medicine, University of Pennsylvania

Title of the talk: "Knocking down blindness: a gene therapy for autosomal dominant retinitis pigmentosa"

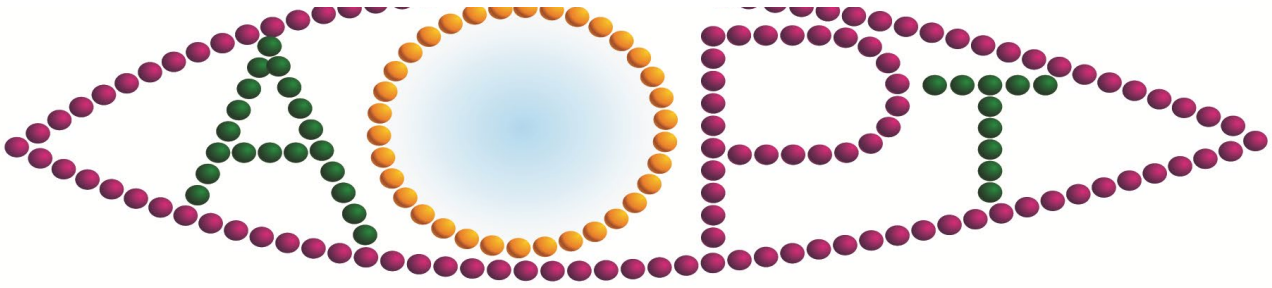
Marina Gorbatyuk, Ph.D., Associate Professor, School of Optometry, Department of Optometry and Vision Science, The University of Alabama at Birmingham

Title of the talk: "Targeting the PERK arm of the Unfolded Protein Response in Retinal Degeneration"

Jiajia Yuan, MD, Tongji Hospital, Huazhong University of Science and Technology, P. R, China

Title of the talk: "Gene therapy for Leber's Hereditary Optic Neuropathy"

Break and Exhibits (14:30-14:50)



Session 5: Young Investigators Session (14:50-16:20)

Session Moderators:

Malinda Fitzgerald, PhD, Professor, Department of Biology, Christian Brothers University; The Neuroscience Institute, The University of Tennessee Health Science Center

Monica Jablonski, PhD, Professor, Department of Ophthalmology, Department of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Hamilton Eye Institute

Session Speakers:

Manas Biswal, PhD, Assistant Professor, University of South Florida

Title of Talk: "AAV delivery of modified erythropoietin (EPO) therapy delays retinal degeneration in a mouse model of geographic atrophy"

Jarel Gandhi, PhD, Postdoctoral Fellow, Mayo Clinic

Title of Talk: "Degradable fibrin scaffolds for induced pluripotent stem cell (iPSC)-retinal pigment epithelium (RPE) Transplantation Using a Pig Model"

Mohamed M Ibrahim, PhD, Postdoctoral Fellow, University of Tennessee Health Science Center

Title of Talk: "Pregabalin Microemulsion Once Daily Eye Drops for Management of Glaucoma"

Fiona McDonnell, PhD, Postdocotoral Fellow, Duke University

Title of Talk: "Vasoregulators mediate distal vessel lumen diameters and outflow facility in human anterior segments"

Bo Yu, PhD, Postdoctoral Fellow, Tulane University

Title of Talk: "A CRISPR-based inducible system for VEGF repression for AMD"

Monika Lakk, PhD, University of Utah

Title of Talk: "TRPV4 -dependent calcium influx regulates strain-induced neurodegenerative pathways in retinal ganglion cells"

Saturday, March 9

Session 6: New Approaches for Treating Age-related Macular Degeneration (AMD) (8:30-10:00)

Session Moderators:

Catherine Bowes Rickman, PhD, Professor, Ophthalmology and Cell Biology, Duke Eye Center, Duke University, Durham NC USA

James Handa, MD, Professor, Ophthalmology, Wilmer Eye Institute, John's Hopkins University School of Medicine

Session Speakers:

Scott Cousins, MD, Professor, Ophthalmology, Duke Eye Center, Duke University, Durham NC USA

Title of Talk: "The Role of Mitochondrial Dysfunction in the Pathogenesis of Dry Age-Related Macular Degeneration: From Concept to Clinic for the Mitochondria-directed Drug, Elamipretide"

Debasish Sinha, PhD, Professor, Ophthalmology, Cell Biology and Developmental Biology, University of Pittsburgh School of Medicine

Title of Talk: "TFEB (transcription factor, EB) as a potential therapeutic target for AMD"

Catherine Bowes Rickman, PhD, Associate Professor, Ophthalmology and Cell Biology, Duke Eye Center, Duke University, Durham NC USA

Title of talk: "The Conundrum of Targeting the Complement Pathway to Treat AMD – Lessons from Animal Models"

James Handa, MD, Professor, Ophthalmology, Wilmer Eye Institute, John's Hopkins University School of Medicine

Title of talk: "A roadmap to find treatment for dry AMD"

Christine Curcio, PhD, Professor, Ophthalmology, University of Alabama School of Medicine.

Title of talk: "Targeting soft drusen in age-related macular degeneration (AMD): rationale and pre-clinical studies of an apolipoprotein mimetic peptide"

Break and Exhibits (10:00-10:20)

Session 7: Current Neuroprotective Therapies for Glaucoma (10:20:11:50)

Session Moderators:

Rebecca Sappington, PhD, Associate Professor, Vanderbilt University

Raghu Krishnamoorthy, PhD, Associate Professor, Pharmacology & Neuroscience, University of North Texas Health Science Center

Session Speakers:

Luca Della-Santina, PhD, Assistant Professor, UCSF

Title of Talk: "Synaptic disassembly and rewiring of the adult retina in a mouse model of glaucoma"

Denise Inman, PhD, Assistant Professor, Northeast Ohio Medical University:

Title of Talk: "Higher Reliance on Glycolysis Limits Responsiveness in Degenerating Glaucomatous Optic Nerve"

Rebecca Sappington, PhD, Associate Professor, Vanderbilt University

Title of Talk: "Interleukin-6 in Retinal Health and Disease"

Luis Alarcon-Martinez, PhD, University of Montreal:

Title of Talk: "Microvascular dysfunction and role of pericytes in glaucoma"

Dorota Stankowska, PhD, Assistant Professor, University of North Texas Health Science Center

Title of Talk: "Alpha B crystallins in glaucoma neuroprotection"

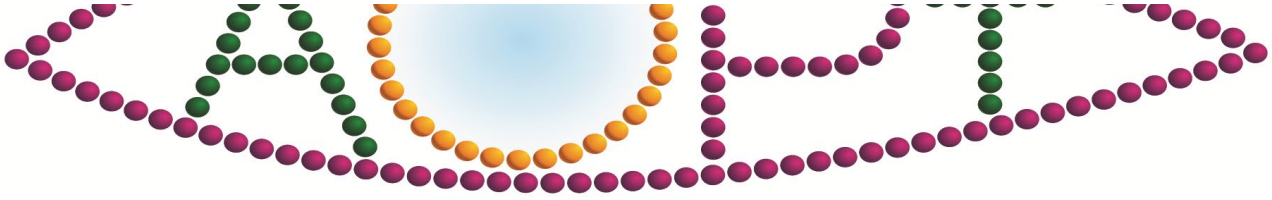
Chenying Guo, PhD, Novartis Institutes for Biomedical Research

Title of Talk: "A simple chronic ocular hypertensive murine model of glaucoma – opportunities for neuroprotection studies"

Lunch-Learn (11:50-13:00)

How to setup and run a new lab in academic setting?

Panel Leaders: Carol Toris, Christine Wildsoet and Vivian Lee



Session 8: Discovery and Development of Novel Ocular Therapies
(13:00-14:30)

Session Moderators:

Carol Toris, PhD, Professor, Department of Physiology & Biophysics, Case Western Reserve University

Iok-Hou Pang, PhD, Professor, North Texas Eye Research Institute, University of North Texas Health Science Center

Session Speakers:

Padmanabhan Pattabiraman, PhD, Department of Ophthalmology and Visual Sciences, Case Western Reserve University

Title of Talk: "Clusterin regulates intraocular pressure by modulating extracellular matrix in trabecular meshwork outflow pathway"

Wei Li, PhD, Associate Professor, Bascom Palmer Eye Institute, University of Miami Health System

Title of Talk: "Ligandomics for retinal angiogenesis drug discovery"

Sanjoy Bhattacharya, PhD, Professor, Bascom Palmer Eye Institute, University of Miami Health System

Title of Talk: "Metabolomics for ocular drug discovery"

Jeffery Gidday, PhD, Professor, Ophthalmology, Neuroscience and Physiology, Louisiana State University

Title of Talk: "Epigenetics-based therapeutics for retinal disease: Using proteomics to identify disease-resilient phenotypes"

Carl Romano, PhD, Executive Director, Regeneron Pharmaceutics

Title of Talk: "21st Century Ocular Pharmacology and Therapeutics: Viral Vectors as Drugs"

Stephen Poor, MRCOphth, Director, Novartis Institutes for BioMedical Research (NIBR)

Title of Talk: "Lessons learned from drugs that fail"

Break and Exhibits (14:30-14:50)

Session 9: Advances in Drug Delivery (14:50-16:20)

Session Moderators:

Christine Wildsoet, OD, PhD, Professor, School of Optometry, University California Berkeley

David Waterbury, Raven Biosolutions LLC.

Session Speakers:

Ilva Rupenthal, PhD, Associate Professor & Director, Buchanan Ocular Therapeutics Unit, Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, Auckland, New Zealand

Title of Talk: "Specific drug targeting to enhance treatment efficacy"

Heather Sheardown, PhD, Professor, Professor and Canada Research Chair in Ophthalmic Biomaterials, Associate Director, Fraunhofer Project Centre for Biomedical Engineering and Advanced Manufacturing, Department of Chemical Engineering, McMaster University, Hamilton ON, L8S 4L7, Canada

Title of Talk: "Can Drug Delivery Enhance the Efficacy of Ocular Therapeutics"

Uday B. Kompella, MPharm, PhD, Professor of Pharmaceutical Sciences, University of Colorado Denver, Aurora

Title of Talk: "Suprachoroidal drug delivery to the eye"

Monica Jablonski, PhD, Professor, Department of Ophthalmology, Department of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Hamilton Eye Institute

Title of Talk: "Novel topical formulation for glaucoma"

Qingguo Xu, PhD Assistant Professor, Virginia Commonwealth University

Title of Talk: "A new nanomedicine method for treating corneal graft rejection"

Keynote Address (17:00-18:20)

Moderator: Dr. Thomas Yorio

Keynote Speaker: Casey Kopczynski, PhD

Chief Scientific Officer and Co-founder

Aerie Pharmaceuticals, Inc.

Title of Talk: "Between a ROCK and a Hardened Meshwork: The Discovery and Development of Rhopressa"

Sunday, March 10

Session 10: Novel Therapies for Corneal Diseases (8:30-10:00)

Session Moderators:

Filippo Drago, MD, PhD, Professor, Chairman of the Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

Claudio Bucolo, PhD, Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

Session Speakers:

Haydee Bazan, PhD, Professor, Neuroscience Center of Excellence, School of Medicine, Louisiana University

Title of Talk: "Novel lipid mediators and neurotrophins targeting cornea nerve integrity"

Graziella Pellegrini, PhD, Center for Regenerative Medicine "Stefano Ferrari", University of Modena and Reggio Emilia, Modena, Italy.

Title of talk: "Cell therapy and gene therapy in eye diseases"

Juana Gallar, MD, PhD, Professor, Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain

Title of Talk: "Old and new cation channel blockers to treat ocular discomfort and pain"

Pedram Hamrah, MD, Professor, Department of Ophthalmology, Department of Bioengineering, Tufts University.

Title of talk: "neuropathic corneal pain: approaches for management"

Simon Kaja, PhD, Department of Molecular Pharmacology and Therapeutics, Department of Ophthalmology, Loyola University Chicago

Title of Talk: "Xanthohumol protects corneal epithelial cells against oxidative stress in vitro"

Corrie, Gallant-Behm, PhD, research scientist, miRagen Therapeutics Inc

Title of Talk: "The miR-29b Mimic Remlarsen as an Anti-Fibrotic Therapeutic in the Eye"

Break and Exhibits (10:00-10:20)

Session 11: Hot Topics from Abstract Submissions to AOPT (10:20-11:50)

Session Moderators:

Ze Zhang, MD, Assistant Professor, Department of Ophthalmology, Tulane University

Rajashekhar Gangaraju, PhD, Assistant Professor, Department of Ophthalmology, The University of Tennessee Health Science Center, Hamilton Eye Institute

Session Speakers:

Rajashekhar Gangaraju, PhD, Assistant Professor, Department of Ophthalmology, The University of Tennessee Health Science Center, Hamilton Eye Institute

Title of Talk: "Exploration of the secretome of adipose stem cells for the design of retinal therapeutics"

Suchismita Acharya, PhD, Research Assistant Professor, University of North Texas Health Science Center

Title of Talk: "Novel topically delivered small molecule with IOP lowering and neuroprotective activity"

Peter Kador, PhD, Professor, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center

Title of Talk: "Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts"

Hongli Catherine Wu, Associate Professor of Pharmaceutical Sciences, College of Pharmacy, University of North Texas Health Science Center

Title of Talk: "More than just a reactive oxygen species scavenger: grapes prevent UV-B radiation-induced cataract by upregulating anti-apoptotic protein XIAP"

Raghu Krishnamoorthy, PhD, Associate Professor, Pharmacology & Neuroscience, University of North Texas Health Science Center

Title of Talk: "The Endothelin Receptor Antagonist Macitentan Attenuates Neurodegeneration in a Rodent Model of Glaucoma and Ameliorates Endothelin-Mediated Vasoconstriction"

Donglei Zhang, Vice General Manager, Director of New Drug development, The School of Pharmacy, He University, P. R. China

Title of Talk: "Protection of kaempferol on oxidative stress-induced retinal pigment epithelial cell damage"

Session 12: Disruptive Technologies: Ophthalmic Tools and Methods that Have Changed the Ways We See the Eye (13:00-14:30)

Session Moderators:

Cheryl Rowe-Rendleman, PhD, Consultant at Omar Consulting Group

Randolph Glickman, PhD, Senderoff Professor of Vision Research/McCoy Professor of Ophthalmology, School of Medicine, University of Texas Health Science Center, San Antonio

Session Speakers:

Steven Becker, Ph.D, Program Coordinator, National Eye Institute, NIH

Title of Talk: "Audacious Goals Initiative: Status and Impact"

Randolph Glickman, PhD, Senderoff Professor of Vision Research/McCoy Professor of Ophthalmology,, School of Medicine, University of Texas Health Science Center, San Antonio

Title of Talk: "Photoacoustic Imaging and Sensing: a New Way to See the Eye"

Francois Binette, Senior Vice President, Globe Head of Product Development, Biotime

Title of Talk: "A Platform to Take on the Entire Progressive Retinal Degeneration Disease Continuum"

Subrata Batabyal, PhD, Senior Technical Officer, Nanoscope Technologies LLC

Title of Talk: "Early stage detection of Glaucoma by monitoring nanostructure and function of RGC layer using Multifractal OCT"

Claire M. Gelfman, PhD, Executive Director Pharmaceutical Development, Adverum Biotechnologies, Inc

Title of Talk: "Preclinical Evaluation of ADVM-022, a Novel Gene Therapy Approach to Treating Wet Age-Related Macular Degeneration"

Panel Discussion: led by Cheryl Rowe-Rendleman, PhD, Consultant at Omar Consulting Group

Break and Exhibits (14:30-14:50)

Session 13: What Every Eye Doctor Needs to Know About the FDA (14:50-16:20)

Session Moderators:

Bing Cai, PhD, Division director, Division of Liquid-Based Products at FDA's Office of Life Cycle Products, Office of Pharmaceutical Quality, Center of Drug Evaluation and Research.

Jayne Weiss, MD, Chairman, Department of Ophthalmology, Director of LSU Eye Center of Excellence, Louisiana State University School of Medicine

Session Speakers:

Wiley A Chambers, MD., FDA/CDER/OND,

Title of Talk: "Advancing Technology Challenges in Ophthalmic Drug Approvals"

Markham Luke, MD, FDA/CDER/OGD:

Title of Talk: "Generic Drugs and Their Role in Bringing Next Generation Products: An FDA Perspective"

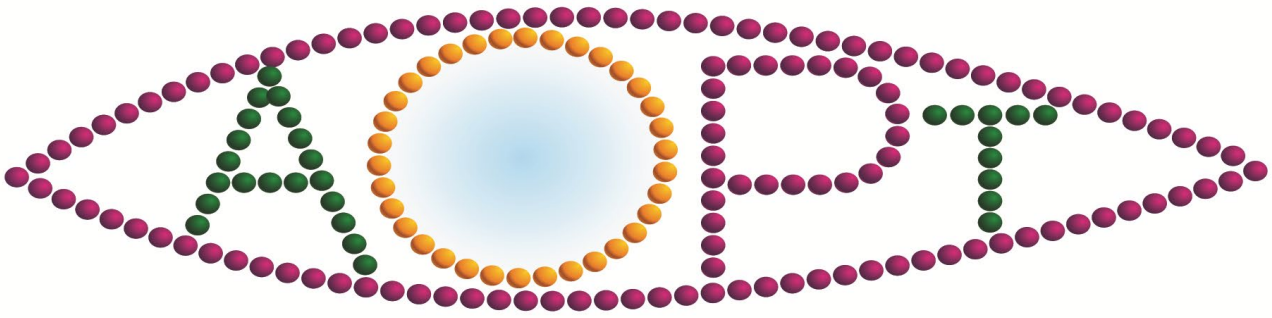
Darby Kozak, Ph.D., FDA/CDER/OGD,

Title of Talk: "Generic Ophthalmic Drug Products, Physical Characteristics and Bioequivalence"

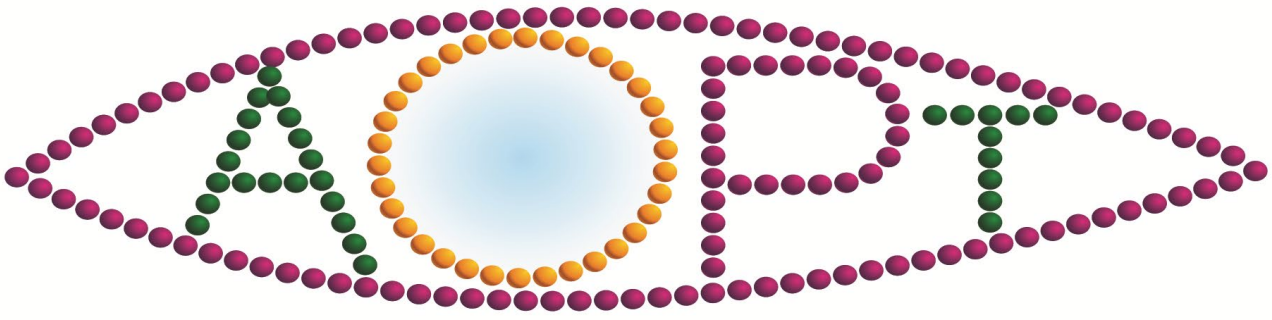
Patricia Onyimba, MS., FDA/CDER/OPQ,

Title of Talk: "How FDA Ensures Quality of Ophthalmic Drug Products"

Closing Remarks (16:20-16:40)



Platform Abstracts



Session 1: NOLA Ophthalmic Research

1.1 LRP-1 targeted retinal neuroprotection in diabetic db/db mice

Partha S Bhattacharjee, Josiah Sherman and Dalia El-Desoky.

Department of Biology, Xavier University of Louisiana, New Orleans, LA 70125

Purpose: Diabetes mellitus accompanies impaired glucose and lipid homeostasis. One cell membrane receptor, low-density lipoprotein receptor-related protein-1 (LRP-1), a protein normally involved in regulating lipid homeostasis, plays a critical role in repairing and/or protecting neurons from injury. Existing evidence suggests that diabetic hyperglycemia suppresses LRP-1 expression in the retina, resulting in receptor desensitization of LRP-1. In a setting of diabetic hyperglycemia, the role of LRP-1 in neuronal homeostasis is unknown.

Methods: Diabetic db/db mice homozygous for the spontaneous mutation $Lepr^{db}$ (BKS.Cgm +/+ $Lepr^{db}$/J) and their age-matched non-diabetic heterozygous controls (*db/m*) were intravitreally treated with 100μM apoEdp (LRP-1-ligand) in sustained release formulation vehicle. Treatment started at 12 weeks of age and mice were sacrificed at 24 weeks. Western blot and immunohistochemical analyses were done to study neuronal apoptosis and relevant LRP-1-targeted signaling pathways.

Results: Western blot analysis of retinal extract suggests that LRP-1 activation by apoEdp treatment resulted in (a) the inactivation of cytoplasmic protein phosphatase PP2A (b) increase in intracellular kinase activity of PI3K/Akt and ERK1/2 pathways, and (c) inhibition of pro-apoptotic p-Gsk3β, BAD and cleaved caspase 3. Intravitreal apoEdp treatment resulted in the significant reduction in retinal ganglionic cell (RGC) death compared to vehicle treatment as determined by immunohistochemical analysis of retinas for TUNEL and cleaved caspase 3.

Conclusions: Our results suggest LRP-1 is a novel therapeutic target for the treatment of DR and may be considered for other retinal neurodegenerative diseases of RGC apoptosis.

1.2 Antisense and Gene Therapy Rescues Hearing, Balance and Vision in Usher syndrome

Katelyn Robillard[1], Russell J. Amato[1], Robert F. Rosencrans[1], Marianne Hathaway[1], Abhilash Ponnath[1], Bhagwat Alapure[1], Francine M. Jodelka[2], Bifeng Pan[3], Carl Nist-Lund[3], Nicolas G. Bazan[1], Hamilton E. Farris[1], Frank Rigo[4], Michelle L. Hastings[2], Gwenaëlle G.S. Géléoc[3], Jennifer J. Lentz[1]

[1]Neuroscience Center of Excellence, LSU Health Sciences Center, New Orleans, LA, USA. [2]Dept. of Cell Biology and Anatomy, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA. [3]Dept. of Otolaryngology, F.M. Kirby Center for Neurobiology; Boston Children's Hospital, Harvard Medical School, Boston, MA, USA. [4]Ionis Pharmaceuticals, Carlsbad, CA, USA.

Purpose: Usher syndrome (Usher) is the most common genetic cause of concurrent deaf-blindness. Transgenic mice with the USH1C c.216G>A mutation (216A), which causes Usher in humans, have profound hearing loss, and vestibular and visual deficits similar to patients. The 216A mutation causes aberrant splicing, which produces a truncated protein. We aim to develop therapeutic approaches for the treatment of hearing, balance and vision loss in Usher using antisense oligonucleotides and gene replacement therapy.

Methods: 216AA-Usher and control mice were treated with 216A-targeted ASOs or AAV-Ush1c vectors using various delivery and timing strategies followed by assessments of hearing, balance and vision. Ush1c mRNA and harmonin protein expression were also evaluated in cochlear and retinal tissues.

Results: Systemic and local treatment of 216AA-Usher mice at various ages with ASOs blocks aberrant splicing and leads to improvements in Ush1c mRNA and harmonin protein expression in cochlear and retinal tissues, and rescues hearing, balance and vision. Treatment with AAV-Ush1c vectors rescues hearing and balance. Treatment with retinal-specific AAV-Ush1c studies are on-going.

Conclusions: Antisense and gene therapy-based therapeutics show promise in the treatment of a number of diseases, but have not yet been extensively explored as a treatment for Usher. Our results show that optimized delivery of ASOs to the ear and eye can effectively target Ush1c mutations in the cochlea and retina. These results also demonstrate the potential for long-term benefits to hearing and vision with ASO treatment in patients who carry this USH1C mutation, and in hearing and vision loss in general.

1.3 Molecular organization of lipids in the human macula and retinal periphery

William C. Gordon, Bokkyoo Jun, Nicolas G. Bazan, Louisiana State University Health New Orleans, School of Medicine, Neuroscience Center of Excellence, New Orleans, LA, USA

Purpose: Photoreceptor phospholipids (PLs) containing VLC-PUFAs derived from docosahexaenoic acid (22:6) are critical for retinal function. Therefore, we asked if changes in VLC-PUFA-containing phosphatidylcholine (PC) species accompanies macular degeneration (MD). Here we describe the distribution and molecular organization of these PCs in normal and MD donor retinas.

Methods: Twenty µm-thick-sections of normal, early and advanced MD eye donors were MALDI imaged and lipids characterized. VLC-PUFAs in normal and MD macula and peripheral retinas were then analyzed and quantitated by LC-MS/MS.

Results: PCs were differentially distributed within normal retina, and regionally abundant species identified, but MD total PC and VLC-PUFA PC distribution was altered. Overall, n-3 PC species were affected by disease; n-6 species remained constant. Total MD VLC-PUFAs were significantly depressed in both macula and periphery; 54 C and 56 C PCs (containing VLC-PUFAs and 22:6) within normal retina were most abundant (56 Cs more so), but exhibited significant reduction within MD total retinas. In normal retinas, 54 C and 56 C PCs were most abundant within periphery. Generally, this was also found in MD retinas. However, greatest loss occurred within peripheral MD retinas.

Conclusions: This suggests that changes in VLC-PUFA abundance within peripheral retina may contribute to retinal degeneration.

Funding: NEI EY005121 and EENT Foundation.

1.4 Mechanisms by which ciliary neurotrophic factor (CNTF) protects rods and cones

Minghao Jin[1,2], Songhua Li[1], William C. Gordon[1,2], Nicolas G. Bazan[1,2]

[1]Neuroscience Center and [2]Department of Ophthalmology, LSU School of Medicine, New Orleans, LA, USA

Purpose: Exogenous CNTF has shown multiple controversial effects on therapy of photoreceptor degeneration, thereby increasing difficulty in defining the primary function of CNTF. Using CNTF-deficient mice, we tried to identify the primary role of CNTF in regulating the visual system function and neuroprotection.

Methods: All experiments were performed using 129S2/Sv (wild-type) and *Cntf*-/- mice with homologous Leu450 *Rpe65* alleles. Retinal morphologies and proteins were analyzed by light microscopy, immunohistochemistry or immunoblot analysis. Retinoids involved in the visual cycle were measured by high performance liquid chromatography. Visual function was evaluated by scotopic, photopic or flicker electroretinographies. Retinal photodamages were induced by exposing mice to 12000 lux light for 30~75 min.

Results: *Cntf*-/- retinas displayed higher expression levels of rod and cone opsins accompanied by increase in the thickness of the outer nuclear layers and the lengths of the photoreceptor outer segments. Retinoid isomerase activity as well as expressions of RPE65 and lecithin:retinol acyltransferase (LRAT), which synthesizes the RPE65 substrate, were also significantly increased in the *Cntf*-/- RPE. Upregulated RPE65 and LRAT accelerated both the visual cycle rates and recovery rates of rod light sensitivity in *Cntf*-/- mice. Rod *a*-wave and cone *b*-wave amplitudes were increased in *Cntf*-/- mice, but rod *b*-wave amplitudes were unchanged compared with those in wild-type mice. Of note, rods and cones in *Cntf*-/- mice exhibited hypersusceptibility to light-induced degeneration.

Conclusions: CNTF is a common extracellular signal that prevents excessive production of opsins, the photoreceptor outer segments, and visual chromophores to protect rods and cones from photodamage.

1.5 Neuroprotection by novel lipid mediators: significance in retinal degenerations

Nicolas G. Bazan, Louisiana State University Health New Orleans, School of Medicine, Neuroscience Center of Excellence and Department of Ophthalmology, New Orleans, LA, USA

Purpose: The pathophysiological significance of the essential fatty acid docosahexaenoic acid (DHA) and of its bioactive mediators - docosanoids and elovanoids (ELVs) - are being explored.

Methods: Our approach includes genetic ablation of proteins, cell-specific conditional KOs of key genes, ERG, OCT, MS-Molecular imaging, LC-MS/MS-based mediator lipidomic analysis, AMD retinas and human RPE cells in primary culture.

Results: The preferential PRC uptake and DHA metabolism in PRC depends on the Adiponectin receptor 1. We found that: a) NPD1 mediates preconditioning rescue of RPE and PRC; b) decreased ability to biosynthesize NPD1 in hippocampus of early stages of Alzheimer's disease; c) ELongation of Very Long chain fatty acids-4 provides precursors for ELV biosynthesis; d) ELVs protect RPE and PRC; e) Oligomeric Aβ peptide-induced retinal degeneration was counteracted by ELVs, restoring RPE morphology and homeostasis; f) ELVs reduced expression of inflammatory, senescence and AMD-related genes triggered by OAβ.

Conclusions: The identification of early mechanisms of PRC survival mediated by ELVs and docosanoids contributes to understanding pro-homeostatic modulation, inflammatory responses, and innate immunity. ELVs protect RPE and retina from OAβ damage targeting the transcriptome of senescence, AMD, inflammation-associated gene expression, and by preserving the expression of RPE-functional genes, resulting in restored PRC. These mechanisms open avenues for prevention and therapeutic applications in glaucoma, AMD (particularly dry form) and other retinal degenerative diseases.

Funding: NEI EY005121, NINDS NS046741/NS2302, NIGMS P30 GM103340, and EENT Foundation.

Session 2: Therapeutic Modalities in Ophthalmology

2.1 AAV-mediated gene therapy for long-term effective intraocular pressure (IOP) control in a canine open-angle glaucoma (OAG) model

András M. Komáromy[1], Kristin L. Koehl[1], Christine D. Harman[1], Annie Oh[1,2], Sanford L. Boye[3], Juan P. Steibel[4], Leandro B. Teixeira[5], Carol B. Toris[6], Sayoko E. Moroi[7], William W. Hauswirth[8], Shannon E. Boye[8]

[1]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA; [2]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA; [3]Department of Pediatrics, University of Florida, Gainesville, FL, USA; [4]Department of Animal Science & Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA; [5]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA; [6]Case Western Reserve University Department of Ophthalmology and Visual Sciences, Cleveland, OH, USA; [7]Department of Ophthalmology and Visual Sciences, University of Michigan, Kellogg Eye Center, Ann Arbor, MI, USA; [8]Department of Ophthalmology, University of Florida, Gainesville, FL USA

Purpose: To achieve long-term, stable IOP control by AAV-mediated gene replacement therapy of the aqueous humor outflow pathways (AHOP) in a well-established canine OAG model with a mutation in ADAMTS10.

Methods: Single stranded, capsid mutated AAV2 vectors were evaluated for their ability to target the AHOP of normal (n=11) and ADAMTS10-mutant dogs (n=4) using green fluorescent protein (GFP) reporter gene. 109-1012vg/50µL were administered intracamerally (IC), and GFP expression evaluated clinically and by immunohistochemistry (IHC). Subsequently, ADAMTS10-mutant dogs (n = 19) at various stages of OAG were IC injected unilaterally with AAV2(Y444F)-smCBA-hADAMTS10, with 1011-1012vg in 50-200µL. Outcome measures included weekly diurnal IOP, outflow facility, aqueous humor flow, retinal and optic nerve head (ONH) function and morphology, and hADAMTS10 transgene expression.

Results: Single residue capsid mutated AAV2(Y444F) provided consistent GFP expression in the canine AHOP, with clinically observable fluorescence at the 1012vg dose. hADAMTS10 transgene expression was identified by qPCR for at least 35 months post injection. A lower dose (1011vg) of AAV2(Y444F)-smCBA-hADAMTS10 did not result in a detectable IOP decrease. The use of higher doses (1012vg; n=6) resulted in a significant IOP decrease (13.9±1.8 vs. 28.6±4.2 mmHg; $p<0.01$) and increased outflow facility (0.35±0.15 vs. 0.24±0.9; $p<0.01$) with preservation of the ONH over the 12-24-month observation period in 4 of 6 ADAMTS10-mutant dogs treated.

Conclusions: In an OAG large animal model, we provide proof-of-concept that long-term IOP control and ONH protection is achieved by targeting transgene expression to the AHOP with a single stranded AAV2-based vector.

Funding Sources: NIH R01-EY025752, R01-EY024280, P30-EY007003, P30EY021721, TRR018411C, Glaucoma Research Foundation, MSU Discretionary Funding Initiative (DFI), Research to Prevent Blindness, Foundation Fighting Blindness, Edward Sheppard and family.

2.2 Bimatoprost Sustained-Release Implants for Glaucoma Therapy

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Purpose: A biodegradable bimatoprost sustained-release implant (BimSR) was developed to address nonadherence in the glaucoma population. A clinical study evaluated the safety and intraocular pressure (IOP)–lowering effect of BimSR.

Methods: BimSR was designed to release drug in the eye for 4 to 6 months. In a Phase 1/2, 24-month, paired-eye trial in 75 patients, BimSR (6-, 10-, 15-, or 20-µg dose strength) was administered intracamerally in the study eye; the fellow eye was treated with topical bimatoprost 0.03% QD. Patients were permitted rescue topical medication or a single repeat treatment with BimSR.

Results: Mean IOP was reduced by all BimSR dose strengths. IOP was controlled without rescue or retreatment in 68%, 40%, and 28% of BimSR-treated eyes up to 6, 12, and 24 months, respectively.

Conclusions: BimSR demonstrated IOP-lowering efficacy with effects lasting for more than 6 months in the majority of patients and up to 24 months in a subset of patients. The mechanism of action of prostaglandin analogs (PGAs) is upregulation of matrix metalloproteases (MMPs) in target tissues, which increases aqueous outflow. Published studies have demonstrated that MMP expression is PGA dose-related. Since BimSR delivers a 4-fold higher drug concentration in the ciliary body compared with topical dosing, we hypothesize that the sustained IOP lowering with BimSR beyond the implant drug elution period of 4 to 6 months could involve more durable MMP-mediated remodeling of aqueous outflow pathways. The results support further clinical development of BimSR; phase 3 trials are ongoing.

2.3 Activation of PPARa, a Potential therapeutic strategy for Age-Related Macular Degeneration

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PURPOSE: The purpose of this study was to evaluate therapeutic effects of fenofibric acid (Feno-FA), a peroxisome proliferator-activated receptor-alpha (PPARa) agonist, on neovascularization (NV) in models recapitulating neovascular age-related macular degeneration (AMD), and elucidate its mechanism of action.

METHODS: Laser-induced choroidal NV (CNV) in rats, very low-density lipoprotein receptor knockout (Vldlr-/-) and Ppara-/- mice were used as animal models. Vascular leakage was examined by fundus fluorescein angiography and permeability assay. CNV was evaluated by CNV areas and volume. In Vldlr-/- mice, sub-retinal NV (SRNV) and intra-retinal NV (IRNV) were quantified. Inflammation was evaluated using quantification of inflammatory factors and leukostasis in the retina. Endothelial progenitor cells (EPC) were isolated from bone marrow. EPC mitochondrial function was measured using Seahorse analyzer.

RESULTS: Systemic administration of Feno-FA significantly reduced vascular leakage and CNV volume, and suppressed SRNV and IRNV in CNV rats and Vldlr-/- mice. Feno-FA down-regulated the expression of VEGF, TNF-a and ICAM-1 in the eyecups of CNV rats and decreased adherent retinal leukocytes in Vldlr-/- mice. Furthermore, Ppara-/- mice developed more severe CNV compared with wild-type mice, and PPARa knockout abolished the beneficial effects of Feno-FA on CNV. Isolated EPC from Vldlr-/- mice showed over-activated Wnt signaling, correlating with elevated mitochondrial function and increased mitochondrial mass and DNA copy numbers in EPC, compared to wild-type EPC. Feno-FA down-regulated Wnt signaling in EPC and decreased EPC numbers and mitochondrial function.

CONCLUSIONS: PPARa activation has therapeutic effects on CNV through inhibition of Wnt signaling in EPC via modulation of mitochondrial function.

2.4 Development of Luxturna™ (voretigene neparvovec-rzyl): Gene Therapy for *RPE65* Biallelic Mutation Associated Inherited Retinal Disease

Daniel C. Chung, DO, MA, Clinical Ophthalmic Lead, Spark Pharmaceuticals

Purpose: Several early-phase human trials provided preliminary evidence of safety and efficacy for adeno-associated virus-mediated human *RPE65* gene augmentation for *RPE65*-biallelic mutation-associated inherited retinal dystrophy. We report the clinical development, of Luxturna (voretigene neparvovec-rzyl) the first FDA approved gene therapy for a genetic disease for the treatment of patients with biallelic mutations in the *RPE65* gene associated retinal dystrophy with remaining viable retinal cells.

Methods: Thirty-one subjects with disease-causing biallelic *RPE65* mutations were randomized 2:1 to intervention or control. Eligibility criteria included age ≥3 years-old; bilateral visual acuity worse than 20/60 and/or visual field less than 20 degrees in any meridian; evidence of sufficient viable retinal cells by fundus photography and optical coherence tomography; ability to be evaluated on mobility testing; and willingness to provide consent or parental permission and assent, where appropriate. Subjects in the intervention group received subretinal injections of Luxturna sequentially to each eye within an 18-day window. Control subjects did not receive Luxturna for at least 1 year from baseline, but completed the same testing regiment as those in the intervention arm. Using a standardized subretinal delivery procedure and under general anesthesia, 1.5E11 vector genomes/eye were delivered in a total volume of 300 µl. A novel, standardized multi-luminance mobility test was the primary efficacy endpoint, with
secondary endpoints including full field light sensitivity testing, assigned first eye mobility change score and visual acuity, and additional prespecified endpoints including visual field

Results: All subjects completed Year 1 follow-up testing. Phase 3 study results include demographics, safety information, and mobility testing change score (performance at 1 year compared with baseline), and secondary endpoints of full field tight sensitivity testing, assigned first eye mobility change score and visual acuity. A separate study analyzing mobility test data in untreated normal and retinal dystrophy cohorts was used to validate the mobility test's ability to distinguish low vision from normal-sighted populations, differentiate a range of performance in low vision subjects, and confirm changes in functional vision over time. The trial of 31 subjects met with statistical

significance its primary endpoint, the bilateral mobility test change score ($p = 0.001$), as well as the first two of three secondary endpoints, specifically full-field light sensitivity threshold testing, or FST ($p < 0.001$), and the assigned first eye mobility test change score ($p = 0.001$). Statistical significance was not achieved for the third secondary endpoint, visual acuity ($p = 0.17$). Serious side effects reported in the US Full Prescribing Information include endophthalmitis, that may lead to blindness, permanent visual acuity loss or retinal changes causing vision loss, Other potential side effects that can be associated with Luxturna treatment include hyperemia, cataracts, increased intraocular pressure, retina tears, epiretinal membrane, corneal dellen, macular hole, subretinal deposits, conjunctival edema, eye irritation or pain.

Conclusions: The clinical development of Luxturna (vortigene neparvovec-rzyl) including the primary endpoint of mobility testing and 2 of the 3 secondary endpoints in the Phase 3 trial provided sufficient evidence of the efficacy and safety of Luxturna, a surgically administered gene therapy, which has received approval from the FDA (Dec 2017) and the European Commission (Nov. 2018).

2.5 Small molecule ligand targeting of locked nucleic acids to enable corneal delivery

Kameron V. Kilchrist[1,2] **(Travel Awardee),** Martin G. Nussbaumer[2], Ashwath Jayagopal[2]

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[2]Roche Innovation Center Basel, Pharma Research and Early Development, F. Hoffmann-La Roche, Ltd., Basel, Switzerland.

Purpose: Synthesize and test small molecule ligands (SML) for locked nucleic acid (LNA) delivery to the cornea. The development of LNA delivery technologies would allow any gene to be inhibited, enabling a new generation of corneal therapeutics.

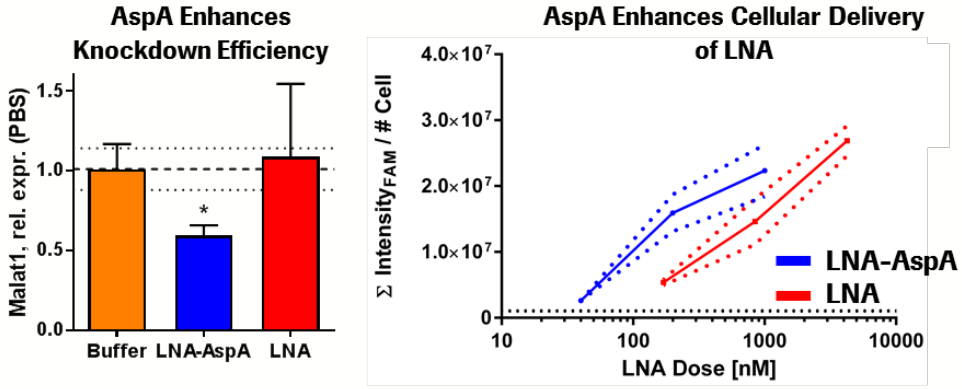
Methods: Phosphorothioated LNA were synthesized by standard phosphoroamidite chemistry and terminated with a 5' hexylamino linker. The SML 1,2-dithiolane-4-carboxylic acid (asparagusic acid, AspA) was conjugated to the LNA amine via EDC/NHS coupling and purified by 2-propanol precipitation and HPLC. The LNA-AspA conjugate was applied to ARPE-19 and HCE-T cells for 30 minutes in PBS then washed with full serum media, simulating instillation via eye drops and challenge by tear proteins.

Results: Treatment with 40 nM LNA-AspA resulted in a significant reduction (-41%) of the model gene MALAT1, while the untargeted LNA resulted in a slight increase (+8%) in MALAT1 transcript levels (Fig 1A). AspA targeting resulted in a ~10-fold increase of cellular uptake of LNAs relative to untargeted controls (Fig 1B). Further, microscopy shows that treatment with LNA or LNA-AspA does not adversely affect cell monolayer morphology, suggesting nontoxicity.

Conclusions: We have synthesized SML LNA conjugates showing proof-of-concept data that SMLs can enable corneal LNA delivery. This supports further inquiry into this unique class of molecules that enable nontoxic LNA delivery to corneal cells

Figure 1.

A. B.


AspA Enhances Knockdown Efficiency
Malat1, rel. expr. (PBS)
1.5
1.0
0.5
0.0
*
Buffer
LNA-AspA
LNA
AspA Enhances Cellular Delivery of LNA
Σ Intensity FAM / # Cell
4.0×10^7
3.0×10^7
2.0×10^7
1.0×10^7
0
10
100
1000
10000
LNA Dose [nM]
LNA-AspA
LNA

Session 3: Inflammation in Retinal Degenerative Diseases: Immune Therapy

3.1 HTRA1 inactivates TSP1 mediated subretinal immune-suppression

Fanny Beguier[1]*, Michael Housset[1]*, Christophe Roubeix[1]*, Sebastien Augustin[1], Mustapha Benchaboune[2], Jean-François Girmens[2], José-Alain Sahel[1,2], Xavier Guillonneau[1], **Florian Sennlaub1†**

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CD47 activation by Thrombospondin 1 (TSP-1) is essential in maintaining the subretinal immunosuppressive environment and prevents the subretinal accumulation of Mononuclear Phagocytes (MPs), a family of cells that include monocytes and macrophages. Age-related Macular Degeneration (AMD), a highly heritable, major cause of blindness, is characterized by the breakdown of the immune-suppression and an accumulation of pathogenic MPs. Of all genetic factors, a variant of Complement factor H (CFH) and a risk haplotype of 10q26 are associated with greatest linkage to AMD. We recently showed that complement factor H (CFH) and in particular the AMD-associated CFH variant, curbs Thrombospondin 1 (TSP-1) activation of CD47. We now demonstrate that Mo from homozygous carriers of the major AMD-risk haplotype of the 10q26 locus significantly overexpress the High-Temperature Requirement A Serine Peptidase 1 (HTRA1). Mechanistically we demonstrate that HTRA1 hydrolyses TSP-1, preventing its ability to activate CD47 and induce MP elimination. Our study reveals a comprehensive mechanism how CFH and HTRA1 participate in the pathogenesis of AMD and opens new therapeutic avenues to restore subretinal immunosuppressivity and inhibit the pathogenic subretinal inflammation.

3.2 An immune target for neuroprotection in glaucoma

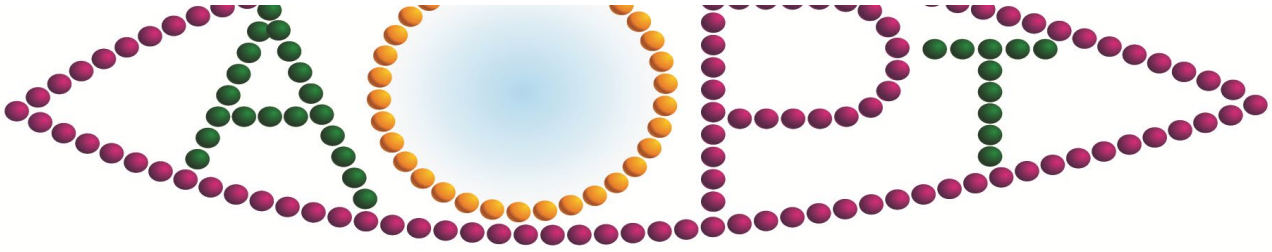
Dong Feng Chen. Schepens Eye Research Institute of Massachusetts Eye and Ear, Department of Ophthalmology, Harvard Medical School, Boston, MA. Department of Ophthalmology, Second Xiangya Hospital of Central South University and Institution of Ophthalmic Center, Changsha, Hunan Province, China.

Purpose: Glaucoma is a leading cause of blindness worldwide and is characterized by progressive optic nerve damage and retinal ganglion cell (RGC) death. However, the mechanisms underlying retinal neurodegeneration and vision loss in glaucoma are not fully understood.

Methods: Glaucoma was induced in adult (10 - 16 weeks old) male and female C57BL/6J (B6), Rag1-/-, and TCRa-/-, and germ-free mice by microbead injection into the anterior chamber. RGC and axon loss was quantified in retinal flat-mounts and semi-thin optic nerve sections, respectively. Heat shock protein (HSP)-specific T cell responses were determined by ELISPOT assays.

Results: Using both inducible and spontaneous glaucomatous mouse models, our data showed that a transient elevation of intraocular pressure for less than three weeks induced T cell infiltration into the retina and HSP-specific T cell responses. This T cell response is responsible for a prolonged phase of RGC and axon loss after IOP returned to a normal range. Mice deficient in T cell functions displayed an attenuated optic nerve and RGC loss; whereas, adoptive transfer of diseased or HSP-responsive T cells facilitated neurodegeneration in glaucomatous mice. Importantly, mice raised in the absence of commensal microflora (germ-free) did not develop HSP-specific T cell responses, nor did they exhibit RGC and axon loss under the elevated IOP.

Conclusions: These studies revealed an autoimmune mechanism associated with the neural damage in glaucoma. They provide compelling evidence suggesting an essential role of adoptive immunity, especially T cells that are pre-sensitized by commensal bacteria, in glaucomatous neurodegeneration.



3.3 Role and regulation of the innate inflammatory system in diabetic retinopathy

Patrice E. Fort, Ophthalmology and Visual Sciences, Molecular & Integrative Physiology, Kellogg Eye Centre

Systemic and local inflammation have been associated with many ocular diseases but the role of retinal tissue inflammation in diabetic retinopathy (DR) has only emerged relatively recently. In particular the role of innate inflammation while widely associated with age-related macular degeneration, had received little attention in DR. Use of discovery approaches and associated pathway analysis on retinal tissues from both animal models and human donors with diabetes revealed a central role for retinal inflammation, particularly the complement system. By combining discovery and orthogonal approaches, we further assessed the inflammatory response association with diabetes and the onset and progression of DR. Our results clearly support that the activation of several inflammatory pathways, including the complement system are specifically associated with DR. Of note, the cellular localization of the innate immune activation strongly demonstrates the extra-vascular nature of this immune response in diabetic retinopathy donors without apparent major vascular disruption. This study is consistent with a key role of inflammation and the innate immune response in the onset and progression of DR, and suggests a role of inflammation in neuroretinal disruption such as gliosis and neurodegeneration, potentially independently from advanced vascular alterations. (Supported by NIH EY020895 and P30 EY007003, Fight for Sight Grant-in-Aid, Eversight research grant).

3.4 Monocyte-Derived Macrophages in Diabetic Retinopathy

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Purpose: Diabetic retinopathy (DR) is associated with monocyte (Mo) retinal infiltration. Dyslipidemia is a risk factor of DR. We hypothesized that Mos exposure to systemic diabetic environment promote their differentiation into pro-inflammatory macrophages (Mφs). We analyzed the differentiation of Mos from a cohort of DR patients and compared it to the differentiation of naive Mos exposed to high glucose and/or high lipid concentration.

Methods: Mos from controls, T2DM (patients with no sign of DR), NPPDR (non-proliferative DR) and PDR (proliferative DR) were isolated from blood and allowed to differentiate into Mφs for 18 h. Naive Mos were differentiated in different glucose concentrations and palmitate (PA). Cytokine expression was quantified by RT-qPCR. Mφs-Secretome was tested for angiogenic activity in rat aortic rings.

Results: IL-6 and IL-8 are elevated in Mφs from the 3 diabetic groups and Mφs from DR groups exhibited elevated levels of VEGF and CCL2. Naive Mφs differentiated in Low Glucose, Normo Glucose or High Glucose conditions shown no difference in cytokine expression. Control Mφs differentiated in the presence of PA expressed elevated levels of all pro-inflammatory cytokines, but no VEGF. PA-treated Mφs secretome had a potent anti-angiogenic effect on rat aortic rings model.

Conclusions: Mφs differentiated from DR patients exhibited a specific inflammatory pattern. PA, but not HG, treatment of naive Mos partly replicates their differentiation. Secretome from PA-treated Mφs has a potent anti-angiogenic effect. Our data suggest that Mos/Mφs polarized by dyslipidemia are potential key players in initial retinal vessel loss.

3-5 Immune suppression as an alternative approach to control retinal angiogenesis

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Purpose: To investigate the therapeutic potential of the immune suppressive drug, tacrolimus, in angiogenic retinal diseases.

Methods: Bone marrow-derived macrophages (BMDMs) were cultured from C57BL/6 mice. The cells were then treated with either tacrolimus (4ng/ml, and 20ng/ml) or dexamethasone (0.1μM) under hypoxic (1% oxygen) or normoxic conditions for 24h. The mRNA expression of inflammatory cytokines (IL-1β, TNF-a, IL-6), chemokines (CCL2, CCL22), proangiogenic factors (VEGF, TGF-β, bFGF, PDGF, IGF) and anti-angiogenic factors (PEDF, TSP, TIMP, angiostatin, endostatin) were analysed by real-time RT-PCR. The therapeutic effect of tacrolimus in retinal angiogenesis was tested in an in vivo model of laser-induced choroidal neovascularisation (CNV) using a 0.02% tacrolimus/Semi Fluorinated Alkanes (SFAs) eyedrop. Mice treated with 0.1% dexamethasone eyedrop and anti-mouse VEGF (intravitreal) were used as controls.

Results: Tacrolimus reduced the expression of pro-angiogengic genes including VEGF, FGF2, TGFβ, PDGF and IGF in BMDMs in both normoxic and hypoxic conditions. Interestingly, tacrolimus also reduced the expression of anti-angiogenic genes including TIMP1, TSP1 and PEGF. Dexamethasone reduced VEGF expression but increased FGF2 and IGF expression in BMDMs under hypoxic condition. Dexamethasone also increased the expression of anti-angiogenic genes including TIMP1, TSP-1, PEDG and Col18a1 in BMDMs. Both 0.02% tacrolimus/SFA and 0.1% dexamethasone eyedrops significantly reduced laser-induced CNV. 0.02% tacrolimus/PBS suspension had no significant effect on laser-induced CNV.

Conclusions: Tacrolimus and dexamethasone suppress retinal angiogenesis through different mechanisms. Tacrolimus reduces the expression of pro-angiogenic growth factors, whereas dexamethasone enhances the expression of anti-angiogenic growth factors in macrophages.

Session 4: Gene Therapy Approaches in Treating Eye Diseases

4.1 Precision Genome Surgery for Imprecision Medicine

Stephen Tsang, MD, PhD, Department of Pathology and Cell Biology, Columbia University

This presentation will focus on a universal, mutation-independent genome surgery method that could be used to treat patients with autosomal dominant disorders. Our experimental study used a combination gene therapy using both gene ablation and gene replacement in human RHO mutation knock-in mouse models of retinitis pigmentosa (RP). Using dual adeno-associated viruses (AAVs), we (1) destroyed the expression of the endogenous Rho gene in a mutation-independent manner via an improved clustered regularly interspaced short palindromic repeats (CRISPR)-based gene deletion and (2) enabled expression of wild-type protein via exogenous cDNA. Our results suggested that the ablate-and-replace strategy can ameliorate disease progression as measured by photoreceptor structure and function for both of the mutation knock-in models. These results demonstrated the potency of the ablate-and-replace strategy to treat RP caused by different RHO mutations.

4.2 Knocking down blindness: a gene therapy for autosomal dominant retinitis pigmentosa

William A. Beltran[1], Artur V. Cideciyan [2], Valérie L. Dufour [1], Malgorzata Swider [2], William W. Hauswirth[3], Samuel G. Jacobson [2], Alfred S. Lewin [4], Gustavo D. Aguirre [1]. [1]Division of Experimental Retinal Therapies, Department of Clinical Sciences & Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104 USA; [2]Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania 19104 USA; [3]Department of Ophthalmology, University of Florida, Gainesville, Florida 32610; [4]Department of Molecular Genetics & Microbiology, University of Florida, Gainesville, Florida 32610.

Purpose: To use a light-sensitive canine model of *RHO*-adRP to validate a newly developed allele-independent corrective gene therapy approach that combines knockdown and replacement of *RHO* mRNA in a single AAV2/5 vector.

Methods: The AAV2/5 construct contains both an shRNA (*shRNA*$_{820}$, under H1 promoter control) that targets a homologous region of both human and dog *RHO*, and a resistant codon-modified human *RHO* cDNA (*RHO*$_{820}$) under control of a human opsin (hOP) promoter. This vector was subretinally-injected in one eye of 4 RHO$^{T4R/+}$ dogs (contralateral eyes with BSS). At 12, 24, 36 and 48 wks post-injection the retinas were challenged by an acute light exposure protocol that is used in this model to rapidly assess the effect of therapeutic intervention in preventing light-induced loss of rods. OCT and ERG were conducted during the course of the study. At termination (50 wks PI) retinal tissues were processed for immunohistochemistry.

Results: OCT analysis showed preservation of ONL thickness in the AAV-treated areas while no protection was seen in untreated areas nor in the BSS-treated regions. *In vivo* results were confirmed by IHC that showed preservation of ONL and both rod and cone inner and outer segments. ERGs showed significantly better rod- and cone-mediated function in the AAV- than in the BSS-treated eyes.

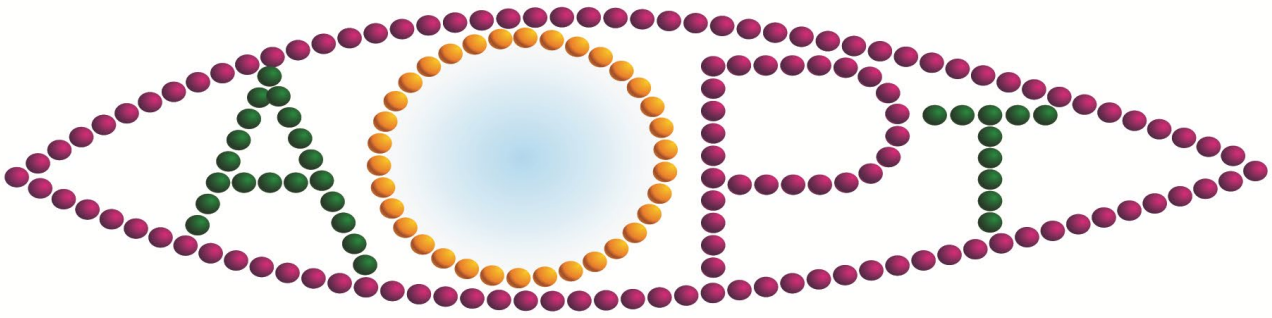
Conclusions: This long-term study (50 wks) expands our recent findings (Cideciyan et al. *PNAS*, 2018) that showed that scAAV2/5-hOP-*RHO*$_{820}$-H1-*shRNA*$_{820}$ prevents the onset of retinal degeneration in a naturally occurring large animal model of *RHO*-adRP, thus setting the stage for IND-enabling studies.

Support: Ophthotech Corp., NIH/NEI RO1-EY06855, Foundation Fighting Blindness.

4.3 Targeting the PERK arm of the Unfolded Protein Response in Retinal Degeneration

Marina Gorbatyuk, School of Optometry, Department of Optometry and Vision Science, The University of Alabama at Birmingham

PERK activates an Integrated Stress Response (ISR) resulting in eIF2a phosphorylation (p), temporal translational attenuation and selective ATF4 up-regulation. Notably, chronic ISR triggers apoptosis through GADD34. We previously reported that p-eIF2a, ATF4, CHOP and GADD34 up-regulation in rd16, T17M RHO and rd10 mice is associated with translational inhibition. Therefore, we aimed to validate p-eIF2a as a therapeutic target to interfere with retinal degeneration. The study used rd16 mice. Ablation of GADD34 and PERK were obtained by breeding the rd16 mice with GADD34 KO, PERKf/f and iCre mice. The role of PERK and eIF2a was also assessed using a PERK inhibitor, GSK2606414. We analyzed mice at postnatal day (P)18 using the scotopic ERG and at P15 using the SUnSet method, IHC, and protein analysis to assess p-eIF2a. rd16 GADD34-/- retinas demonstrated no detectable changes in ERG recording, substantial elevation of p-eIF2a by 68%, and diminished apoptosis by 40%. This diminishing was not associated with drastic changes in translational rate, suggesting no further correlation between p-eIF2a up-regulation and translational attenuation. On the other hand, PERK inhibition resulted in a 48% p-eIF2a decline and a small increase in translational rate. In addition, genetic PERK ablation in rd16 did not provoke dramatic ERG changes. Therefore, GADD34 ablation most likely caused preferential decline of the pro-apoptotic proteins, with no effect on the overall translational rate. Because p-eIF2a downregulation did not result in a full translational recovery, we hypothesize that p-eIF2a may have limitations for controlling protein synthesis under chronic ISR activation.



4.4 Gene therapy for Leber's Hereditary Optic Neuropathy

Jiajia Yuan[1], Bin Li[1,2], Yong Zhang[2], Hongli Liu[1], Yangyang Du[1], Dan Wang[1], Zhen Tian[2], Xin Li[2]. [1]Department of Ophthalmology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China,[2]Department of Ophthalmology, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei Province, China;

Purpose: To study the safety and efficacy of gene therapy for Leber's hereditary optic neuropathy.

Methods: More than 100 patients with Leber's hereditary optic neuropathy mutation at site 11778G>A were recruited, including 3 Taiwan compatriots and 10 patients from Argentina. AAV2-ND4 was injected into the vitreous cavity and followed up at 1,3,6,12 months after treatment. Eye examination includes BCVA, visual field, and so on. Periodic results were obtained from March 2017 to January 2019, and the current clinical results are now reported.

Results: There is almost no complication occurred in our team's gene therapy for the more than 100 patients. Currently, 100 patients have been re-examined six months after treatment and he total effectivity is about 50%. Both the BCVA of the patients within 24 months($p<0.01$) and over 24 months($p<0.01$) were improving. None of the 10 patients in Argentina had any complication after treatment, and the BCVA of all 10 patients have improved significantly.

Conclusion: Gene therapy for Leber's hereditary optic neuropathy is safe and effective.

Session 5: Young Investigators Session

5.1 AAV delivery of modified erythropoietin (EPO) therapy delays retinal degeneration in a mouse model of geographic atrophy

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Purpose: We established a mouse model of retinal degeneration by inducing oxidative stress in the retinal pigment epithelium (RPE). The purpose is to determine whether delivery of modified form of erythropoietin (EPO-R76E) using an adeno-associated virus (AAV) can prevent retinal degeneration seen in these mice.

Methods: Mouse model of RPE (retinal pigment epithelium) oxidative stress was induced by exon specific deletion of the protective enzyme MnSOD (Sod2) by Cre/Lox mechanism. Retinal degeneration was monitored by electroretinography (ERG) and spectral domain optical coherence tomography (OCT) over a period of 9 months. Experimental mice (six weeks) received subretinal injection of a recombinant AAV serotype 1 vector expressing modified erythropoietin (EPO-R76E) in the right eye and AAV1-GFP in left eye.

Results: Following doxycycline induction of Cre, mice demonstrated increased signs of oxidative stress in RPE and a gradual decline in the ERG response and thinning of the outer nuclear layer (by SD-OCT) which were statistically significant by 6 months. EPO-R76E over- expression in RPE by the AAV vector delayed the progressive retinal degeneration as measured by ERG responses and outer nuclear thickness by SD-OCT in RPE specific Sod2 knockout mice at 6 months and 9 months of age.

Conclusions: Deletion of Sod2 in the RPE leads to some of the salient features of dry AMD. Subretinal AAV1 delivery of EPO-R76E led to RPE specific expression. Delivery of EPO-R76E vector can be used as a tool to prevent retinal degeneration seen in this mouse model.

5.2 Degradable fibrin scaffolds for induced pluripotent stem cell (iPSC)-retinal pigment epithelium (RPE) Transplantation Using a Pig Model

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Purpose: We previously demonstrated in vitro that human fibrin can produce biodegradable scaffolds capable of supporting growth and differentiation of iPSC-RPE monolayers. As a precursor to a pre-clinical safety trial of iPSC-RPE+fibrin, we first must demonstrate that the fibrin is safely degradable within the subretinal space.

Methods: Fibrin gels were cast by mixing human fibrinogen (40mg/mL) and thrombin (50U/mL) solutions in a custom mold, punched to produce 1.5mm x 5.0mm implants, and stained with trypan blue for visualization. Scaffold degradation was assessed following implantation in the subretinal space of the domestic pig. Post-surgery, animals were monitored up to 12 weeks, with follow-up indirect exams, fundus photos, OCT, and postmortem histology.

Results: After implantation, the fibrin gel, 204±32µm thickness, was visible under the retina. After 2 days, the retina is flattened but appears elevated above the implant; histology confirmed the gel was placed successfully within the subretinal space without damage to the surrounding tissue. The scaffolds lost their blue color within 3 days, and the gel shape began to shrink after 7 days. By the 8th week, there were no detectable signs of the scaffold (n=4). Histology at 8 weeks shows mild inflammation at the retinotomy consistent with cautery use. No evidence of the implant or histological changes was present in the region of the implant.

Conclusion: Fibrin hydrogel scaffolds implanted in the subretinal space degrade within 8 weeks without any detectable adverse effects. Fibrin gels offer a bio-degradable scaffold for use in RPE transplantation, improving upon current long-lasting scaffolds.

5.3 Pregabalin Microemulsion Once Daily Eye Drops for Management of Glaucoma

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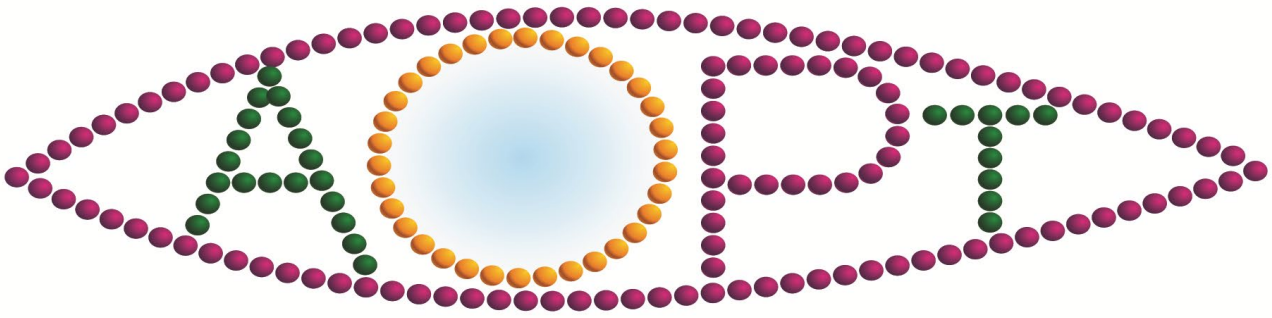
Purpose: Our study was designed to develop a novel pregabalin-loaded topical ophthalmic microemulsion-based formulation as once-daily IOP-lowering drops to overcome the drawbacks of currently available glaucoma therapies.

Methods: We engineered mucoadhesive W/O/W microemulsion containing pregabalin and characterized it using multiple in-vitro and in-vivo evaluations including: particle size, zeta potential, viscosity and mucoadhesion, drug release, corneal permeability, cytotoxicity, in-vivo safety and efficacy studies.

Results: Our microemulsion was engineered using highly biocompatible components with in-situ gelling properties that improve viscosity and bioadhesion, enhance corneal penetration and provide continuous drug release for 24h. Because our microemulsion has miniscule particle size (< 20nm), it is transparent and does not blur vision. Our formulation is safe to the eye, as demonstrated with MTT assay and slit-lamp biomicroscopic exams, which showed that there is no any sign of irritation. Also, our formulation markedly enhances pregabalin efficacy. Using Dutch belted rabbits, we effectively demonstrate that a single drop of our microemulsion can induce 42.3% reduction in IOP that returned to baseline at after 34h. In the absence of our microemulsion, the same drug produced only 29% IOP reduction that returned to baseline after 10h.

Conclusions: We have engineered novel topical ophthalmic formulation that supported once daily dosing of pregabalin as a novel IOP-lowering therapy. Our formulation greatly improves pregabalin IOP-lowering efficacy both in terms of amplitude and duration of response, which is supported by improved mucoadhesion, increased corneal permeability, penetration enhancing ability and sustained release behavior. If replicated in prospective clinical trials, our formulation could revolutionize glaucoma therapy.



5.4 Vasoregulators mediate distal vessel lumen diameters and outflow facility in human anterior segments

Fiona McDonnell[1], Alex A. Huang[2], Joseph M. Sherwood[3], W. Daniel Stamer[1,4]

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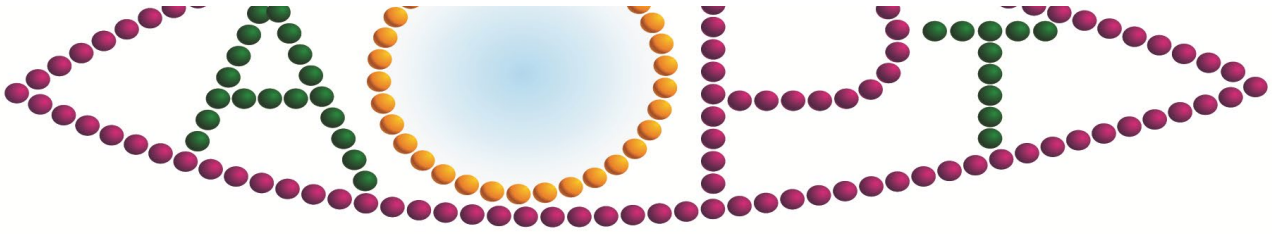
Purpose: The trabecular meshwork (TM) and Schlemm's canal (SC) are well established as the primary source of outflow resistance. We recently investigated the role of vessels distal to SC in outflow resistance and found that they are responsible for up to 50% of total resistance, and that their vasomotion affects outflow facility. Thus, IOP-lowering therapies may be more effective if targeted to both the proximal and distal regions of the conventional outflow pathway. Here we visualize the effects of the vasoregulators, endothelin-1 (ET-1) and diethylenetriamine/nitric oxide (DETA-NO) on distal vessel lumen diameter.

Methods: We used the human anterior segment perfusion model under constant flow conditions (2.5µl/min) while continuously monitoring intrachamber pressure using iOnlyHuman software. Cadaveric eyes were trabeculotomized prior to perfusion and a stable baseline was achieved before treatment. We imaged the vessels by SD-OCT using the Spectralis FLEX and conducted image analysis in ImageJ.

Results: In trabeculotomized anterior segments, we found that ET-1 (100nM) effectively decreased outflow facility, which coincided with a visualized decrease in the lumen diameters of intrascleral vessels (~40% decrease) and collector channels (~35% decrease). In contrast, when the nitric oxide donor, DETA-NO (100µM), was introduced by chamber exchange lumen diameters of intrascleral vessels (~190% increase) and collector channels (~170% increase) increased in conjunction with increased outflow facility.

Conclusions: Our data shows that the endogenous vasoregulators ET-1 and DETA-NO affect distal vessel lumen diameter, which in turn affects outflow facility. Therefore, targeting these regions of the outflow pathway, particularly after MIGs procedures may be therapeutically beneficial.



5.5 A CRISPR-based inducible system for VEGF repression for AMD

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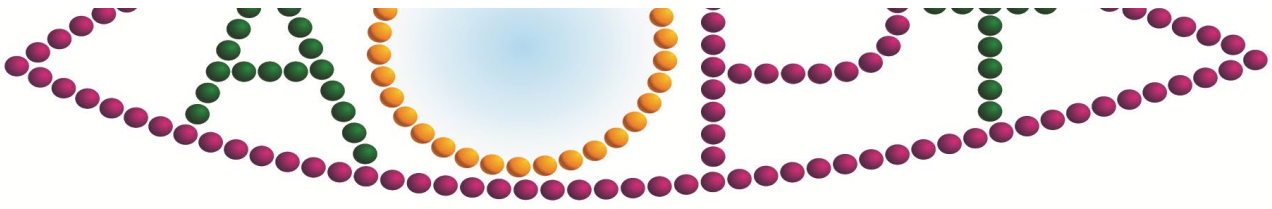
Purpose: Age-related macular degeneration (AMD) is the primary cause of irreversible blindness in the elderly. The current paradigm for wet AMD treatment is monthly intravitreal injection of anti-VEGF antibodies, which poses a significant economic burden to the patients. Continuous blocking of VEGF may have adverse effect in choroid capillaries and the ciliary body. We propose a gene therapy approach to drive inducible and reversible VEGF repression using a CRISPR-based system, which could avoid repetitive intravitreal injection while achieving controllable VEGF repression.

Methods: A series of ARPE-19 cell lines have been established. In these cells, guide RNAs targeting human VEGF promoter were constitutively expressed, while dCas9-Krab expression was reversibly controlled by doxycycline (Dox). Dox induction of dCas9-Krab expression was confirmed by Western blot analysis and immunostaining. Teal-time PCR and ELISA were employed to quantify the VEGF expression before and after Dox treatment. Laser injury model is being used to evaluate the efficacy of the approach *in vivo*.

Results: Induction and dosage-response of CAs9-Krab expression were confirmed by Western blot analysis and immunostaining. Guide RNAs have been optimized to control VEGF expression in ARPE-19 cells. Up to 70% reduction of VEGF mRNA and protein expression has been achieved in the system. Moreover, conditional medium from these cells significantly repressed endothelial cell proliferation and angiogenesis *in vitro*.

Conclusions: We have established a CRISPR-based reversible VEGF repression system, which could be used to treating diseases including AMD and diabetic retinopathy.



5.6 TRPV4 -dependent calcium influx regulates strain-induced neurodegenerative pathways in retinal ganglion cells

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Purpose: Mechanical deformations of the eye can lead to neuronal damage, inflammation and may contribute to optic neuropathies but the identity and properties of mechanotranducers are not well understood. Our goals are to define the molecular mechanisms that drive RGC mechanotransduction in vitro and in vivo.

Methods: Intraocular pressure in C57BL/6, TRPV4-/- and TRPV4flox/Thy1-/- mouse retinas was elevated through injection of microbeads in the ipsilateral eye. RGCs were isolated by magneto-separation (MACS) and exposed to cyclic biaxial strains in the presence/absence of mechanosensitive ion channel inhibitors. Immunolabeling, Ca-imaging and qRT-PCR were utilized to determine the changes in gene and protein expression, and localization.

Results: Radial stretch evoked dose-dependent elevations in [Ca2+]i that were suppressed by TRPV4 channel antagonists and mimicked by TRPV4 agonists. The effects of stretch were associated with altered gene expression/trafficking of ER stress and pro-apoptotic proteins as well as putative mechanotransducers. The fraction of pro-apoptotic cells was markedly lower in preparations isolated from TRPV4-/- retinas, in retinas with conditionally ablated TRPV4 channels and following exposure to TRPV4 antagonists. Conditional KO retinas showed significantly less ER stress and reactive gliosis.

Conclusions: Tensile stretch regulates TRPV4- and calcium-dependent pro-apoptotic pathways previously linked to glaucomatous neurodegeneration. Conditional ablation of TRPV4 showed a protective phenotype that included reduced ER stress and less inflammation. These data support the view that elevated IOP acts on nonaxonal targets within the retina, with pressure-induced signaling within the somatodendritic compartment sufficient to compound RGC injury.

Session 6: New Approaches for Treating Age-related Macular Degeneration (AMD)

6.1 The Role of Mitochondrial Dysfunction in the Pathogenesis of Dry Age-Related Macular Degeneration: From Concept to Clinic for the Mitochondria-directed Drug, Elamipretide

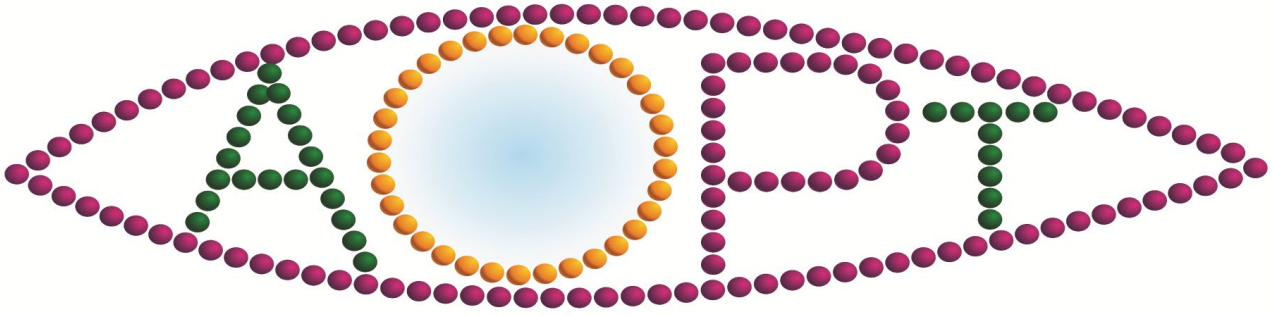
Scott W. Cousins, Duke Eye Center, Duke University

Purpose: Mitochondrial dysfunction has been proposed as one major mechanism for the pathogenesis of drusen and geographic atrophy. This presentation will review rationale and data to support Elamipretide, a novel mitochondrial-directed drug, as a potential therapeutic for dry AMD.

Methods: RPE cell culture, mouse models and human subjects were utilized.

Results: In vitro and in vivo data provided "proof-of-concept" rationale for proceeding into a clinical trial for Elamipretide. Preliminary results of the Phase 1 ReCLAIM trial of subcutaneously administered Elamipretide in drusen and in geographic atrophy patients will be presented.

Conclusions: Mitochondrial directed therapies, and specifically Elamipretide, offer promise as treatment for vision loss associated with drusen and with geographic atrophy.



6.2 TFEB (transcription factor, EB) as a potential therapeutic target for AMD

Debasish Sinha[1,2], Nadezda Stepicheva[1], Sayan Ghosh[1], Stacey Hose[1], Sara Jabalameli[1], Imran A. Bhutto[1], Peng Shang[1], Meysam Yazdankhah[1], Joseph Weiss[1], Gerard A. Lutty[2], J. Samuel Zigler Jr.[2], Leah Byrne[1]

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Purpose: While rapamycin, or rapalogs, have shown potential therapeutic efficacy for age-related pathologies in animal models, significant side effects limit their use in humans. This study was undertaken to test our hypothesis that rejuvenating impaired lysosomal function in the RPE by activating transcription factor, EB (TFEB) will intensify lysosomal biogenesis and thereby delay or reverse the progression of early/dry AMD.
Methods: We engineered AAV2 and AAV8-tyrosine mutant-mediated WT-TFEB and constitutively active TFEB-S210A constructs for gene therapy studies. Primary culture of RPE cells from *Cryba1* KO mice was infected with TFEB-WT-AAV vector or TFEB-S210A-AAV vector for 24 h at a dose of 1 X 10^9 infective units/ml. Western blotting (WB) and quantitative PCR (QPCR) techniques were used to detect target gene expression at either the protein or mRNA level for the CLEAR (Coordinated Lysosomal Expression and Regulation) network. Human AMD and age-matched control sections from the posterior pole were immuno-labeled with antibodies to TFEB and co-stained with DAPI.
Results: Our data showed reduced nuclear TFEB staining in early AMD patient tissues compared to aged-matched controls. Cultured KO RPE cells showed that overexpression of constitutively active TFEB-S210A increased mRNA expression of CLEAR network genes like CTSB (Cathepsin B), LAMP2 and ATP6VOA1 (V-ATPase). Moreover,

WB data indicated increased expression of CTSD (Cathepsin D), along with a decrease in the expression of the autophagosome marker, p62/SQSTM1.
Conclusion: These studies support our hypothesis that TFEB can induce lysosomal activity in the RPE cells, which can delay or reverse the pathologies seen in early/dry AMD.

Acknowledgements: This work was supported by Research to Prevent Blindness (RPB) Catalyst Award for Innovative Research Approaches to AMD (DS), RPB unrestricted grant to the University of Pittsburgh and funds from the Jennifer Salvitti Davis, M.D. Chair Professorship in Ophthalmology (DS).

6.3 The Conundrum of Targeting the Complement Pathway to Treat AMD – Lessons from Animal Models

Michael Landowski, Una Kelly, Mikael Klingeborn, Marybeth Groelle, Jin-Dong Ding, and Daniel Grigsby and **Catherine Bowes Rickman**

Duke Eye Center, Duke University School of Medicine, Durham, NC, USA

Purpose: One of the strongest susceptibility genes for age-related macular degeneration (AMD) is complement factor H (CFH); however, how it contributes to AMD pathobiology is still unclear and clinical trials of complement inhibitors in AMD patients have failed. To help clarify this apparent paradox, the effect of the principal AMD-risk associated CFH variant (Y402H) on the development and progression of age-dependent AMD-like pathologies was determined in vivo.

Methods: Transgenic mice expressing equal amounts of the full-length normal human CFH Y402 (CFH-Y/0) or the AMD-risk associated CFH H402 (CFH-H/H) variant on a Cfh-/- background were aged to 90 weeks and switched from normal diet (ND) to a high fat, cholesterol-enriched (HFC) diet for eight weeks. The resulting phenotype was compared to age-matched controls maintained on ND. ERGs and plasma samples were obtained prior to sacrifice. Eyes were collected to assess retinal pigmented epithelium (RPE) damage by histological examination.

Results: Strikingly, an AMD-like phenotype including vision loss and RPE damage was detected only in aged CFH-H/H mice following the HFC diet. Genotype-dependent changes in plasma and eyecup lipoproteins, but not complement activation were detected and positively correlated with the AMD-like phenotype in aged CFH-H/H mice.

Conclusion: For the first time, we show that aged mice expressing the human H402, but not the Y402 variant, develop AMD-like changes. Interestingly, H402 mice display differences in their systemic and ocular lipoprotein levels, but not complement activation following the HFC diet. These findings support targeting lipoproteins, not complement, for the treatment of AMD.

Funding: We gratefully acknowledge funding from NIH NEI R01 EY026161 (CBR); P30 EY005722 (Core); RPB/IRRF Catalyst Award for Innovative Research Approaches to AMD (CBR), RPB unrestricted grant to the Duke Eye Center and a Fighting Blindness Individual Investigator Award (CBR)

6.4 A roadmap to find treatment for dry AMD

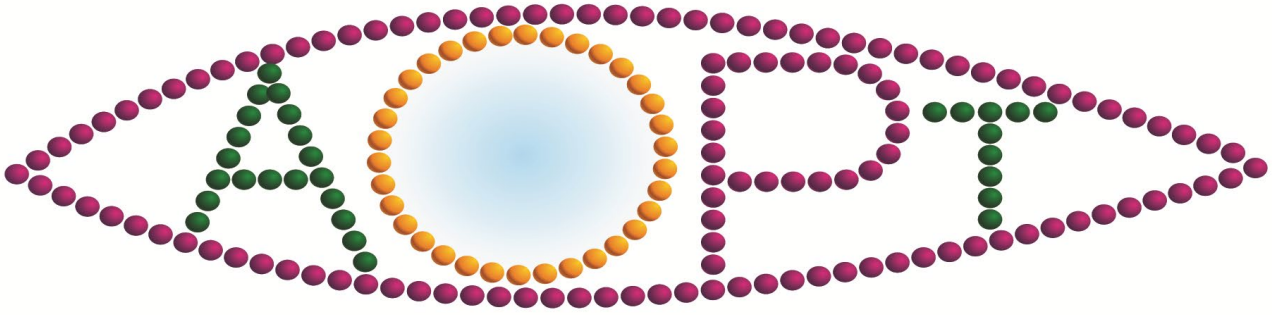
James T. Handa, MD, Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD

Purpose: Despite over 30 years of intensive research, no treatment has been developed that effectively treats dry AMD. The purpose is to describe a roadmap for developing effective AMD treatment.

Methods: Review of critical work on AMD pathobiology

Results: Potential targets for treating AMD will be presented. The potential roadblocks of what prevents these targets from being transitioned to treatment will be discussed, focusing on the need for determining the relative contribution of these pathways, at what disease stage they are most influential, and a strategy for uncovering novel pathways that could be involved in AMD pathogenesis by using human AMD tissue.

Conclusions: Prioritizing the contribution of known dysregulated pathways and when these pathways cause AMD by studying AMD tissue at different disease stages may accelerate the development of effective treatment for dry AMD.



6.5 Targeting soft drusen in age-related macular degeneration (AMD): rationale and pre-clinical studies of an apolipoprotein mimetic peptide

Christine A. Curcio,[1] Yoko Miura[2], Martin Rudolf[2]. Ophthalmology, University of Alabama School of Medicine

Purpose: 1) To review the biology of human soft drusen, the major intraocular risk factor for AMD progression; 2) to present pre-clinical studies of an apolipoprotein (apo) mimetic peptide to clear lipids in Bruch's membrane (BrM).

Methods: 1) literature is summarized (PMID 30357336); 2) an 18-amino acid apoA-I mimetic peptide (L-4F) or a scrambled peptide was delivered intravitreally to

10-11-month-old apoE-deficient mice (single dose) or macaque monkeys >20 years of age (ascending doses over 6 months). Eyes were evaluated via histochemistry, electron microscopy, pharmacokinetics (mouse), and clinical imaging (monkey) (PMID 28972410; IOVS in press)

Results: 1) The main component of soft drusen is 'membranous debris' that represents partly preserved lipoprotein particles accumulating in BrM and backing up towards the retinal pigment epithelium (RPE) throughout adulthood. Peroxidized BrM lipids promote inflammation and neovascularization. Clinical imaging links druse growth to RPE death. Genetics, epidemiology, gene expression, and a layer of deposits in the subretinal space together suggest an intraocular network of lipid genes serving cone- and rod physiology, respectively. 2) Animals receiving peptide L-4F exhibited diminished histochemically detectable esterified cholesterol and a thinner and more compact BrM relative to controls. Fluorescein-tagged peptide accessed murine BrM at 1 day and persisted for 14 days. Macaque fundus lesions represented lipoidal degeneration of RPE, which was unaffected by L-4F treatment while BrM cleared.

Conclusions: Targeting soft drusen to prevent AMD end-stages has strong biologic basis. Pre-clinical tests of an apolipoprotein mimetic peptide are promising. Multimodal imaging is required to confirm soft drusen in animal models.

Session 7: Current Neuroprotective Therapies for Glaucoma

7.1 Synaptic disassembly and rewiring of the adult retina in a mouse model of glaucoma

Luca Della Santina, Alfred Yu, Kelly Mai, Alan Tran, Yvonne Ou.

Department of Ophthalmology, University of California, San Francisco. U.S.A.

Purpose: Synaptic disconnection between retinal ganglion cells (RGCs) and bipolar cells (BCs) is among the earliest events detected in the inner retina following to intraocular pressure (IOP) elevation. It is not known how this process takes place, if there is selectivity in synapse disconnection and whether the adult retina has potential for rewiring of lost synapses.

Methods: Laser photocoagulation of episcleral and limbal vessels was performed unilaterally in adult CD-1 mouse eyes, generating IOP elevation lasting 7 days. Individual RGCs and their PSD95 were labeled biolistically. Ribbons were immunolabeled with CtBP2, Type6 and rod BC were immunlabeled with Synaptotagmin-2 and PKCalpha. Synaptic connectivity was measured by colocalization analysis in 3D using ObjectFinder on confocal stacks at 7, 14, and 30 days after IOP elevation. Longitudinal flash ERG recordings were performed at these same time points.

Results While all types of alpha RGCs (aRGCs) lose ribbons prior to PSD95, ON-sustained aRGCs lose their original BC connectivity pattern while OFF-sustained aRGCs regain it over time. Surprisingly, 30 days after IOP elevation, we found evidence of rewiring of ON-sustained aRGCs with rod bipolar cells. The ERG scotopic b-wave amplitude was initially reduced but eventually recovered to control level 30 days after IOP elevation.

Conclusions: aRGCs show diverse strategies of synaptic disconnection in response to IOP elevation, potentially explaining their different functional changes. ON-sustained aRGCs rewire over time to rod bipolar cells. These data suggest that the adult diseased retina exhibits circuit-level plasticity that can be leveraged for early disease detection and targeted treatment.

7.2 Higher Reliance on Glycolysis Limits Responsiveness in Degenerating Glaucomatous Optic Nerve

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[1]*Department of Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH, USA*

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Purpose: Metabolic dysfunction accompanies neurodegenerative disease. An important step for therapeutic development is to distinguish disease-associated changes from aging effects in metabolism. We found decreases in maximal respiration with aging by examining oxygen consumption rate in ex vivo optic nerve using the Seahorse Analyzer, a novel approach for investigating mitochondrial function in axons.

Methods: Acutely isolated optic nerves (ON) from DBA/2J and DBA/2-*Gpnmb*$^{+}$ mice at 3, 6 and 10 months of age were sectioned and secured in 24-well islet Seahorse plates. ONs were sequentially subjected to an ATP synthase inhibitor, a protonophore, and a Complex III inhibitor, with or without the aconitase inhibitor fluorocitrate. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured.

Results: ECAR, the discharge of protons from lactate release or byproducts of substrate oxidation, was significantly higher in the degenerating ON. In the presence of fluorocitrate, OCR was higher, with more ATP produced, in degenerating compared to aged ON. However, degenerating ON showed lower maximal respiration. With fluorocitrate and challenged with ATPase inhibition, the degenerating ON was incapable of further upregulation of glycolysis to compensate for the loss of oxidative phosphorylation. Inclusion of 2-deoxyglucose as a substrate during ATPase inhibition indicated a significantly higher proportion of ECAR was derived from substrate oxidation than glycolysis in degenerating ON.

Conclusions: These data indicate that degenerating axons possess no additional capacity for times when oxidative phosphorylation would fail, as in anaerobic conditions. The higher ATP output from axonal mitochondria in degenerating ON may compensate for this lack of resiliency.

7.3 Interleukin-6 in Retinal Health and Disease

Rebecca M. Sappington,[1,2,4] Franklin D. Echevarria,[3] Lauren Wareham,[4]

[1] Dept. Ophthalmology and Visual Sciences, [2] Dept. Pharmacology, [3]Neuroscience Graduate Program, Vanderbilt University School of Medicine; [4] Vanderbilt Eye Institute, Vanderbilt University Medical Center

Purpose: Interleukin-6 (IL-6) is a pleiotropic cytokine associated with a variety of ocular disorders and injuries, including retinal ganglion cell (RGC) degeneration. However, IL-6 exhibits both neuroprotective and neurodestructive outcomes for RGCs. Here, we investigated constitutive activities of IL-6 to begin resolving these ambiguities in IL-6 outcomes for RGCs.

Methods: We examined outcomes of structural and functional physiology in the early visual pathway of mice with germline IL-6 deficiency (*IL-6 -/-*). Neonatal (P3, P7, P21) or adult (2-3 mo) male and female *IL-6 -/-* mice (B6.129S2-IL6tm1kopf/J) and respective genomic controls (B6129SF2/J; WT) were used for all experiments. We further examined IL-6 outcomes in purified primary cultures of RGCs treated with colchicine.

Results: In situ hybridization studies indicated that IL-6 is expressed by RGCs throughout post-natal development and in maturity. Neural tracing studies revealed a reduction in the rate of anterograde axon transport in *IL-6-/-* mice, as compared to WT ($p < 0.05$). Transcriptome analysis, protein expression and histological studies revealed that this is accompanied by disruption of microtubules and subsequent motor protein trafficking in *IL-6-/-* mice ($p < 0.05$). *In vitro* studies confirmed a direct effect of IL-6 on microtubule stability in RGCs.

Conclusions: We identify, for the first time, that constitutive IL-6 signaling influences structure and function of RGC axons. Our findings: 1) provide indications for how IL-6 may promote neuroprotection and repair of RGCs and 2) highlight the critical need to consider constitutive functions of inflammatory factors, when assessing their role pathology and potential for therapeutic targeting.

7.4 The role of alpha crystallins and neuroprotection in glaucoma

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Purpose: To determine if the core peptide derived from aB-crystallin (*Peptain-1)* could promote neuroprotection of retinal ganglion cells (RGCs) in rodent models of glaucoma.

Methods : Primary rat RGCs and retinal explants were exposed to either normoxic or hypoxic conditions in the presence of either *Peptain-1* or a scrambled peptide and RGC survival was assessed. Rats were IOP-elevated in one eye using the Morrison's method and intraperitoneally (i.p.) injected with *Peptain-1* three times per week for five weeks and surviving RGCs and axons were counted. Cytochrome c oxidase complex 6b2 (COX6B2) levels were assessed. Ischemia reperfusion (I/R) injury was performed in C57BL/6 mice and *Peptain-1* injections were given 3h prior and immediately after the I/R, surviving RGC were counted 14 days post-I/R injury. In separate experiments, mice were i.p. injected with *Peptain-1*-Cy7 to determine its ability to cross the blood retinal barrier and penetrate into RGCs.

Results: *Peptain-1* treatment decreased (by 60%) hypoxia-induced primary RGC death ($p< 0.001$) and significantly ($p< 0.001$) enhanced RGC survival (3.5-fold) in retinal explants, compared to scrambled peptide control. I.p. injections of *Peptain-1* significantly inhibited RGC death ($p< 0.05$), reduced axonal loss ($p< 0.02$) in rats and showed increased COX6B2 levels. *Peptain-1* enhanced RGC survival by 50% ($p< 0.01$) after I/R injury in mice, compared to those treated with the scrambled peptide. *Peptain-1*-Cy7 was detected in the retina following its i.p. injection.

Conclusions: The i.p injected *Peptain-1* exhibits soma-, axo- and mito- protective properties, which could facilitate neuroprotection against insults that cause RGC death in rodents.

7.5 A simple chronic ocular hypertensive murine model of glaucoma – opportunities for neuroprotection studies

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Purpose: Glaucoma is an optic neuropathy commonly associated with elevated intraocular pressure (IOP) resulting in progressive loss of retinal ganglion cells (RGCs) and optic nerve degeneration. New therapeutic approaches that better preserve the visual field by promoting RGC survival are highly needed. We have developed a new murine glaucoma model to facilitate the assessment of novel neuroprotective agents (Guo et al., 2018 PMID: 29949582).

Methods: Photopolymerizable hyaluronic acid glycidyl methacrylate (HAMA), mixed with microbeads, was injected into the anterior chamber and cross-linked to the iridocorneal angle by UVA light to impede the aqueous humor outflow. IOP was measured by a TonoLab under isoflurane. RGC loss and glial cell reactivities were assessed in retinal flatmounts immunostained with BRN3A, GFAP and IBA1. Axon loss was quantified in optic nerve cross sections. Total retinal RNA was purified for RNAseq to detect transcriptomic changes induced by ocular hypertension.

Results: HAMA/microbeads crosslinking induced sustained IOP elevation ~45% above baseline for >4 weeks. Significant RGC death and optic nerve degeneration were noted within one month (~30% RGC/axon loss). Activation of glial cells appeared to be an early event preceding detectable RGC degeneration. Transcriptome profiling revealed differential expression of genes involved in neuroinflammation, mitochondrial and apoptosis pathways, as well as genes regulating RGC survival and neurite regeneration.

Conclusions: This new murine model has demonstrated molecular and cellular features recapitulating key pathophysiological changes known to occur in human glaucoma and in other preclinical models. It is envisioned that this preclinical model can be used for glaucoma neuroprotective studies.

Disclosure: Authors are all employees of Novartis Institutes for Biomedical Research (NIBR) and funding for this work was provided by NIBR. Authors may own stock of Novartis.

Session 8: Discovery and Development of Novel Ocular Therapies

8.1 Clusterin regulates intraocular pressure by modulating extracellular matrix in trabecular meshwork outflow pathway

Padmanabhan Pattabiraman[1], Sai Supriya Vuda[1,2], Jaeyoung Heo[1]. [1]Department of Ophthalmology and Visual Sciences; [2]Case Undergraduate Program (Chemical Biology); [3]Case Western Reserve University, Cleveland, Ohio, USA.

PURPOSE: To understand the functional role of the secretory chaperone protein clusterin in the regulation of intraocular pressure.

METHODS: Effect of adenovirus-mediated overexpression of clusterin (AdCLU) expression in outflow pathway tissue on IOP and ECM changes assessed in human anterior segments and in wild-type mice (C57BL6-SVJ129). Effect of AdCLU on actin, extracellular matrix (ECM) like collagen-1A and fibronectin and cysteine protease cathepsin k in HTM cells were determined by immunofluorescence, qPCR, immunoblotting, and ELISA analyses. Students t-tests were used for statistical analyses and results were significant if $p<0.05$ with a sample size of N=4-8 in each experiment.

RESULTS: Perfusion of AdCLU for 24h in cadaveric human anterior segments (N=5) or AdCLU injection into the anterior chamber lowered IOP significantly. The maximum decrease in IOP was 29.2±5 % (Mean±SEM). The IOP lowering effect in mice was found after 3 days post-injection and lasted up to 10 days. The IOP decrease corroborated with a lower staining for the collagen1A and fibronectin in TM-JCT tissue both ex vivo and ex vivo. Increase in clusterin secretion due to AdCLU was confirmed by elevated levels of clusterin in pooled (N=6) mice AH detected by ELISA. AdCLU increased clusterin expression and secretion led to rounded cells with intact nuclei, decreased actin stress fibers, ECM (collagen1A and fibronectin) indicating the loss in cell tension, and the cysteine collagenolytic cathepsin k (CTSK) mRNA, protein and activity.

CONCLUSION: We have identified a novel role of clusterin in maintaining optimal cell-matrix interactions via its effector CTSK to regulate IOP homeostasis.

ACKNOWLEGEMENTS: Funding from The Glaucoma Research Foundation (Shaffer Grant), and EVERSIGHT Eye and Vision Research Grant to PP, SOURCE Summer Fellowship and Sigma Xi Grant-In-Aid to SSV, Departmental RPB Support and P30 Core Grant (EY11373).

8.2 Ligandomics for retinal angiogenesis drug discovery

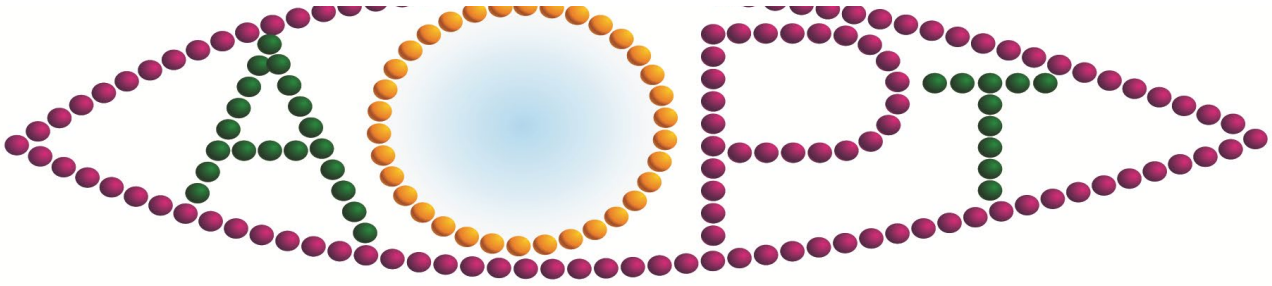
Wei Li[1], Tian Hong[2]. [1]Bascom Palmer Eye Institute, Department of Ophthalmology, University of Miami School of Medicine, Miami, Florida, USA. [2]LigandomicsRx, LLC, Miami, Florida, USA.

Purpose: Cellular ligands are traditionally identified on a case-by-case basis with technical challenges. It is even more daunting to identify ligands with pathogenic role and therapeutic potential. The purpose of this study is to develop an innovative technology of "ligandomics" for systematic identification of disease-selective angiogenic factors and development of ligand-guided targeted anti-angiogenic therapies for retinal diseases.

Methods: We developed ligandomics as the only technology to globally map cell-wide ligands with simultaneous binding activity quantification. We applied "comparative ligandomics" to live mice with or without diabetic retinopathy (DR) for systematic profiling of diabetes-selective retinal endothelial ligands. Identified ligands were characterized for their functional activity using *in vivo* corneal angiogenesis and retinal vascular leakage assays. We developed ligand-specific neutralizing monoclonal antibodies (mAbs) and characterized their therapeutic activity to alleviate DR leakage and pathological neovascular iz

Results: Comparative ligandomics identified secretogranin III (Scg3) as a DR-selective endothelial ligand that preferentially bound to diabetic but not healthy retinal vessels. *In vivo* functional assays revelated Scg3 to selectively stimulate corneal angiogenesis and retinal vascular leakage in diabetic but not healthy mice. In contrast, VEGF bound to both diabetic and control retinal vessels, and promoted corneal angiogenesis in both diabetic and healthy mice. Intravitreally injected Scg3-neutralizing mAbs antibodies ameliorated DR leakage in diabetic mice and pathological retinal neovascularization in oxygen-induced retinopathy (OIR) mice with minimal side effects.

Conclusions: These findings suggest that anti-Scg3 mAb is a highly disease-selective angiogenesis blocker with high safety profile and that ligandomics is a powerful technology for drug target discovery.



8.3 Metabolomics for ocular drug discovery

Sanjoy K. Bhattacharya[1], Mathew Merritt[2], Ram Khattri[2], Chris Beecher[3], Ciara Myer[1], Muhammad Zain Chauhan[1] and Anna K. Junk[1]. [1]Bascom Palmer Eye Institute, University of Miami, Miami, Florida, 33136; [2]Department of Biochemistry & Molecular Biology, University of Florida, Gainesville, FL 32610; [3]Miami VA Healthcare System, 1201 NW 16th St, Miami, FL 33125

Purpose: To perform metabolomics analysis of aqueous humor (AH) from control and primary open angle glaucoma subjects.

Methods: We performed metabolomics analysis of AH using 1D NMR and mass spectrometry based isotopic ratio outlier analysis (IROA). In our pilot analysis, control and glaucoma subjects (n= 10 each) derived aqueous humor (AH) samples were subjected to 1D NMR. Metabolites were confirmed using heteronuclear single quantum coherence (HSQC) and total correlation spectroscopy (or TOCSY) experimental analysis. The same samples were then subjected to IROA using established standards and were analyzed using Clusterfinder software.

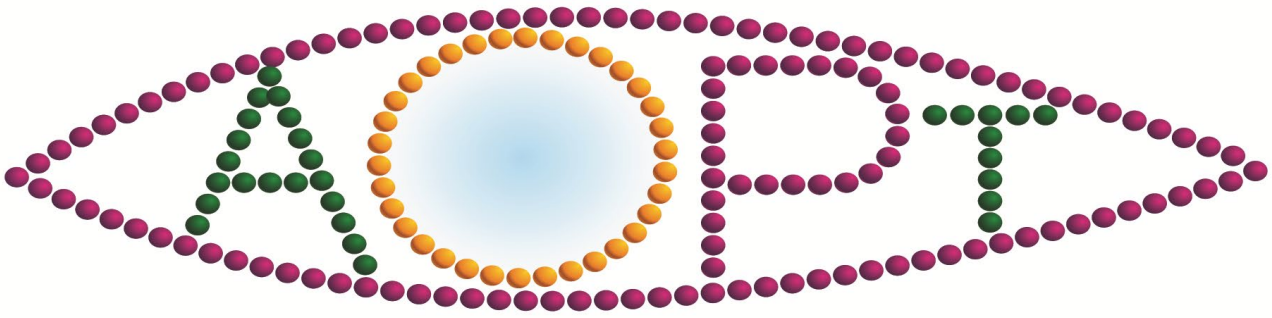
Results: We were able to assign several aliphatic and aromatic compounds in the spectra of AH samples. We were successful in determining the concentration of metabolites in the AH using NMR analysis.

Conclusions: Our pilot analysis identified several metabolite signatures in the NMR spectrum. We identified a few potential biomarkers in the 1D and 2D NMR spectra. Identification of these compounds may open up their use in drug discovery. The accumulation of these metabolites in the AH provides potential therapeutic intervention avenues.

8.4 Epigenetic Modulators as Novel Therapeutics: Translational Perspectives on 20 Years of Preclinical Success

Jeff M. Gidday, Departments of Ophthalmology, Physiology, and Neuroscience, Louisiana State University School of Medicine, New Orleans, LA 70112

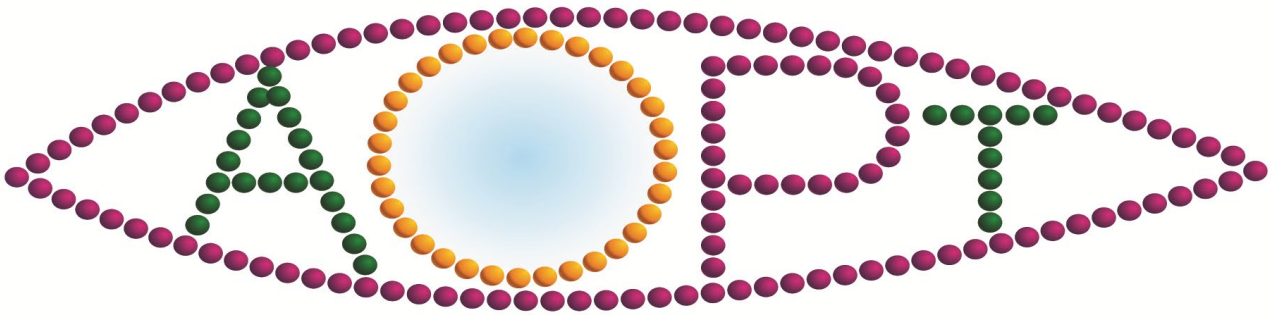
The ability to epigenetically induce new phenotypes in animals by pharmacologic or physiologic stimuli that modulate gene expression is well established. Depending on the nature of the stimulus, the resultant epigenetic response can be an adverse one promoting disease, or a protective one resulting in disease resilience. Studies of the latter birthed an entire field of study known as "pre-conditioning". Over the last two decades, preclinical studies have validated the efficacy of a variety of conditioning stimuli as retinal therapeutics for ischemia and phototoxicity, and have made significant advances probing underlying mechanisms. Newer insights into this "adaptive epigenetics" approach to therapeutics reveal that presenting the conditioning stimulus at distinct times after the acute injury ("post-conditioning") still reduces tissue injury, and that protection can also be realized by conditioning nonretinal tissues such as limb skeletal muscle ("remote conditioning"). In addition, following the discovery that repetitive conditioning increases the duration of the injury-resilient phenotype well beyond the last stimulus, such a protocol was used to protect against the chronic retinal degeneration that defines animal models of glaucoma, diabetic retinopathy, and retinitis pigmentosa. The limits of this response are currently unknown, but evidence from our lab and others indicates that protective phenotypes can even be inherited across generations. In the cardiac and cerebral fields, epigenetics-based remote conditioning serves as a high-safety profile treatment for hundreds of Phase II and III clinical trials. The robustness and reproducibility of the preclinical findings in adaptive epigenetics-based therapies for retinal injury warrant clinical trials of our own.



8.5 21st Century Ocular Pharmacology and Therapeutics: Viral Vectors as Drugs

Carl Romano. Regeneron Pharmaceuticals, Tarrytown, NY, USA

There is one approved gene therapy product for a serious eye disease, and more in the pipeline. Creating a successful gene therapy is not all that different than a conventional pharmacotherapeutic, in principle. To have an efficacious agent, a deep understanding of the biology and where to intervene are extremely helpful. Efficacy also depends on bioavailability to the site of action in sufficient concentration. And it must be safe—there is a risk benefit ratio to all therapies. In this talk, I will review what we have learned of in vitro and in vivo biology of the photoreceptor protein retinoschisin and how we have been approaching the creation of a gene therapy for X-linked retinoschisis.



8.6 Lessons learned from LHA510, a topical vascular endothelial growth factor inhibitor extensively optimized for topical delivery but failed to yield clinical efficacy in a neovascular AMD (nv AMD) PoC Trial

Stephen H Poor, Christopher M Adams, Guillaume Normand, Michael Ferriere, Georges Weissgerber, Cynthia Grosskreutz. Novartis Institutes for Biomedical Research, Cambridge, Ma, USA

Purpose: Clinical trials in wet macular degeneration of oral VEGF inhibitors approved for oncology, reformulated as eye drops, have to date failed due to lack of efficacy. We share results of a randomized, double-masked, vehicle-controlled, Proof-of-Concept study for topically delivered LHA510 in patients with nv AMD.

Methods: LHA510 is a low molecular weight vascular endothelial growth factor receptor inhibitor. In contrast to oncology drugs reformulated as eye drops that failed to deliver topical efficacy in clinical trials (pazopanib and regorafenib), LHA510 was comprehensively optimized for topical application for retina indications. The program flow chart included synthesis of over 2,000 compounds with an iterative optimization for topical efficacy, low systemic exposure, solubility and developability. This study evaluated whether topical LHA510, compared with topical vehicle, could suppress the need for intravitreal (IVT) anti-vascular endothelial growth factor (VEGF) therapy over a 12-week period, in treatment experienced patients with nv AMD who require IVT anti-VEGF every 8 weeks or more frequently to prevent or treat disease recurrence.

Results: The study did not meet the primary efficacy hypothesis of lower retreatment need in the LHA510 group versus the vehicle group. The key secondary efficacy endpoints were not suggestive of advantages for LHA510 relative to vehicle

Conclusions: Effective topical ocular pharmacotherapy may be out of reach for nv AMD.

Session 9: Advances in Drug Delivery

9.1 Specific drug targeting to enhance treatment efficacy

Ilva D. Rupenthal[1], Frazer P. Coutinho[1], Di Huang[1], Ying-Shan Chen[1], Monica L. Acosta[2], Colin R. Green[3]. [1]Buchanan Ocular Therapeutics Unit, Department of Ophthalmology, [2]School of Optometry and Vision Science; [3]Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, New Zealand

Purpose: Current treatments for retinal diseases such as age-related macular degeneration (AMD) and diabetic retinopathy (DR) are not cell-specific and may thus impair mechanisms required for tissue recovery leading to potential off-target effects. We have identified two cell surface receptors, Syndecan-4 and CD44, which are upregulated in retinal disease offering the potential for cell-specific drug targeting. This presentation will give an overview of connexin43 hemichannel blockers, which act upstream in the disease process to reduce inflammation and enable vessel normalisation, targeted specifically to diseased retinal cells.

Methods: The intracellularly acting connexin43 hemichannel blocker, Gap19, was conjugated to the cell penetrating peptide Xentry (XG19), which targets Syndecan-4 receptors, while extracellularly acting Peptide5 was incorporated into hyaluronic acid coated nanoparticles (HANP) binding to CD44 receptors. Both systems were tested for increased cell uptake and tissue targeting in vitro and ex vivo before being evaluated in a mouse model of choroidal neovascularisation (CNV) and a rat retinal ischaemia-reperfusion (IR) model, respectively.

Results: XG19 uptake was significantly increased under hypoxic and inflammatory conditions correlating with increased Syndecan-4 expression, while HANP localised primarily on CD44 expressing retinal pigment epithelial cells. Both targeted systems achieved significantly better treatment outcomes than their non-targeted counterparts, resulting in reduced lesion volume on day 7 (XG19; CNV model) and reduced retinal thinning at week 8 (HANP; IR model), respectively.

Conclusions: Targeting therapeutics specifically to cell surface receptors upregulated in disease may enhance treatment efficacy while minimizing any off-target effects in healthy cells and tissues.

9.2 Can Drug Delivery Enhance the Efficacy of Ocular Therapeutics

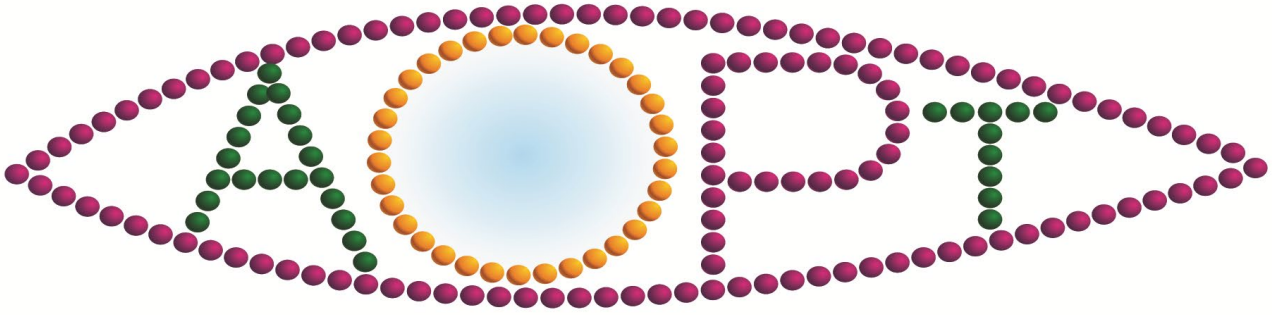
Heather Sheardown, Francis Lasowski, Ben Muirhead, Talena Rambarran, Naveed Yasin. Department of Chemical Engineering, McMaster University, Hamilton ON Canada

Purpose: To evaluate the toxicity and efficacy of drug delivery systems for treating ocular disease.

Methods: Three drug delivery systems, a micelle based system and two injectable in situ gelable systems were evaluated for toxicity and efficacy in vitro and in appropriate animal models. The micelle based systems was examined for the potential to deliver cyclosporine A to treat dry eye disease. The injectable systems were evaluated for the ability to release protein therapeutics for the treatment of conditions of the back of the eye.

Results: Efficacy results with the micelle based system showed that delivery of the drug once every three days was superior in terms of disease resolution when compared to the current clinical standard, Restasis®. Higher concentrations of cyclosporine A were examined with no effects on the animals in terms of tolerability. For back of the eye treatment, it was shown that a system based on POEGMA was able to release a model antibody drug for periods of up to 6 months and showed good tolerability in the eye. It was possible to resolve laser induced choroidal neovascularization with this delivery system and Avastin. A PNIPAAM based system showed excellent tolerability but shorter release durations and was deemed to be more suitable for anterior segment applications.

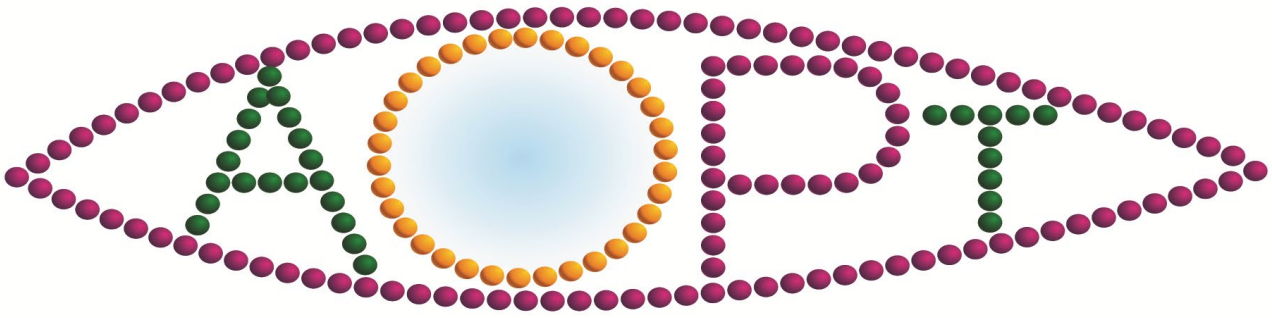
Conclusions: Drug delivery holds significant promise for the treatment of ocular disease, providing more convenient and more efficacious treatment than conventional methods with less inconvenience to the patient and the physician.



9.3 Suprachoroidal drug delivery to the eye

Uday B. Kompella, PhD, Pharmaceutical Sciences, Ophthalmology, and Bioengineering, University of Colorado Denver, Aurora, Colorado, USA.

Suprachoroidal space is a virtual space behind the sclera and above the choroid, where drug products can be administered. Since this is an invasive method of drug administration, ideally, the procedure should be limited to low frequency dosing. Evidence to date suggests rapid clearance of conventional small molecules as well as large molecules from the suprachoroidal space, when the drugs are presented in their molecular form in solutions. Thus, there is a need to employ slow release dosage forms such as pure drug suspensions, other depot dosage forms, or chemical modifications to sustain drug delivery. Additionally, drug products administered to the suprachoroidal space can be targeted better to the back of the eye tissues by using either a cannula or other physical means including particle density driven delivery or even iontophoresis. Special devices or unique injection approaches may be needed to place the drug product in the suprachoroidal space. While hemorrhage at the site of administration is a concern, advanced clinical studies to date suggest reasonable safety for suprachoroidal injections. This presentation will provide an overview of advances in suprachoroidal drug delivery based on preclinical and clinical studies to date.



9.4 Novel topical formulation for glaucoma

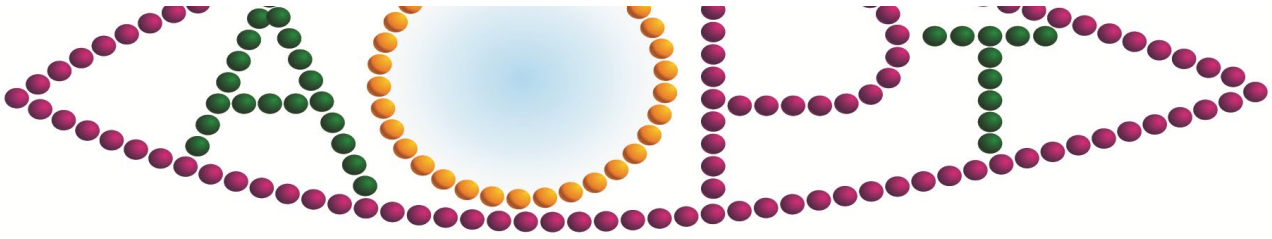
Monica M. Jablonski[1,2,3]; Sumana R. Chintalapudi[1,2], Doaa N. Maria[1,3]; Janey L. Wiggs[4]; Robert W. Williams[5], Mohamed Ibrahim[1]. [1]Department of Ophthalmology, The Hamilton Eye Institute; [2]Department of Anatomy and Neurobiology; [3]Department of Pharmaceutical Sciences; [5]Department of Genetics, Genomics and Informatics, The University of Tennessee Health Science Center, Memphis, TN, USA; [4]Department of Ophthalmology, Harvard Medical School, and Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, USA

Purpose: Glaucoma is a leading cause of blindness and IOP is the only modifiable risk factor. In this study, we combined systems genetics with human GWAS and pharmacology to define and validate a gene modifier of IOP. We also engineered a topical formulation of a new IOP-lowering drug.

Methods: IOP was measured in aged BXD mice. A genomic region was identified using QTL analyses. Stringent refinement based on correlation analyses and SNPs identified Cacna2d1 as the candidate gene. Subcellular localization was determined by immunohistochemistry. The GLAUGEN/NEIGHBOR consortium database was used to identify SNPs within the candidate gene in humans. The IOP lowering effect of pregabalin, a specific antagonist of CACNA2D1, was evaluated as eye drops. After confirmation of its IOP-lowering response, a novel topical delivery system was engineered.

Results: A single eQTL on Chr 5 was identified with a significant LRS. Cacna2d1 was identified as a cis-regulated candidate gene. CACNA2D1 is expressed in the ciliary body and trabecular meshwork. GLAUGEN/NEIGHBOR POAG meta-analyses revealed an imputed SNP (rs2299184 [A]) nominally associated with POAG (p=0.001). Pregabalin, an antagonist specific for CACNA2D1, reduced IOP. Incorporation of pregabalin in a novel microemulsion markedly increased the efficacy of pregabalin.

Conclusions: We combined systems genetics, bidirectional studies, and pharmacology to identify and validate a genetic modulator of IOP: CACNA2D1. To increase the efficacy of pregabalin, we engineered a novel topical formulation that greatly improves IOP-lowering efficacy of the drug. The formulation is biocompatible and supports once daily dosing of drug as an IOP-lowering therapy.



9.5 A new nanomedicine method for treating corneal graft rejection

Qingguo Xu[1], Ming Chen[2], Qing Pan[2], Walter J. Stark[2], Justin Hanes[2]. [1]Pharmaceutics & Ophthalmology, Virginia Commonwealth University, Richmond, VA, USA; [2]Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Purpose: Immunologic rejection is a main cause for corneal graft failure. We developed biodegradable nanoparticles that can be administered by subconjunctival injection (SCT) at the time of surgery and provide sustained release of dexamethasone sodium phosphate (DSP), thus potentially removing the compliance burden on patients.

Methods: DSP-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles (DSP-NP) were prepared by a zinc ion-bridging nanoprecipitation method. DSP-NP were characterized on particle size, morphology, drug loading, drug release, pharmacokinetics and safety. The efficacy of SCT DSP-NP (100μg DSP) to both prevent and rescue corneal allograft rejection was investigated at a rat model.

Results: DSP-NP were ~200nm spheres with ~8wt% drug loading, and sustained drug release over 7days in-vitro. DSP-NP provided sustained DSP levels in aqueous and vitreous for at least 7days. SCT DSP-NP successfully prevented corneal allograft rejection in rats over at least 9weeks at current study. In comparison, corneal rejection in control groups of either placebo particles, saline or free drug occurred within 4weeks accompanied by severe corneal edema, neovascularization and opacity. Most importantly, we observed single SCT DSP-NP effectively reversed early corneal graft rejection (occurred 3 days after transplantation) into clear grafts within 1 week after SCT DSP-NP treatment. All grafts treated with vehicle or free DSP were rejected within 2 weeks in this model. The SCT DSP-NP did not induce increased IOP and showed no ocular toxicity.

Conclusions: Sustained release DSP-NP formulation through SCT injection effectively prevented corneal allograft rejection in rats, and even successfully treated early corneal graft rejection to reverse the rejection.

Session 10: Novel Therapies for Corneal Diseases

10.1 Novel lipid mediators and neurotrophins targeting cornea nerve integrity

Haydee E.P. Bazan, *Louisiana State University Health New Orleans, School of Medicine, Neuroscience Center of Excellence and Department of Ophthalmology, New Orleans, LA, USA*

Purpose: Cornea innervation is important to maintain the homeostasis of the ocular surface. Damage to corneal nerves decreases tear production and impairs wound healing. We have shown that treatment of corneas with pigment epithelium-derived factor (PEDF) plus the ω-3 fatty acid docosahexaenoic acid (DHA) stimulates nerve regeneration. Both molecules are needed for biological activity.

Methods: Mouse corneas (right eye) were injured to damage nerves of epithelium and anterior stroma. Corneas were topically treated with PEDF+DHA, the Resolvin D1 (RvD1) isomer, or Aglistatin. Tears and cornea tissue were collected at different times. Gene expression, immunostaining of the nerves, western blot and lipidomics was performed.

Results: PEDF+DHA action requires the activation of the PEDF receptor (PEDF-R) with Ca2+ independent phospholipase A2 activity that releases DHA from membrane lipids. There is also gene induction of the *bdnf, ngf and sema7a*. Their products, the neurotrophins brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), and the axon growth promoter semaphorin 7A (Sema7A) are released in tears.

Aglistatin, an inhibitor of the PEDF-R, decreases BDNF and Sema7A secretion in tears and inhibits corneal nerve regeneration. In addition, PEDF+DHA treatment activates the synthesis of a new DHA lipid mediator released in tears. This docosanoid has fragmentation pattern and UV spectrum matching RvD6 but different retention time when analyzed by LC-UV-MS/MS. Treatment of injured corneas with the RvD6 isomer stimulates wound healing, corneal sensitivity and nerve regeneration.

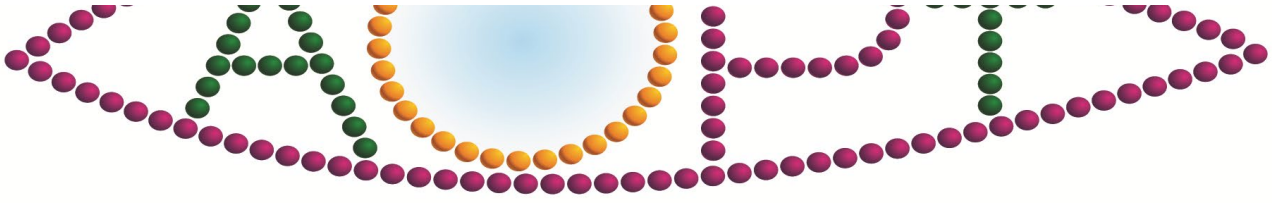
Conclusions: Our results demonstrate novel mechanism of the action of PEDF+DHA in modulating nerve regeneration. (Supported by NIH-NEI grant EY091465)

10.2 Cell therapy and gene therapy in eye diseases

Graziella Pellegrini[1], Paolo Rama[5], Tobias Hirsch[2], Tobias Rothoeft[3], Norbert Teig[3], Eustachio Attico[1], Laura De Rosa[1], Johann W. Bauer[4] and Michele De Luca[1]. [1] University of Modena and Reggio Emilia Modena, Center for Regenerative Medicine "Stefano Ferrari", Modena, Italy. [2] Department of Plastic Surgery, Burn Centre, BG University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, Germany. [3] Department of Neonatology and Pediatric Intensive Care, University Children's Hospital, Ruhr University Bochum, Bochum, Germany. [4] EB House Austria and Department of Dermatology, University Hospital of the Paracelsus Medical University, Salzburg, Austria. [5] Cornea and Ocular Surface Unit, San Raffaele Scientific Institute, Milan, Italy

Regenerative medicine has generated many efforts to explore new therapeutic potentials of both somatic and pluripotent stem cells with many possibilities envisaged for therapeutic applications. Hematopoietic and epithelial cells are extensively adopted for tissue regeneration, due to their high proliferative capacity and their accessibility. 30 years ago, the method for producing epidermis was discovered by cultivation from a small skin biopsy, allowing life-saving treatment of thousands severely burned patients in the following years. The importance of stem cell content was proven for tissues or organs in different pathologies. For instance, recent developments in cell-based therapy for ocular burns provided support for improvement and standardization of the cure for this disabling disease, causing depletion of limbal stem cells. Indeed, biopsies taken from the healthy eye, or other autologous source as oral mucosa in bilateral blindness, can be used for their content of stem cells. Few of these therapies overcame the hurdles related to medicinal product regulation and became available to patients.

The combined use of cell and gene therapy represents a further scientific approach for the treatment of congenital diseases. This approach was proven on hematopoietic cells and has recently been established using genetically modified epidermal cells for life-saving treatment on severe genetic diseases, as epidermolysis bullosa.



10.3 Old and new cation channel blockers to treat ocular discomfort and pain"

Juana Gallar,[1] Susana Quirce,[1] Carolina Luna,[1] David Ares-Suarez,[1] Enrique Velasco,[1] Carlos Belmonte,[1] M. Carmen Acosta,[1] Víctor Meseguer.[1]*Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, San Juan de Alicante, Spain*

Purpose: To evaluate the effects of sodium and potassium channel blockers on the spontaneous and stimulus-evoked activity of cold nerve terminals (CNTs) from intact and injured corneas, using pharmacological and opto-pharmacological approaches.

Methods: Electrical activity of CNTs was recorded in intact and dry eye (DE) guinea-pig corneas superfused at 34ºC. Characteristics of the spontaneous (ongoing activity, OA) and stimulus-evoked activity were analysed. Thermal (ramps to 20ºC or to 50ºC), mechanical (von Frey hairs) and chemical stimulation (98% CO_2 pulses) were applied before and after treatment with different sodium channel blockers (amitriptyline, carbamazepine, gabapentine, lacosamide, lamotrigine, lidocaine, and phenytoin; at different concentrations). In a separate set of experiments, modulation of CNTs neural activity by a new photo-isomerizable small molecule DENAQ was tested in darkness (when HCN and Kv channels are blocked) and under 460nm light illumination (channels unblocked).

Results: CNTs of DE corneas showed higher OA at basal temperature, and lower threshold and enhanced discharge rate in response to stimulation than those of intact corneas. Na-channel blockers reduced in a dose-dependent manner both, OA and stimulus-evoked activity of CNTs in intact and DE eyes.

OA of cold thermoreceptors in the dark was significantly lower in DENAQ pre-incubated corneas than in naïve corneas. Additionally, their OA was significantly reduced under blue light in intact and DE corneas.

Conclusions: Nerve activity increases in dry eye due to changes in the expression of sodium and potassium channels, making Na+- and K+-channel blockers potential tools to pain consecutive to abnormal activity of regenerating corneal nerves.

10.4 neuropathic corneal pain: approaches for management"

Pedram Hamrah, MD, Department of Ophthalmology, Department of Bioengineering, Tufts University

Neuropathic pain is caused by a primary lesion or dysfunction of the nervous system and can occur in the cornea. However, neuropathic corneal pain (NCP) is currently an ill-defined disease. Patients with NCP are extremely challenging to manage, and evidence-based clinical recommendations for the management of patients with NCP are scarce. The objectives of this review are to provide guidelines for diagnosis and treatment of patients with NCP and to summarize current evidence-based literature in this area. We performed a systematic literature search of all relevant publications between 1966 and 2017. Treatment recommendations are, in part, based on methodologically sound randomized controlled trials (RCTs), demonstrating superiority to placebo or relevant control treatments, and on the consistency of evidence, degree of efficacy, and safety. In addition, the recommendations include our own extensive experience in the management of these patients over the past decade. A comprehensive algorithm, based on clinical evaluation and complementary tests, is presented for diagnosis and subcategorization of patients with NCP. Recommended first-line topical treatments include neuroregenerative and anti-inflammatory agents, and first-line systemic pharmacotherapy includes tricyclic antidepressants and an anticonvulsant. Second-line oral treatments recommended include an opioid-antagonist and opiate analgesics. Complementary and alternative treatments, such as cardiovascular exercise, acupuncture, omega-3 fatty acid supplementation, and gluten-free diet, may have additional benefits, as do potential noninvasive and invasive procedures in recalcitrant cases. Medication selection should be tailored on an individual basis, considering side effects, comorbidities, and levels of peripheral and centralized pain. Nevertheless, there is an urgent need for long-term studies and RCTs assessing the efficacy of treatments for NCP.

10.5 Xanthohumol protects corneal epithelial cells against oxidative stress in vitro"

Simon Kaja[1,2,3,4,5],Harsh N Hariani[1], Karoline A Orloff[2], Samatha Ankireddy[2,3], Jenni J Hakkarainen[5], Anita K Ghosh[1,2,3,4]. [1]Graduate Program in Neuroscience, [2]Department of Molecular Pharmacology and Therapeutics, [3]Department of Ophthalmology, Loyola University Chicago, Health Sciences Division, Maywood, IL, USA, [4]Research Service, Edward Hines Jr. VA Hospital, Hines, IL, USA, 5Research & Development Division, Experimentica Ltd., Kuopio, Finland

Purpose: Dry-eye disease (DED) is a multifactorial disorder that affects the tear film and the ocular surface. Inflammation and oxidative stress are generally considered the primary contributors to disease progression. Topical administration of antioxidants can provide benefits for slowing or reversing disease progression. The goal of this study was to assess the anti-oxidative properties of Xanthohumol, a naturally occurring antioxidant found in the hops plant (*Humulus Lupulus*) in corneal epithelial cells.

Methods: Human corneal epithelial cells (HCE-T; Riken; Japan) were exposed to concentrations from 1 nM to 100 μM Xanthohumol for 24 h in order to determine cytotoxicity; cells were exposed to chemically-induced oxidative stress using *tert*-butyl hydroperoxide (tBHP) for 6 h, having been pretreated for 24 h with either 0.1 or 0.5 μM Xanthohumol, vehicle (0.1% DMSO) or remained untreated. MTT and lactate dehydrogenase release assays were used to quantify cell viability. Calcein-AM uptake assays were conducted to determine effects on permeability glycoprotein 1 (P-gp). Immunoblotting and immunocytochemistry were performed using previously validated primers and antibodies.

Results: Xanthohumol was not cytotoxic at concentrations up to 10 μM and resulted in a statistically significant dose-dependent protection of HCE-T cells from tBHP-induced oxidative stress. We did not observe any effect of Xanthohumol on P-gp function. The changes in expression of components of the endogenous antioxidant system elicited by tBHP and Xanthohumol treatment are currently being investigated.

Conclusions: Our *in vitro* findings support testing Xanthohumol in preclinical models for DED, either as monotherapy or in combination with established anti-inflammatory treatment modalities.

10.6 The miR-29b Mimic Remlarsen as an Anti-Fibrotic Therapeutic in the Eye"

Corrie L. Gallant-Behm, Stephanie Propp, Aimee L. Jackson. miRagen Therapeutics Inc., Boulder CO, USA

Purpose: Corneal and retinal fibrosis occur following injury or as a result of progressive disease. There are currently few therapies available to prevent or treat fibrosis in either ocular compartment, making this an area of high unmet medical need. miR-29 is a potent antifibrotic microRNA that inhibits expression of collagens and other fibrotic markers. Endogenous miR-29 expression is downregulated in numerous fibrotic diseases, indicating an opportunity for therapeutic intervention. miR-29 mimics including remlarsen, a clinical stage compound, have been shown to reduce fibrosis in the skin, lung, and other organs. This study investigated the anti-fibrotic effects of remlarsen in the eye.

Methods: Remlarsen was administered via topical drops to the cornea in the context of an alkali burn in the rat, and as intravitreal injections in a rabbit model of proliferative vitreoretinopathy and in a rat model of diabetic retinopathy. Fibrosis was assessed histologically and expression of collagens and other pharmacodynamic biomarkers were assessed using quantitative RT-PCR.

Results: In the rat alkali burn model, topical remlarsen treatment resulted in decreased corneal hazing and scarring, decreased stromal thickness, and decreased expression of multiple pharmacodynamic biomarkers. In the rabbit PVR model and in the diabetic rat, remlarsen intravitreal injection also resulted in decreased pharmacodynamic biomarker expression in the retina. In the diabetic rat, fewer epi-retinal fibrotic foci were also observed.

Conclusions: These studies demonstrate that remlarsen functions as an anti-fibrotic therapeutic in the cornea and retina and that these effects are conserved regardless of the etiology of the injury/insult to the eye.

Session 11: Hot Topics from Abstract Submissions to AOPT

11.1 Exploration of the secretome of adipose stem cells for the design of retinal therapeutics

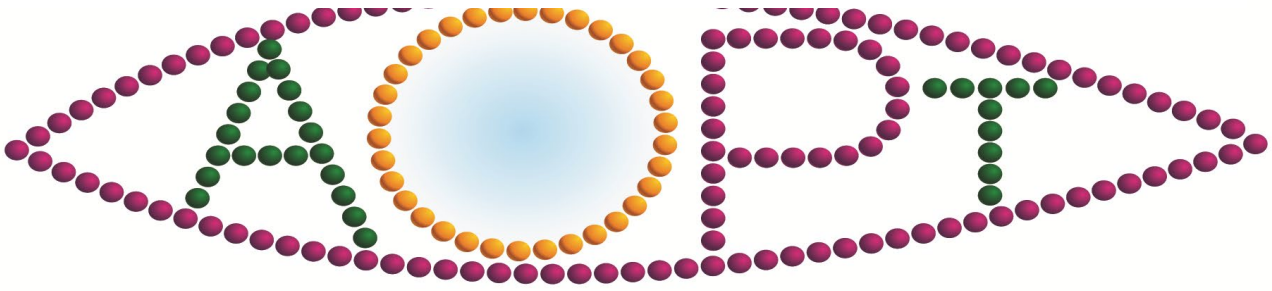
Rajashekhar Gangaraju[1,2], Sally L. Elshaer[1], Kumar Abhiram Jha[1], Mickey Pentecost[3], Raji Lenin[1], William Evans[1], Ramesh Periasamy[1], Lada Klaic[3], Jordy Gentry[1], Samuel M. Thomas[1], Anton Reiner[1], Veronique Jotterand[3], Nicolas Sohl[3]. [1]Ophthalmology, [2]Anatomy, and Neurobiology, University of Tennessee Health Science Center, Memphis, TN, USA. [3]Cell Care Therapeutics, Inc., Monrovia, CA, USA.

Purpose: In retinal diseases, the complex pathways involved at different stages of these progressive diseases poses an ongoing challenge for drug discovery and development. Regenerative medicines using adult stem cells have shown great promise in animal models, though their potential to translate into the clinic is uncertain. This presentation explores the possibility of utilizing the secretome of adipose stem cells as an alternative to complex stem cell therapies in retinal diseases.

Methods: A unique population of adult stem cells isolated from human adipose tissue was used to prepare a protein biologic (ASC-biologic) that harnesses the immunomodulatory and anti-inflammatory properties of mesenchymal stem cells. Intravitreal injection of the ASC-biologic in the Ins2Akita mouse model of T1DM and visual deficits model of mild traumatic brain injury was followed up with visual function assessment and histological analyses.

Results: Post-intravitreal injections, the ASC-biologic was found to be non-toxic and safe during the follow-up period of 3-4 weeks. In vitro, the ASC-biologic was found effective against microglial activation as well as the endothelial activation, the primary sources for disruption of retinal blood barrier integrity, leading to severe inflammation, vascular leakage, and subsequent neurodegeneration. In vivo, the ASC-biologic improved visual acuity and contrast sensitivity, prevented vascular leakage, and suppressed retinal inflammation.

Conclusions: Our data advocate for the use of secretome-based stem cell biologics as a cell-free regenerative medicine alternative for retinal diseases that may not only be safe and effective but may also overcome the handling barriers associated with regenerative medicine products consisting of living cells.



11.2 Novel topically delivered small molecule with IOP lowering and neuroprotective activity

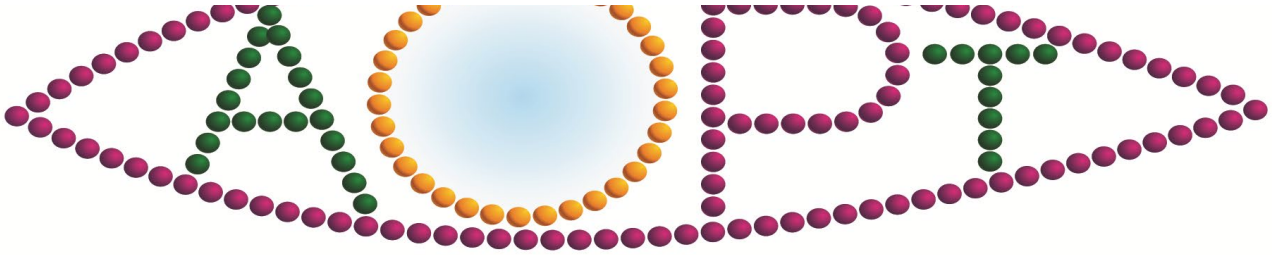
Suchismita Acharya[1,2,3], Dorota L Stankowska[1,2], Bindu Kodati[1,2], Tam P Nguyen[4], Linya Li[1,2,3], Dorette Z Ellis[1,2,3], and Raghu Krishnamoorthy[1,2]. [1]Department of Pharmacology and Neuroscience, [2]North Texas Eye Research Institute, [3]Department of Pharmaceutical Sciences, University of North Texas Health Science Center, Fort Worth, Texas, USA; [4]Department of Bioengineering, The University of Texas at Arlington, Arlington, Texas, USA.

Purpose: To demonstrate that, PLGA nanoencapsulated small hybrid molecule **SA-2NP** eye drop will be bioavailable to retina, lower intraocular pressure (IOP) and prevent retinal ganglion cell (RGC) death in rodent models.

Methods: *Biodistribution study*: Cornea, aqueous humor, vitreous humor (VH), retina, choroid and sclera were collected after 1 h of administration of a single eye drop (30µL) of **SA-2NP** (2%w/v) formulated in PBS to rat (n=6) eyes. Compound **SA-2** was quantified using HPLC/MS. *IOP lowering study:* Single **SA-2NP** (2%) eye drop was instilled to the IOP elevated Morrison's model of glaucoma in rats (n = 3 per group). IOP was measured at time points up to 72 h and repeated in triplicate. *Protection of RGC death:* Optic nerve crush (ONC) was performed on left eye of the mice (C57BL6, 12 weeks, n=5) followed by intravitreal injection of 2ul of 2% **SA-2** in PBS or only 2ul PBS to left eye (control group) at days 0 and 3. At day 7, pattern ERG and quantification of RGC was performed.

Results: Maximum concentration of **SA-2** (~10 pg/mg) was detected in VH and retina. **SA-2-NP** statistically (Rank Sum test, $p<0.001$) lowered the IOP in treated eyes by 21-23% from 3h-72h range in comparison to contralateral control eyes. Compound **SA-2** treatment was significantly (t-test, $p<0.001$) effective in protecting RGC against ONC induced death.

Conclusion: This novel class of hybrid compound when formulated as PLGA encapsulated nanoparticle is bioavailable to posterior chamber via topical ocular delivery, decrease IOP and is neuroprotective.



11.3 Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts

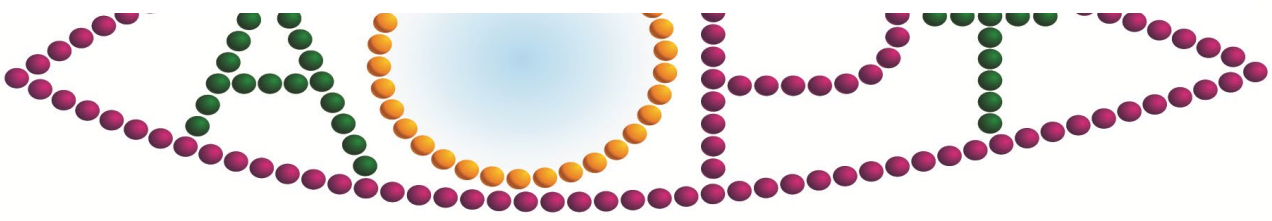
Peter F. Kador[1,2,3] Damian M. Daszynski[1], Puttur Santhoshkumar[4,5], Ashutosh S. Phadte[4,2], K. Krishna Sharma[4,5], Haizhen A. Zhong[6], and Marjorie F. Lou[2,3]. [1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE; [2]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska at Lincoln, Lincoln, NE; [3]Department of Ophthalmology, School of Medicine, University of Nebraska Medical Center, Omaha, NE; [4]Department of Ophthalmology, University of Missouri–Columbia School of Medicine, Columbia, MO; [5]Department of Biochemistry, University of Missouri–Columbia School of Medicine, Columbia, MO; [6]Department of Chemistry, University of Nebraska at Omaha, Omaha, NE

Purpose: The paradigm that cataracts are irreversible and that vision from cataracts can only be restored through surgery has recently been challenged by reports that oxysterols such as lanosterol and 25-hydroxycholesterol can restore vision by binding to aB-crystallin chaperone protein to dissolve or disaggregate lenticular opacities. To confirm this premise, *in vitro* rat lens studies along with human lens protein solubilization studies were conducted.

Methods and Results: Cataracts were induced in viable rat lenses cultured for 48 hours in TC-199 bicarbonate media through physical trauma, 10 mM ouabain, or 1mM of an experimental toxic glycoprotein chaperone. Subsequent 48-hour incubation with 15 mM of lanosterol liposomes failed to either reverse these lens opacities or prevent the further progression of cataracts to the nuclear stage. Similarly, 3-day incubation of 47-year old human lenses in media containing 0.20 mM lanosterol of 60-year-old human lenses in 0.25 and 0.50 mM 25-hydroxycholesterol failed to increase the levels of soluble lens proteins or decrease the levels of insoluble lens proteins. These binding studies were followed up with

in silico binding studies of lanosterol, 25-hydroxycholesterol, and ATP as a control to two wild type (**2WJ7** and **2KLR**) and one R120G mutant (**2Y1Z**) aB-crystallins using standard MOE™ (Molecular Operating Environment) and Schrödinger's Maestro software. Results confirmed that compared to ATP, both oxysterols failed to reach the acceptable threshold binding scores for good predictive binding to the aB-crystallins.

Conclusion: All three studies failed to provide evidence that lanosterol or 25-hydroxycholesterol have either anti-cataractogenic activity or bind aggregated lens protein to dissolve cataracts.



11.4 More than just a reactive oxygen species scavenger: grapes prevent UV-B radiation-induced cataract by upregulating anti-apoptotic protein XIAP

Wu, Hongli[1,2]; Liu, Xiaobin[2]; Wang, Duenshian[2]; Aguilera Garcia, Luis[5]; Li, Yousong[2,3]; Yu, Yu2,4; Ssentamu, Frank[5]. [1]North Texas Eye Research Institute, [2]Department of Pharmaceutical Sciences, [5]College of Pharmacy, University of North Texas Health & Science Center, Fort Worth, TX, United States; [3]Department of Traditional Chinese Medicine, Shanxi Dayi Hospital, Taiyuan, Shanxi, China; [4]Department of Obstetrics and Gynecology, The 2nd Hospital of Dalian Medical University, Dalian, Liaoning, China

Purpose: To investigate if grapes could protect against in vivo ultraviolet B (UV-B) radiation-induced cataract and to study its mechanism of action by identifying its molecular targets in the lens.

Methods: The grape powder was provided by the California Table Grape Commission (CTGC). C57BL/6J mice were fed with the regular diet, regular diet supplemented with glucose and fructose, or the grape diet (regular diet supplemented with 5%, 10%, and 15% grape powder) for 3 months. The animals were then exposed to 20.6 kJ/m2 UV radiation for 15 min to induce cataracts. Two days later, the degree of the cataract and lens morphology was evaluated under the dissecting microscope. Glutathione (GSH), free protein thiol (PSH), protein glutathionylation (PSSG), and redox potential levels were measured to reflect the oxidative markers. To explore its anti-cataractogenic mechanism, agilent literature search (ALS), a data-mining tool, was used to pull the protein targets of grape powder.

Results: We found that 10% and 15% grape powder diet could significantly inhibit the onset as well as the severity of UV-B-induced cataracts. In the 15% grape powder diet group, the majority of lenses remained largely transparent. The GSH and PSH levels were much higher in the 15% grape powder diet group compared with that of the regular diet control group. The accumulation of PSSG, a marker for protein thiol oxidation, was largely inhibited in the grape powder diet groups. The redox potential in grape powder diet groups was much lower as compared with that of regular diet mice. For target prediction, a total of 145 proteins regulated by grapes were identified through ALS and were visualized by protein network. Among these protein targets, X-linked inhibitor of apoptosis (XIAP) was correlated with all of the active ingredients of grapes, indicating anti-apoptotic protein XIAP might be one of the most critical molecular targets of grapes. Our in vivo data confirmed that anti-apoptotic proteins XIAP and Bcl2 were upregulated whereas levels of pro-apoptotic proteins Bax and caspase 3 were significantly less in grape powder diet groups.

Conclusions: Grape powder dose-dependently protected the lens from UV radiation-induced cataract development in mice. Its protective effects may involve not only directly scavenging free radicals but also activating the XIAP-mediated antiapoptotic pathway.

11.5 The Endothelin Receptor Antagonist Macitentan Attenuates Neurodegeneration in a Rodent Model of Glaucoma and Ameliorates Endothelin-Mediated Vasoconstriction

Raghu R. Krishnamoorthy, Nolan R. McGrady, Hayden Jefferies, Wei Zhang, Sai Chavala and Dorota L. Stankowska. Department of Pharmacology and Neuroscience, North Texas Eye Research Institute, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth TX 76107

Purpose: The purpose of this study was to determine if dietary administration of the dual ETA/ETB receptor antagonist, macitentan, could attenuate neurodegenerative changes following IOP elevation in Brown Norway rats and attenuate endothelin-1 (ET-1) mediated vasoconstriction.

Methods: Following IOP elevation, Brown Norway rats were either untreated or treated for 1 month with macitentan (5 or 10 mg/kg/body weight) in dietary gels. Retinal flat mounts from the rats were imaged and surviving RGCs quantitated. RGC function was assessed by pattern ERG analysis following 2 and 4 weeks of IOP elevation and macitentan treatment.

In different set of experiments, adult male and female Long Evans and Brown Norway rats were either untreated or treated with macitentan (5 mg/kg body wt) once a day for 3 days followed by intravitreal injection of 4 □l of 500 □M ET-1 or vehicle in one eye and imaging of the retinal vasculature was carried out using fluorescein angiography.

Results: IOP elevation for 4 weeks produced a 42-61% loss of RGCs which was significantly attenuated in macitentan-treated rats. Pattern ERG analysis revealed that macitentan treatment significantly protected against IOP-mediated decline in pattern ERG amplitude. Vasoconstrictive effects following intravitreal ET-1 injection were greatly reduced in rats administered with macitentan in the diet prior to the ET-1 administration.

Conclusions: The endothelin receptor antagonist, macitentan, has neuroprotective effects by enhancing RGC survival without affecting IOP and blocks ET-1 mediated vasoconstriction which could prevent ischemia.

11.6 Protection of kaempferol on oxidative stress-induced retinal pigment epithelial cell damage

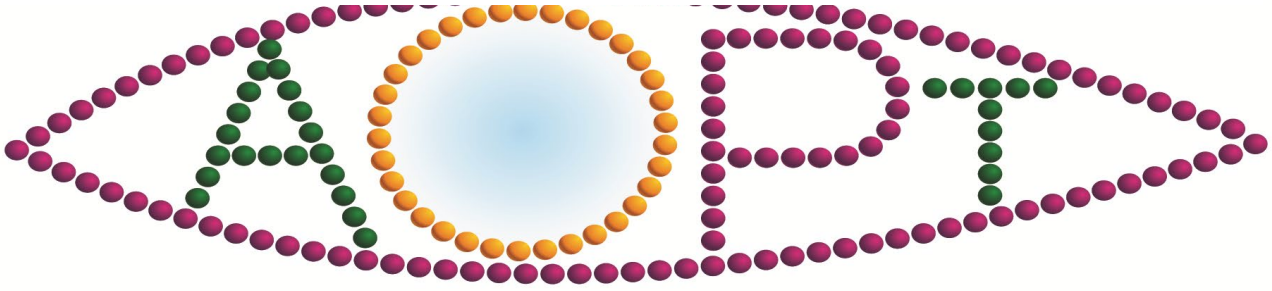
Donglei Zhang[1], Weiwei Du[1], Yuanlong An[1], Xiangdong He[1], Wei He[1,2]. [1]The School of Pharmacy, He University, Shenyang, Liaoning, 110163, China; [2]Shenyang Industrial Technology Institute of Ophthalmology, Shenyang, 110163, China

Purpose: Kaempferol is a natural flavonoid widely distributed in many edible plants, fruits and traditional medicines, and has been reported to have antioxidant, anti-inflammatory, anticancer and antimicrobial activities. In the present study, we have investigated if kaempferol has the protective effects on oxidative stress-induced retinal pigment epithelial cell damage and the possible mechanisms.

Methods: Hydrogen peroxide (H2O2) - induced oxidative stress in vitro cell-based model and sodium iodate-induced retinal degeneration rat in vivo model have been used in this study with or without kaempferol treatment.

Results: Our data indicates that kaempferol protects ARPE-19 cells from H2O2-induced oxidative cell damage and apoptosis. Kaempferol also inhibits the up-regulated vascular endothelial growth factor (VEGF) mRNA expression levels induced by H2O2 in ARPE-19 cells, and affects the oxidation and antioxidant imbalanced system in ARPE-19 cells treated by H2O2 through the regulations of both the activities of reactive oxygen species (ROS) and superoxide dismutase (SOD), meanwhile, the expression of NRF2 and antioxidant genes were notably decreased after H2O2 treatment, treatment with kaempferol significantly counteract these changes. Furthermore, our in vivo experimental results show that in sodium iodate-induced retinal degeneration rat model, kaempferol could protect sodium iodate-induced pathological changes of retina tissue and retinal cells apoptosis, as well as the up-regulated VEGF protein expression in RPE cells.

Conclusions: kaempferol could protect oxidative stressed-human RPE cell damage through its antioxidant activity and anti-apoptosis function, suggesting that kaempferol has a potential role in the prevention and therapeutic treatment of AMD or other retinal diseases mediated by oxidative stress.



Session 12: Disruptive Technologies: Ophthalmic Tools and Methods that Have Changed the Ways We See the Eye

12.1 Audacious Goals Initiative: Status and Impact

Steven M. Becker, National Eye Institute, National Institutes of Health, 31 Center Dr. Bethesda, MD USA 20892

Purpose: The National Eye Institute (NEI) is catalyzing the translation of ocular stem cell therapies with its Audacious Goals Initiative for Regenerative Medicine.

Methods: Since 2015, NEI has organized three consortia to catalyze stem cell-based therapies.

Results: The first focuses on developing functional imaging technologies which can enable non-invasive, in vivo monitoring of activity of individual retinal neurons. The second consortium is attempting to identify novel neural regeneration factors in the visual system. The third, funded in September of 2018, aims to generate translation-enabling models that mimic human eye disease and will evaluate the survival and integration of regenerated neurons in the visual system.

Conclusions: This talk will highlight a few of the projects and show their potential to advance regenerative medicine strategies and increase our understanding of the pathobiology of retinal disease.

12.2 Photoacoustic Imaging and Sensing: a New Way to See the Eye

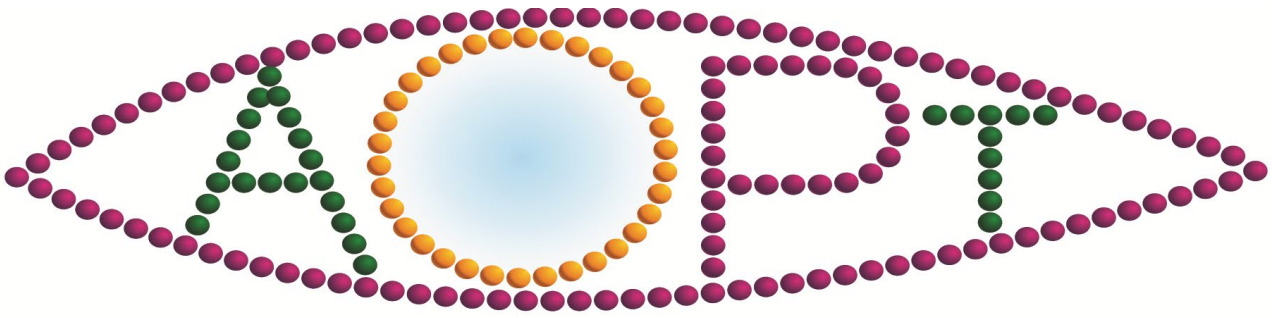
Randolph D. Glickman, Department of Ophthalmology, UT Health – San Antonio, Texas)

Purpose: Photoacoustics (optoacoustics) is a hybrid technology utilizing light excitation of acoustic responses in targets of interest. It has found numerous applications in biomedicine, including eye research, because of its ability to report both morphological and functional data about the interrogated tissue. This presentation will give an overview of current applications.

Methods: Wavelength-dependent absorption of light in a tissue chromophore causes local heating leading to a thermoelastic expansion – contraction cycle. If nanosecond pulses of light are used to excite this process, the resulting pressure wave is an ultrasound signal propagating through the tissue and detectable at the tissue surface. This is highly advantageous, because of the known properties of ultrasound propagation in tissue and the ability to use standard medical ultrasound equipment for detection. The time of arrival and amplitude of the ultrasound signals provide information about the location and nature of the absorber.

Results: Due to the wavelength dependence of the photoacoustic response, functional and physiological applications are possible. For example, retinal oximetry can be determined from the different absorption properties of oxy- and deoxyhemoglobin. Multispectral imaging of the posterior segment can identify pigments such as melanin or lipofuscin. Drug uptake can be assessed by selective photoacoustic excitation. The temperature dependent nature of the thermoelastic response enables thermographic measurements. Use of targeted nanoparticle contrast agents facilitates detection of specific molecular species.

Conclusions: Photoacoustic technology is a powerful, noninvasive tool for ocular research to study ocular morphology, fundamental physiological parameters, cellular responses, and molecular expression.



12.3 A Platform to Take on the Entire Progressive Retinal Degeneration Disease Continuum

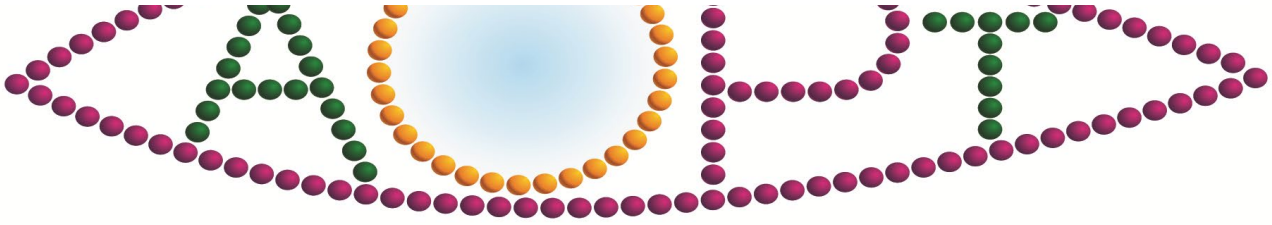
Francois Binette, and Igor O. Nasonkin. BioTime, Inc., Alameda, CA, United States.

Purpose: Blindness caused by retinal degenerative diseases is a major source of disability affecting millions of people. Visual function depends on the structural organization and interactions of various cell layers of the retina. Therefore, replacing degenerated retinal tissue with a new mutation-free retinal implant, consisting of retinal cellular components grown in a dish from human pluripotent stem cells [HPSCs] is a reasonable strategy for the design of universal approaches to treat blindness independent of the etiology of PR cell death.

Methods: BioTime is deriving retinal cells (PRs, RPE) and retinal tissue (organoids) from HPSCs, combined with various scaffold supports to engineer transplantable retinal patches for testing in small and large eye models. To date, we successfully generated subretinal grafts of HPSC-derived retinal tissue in rats and cats using a transvitreal approach, tracked the grafts in vivo by fundus examination (RetCam), and Optical Coherence Tomography, and by immunohistochemistry with retinal and human-specific antibodies after sacrificing the animals.

Results: We demonstrated tumor-free survival, axonal integration of grafts into host retina and in some cases, improvement of vision.

Conclusions: BioTime is developing a range of therapeutic solutions based on its proprietary retinal organoid and pure retinal cell (RPE and PR) technologies to address many retinal diseases irrespective of genetic background. Though challenges remain for designing a product with the optimal cellular composition, and organizational structure, we expect that our biological "toolbox" will allow us to address most of the challenges designing products with defined composition and for predictable improvement of visual function.



12,4 Early stage detection of Glaucoma by monitoring nanostructure and function of RGC layer using Multifractal OCT

Subrata Batabyal, Kissaou Tchedre, Sourajit Mustafi, Bahram Jozi, Weldon Wright, Samarendra Mohanty. Nanoscope Technologies LLC

Purpose: Glaucoma is a leading eye disorder which has few symptoms and difficult to detect in early stages. Currently, more than ~3 million Americans are suffering from glaucoma. The current treatment for glaucoma is to lower intraocular pressure (IOP), which is achieved by eye drops, laser, or incisional surgery. To date, there is no neuroprotective agent approved for commercial use. The most effective way to tackle glaucoma is via early stage detection. However, early stage detection remains challenging due to variable and slowly progressive nature of the disease, measurement variability, and lack of a commonly acceptable reference standard.

Method: We have developed multifractal OCT based analysis of layer specific activity (e.g. RGCs) during visual stimulation to assess the functional state of retina to detect glaucoma in early stage. We have utilized time varying intensity as well as phase fluctuations of OCT signal and implemented multifractal alongwith artificial intelligence analysis for quantitative estimation of functional characteristics. Also locally connected fractal mapping shows nano/micro-structural alterations associated with RGC-loss/dysfunction.

Results: Using Multifractal OCT, we were able to detect retinal layer specific cellular activities upon visual stimulation. Our interferometry-based optical method coupled with in-depth multifractal analysis differentiated retinal activities between wild-type and RGC-dysfunctional mice. We have successfully characterized the loss of RGC activity in acute IOP elevated glaucoma mice model.

Conclusion: The clinical translation of the multifractal OCT technology, capable of early stage diagnosis of glaucoma, will be beneficial to the people at greater risk for developing glaucoma for timely therapeutic intervention and prevention.

12.5 Preclinical Evaluation of ADVM-022, a Novel Gene Therapy Approach to Treating Wet Age-Related Macular Degeneration

Claire M. Gelfman[1], Ruslan Grishanin[1], Judith Greengard[1], Szilard Kiss[2], and Mehdi Gasmi.
[1]Adverum Biotechnologies, Inc., Menlo Park, CA; [2]Weill Cornell Medical College, New York, NY

Purpose: Inhibition of vascular endothelial growth factor (VEGF) is the mode of action of several approved therapies, including aflibercept, for the treatment of wet AMD. Lack of compliance due to the frequent intravitreal dosing requirements may result in recurrence of disease, leading to irreversible vision impairment. Gene therapy providing sustained anti-VEGF levels in the retina following a single injection could drastically reduce the treatment burden and improve visual outcomes.

Methods: ADVM-022, an adeno-associated virus vector encoding aflibercept, has been optimized for intravitreal delivery and strong protein expression. Long-term expression and efficacy of ADVM-022-derived aflibercept was evaluated in a laser-induced choroidal neovascularization model in non-human primates. Ocular safety was evaluated following long-term suppression of VEGF by clinical scoring (inflammatory parameters) as well as OCT (retinal thickness).

Results: Intravitreal administration of ADVM-022 was well tolerated and resulted in sustained ocular aflibercept levels in aqueous and vitreous humor, as well as retina and choroid. In addition, ADVM-022 administration 13 months prior to the laser prevented the occurrence of clinically relevant choroidal neovascularization lesions, to the same degree as a bolus of aflibercept delivered at the time of laser.

Conclusions: These results demonstrate that a single intravitreal administration of ADVM-022 may provide a safe and effective long-term treatment option for wet macular degeneration and may ultimately improve patients' visual outcomes.

Session 13: What Every Eye Doctor Needs to Know About the FDA

13.1 Advancing Technology Challenges in Ophthalmic Drug Approvals

Wiley A. Chambers, *Office of New Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland 20993, United States*

Abstract: What has been described as the "low hanging fruit" is gone from ophthalmology. We have products which dilate and constrict the pupil, reduce aqueous production, increase aqueous outflow, kill common infecting organisms, reduce inflammation, and stop the growth of abnormal blood vessels. We have instruments which allow us to noninvasively look at individual layers of the cornea and retina and to follow red blood cells as they travel through capillaries. We can substitute genetic code materials where defects would have lead to a loss of visual function. But individuals still go blind. Current successes suggest that it may be time to upgrade our paradigm from treatments to cures.

13.2 Generic Drugs and Their Role in Bringing Next Generation Products: An FDA Perspective

Markham Luke, Darby Kozak, Andrew Babiskin, Sam Raney, Rob Lionberger.

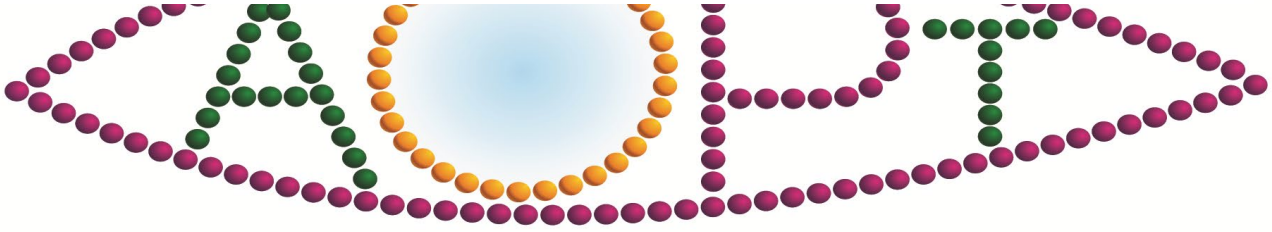
Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland 20993, United States

Purpose: To understand the role of generic drugs in moving the marketplace and technological space forward. Generic drugs as envisioned by the crafters of the respective legal framework cost less to develop and allow for greater accessibility of quality drugs in a given area for the American public.

Methods: The Office of Generic Drugs and its Office of Research and Standards have strategically approached the regulatory approach to the marketing of generic drugs to enable high standards for these drugs and at the same time making these drugs more accessible. This is done in the background of the current regulatory and economic environment which will be broadly described.

Results: Generic drugs constitute roughly 90% of all prescribed drugs currently. Certain drugs present greater challenges to development of generic counterparts. These typically fall into the category of "complex drugs". To facilitate the development of these complex drug products, FDA has sought to provide product specific guidances "PSGs" and outlined consistent regulatory pathways to market for these products. These include in vitro methods. Generic drugs have enabled better access to effective treatment regimens for patients and also pushed technological innovation for new drugs to come to market.

Conclusions: FDA's generic drug user fee funded research environment is not static and continues to evolve to meet the challenges presented by new reference products and new technologies to bring accessible drugs to market.



13.3 Generic Ophthalmic Drug Products, Physical Characteristics and Bioequivalence

Darby Kozak[1], Xiaoming Xu[2], Andrew Babiskin[1], Yan Wang[1], Rob Lionberger[1]

1. *Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland 20993, United States*

2. *Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland 20993, United States*

Purpose: Provide an overview of the regulatory framework that generic ophthalmic drug products are subject to, and approved under, as well as briefly discuss the different in vivo and in vitro studies that are recommended to demonstrate the bioequivalence (BE) of a generic ophthalmic product to its corresponding reference listed drug (RLD).

Methods: We reviewed the U.S. FDA Orange Book, Code of Federal Regulations, Product-Specific Guidances, and Generic Drug User Fee Amendments (GDUFA) funded research on topical ophthalmic drug products. Studies included characterization of the critical quality attributes (CQAs) of complex ophthalmic products and PBPK modeling to bridge the understanding of how these CQAs may affect ocular bioavailability.

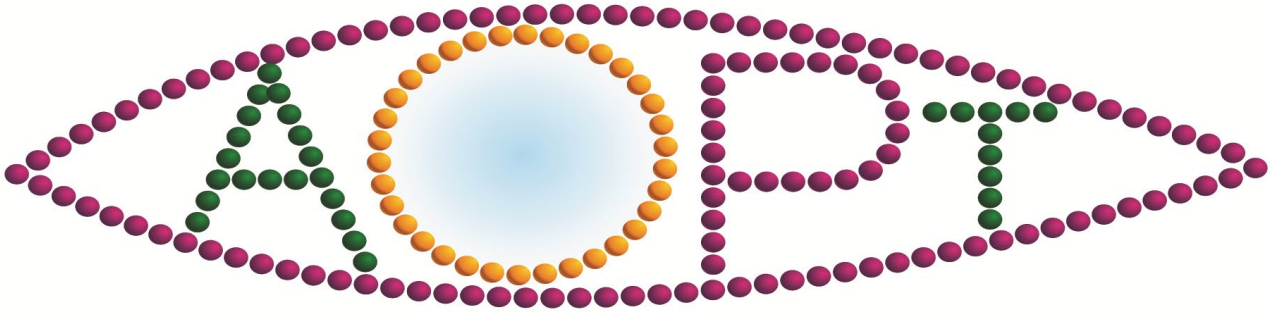
Results: There are over 140 approved topical ophthalmology RLD product. Currently only 47% of these RLDs have an approved therapeutically equivalent (TE) generic product. To be designated as TE, a generic product must demonstrate it is pharmaceutically equivalent (e.g. contain the same active, dosage form, and be similarly formulated) and BE to the RLD. In general, BE studies compare the generic to the reference product and can include comparative in vivo aqueous humor PK and/or comparative clinical endpoint studies. In addition, in some instances, comparative in vitro studies of the product CQAs may be used to demonstrate BE. Ultimately, the selected study must provide an accurate, sensitive, and reproducible measure to ensure BE.

Conclusions: FDA conducts research and provides guidance to facilitate Americans access to safe, effective, and high-quality generic drug products.

13.4 How FDA Ensures Quality of Ophthalmic Drug Products

Patricia Onyimba, *Office of Lifecycle Drug Products, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland 20993, United States*

Abstract: FDA plays a critical role in protecting the public from poor quality products. The Office of Pharmaceutical Quality (OPQ) within CDER/FDA assures through review, inspection, surveillance, policy and research that ophthalmic drug products available to the American people are of high quality and safe. This presentation provides insight on how FDA uses a risk-based, scientific and regulatory approach to evaluate the information submitted by firms seeking approval of ophthalmic drug products, to ensure that quality is designed into the product and the product is robust and will meet predetermined acceptance criteria throughout product shelf-life. A brief introduction of OPQ and the regulations governing the quality of ophthalmic drug products will be provided. The presentation will also discuss the type of Chemistry, Manufacturing and Controls (CMC) information submitted by applicants and evaluated by the FDA prior to approval of both brand and generic ophthalmic products to ensure that both products meet similar quality standards and are comparable. Finally, a brief mention of how the FDA assures that ophthalmic products continue to maintain their quality standards after approval and during commercialization will be provided.



Poster Session

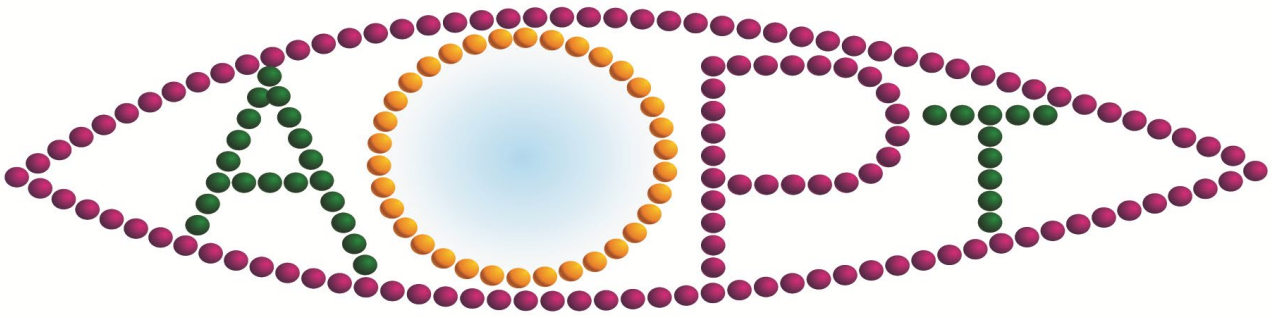
We would like to thank National Eye Instute, BrightFocus Foundation, Cooper Vision, Foundation Fighting Blindness and OMAR Consulting Group to sponsor the Awardees in the Poster Session.

Special thanks to Foundation Fighting Blindness for sponsoring the beverage for the session.

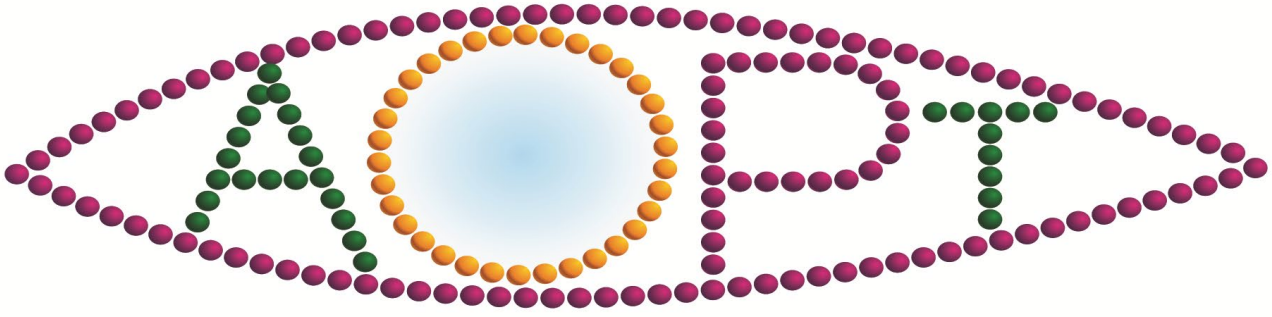
Poster Session Moderators:

Maria Reinoso, MD, Associate Professor, LSU Eye Center, Louisiana State University School of Medicine

Juana Gallar, MD, PhD, Professor, Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain



Poster Abstracts



Poster #1 Trabecular Meshwork Swelling and Osmoregulation

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Purpose: Trabecular meshwork (TM) plays a pivotal role in maintenance of intraocular pressure (IOP) within physiological ranges. Increase in TM resistance to aqueous humor outflow results in IOP elevation, optic neuropathy and vision loss in hypertensive glaucoma. TM outflow resistance is regulated dynamically but mechanisms are unclear. We hypothesize that mechanosensitive and swelling sensitive ion channels in transient receptor potential vanilloid (TRPV) and melastatin (TRPM) channel families interact with Na-K ATPases and aquaporins to regulate dose-dependent swelling of TM cells.

Methods: Immunostaining, calcium, sodium, potassium imaging, cell volume assays, whole cell patch-clamp electrophysiology and shRNA cell transfections demonstrated functional expression of TRPV, TRPM and Na-K ATPase family isoforms in primary and immortalized TM cells. Hypotonic stimulation and/or pharmacological agonists/antagonists were used to quantify the impact of mechanotransduction channels and pumps on the swelling response.

Results: Transmembrane current amplitudes and calcium signals showed dose-dependent swelling responses. Hypotonic stimulation induced structural changes by decreasing F-actin filament expression intensity. Intracellular calcium increases due to hypotonic stimulation required TRPV4 and TRPM4 activation for optimal responses. Hypotonic stimulation induced currents composed of intracellular calcium spikes and slower sodium currents associated with regulatory volume decrease. Cell current/voltage signals and calcium/sodium responses to hypotonic stimulation were attenuated varyingly by specific TRP channel antagonists.

Conclusions: These results identify TRPV4/TRPM4 interaction as a mechanism for TM osmosensing and subsequent cell swelling responses. Calcium signaling pathways, ideal ionic gradients for swelling through aquaporins, and whole TM tissue constriction/relaxation are likely mediated by these channels for regulating aqueous humor outflow.

Poster #2 Evaluating the safety of pharmaceutical drugs in a dual model of cell viability and barrier integrity

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Purpose: The aim of this work is to evaluate the safety of pharmaceutical drugs currently applied at a clinical level.

Methods: An in vitro corneal epithelial model was generated from human normal limbal cells grown on 1.12 cm^2 Transwell inserts and cultured for 7 days under air-lift conditions. Corneal toxicity and barrier disruptions were studied upon application of selected pharmaceutical drugs. Corneal toxicity was assessed via MTT assay while corneal barrier disruptions were assessed via variations in Trans-epithelial electrical resistance (TEER) before and after 30 minutes drug exposure and 120 minutes post-incubation in culture media. Toxicity and barrier disruptions were classified by a reduction in cell viability or barrier integrity in 40% or more.

Results: None of the evaluated ophthalmic drugs decreased cell viability in more than 10% overall, therefore, all formulations were classified as non-toxic. However, barrier integrity was highly affected upon application of several compounds, with decrements of almost 60% in initial TEER values.

Conclusions: The use of complementary test to cell viability via MTT could stablish new criteria for classification of current pharmaceutical products, relying not only on cell viability but in the barrier disruption potential. This test could be useful in distinguishing time-dependent and reversible damage to the ocular surface that is currently undervalued in current protocols for pharmacological safety.

Poster #3 ET-1 Treatment Reduces Expression of ATP5H and Cox17 in Retinal Ganglion Cells

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Purpose: Endothelin-1 (ET-1) treatment has been shown to promote apoptosis of retinal ganglion cells (RGCs), however, the precise mechanisms underlying these effects are still unknown. The purpose of the study was to assess the changes in gene expression at the level of translatome, occurring during endothelin-mediated neurodegeneration of RGCs.

Methods: Primary RGCs isolated from post-natal day 5 rat pups were treated with ET-1 (100 nM) for 24 h in trophic factor-free medium. Polysomal RNA was isolated and libraries for RNA-Seq were prepared. Trimmed mean of M-values (TMM) was used to normalize the gene expression. Genes with expression changes more than 1.5 fold with $p < 0.05$ were considered differentially expressed. Rats were intravitreally injected in one eye with 2 nmole of ET-1 and retina sections obtained were analysed for expression of ATP5H and Cox17.

Results: The STRING network analysis revealed mitochondrially relevant genes and out of the 156 differentially expressed genes, 23 genes were identified with known or predicted mitochondrial function. An increase in the expression of key mitochondrial genes including cytochrome c oxidase copper chaperone (Cox17) and ATP synthase, H^+ transporting mitochondrial F0 complex (ATP5H) was observed. However, a decrease in expression of ATP5H and Cox17 was found both in cultured RGCs treated with ET-1 as well as in retinal sections (primarily in the RGC layer) from rats eyes injected with ET-1.

Conclusions: ET-1 treatment produced changes in expression of key regulators of mitochondrial bioenergetics and oxidative metabolism which could be indicative of their involvement in neurodegeneration in glaucoma.

Poster #4 CX43 knockout human iPSC fail to generate normal retinal cups

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Purpose: Connexin43 (CX43) is a major protein that forms gap junction channels in embryonic stem cells. Mutations in the gene *Gap Junction Protein Alpha 1* (*GJA1*) expressing CX43 were identified in oculodentodigital dysplasia (ODDD) syndrome families. Symptoms include eye abnormalities such as microphthalmos. We established a model of ODDD using human iPSC-derived retinal cups. These may be useful to investigate if microphthalmos can be rescued by small molecules or gene therapy.

Methods: *GJA1* was disrupted using CRISPR/Cas9. The established lines were characterized by immunofluorescence and qPCR. The *GJA1* knockout cell lines and wild-type iPSC were differentiated into retinal organoids. Expression levels of neural makers and retinal markers were identified by qPCR and immunostaining.

Results: Using CRISPR/Cas9 genome editing, we successfully obtained multiple iPSC *GJA1* knockout clones. *GJA1*$^{-/-}$ iPSC remained undifferentiated and morphologically indistinguishable from wild-type iPSC. They remained typical iPSC morphology and had high expression level of pluripotency markers such as *OCT4*, *SOX2* and *NANOG*. *GJA1*$^{-/-}$ iPSCs had no defects in self-renewal and pluripotency state in primed states. However, after 26 days(d) differentiation, *GJA1*$^{-/-}$ iPSC failed to generate thick neuroepithelium in retinal organoids culture, which resulted in smaller retinal cups and thin neural retina. At d26 the neural identity marker *PAX6* was significantly lower than in the wild-type. Increased apoptotic cell death in *GJA1*$^{-/-}$ retinal cups were found at d50 as determined by immunoreactivity of CASPASE 3.

Conclusions: *GJA1* is not required for pluripotency in iPSC in primed states, but is required for iPSC to develop into normal retinal cups.

Poster #5 Therapeutic targeting of fibrosis and inflammation by novel aryl hydrocarbon receptor (AhR) ligands in neovascular age-related macular degeneration

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Purpose: AhR is a transcriptional regulator with pleiotropic functions in xenobiotic and lipid metabolism, vascular development, and cancer. Previously, we reported that loss of *AhR in vivo* exacerbates the severity of laser-induced choroidal neovascular (CNV) lesions. This led us to test the effect of AhR activation on angiogenesis and fibrosis, pathways associated with development and progression of CNV.

Methods: We screened AhR-active ligands (n=12) for their ability to activate AhR in choroidal endothelial cells (chEC), assessed their effect in *in vitro* functional angiogenesis assays and the *in vivo* laser-induced CNV model (C57BL/6J, 10-12 months, n=10 per group). Circulating cytokine (n=62) levels were measured by Mouse Cytokine ArrayC3. Based on the results from *in vitro* and *in vivo* studies, we designed and synthesized novel AhR ligands (n=5) and tested their activity, solubility, and toxicity *in vitro* and *in vivo*.

Results: We found that AhR-active ligands induced AhR promoter activity (5-12 fold), expression of AhR target genes (*CYP1A2*, *CYP1B1*), and inhibited VEGF-induced endothelial migration and tube-formation. Daily intraperitoneal treatment with AhR-active ligands alleviated the severity of laser-induced lesions (55-57%), compared to vehicle. AhR activation resulted in a decrease (33-37%) in collagen type IV accumulation in the CNV lesions, and reduction in the circulating levels of proinflammatory cytokines (n=8; $p < 0.01$).

Conclusions: Collectively, these findings validate the role of the AhR pathway in regulating the pathogenesis of CNV lesion formation and support our hypothesis that pharmacologic targeting of AhR may be used as potential therapy for the treatment of neovascular AMD.

Poster #6 Protecting Photoreceptor Function in Retinitis Pigmentosa with the Sphingolipid Analogue, FTY720

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Purpose: Retinitis Pigmentosa (RP) is an incurable and untreatable group of heterogeneous retinal neurodegenerative (RD) diseases that cause progressive dysfunction and death of retinal photoreceptor cells. A critical barrier for developing a therapy for RP is its heterogeneous nature, as it involves many pathways and genes. We've identified retinal ceramide, the signaling sphingolipid that is a potent mediator of cell death, increases in the retina and facilitates the retinal cell death process in many heterogeneous forms of degenerations. We hypothesized, regardless of the causal factor/gene, ceramide could be targeted to prevent and/or delay photoreceptor cell death. The purpose of this study was to test a Ceramide metabolic inhibitor for protection from retinal degeneration in mouse models of RP.

Methods: RD10 mice were given intraperitoneal injections with 2.5 mg/kg of FTY720 or Vehicle from 10 days (P10) and continued until the end of the experiment P60. Functional vision was measured by Optokinetic Tracking (OKT) at P30, P45, and P60. Likewise, photoreceptor function was measured by Electroretinogram (ERG) and retinal structure was measured in histological assays at P30, P45, and P60.

Results: Systemic delivery of ceramide inhibitor, FTY720, protected functional vision (OKT) in RD10 mice significantly by delaying the death of photoreceptor cells as observed in ERG and histological analyses.

Poster #7 TRPM8 Antagonist AMTB Impairs the Ability of Cold Thermosensitive Trigeminal Neurons Innervating the Ocular Surface to Encode Cold Stimulus Intensity

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Purpose: In the present work, we explored the effects of TRPM8 antagonist N-(3-aminopropyl)-2-{[(3-methylphenyl) methyl]oxy}-N-(2-thienylmethyl) benzamide hydrochloride salt (AMTB) on the background and stimulus-evoked activity of TG low threshold mechanosensory neurons (LTM), cold thermoreceptor, and mechano- and polymodal nociceptor neurons innervating the ocular surface (OS) of the anesthetized adult rat.

Methods: Impulse activity of TG neurons was recorded extracellularly with tungsten electrodes (1-2MΩ) and stored for off-line analysis with dedicated software. A microprobe thermometer was placed on the corneal surface for simultaneous temperature recording. Background impulse activity (BA) and the impulse response to cold (-0.1 to -20ºC), heat (+0.1 to +20ºC), chemical (hyperosmolar solutions, CO_2) and mechanical stimulation were analyzed in the different types of TG neurons innervating the OS. Background and stimulus-evoked activity before and 15 min after topical treatment with 1mM AMTB were compared.

Results: BA of cold thermoreceptor neurons was significantly reduced after ATMB (from 5.16±0.71 to 3.04±0.75 imp/s; $p<0.05$, n=15). Their response to cold stimulation was abolished by AMTB in 58.7% of recorded neurons, although when present the response to cooling stimuli did not encode the intensity of the cold stimulus. AMTB did not affect mechanical responsiveness of LTM neurons (n=6). Spontaneous and stimulus-evoked activity of mechano- and polymodal nociceptor neurons (n=5) was not significantly affected by AMTB.

Conclusions: Results confirms that cold thermosensitive TG neurons innervating the cornea and conjunctiva encode the intensity of temperature changes occurring on the ocular surface. This ability to encode cooling stimulation is mediated mainly by TRPM8, being abolished by AMTB.

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Poster #8 L-serine administration protects against neurovascular dysfunction in a mouse model of retinopathy of prematurity

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Purpose: Retinopathy of prematurity (ROP) is a vision-threatening neurovascular disorder and the leading cause of blindness in children worldwide. Emerging evidence indicate that neuronal energy demands drive the vascular development, particularly in photoreceptors which have the highest density of mitochondria in the body. Our knowledge about photoreceptor energy fuel requirement is very limited, particularly with respect to L-serine, although it has been shown to be critical to other highly metabolically active cancer cells. We aim to investigate the impact of L-serine on retinal neurovascular function in ROP.

Methods: In a mouse model of oxygen-induced retinopathy (OIR), we examined the retinal neuronal activity with L-serine or vehicle administration by electroretinography, photoreceptor structure with immunohistochemistry, and retinal vascular pathology with isolectin staining. Retinal use of L-serine as energy fuel was tested by Seahorse analysis. We also examined L- serine levels with ELISA, and retinal endogenous serine synthetic enzymes with Single-cell transcriptomics.

Results: In OIR, L-serine protected against cone dysfunction and normalized cone structure. L- serine also inhibits retinal neovascularization and this inhibition was abolished in photoreceptor- degenerating mice. L-serine increased mitochondrial oxygen consumption in isolated retinal punches *ex vivo* and photoreceptor cells *in vitro*. Circulating L-serine levels were induced in OIR versus normal mice and retinal serine synthetic enzymes were mostly expressed in Müller cells by Single-cell transcriptomics. Serine synthetic enzymes were highly induced in primary Müller cells isolated from OIR versus normal mouse retinas.

Conclusions: Our findings suggest that Müller-cell-derived L-serine may be energy fuel for photoreceptor metabolism and decrease neurovascular changes in ROP.

Poster #9 Loxl1 knockdown in primary optic nerve head astrocytes results in molecular and cellular phenotypes associated with reactive astrocytosis and elastinopathy

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Purpose: Exfoliation glaucoma is a common type of secondary open-angle glaucoma linked to single nucleotide polymorphisms in the lysyl oxidase-like 1 (*LOXL1*) gene. Non-coding variants in the *LOXL1* gene can result in reduced expression levels of Loxl1 protein in multiple ocular tissues, including the optic nerve head. The purpose of this study was to determine the effect of *Loxl1* down-regulation on optic nerve head astrocytes (ONHA).

Methods: Loxl1 knockdown was achieved by transfection of rat *Loxl1* gene-specific siRNA. For some experiments, a reactive astrocytic phenotype (reactive astrocytosis) was induced by exposure of ONHAs to either a humidified hyperbaric (20 - 25 mm Hg) atmosphere or to 10% static stretch. Quantitative PCR, immunoblotting and immunocytochemistry were performed using previously validated primers and antibodies.

Results: *Loxl1* expression was reduced by ~55% following siRNA transfection. Expression levels of GFAP (n = 5, $P < 0.001$) and voltage-gated calcium channel subunits (n = 3-4; $Ca_V\alpha$, $P < 0.01$; $Ca_V2.1$, $P < 0.01$) were significantly increased following *Loxl1* knockdown, suggestive of reactive astrocytosis. Concurrently, expression levels of elastin and collagen VI were significantly decreased, suggestive of cellular elastinopathy. Notably, induction of reactive astrocytosis by *Loxl1*-independent means resulted in almost identical molecular signatures.

Conclusions: *LOXL1* variants associated with exfoliation glaucoma may sensitize the optic nerve head to the deleterious consequences of elevated IOP by inducing reactive astrocytosis and cellular elastinopathy. Our data tentatively suggest a conserved a mechanism underlying reactive astrocytosis in exfoliation glaucoma and other subtypes of open angle glaucoma.

Poster #10 AAV delivery of alternative splice product of Complement factor H (CFH), FHL-1 to liver affects vision in *Cfh* knockouts.

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Purpose: Variants of Complement factor H (CFH) and its alternative splice form, FHL-1, are major genetic risk factors for AMD but the relative role/contribution of each form to AMD development is unknown. We used AAVs expressing FHL-1 to examine the function of FHL-1 in *Cfh* knock out (Cfh-/-) mice. We tested the hypothesis that FHL-1 directly contributes to AMD risk.

Methods: AAV vectors were designed for systemic expression of FHL-1, using a liver-specific promoter and delivered by tail vein injection in *Cfh-/-* mice. FHL-1 expression was measured on Westerns, and complement activity was assessed by Western blot of complement proteins C3, FB, and C5. FHL-1's effect on ocular phenotypes was assessed in aged (>90 weeks) *Cfh-/-* mice fed a high fat, cholesterol-enriched (HFC) diet (*Cfh-/-*~HFC). Visual function was measured by electroretinography (ERG).

Results: *Cfh-/-* mice injected with the AAV constructs had detectable levels of FHL-1 in their plasma. Plasma concentrations of C3 and FB were unchanged by FHL-1 expression. An increase in uncleaved C5 was detectable in young, but not aged, *Cfh-/-* mice fed an HFC diet. Visual function of *Cfh-/-*~HFC was affected by expression of FHL-1, resulting in an ERG b-wave deficit normally absent in this genotype.

Conclusions: FHL-1 expressed in the liver affects systemic levels of intact C5, indicating functionality of the construct. Development of a visual deficit with liver expression of FHL-1 supports a role of circulating FHL-1 in the development of phenotypes in our mouse model, which has implications for AMD therapies targeting the complement system.

Poster #11 Astrocyte Subtypes Determine the Fate of Ganglion Cells

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Purpose: Endothelin-1(ET-1) and its receptors are involved in the etiology of glaucoma. Previously, we reported that ET-1 treatment induced reactivation of astrocytes (ASTs) and apoptosis of retinal ganglion cells (RGC). However, ET-mediated subtype changes of ASTs and their effect on RGC survival are largely unknown. This study aimed to studying the roles of ET-1 in the interaction between RGCs and AST subtypes.

Methods: Primary rat RGCs were isolated from rat pup retinas and ASTs from optic nerve. Contact and non-contact RGC-AST co-cultures were treated with ET-1. ET-1-mediated intracellular calcium was monitored in RGCs, ASTs and co-culture of RGCs and ASTs using Fura-2 AM calcium imaging. Cell death and survival was detected using LIVE/DEAD assay. Expression of astrocytic markers in cultured ASTs and rat retina was determined by immunocytochemistry.

Results: ET-1-induced elevation of $[Ca^{2+}]_i$ was significantly attenuated in co-culture of RGCs and ASTs, and accordingly less cell death was also observed in both co-culture systems. More synapse formation was detected in RGC-AST co-cultures. The longer co-culture time produced more synapses. ET-1 treatment or c-Jun overexpression in ASTs changes the gene and protein expression profile of astrocytic markers. ET-1 intravitreal injection in rats also upregulated GFAP, Serping1 and CD14.

Conclusions: ASTs co-cultured with RGCs stimulate more synapse formation in RGCs and decrease RGC death. ET-1 treatment induces not only the reactivation of ASTs but also the switch of astrocyte subtypes, which could lead to dysfunction of axon transport in the optic nerve and affect RGC survival.

Poster #12 Dysregulation of ATX/LPA Signaling Axis in the Aqueous Humor of Human Glaucoma Patients

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Purpose: Lysophosphatidic acid (LPA), a bioactive lipid and product of autotaxin (ATX), has been shown to modulate the aqueous humor (AH) outflow and intraocular pressure (IOP). To seek possible etiological significance of dysregulated ATX/ LPA signaling axis in primary open angle glaucoma (POAG), here, we determined the levels of LPA and ATX in the AH of POAG patients and investigated the regulation of ATX expression in human primary trabecular meshwork (TM) cells.

Methods: The levels of ATX, LPA, and ATX substrate-lysophosphatidylcholines (LPC) in AH derived from POAG and cataract patients (n=25) were measured by ELISA and mass spectrometric analyses. The regulation of ATX protein and mRNA, in TM cells (n=6) using different physiological agents, siRNA and inhibitors was evaluated by immunoblot analysis.

Results: Significant increases in ATX protein levels was found in the AH of POAG patients compared to age and gender matched cataract patients and they exhibited a positive correlation with IOP values in POAG patients. Both LPA and LPC levels in the POAG samples were significantly elevated compared to cataract samples. ATX protein levels were significantly elevated in TM cells in response to the treatment with dexamethasone, TNF-α, and IL-1β via ERK, NF-κB or SMAD2/3, and glucocorticoid receptors. ATX deficiency and inhibition suppressed the fibrogenic activity in TM cells.

Conclusions: Collectively, these results reveal that the dysregulated levels of ATX, LPA and LPC in AH associate with the etiology of ocular hypertension in POAG patients, and ATX as a promising therapeutic target to lower IOP in glaucoma patients.

Poster #13 REV-ERBa Regulates RPE Function and Age-related Degeneration in Mice

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Purpose: Retinal pigment epithelium (RPE) dysfunction and atrophy is observed in patients with dry form of age-related macular degeneration (AMD). This often leads to further degeneration of nearby photoreceptors, which ultimately causes blindness. Dysfunction of RPE cells during aging is associated with oxidative stress, dysregulated cellular metabolism and inflammation during aging. Here we investigated the role of the nuclear receptor REV-ERBa, a redox sensitive transcriptional regulator of metabolism and inflammation, in regulating RPE function in AMD pathogenesis.

Methods: REV-ERBa deficient (*Rev-erba*$^{-/-}$) mice and littermate wild type (WT) controls were analyzed at various time points during aging. Morphological features were analyzed using fundus imaging and RPE/choroid complex flat mounts and cross-sections. Ultrastructure of RPE, retina and Bruch's membrane were characterized by electron microscopy. RPE phagocytosis was measured by fluorescent microbeads uptake. Expression levels of *Rev-erba* and relevant metabolic and inflammatory genes were analyzed in isolated *Rev-erba*$^{-/-}$ and WT RPE.

Results: REV-ERBa expression levels were decreased with age in WT RPE. Aged *Rev-erba*$^{-/-}$ mouse eyes showed abnormal lipid-enriched subretinal lesions, substantially decreased visual function, patchy areas of RPE degeneration with disrupted tight junctions and decreased expression of junctional genes. Moreover, phagocytic activity was significantly diminished in isolated *Rev-erba*$^{-/-}$ RPE cells. *Rev-erba*$^{-/-}$ RPE also exhibited a decrease in metabolic gene expression and an increase in inflammatory gene expression including complement components.

Conclusions: Our findings suggest that REV-ERBa is a novel regulator of RPE function and degeneration in AMD pathogenesis, and may serve as a potential molecular target for developing future therapeutics.

Poster #14 Aberrant BMAL1 dependent claudin-5 cycling induces geographic atrophy

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Purpose: Circadian rhythms involvement in retinal function is not fully understood. Here, we examined the role of circadian rhythms in the regulation of the inner blood-retinal barrier (iBRB) function that we show plays a role in the development of geographic atrophy (GA), the end stage of 'dry' age-related macular degeneration (AMD).

Methods: iBRB integrity was characterised *in vitro* by western blotting and qPCR and *in vivo* using fundus fluorescein angiography (FFA) and contrast enhanced magnetic resonance imaging. Mice were sub-retinally injected with adeno-associated virus targeting claudin-5 expression and placed onto a cholesterol-enriched diet. Eyes were enucleated and immunohistochemical analysis undertaken.

Results: Retinal claudin-5 expression is tightly regulated by *BMAL1* and the circadian clock leading to phenotypically more permeable retinal vessels in the evening. Persistent suppression of claudin-5 expression in mice exposed to a cholesterol-enriched diet induces retinal pigment epithelium (RPE) cell atrophy. A similar phenotype is observed in non-human primates where persistent claudin-5 suppression in the macular region induced RPE atrophy. FFA in human subjects showed increased retinal vascular permeability in the evening compared to morning.

Conclusion: Our findings show the iBRB is highly dynamic and implicates an inner retina derived component to the early pathophysiological changes observed in AMD. Circadian regulation of claudin-5 facilitates material exchange between blood and the neural retina allowing replenishment of 'spent' photoreceptor outer segments by the RPE. These results suggest re-establishing claudin-5 cycling at the iBRB may represent a novel therapeutic target for the prevention and treatment of GA secondary to dry AMD.

Poster #15 Schlemm's canal imaging, pressure measurement, catheterization and substance delivery in live monkeys

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Purpose

1) We describe a real time technique to measure pressure in Schlemm's canal (SC) that may help understanding glaucoma pathophysiology and evaluating potential therapeutics.

2) We describe a gene delivery technique allowing injection of smaller volumes and lower titers directly into SC, minimizing off-target effects.

Methods: 1) A needle, connected to a pressure transducer and computer, is placed into the anterior chamber to continuously record and control IOP. Concurrently, SC is imaged using an endoscope attached to a digital camera in turn connected to a computer. The images obtained are time synchronized with the collection of IOP data. 2) With gonioscopic/microscopic visualization of SC, a microcatheter is inserted into the canal circumferentially ab interno. The vector along with trypan blue dye is injected while withdrawing the catheter and visualized in the SC with an endoscope over 360°.

Results: 1) SC fills with venous blood and blanches as IOP is lowered and raised, respectively. The range of IOP over which these changes are noted is wider and higher than predicted (~5-22mmHg). Sausaging of the blood column in SC is observed indicating segmental differences. 2) 360° catheterization of SC was routinely executed. The vector was injected and visualized with an endoscope as a bright blue band within SC.

Conclusion

1) Recording canalicular morphology and IOP, with synchronized time stamping, will provide a precise description of pressure-related canalicular dynamics.

2) Placing a microcatheter directly into SC may facilitate development of gene therapies targeting conventional outflow pathway structures.

Poster #16 Role of miR-29c-3p in regulation of extracellular matrix synthesis

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Purpose: To investigate changes in microRNA (miRNA) expression in lamina cribrosa (LC) cells treated with transforming growth factor beta 2 (TGFβ2).

Method: Non-glaucomatous primary human LC cells were grown to 100% confluency and treated with TGFβ2 (5ng/ml) or control for 24hours. Differences in expression of miRNAs were analysed by miRNA Q-PCR array. LC cells were transfected with miR-29c-3p (10nM) mimic, inhibitor or non-targeting controls and analysed by Q-PCR to confirm overexpression or knockdown of miR-29c-3p. mRNA targets of miR-29c-3p were determined through protein expression analysis by immunocytochemistry. The effects of miR-29c-3p and TGFβ2 on collagen type (COL) I and IV protein expression were evaluated in cells transfected with miR-29c-3p mimic, inhibitor or control and treated with TGFβ2 expression.

Result: miRNA PCR arrays showed that TGFβ2 treatment downregulated the expression of miR-29c-3p in LC cells. Transfection of miR-29c-3p mimic or inhibitor showed upregulation and downregulation of miR-29c-3p respectively, confirming transfection efficiency. Immunocytochemistry analysis showed that miR-29c-3p regulates the expression of COL I and IV. Overexpression of miR-29c-3p decreased TGFβ2 induced COL I and IV expression. Inhibition of miR-29c-3p exacerbated the effects of TGFβ2 on COL I and IV expression.

Conclusion: TGFβ2 induced the downregulation of miR-29c-3p, an anti-fibrotic miRNA, which may stimulate a pro-fibrotic response and pathogenic remodelling of the optic nerve head. The inhibitory effects of miR-29c-3p on TGFβ2, suggest that miR-29c-3p regulates ECM protein synthesis and restoring the expression of miR-29c-3p may preserve the microarchitecture of the LC.

Poster #17 Sodium 4-phenylbyrate rescues glucocorticoid-induced ocular hypertension by reducing abnormal ECM deposition in the TM via activation of MMP9

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Purpose: Ocular hypertension (OHT) is a serious side effect of glucocorticoid (GC) therapy and if left undiagnosed, it leads to glaucoma. Previously, we have shown that, sodium-4-phenylbutyrate (PBA) rescues GC-induced glaucoma. However, the exact mechanism behind PBA mediated reduction of GC-induced OHT is not completely understood. Here, we examined whether PBA rescues GC-induced OHT by reducing abnormal ECM deposition via activating matrix metalloproteinases (MMP).

Methods: GC-induced OHT mice were topically instilled 1% PBA or vehicle twice daily. IOPs were measured every week and outflow facility was analyzed after 5-weeks of treatment. Immunostaining, Western blot and qPCR were performed to analyze the effect of PBA on Dex-induced ECM deposition and ER stress in primary human TM cells, mouse and ex-vivo cultured human TM tissues. Zymography used to study the effect of PBA on MMP activity.

Results: Topical PBA (1%) eye-drops reduced GC-induced OHT and also improved outflow facility significantly. PBA reduced GC-induced intracellular and extracellular ECM accumulation and also prevented induction of ER stress in primary human TM cells, ex-vivo human cultured TM tissues and mouse TM tissues. Interestingly, we observed that PBA can reduce existing ECM deposition when primary TM cells were grown on decellularized GC-treated ECM. Gene expression, Western blot and zymography assays revealed that PBA induces increased expression and activity of MMP-9 over control in glaucomatous cells. Inhibition of MMP-9 activity by chemical-inhibitors abrogated PBA's effect on ECM reduction.

Conclusions: PBA can be a very attractive treatment for general POAG via targeting abnormal ECM accumulation in the TM.

Poster #18 Vitamin B12 deficiency caused by metformin is associated with the risk of diabetic retinopathy

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Purpose: Metformin is the cornerstone drug treatment for type 2 diabetes. Recent studies show that this treatment is associated with deficiency of vitamin B12, which is thought to be caused by impaired vitamin B12 absorption. The aim of this study was to evaluate the associated risk of the vitamin B12 deficiency in metformin-treated patients with and without diabetic retinopathy.

Methods: We enrolled 384 diabetic retinopathy patients treated for at least 3 years with metformin and 170 diabetic patients managed with diet or insulin or other treatments. Circulating vitamin B12 levels along with total cholesterol, HDL cholesterol, triglycerides, glycated hemoglobin, homocysteine and folate were evaluated. Prevalence, predictive values, sensitivity and specificity were assessed by Bayes' theorem.

Results: Vitamin B12 deficiency in metformin-treated patients with diabetic retinopathy showed positive predictive value 85%, negative predictive value 70%, sensitivity 87%, specificity 67%, prevalence 69%.

Conclusions: Long term of metformin treatment causes vitamin B12 deficiency that is associated with the risk of diabetic retinopathy. Therefore, we suggest that vitamin B12 supplementation could be useful in patients under long-term metformin treatment.

Poster #19 Assessment of soluble epoxide hydrolase and role of lipid mediators in choroidal neovascularization

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Purpose: Choroidal neovascularization (CNV) is a major pathological feature of age-related macular degeneration (AMD), and new therapies are still needed. Previously, we identified soluble epoxide hydrolase (sEH) as a binding target of an antiangiogenic small molecule, SH-11037. Here, we aimed to investigate sEH expression in the eye and the effects of the lipid substrate and product of sEH in CNV.

Methods: The expression localization, and activity of sEH in eyes of laser-induced choroidal neovascularization (L- CNV) mice were evaluated by immunohistochemistry and an ex vivo enzymatic assay. The sEH inhibition by SH-11037 and mode of inhibition was assessed by recombinant sEH activity assay. Also, sEH substrate 19,20-epoxy docosapentaenoic acid (EDP) and product 19,20-dihydroxy docosapentaenoic acid (DHDP) were injected intravitreally in L-CNV mice, and effects were assessed by imaging and immunofluorescence.

Results: sEH was upregulated in the rod photoreceptors in L-CNV mice compared to control mice and it did not co- localize with other retinal cell type markers. Correspondingly, sEH activity was increased in L-CNV eyes and was normalized by SH-11037 or a known sEH inhibitor. SH-11037 inhibited recombinant sEH activity, with IC_{50} = 0.15 µM. Enzyme kinetics analysis demonstrated that SH-11037 decreased V_{max} and increased K_M, revealing SH-11037 as a mixed-type inhibitor of sEH, with K_i = 1.73 ± 0.45 µM. Intravitreal 19,20-EDP reduced CNV lesion volume compared to vehicle and 19,20-DHDP.

Conclusions: Our studies confirm the relevance of sEH and its lipid metabolites for choroidal neovascularization, which these mediators affect angiogenesis will be critical.

Poster #20 Activation of TRPV4 channels reduces IOP and improves outflow facility by regulating eNOS dependent NO release from the TM

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Purpose: Nitric oxide (NO) is known to reduce intraocular pressure (IOP) by relaxation of the trabecular meshwork (TM) and distal vessels of the conventional outflow pathway. Nonetheless, the intrinsic pathways by which outflow pathway tissues regulate NO production is yet to be elucidated. In vascular endothelium, activation of mechanosensory transient receptor potential vanilloid 4 (TRPV4) channels results in endothelial nitic oxide synthase (eNOS) mediated NO release, which in turn promotes vasodilation. Here, we explored whether TRPV4 activation regulates NO release in the conventional outflow pathway.

Methods: In wildtype and glucocorticoid-induced ocular hypertensive C57BL/6J mice, the effect of TRPV4 agonist GSK1016790A on IOP and outflow facility was determined using rebound tonometry and constant-flow infusion method respectively. Effect of TRPV4 agonist on eNOS activation and NO production was determined using Western blot and DAF-FM assay in primary human TM cells and *ex vivo* cultured human TM donor tissues. We developed a novel method for electrochemical measurement of NO using NO electrodes in human anterior segment tissues.

Results: Topical administration of TRPV4 agonist significantly reduced IOP and increased outflow facility. TRPV4 activation increased eNOS phosphorylation and NO production in primary human TM cells and *ex vivo* cultured human TM. Treatment of human anterior segments with TRPV4 agonist resulted in increased production of NO as detected electrochemically. Nonselective inhibition of NOS by L-NAME abrogated the IOP lowering effect of TRPV4 agonist in mice and reduced NO production in outflow pathway cells and tissues.

Conclusion: TRPV4 activation improves IOP and outflow, perhaps by NO regulation.

Poster #21 Non-invasive monitoring of suprachoroidal, subretinal and intravitreal biodegradable implants using confocal laser scanning ophthalmoscope (cSLO) and optical coherence tomography (OCT)

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Purpose: Our long-term goal is to facilitate continuous noninvasive monitoring of drug delivery in the eye. Although injectable implants are reality for the vitreous humor, we are not aware of similar efforts in the suprachoroidal and subretinal spaces. To address these gaps, our objective was to inject implants into suprachoroidal, subretinal, and intravitreal spaces and monitor their length, diameter, and volume noninvasively.

Methods: Biodegradable poly(lactide-co-glycolide) (PLGA) implants of various lengths and diameters were injected in isolated bovine eyes at suprachoroidal, subretinal, and intravitreal locations. The implants were imaged noninvasively using cSLO and OCT modes of Heidelberg Spectralis HRA+OCT instrument after adjusting the corneal curvature for the bovine eye.

Results: Simultaneous cSLO and OCT images identified implants in different regions. Implant length and diameter were obtained using cSLO images. Volumes for suprachoroidal and subretinal implants were estimated by integrating the bleb areas, at various depths in OCT images or by using the thickness map of 1 mm diameter central circle of ETDRS grid. Intravitreal implant volume was estimated using the dimensions obtained from cSLO images. Image-based measurements of length, diameter, and volume correlated well with the values prior to injection. The accuracy for noninvasive measurement of PLGA implant for length, diameter, and volume ranged from 93-104%, 75-118%, and 58-171%, respectively, for the various routes and approaches.

Conclusions: Suprachoroidal, subretinal, and intravitreal implants can be monitored for their length, diameter, or volume using cSLO and OCT imaging. Such measurements may be useful in monitoring implant degradation and drug release in vivo.

Poster #22 Computational systems biology approach to identify novel pharmacological targets for diabetic retinopathy

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Purpose: Diabetic retinopathy was included by the World Health Organization in the eye disease priority list. Up to now, only proliferative diabetic retinopathy can be treated with approved drugs, such as intravitreal anti-vascular endothelial growth factor (VEGF) agents or steroids. In this perspective, there is the urgent need to explore novel pharmacological targets for treatment of diabetic retinopathy.

Methods: We applied a systems biology approach to identify novel drug targets for diabetic retinopathy. Transcriptomic data were retrieved from Gene Expression Omnibus Dataset repository (GEO) datasets. Analysis of GEO datasets was carried out with an enrichment-information approach, which gave as output a series of complex gene-pathway and drug-gene networks. Networks were analyzed through Cytoscape. Bioinformatic data have been integrated with literature search and data mining through clinicaltrial.gov.

Results: Bioinformatic analysis predicted 102 putative pharmacological targets. No current references are available regarding 19 predicted targets, while, only 15 targets have been investigated in clinical trials for treatment of diabetic retinopathy. Our computational analysis has revealed 19 novel potential pharmacological targets for diabetic retinopathy: *SLC13, CFLAR, SCTR, FSHB, RLN3, ADR1D, ADR2C, CHRM5, LPAR2, FSHR, THRB, GLRA3, PRSS3, KCNJ16, KCNE2, ATP4A, ATP1B4, ADCY7, PTGES.*

Discussion: Analysis of these networks identified genes and biological pathways related with inflammation, fibrosis and G protein-coupled receptors that are potentially involved in development of the disease. This analysis provided new clues on novel pharmacological targets, useful to treat diabetic retinopathy.

Poster #23 Crosstalk Between Transforming Growth Factor Beta-2 and Toll Like Receptor 4 in the Trabecular Meshwork

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Purpose: One of the primary risk factors for primary open angle glaucoma (POAG) is elevated intraocular pressure (IOP). Discovering potential new targets to lower IOP is necessary for effective drug therapies. We recently discovered a novel molecular mechanism involved in the development of glaucomatous trabecular meshwork (TM) damage and elevated IOP. We have identified TGFβ2 and toll-like receptor 4 (TLR4) signaling crosstalk regulates changes in the TM ECM and mutation in *Tlr4* rescues TGFβ2 -induced ocular hypertension in mice. Here, we investigated the role of an endogenous TLR4 ligand, FN-EDA, and a downstream signaling molecule, NFκB, in TGFβ2-induced ocular hypertension in mice.

Methods: B6.FN-EDA$^{-/-}$, B6.FN-EDA$^{+/+}$/TLR4$^{-/-}$, B6.FN-EDA$^{-/-}$/TLR4$^{-/-}$, and C57BL/6J mice were intravitreally injected with 2.0µL Ad5.TGFβ2 (2.5x10^{7}pfu) in one eye and the contralateral eye was uninjected. Likewise, we tested mice lacking the p50 subunit of NFκB (B6.Cg-*NFκB1tm1Bal*/J) and C57BL/6J mice. IOP was measured once per week using a rebound tonometer on isoflurane-anesthetized mice. Significance determined by one-way ANOVA at each time point. At 6-week post-injection eyes were harvested, fixed, and sectioned for immunohistochemistry to assess total fibronectin and FN-EDA isoform expression.

Results: Ad5.TGFβ2 significantly induced ocular hypertension in C57BL/6J mice. Mutations in *Tlr4, FN-EDA*, and *NFκB* blocked Ad5.TGFβ2 induced ocular hypertension with no significant IOP elevation. Total FN and FN-EDA isoform expression increased in Ad5.TGFβ2 injected C57BL/6J mice.

Conclusions: TLR4, FN-EDA, and NFκB are necessary for TGFβ2 induced ocular hypertension in mice. These data provide potential new targets to lower IOP.

Poster #24 Identification of the functional complex between hnRNPL and the glaucoma-associated long noncoding RNA, PEXpress

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Purpose: Pseudoexfoliation (PEX) glaucoma is a common and aggressive form of glaucoma with distinct clinical manifestations of fibrillary material deposition in the conventional outflow pathway. The long noncoding RNA, *PEXpress*, is highly associated with risk of PEX glaucoma, however the mechanism by which it regulates molecular changes in the tissues of the outflow pathway is unknown.

Methods: Fluorescence in-situ hybridization probes targeted to *PEXpress* localized *PEXpress* subcellularly. Interacting proteins were identified using a streptavidin pulldown assay with biotinylated *PEXpress* in conjunction with mass spectrometry. RNA-seq identified and quantified downstream gene targets of *PEXpress*. Adenovirus encoded with targeted shRNAs was used to knock down *PEXpress* in Schlemm's canal (SC) cells. Signaling molecules AKT, MAPK and FAK were analyzed for their phosphorylation status by Western blot, and cellular morphology was assessed in SC cells using Nikon software.

Results: *PEXpress* localized predominantly to the nuclei of cells and bound the mRNA regulatory protein, hnRNPL. In immortalized human lens (HLE-B3) cells, knockdown of *PEXpress* led to dysregulation of over 450 target genes, while overexpression of *PEXpress* significantly altered the expression of ~100 other genes. When *PEXpress* abundance was manipulated, phosphorylation status of MAPK, AKT and FAK was not changed in HLE-B3 cells. Interestingly, knockdown of *PEXpress* in SC cells led to a significant increase in the ratio of pAKT/AKT, which was coincident with a significant change in cellular morphology.

Conclusions: *PEXpress* regulates gene expression, AKT signaling, and cellular morphology in glaucoma relevant cells, making it a viable therapeutic target for PEX glaucoma.

Poster #25 Assessment of ocular blood flow regulation using laser speckle flowgraphy in healthy subjects

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Purpose: Ocular blood flow (OBF) regulation during changes in ocular perfusion pressure (OPP) or during stimulation with flicker light has been extensively investigated in the recent years using various techniques. The aim of the present studies was to evaluate whether Laser speckle flowgraphy (LSFG), a commercially available technique for measuring OBF, is capable to assess blood flow regulation.

Methods: In 27 subjects, measurements of optic nerve head (ONH) and retinal blood flow were performed during isometric exercise and in 20 subjects, blood flow was assessed before and during stimulation with flicker light. Mean blur rate (MBR) in the ONH (MBR_{ONH}), relative flow volume (RFV) in retinal arteries (RFV_{ART}) and veins (RFV_{VEIN}) were assessed using LSFG.

Results: In the isometric exercise experiments, the relative increase in OPP (78.5 ± 19.8%) was more pronounced than the increase in OBF parameters (MBR_{ONH}: 18.1 ± 7.7%, RFV_{ART}: 16.5 ±12.0%, RFV_{VEIN}: 17.7 ± 12.4%) indicating for an autoregulatory response of the vasculature. Retinal stimulation with flicker light induced a significant increase in MBR_{ONH} by +17.5%±6.6% ($p<0.01$), as well as in RFV_{ART} by +23.8±10.0% ($p<0.05$) and in RFV_{VEIN} +23.1%±11.0 ($p<0.05$).

Conclusions: As expected, the increase in OBF parameters was less pronounced than the increase in OPP during isometric exercise, while flicker stimulation induced a significant increase in OBF. These results indicate that LSFG is an appropriate method for the quantification of retinal and ONH blood flow during different provocation tests and may be applied as a non-invasive, easy to use tool to assess OBF regulation in humans in the future. Support from the Austrian Science Fund FWF KLI 529 is gratefully acknowledged.

Poster #26 Characterization of RPE Cell Death Induced by 4-HNE

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Purpose: Degeneration of the retinal pigment epithelial (RPE) underscores the pathology in geographic atrophy (GA), a late stage dry age-related macular degeneration (AMD). Oxidative stress and aging are known to contribute to GA pathogenesis. However, how RPE cells die in GA is still controversial. 4-Hydroxynonenal (4-HNE) is an oxidative stress marker produced by lipid peroxidation, which is accumulated in aging cells and could be related to age-related diseases. The goal of the study is to determine the mechanism of 4-HNE-induced RPE cell death.

Methods: ARPE-19 cells were treated with 4-HNE at different concentrations to induce cell death**.** Inhibitors of apoptosis, necroptosis, pyroptosis and ferroptosis pathways, were used to test for their effect on cell viability. Cell morphology and molecular markers (including PYCARD and RIPK3) were examined under a microscope. ATP and ROS levels were measured using standard methods.

Results: We found that 4-HNE (5-10 ug/ml) induces significant RPE cell death, consistent with significantly decreased cellular ATP but increased ROS levels. Necrosis inhibitor Necrostatin-7 and ferroptosis inhibitors liproxstatin-1 and DFO, but not apoptosis inhibitors, prevented 4-HNE-induced RPE cell death. On the molecular level, both inflammasome and necrosome are activated in RPE cells treated with 4-HNE, as shown by PYCARD and RIPK3 visualization.

Conclusions: Ferroptosis and/or necroptosis, but not apoptosis, underlie RPE death induced by 4-HNE. Crosstalk between different cell death pathways may occur when RPE cells are under oxidative stress. The inhibitors from our study may have therapeutic implications for GA.

Poster #27 Cathepsin K, a secreted cysteine protease, regulates transforming growth factor-β2 bioavailability and extracellular matrix in the trabecular meshwork

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PURPOSE: Cysteine protease like Cathepsin K (CTSK) regulates extracellular matrix (ECM) remodeling. CTSK degrades helical and non-helical regions of collagen 1 and regulates transforming growth factor-β(TGFβ) stability. We investigated the role of CTSK on TGFβ2 bioavailability and ECM remodeling in human trabecular meshwork (HTM).

METHODS: CTSK expression, activity and distribution in HTM cell, and in aqueous humor (AH) outflow tissue was assessed. Effects of adenovirus-mediated expression of CTSK (AdCTSK) on changes in actin organization, ECM production and remodeling, and TGFβ2 levels were analyzed using qPCR, immunofluorescence, and immunoblotting. Students t-test was used for statistical analyses and results were significant if $p<0.05$ with a sample size of $N\geq3$ in each experiment.

RESULTS: CTSK is distributed in the TM and juxta-canalicular tissues of AH outflow pathway and CTSK activity is found in HTM cell extracts and culture media. Increasing CTSK expression in HTM cells using AdCTSK resulted in- a) shorter and coalesced actin stress fibers assessed using phalloidin staining, b) decreased collagen-1A and fibronectin assembly and fibril formation as visualized by immunofluorescence, and c) significant decrease in collagen-1A and fibronectin expression ($p<0.05$, $n=3$). AdCTSK significantly decreased TGFβ2 mRNA ($p=0.004$, $n=3$) and total intracellular TGFβ2 levels ($p<0.05$, $n=3$).

CONCLUSION: Together, these preliminary findings identify CTSK as an important ECM modulator in the TM. We believe that CTSK is involved in the IOP homeostasis by regulating the bioavailability of TGFβ2 and maintaining optimal cell-matrix interactions in the AH outflow pathway. Any kind of dysregulation in CTSK functions can lead to elevated IOP.

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Poster #28 Intravenous treatment of choroidal neovascularization by photo-targeted nanoparticles

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ABSTRACT: Choroidal neovascularization (CNV) is the major cause of vision loss in wet age-related macular degeneration (AMD). Current therapies require repeated intravitreal injections, which are painful and can cause infection, bleeding, and retinal detachment. We developed a drug delivery system that can be administered intravenously and accumulate in the back of the eye by light-triggered targeting. Photo-targeted nanoparticles (NP-[CPP]) were formed from PEG-PLA chains modified with cell penetrating peptide (CPP). Cell uptake of NP-[CPP] was inactivated by attaching a photocleavable group DEACM to the CPP, which also placed [CPP] in the core of the nanoparticle, preventing it from interacting with cells. Irradiation with 400 nm (blue) light cleaved DEACM, releasing CPP from the NP core and rendering it active. This system was evaluated in mice with laser-induced CNV. After intravenous injection of NP-[CPP], irradiation at the eye cleaved DEACM, allowing NP accumulation in the choroidal neovascular lesions. NP- [CPP] with irradiation showed greater accumulation in neovascular lesions compared to the same nanoparticles without irradiation or nanoparticles without CPP. In the same mouse CNV model, NP-[CPP] loaded with doxorubicin significantly reduced neovascular lesion size. This phototriggered targeting strategy could allow non-invasive treatment of CNV and similar diseases, and enhance the proportion of drug in diseased areas of the eye vs. other healthy parts of the eye or body.

Poster #29 Does protein acetylation plays crucial role in δ-opioid receptors mediated retinal ganglion cell neuroprotection in glaucoma?

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Purpose: This study determines the role of protein acetylation and its downstream targets that play crucial role in the δ-opioid receptor agonist, SNC-121-mediated retina neuroprotection in glaucoma model.

Methods: Intraocular pressure (IOP) was raised in Brown Norway rats by injecting 2M hypertonic saline into the limbal veins. Animals were administered with SNC-121 (1 mg/kg; i.p) daily for 7 days. Retinas were determined for the changes in the expression of genes and proteins at day 7^{th} using qPCR based array and Western blotting.

Results: IOP was increased significantly ($P<0.05$) in ocular hypertensive (OH) animals. Elevated IOP caused a significant increase in HDAC (Histone Deacetylase) Class I (HDAC 1, 2, 3 and 6) activity by 17.8±5% ($P<0.05$) that was abrogated by SNC-121 treatment. Concomitantly, acetylated histone H3 levels were decreased in OH retinas and restored by SNC-121 treatment. A significant ($p<0.05$) increase in mRNA expression of transcriptional factors STAT3 and decrease in CREB ($p<0.05$) was observed OH animals, which was restored to normal levels by SNC-121 treatment. SNC-121 phosphorylated CREB whereas, SNC-121 inhibited phosphorylation of STAT3 in the OH animals. Additionally, a significant decrease (> 2fold, $P<0.05$) in level of neurotrophic factors (e.g., BDNF, CNTF, and FGF) and increase in pro-inflammatory cytokines (e.g., IL-1β and IL-6) was seen in the retina of OH animals.

Conclusions: Our data suggest that IOP exacerbation disrupts protein acetylation homeostasis, favoring the production of pro-inflammatory milieu in OH eye. Our novel finding suggests that early intervention with SNC-121 reverse such changes, thereby providing neuroprotection against glaucomatous injury.

Poster #30 A guinea pig-based screening of intraocular pressure lowering drugs as a novel avenue for controlling myopia progression

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Purpose: In an already published study, we reported that topical latanoprost was effective in both inducing a sustained (across 24 h) decrease in IOP and slowing myopia progression in myopic guinea pigs. As the first step in a follow-up study, this study examined the efficacy of four different glaucoma drug classes in lowering intraocular pressure in guinea pigs as potential alternative avenues for controlling myopia progression.

Methods: 7-9 months old guinea pigs (GPs) underwent monocular topical glaucoma drug treatment for 4 weeks. 15 GPs were equally divided into five treatment groups of 3 animals that received: 1) Latanoprost QD (Prostaglandin, 0.005%), 2) Timolol QD (Beta blocker, 0.5%), 3) Brimonidine BID (a2 agonist, 0.15%), 4) Brinzolamide suspension BID (Carbonic anhydrase inhibitor, 1%), and 5) Non-preserved artificial tears QD (control). Diurnal intraocular pressures (IOPs) were recorded at baseline (before treatment), and after 2 and 4 weeks of treatment, in each case at 4 time points, using iCare tonometry.

Results: Key results are summarized in Table 1, as changes in interocular differences from baseline. Both latanoprost and timolol induced a sustained lowering of IOP, as reflected in changes recorded at week 4. Brimonidine and Brinzolamide were not as effective in lowering IOP.

Conclusions: As shown previously for latanoprost, daily topical timolol was also effective in lowering IOP over 24 h in healthy guinea pigs. This result contrasts with previous human studies, which reported timolol (sympathomimetic) to be ineffective in lowering IOP during the night. This may reflect the crepuscular nature of guinea pigs.

Table1: Mean interocular (treated-control) differences; ± SEM in mmHg at the end of the treatment (4 weeks) compared to baseline.

Treatment group	9:35 am	3:35 pm	9:25 pm	3:25 am	Grand mean
Artificial tears	3.8 ± 1.45	-3.0 ± 0.12	2.1 ± 0.43	-1.1 ± 0.75	0.45 ± 0.69
Latanoprost	0.22 ± 2.6	-4.7 ± 1.43	-1.4 ± 0.58	-2.6 ± 0.02	-2.12 ± 1.16
Briminodine	-0.44 ± 0.29	2.0 ± 0.49	0.0001 ± 0.77	3.8 ± 1.7	1.34 ± 0.81
Brinzolamide	2.7 ± 3.08	2.3 ± 0.45	-2.0 ± 0.4	-0.22 ± 0.7	0.70 ± 1.16
Timolol	-2.8 ± 0.4	-0.11 ± 1.24	0.22 ± 0.35	-3.7 ± 2.42	-1.60 ± 1.10

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Poster #31 Using Tandem-Mass-Tags to Quantify Sex-Dependent Retinal Proteome Phenotypes Identified by Electroretinography

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Purpose: A wide array of biological and technical variables can affect electroretinogram (ERG) outcomes, including gender. In turn, the prevalence of many retinal diseases exhibit strong gender dependencies. When we examined mouse scotopic ERG data by sex, trends were obvious under resting conditions. Therefore, we used a mass spectrometry (MS) based approach to characterize potential sex-based differences in the retina of the normal adult mouse.

Methods: 30-week-old outbred Swiss-Webster ND4 mice were used. Scotopic ERGs were obtained in 8 male and 8 female ketamine-xylazine anesthetized mice. Retinae from 5 male and 5 female mice were processed for MS3 analysis on a Fusion Orbitrap Mass Spectrometer (Thermo Fisher) using Tandem-Mass-Tags for quantification. Statistical analysis was performed using Stata$_{SE15}$, and bioinformatics by Ingenuity Pathway Analysis (Qiagen).

Results: Male mice have significantly higher ERG b-wave amplitudes. MS identified 4,264 proteins, 4,093 of which were quantified. 68 proteins were differentially expressed, between genders, by at least 1.5-fold and 32 of those by at least 2-fold. Two identified pheromone proteins, previously unreported in retina, differed more than 4-fold by gender. Additionally, 17 crystallin proteins were quantified, 12 of which were differentially expressed, with 10/12 more abundant in males.

Conclusions: Distinct molecular phenotypes likely contribute to the significantly greater resting scotopic ERG amplitudes observed in male mice, given that the expression levels of a large number of proteins involved in structure, development, signaling, and metabolism exhibited sex-dependent differences. Our findings may provide phenotypic insights into retinal function, as well as why many retinal diseases exhibit unique gender disparities.

Poster #32 Stabilization of hypoxia-inducible transcription factor-1a (HIF-1a) in rat glaucomatous optic nerve

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Purpose: This study was designed to determine the stabilization of hypoxia-inducible transcription factor-1a (HIF-1a) and production of pro-inflammatory cytokines in rat glaucomatous optic nerves.

Methods: Brown Norway rats were used to elevate intraocular pressure (IOP) by injecting 50 µL of 2 M hypertonic saline into the circumferential limbal veins. IOP was recorded prior to surgery (baseline IOP) and weekly after injury. HIF-1a inhibitor (KC7F2 0.05- 2 mg/kg; i.p.) was administered right after injury and subsequently daily for 28 days. The changes in the level of pro-inflammatory cytokines, GFAP, and HIF-1a were measured by RT-PCR, Western blotting, and immunohistochemistry.

Results: Intraocular pressure (IOP) elevation stabilizes HIF-1a at day 7th and 28th, post ocular injury. HIF-1a protein expression was increased by 45±4 (P<0.05) and 75±7 (P<0.05) in the optic nerve of ocular hypertensive animals at day 7th and 28th, respectively. Both HIF-1a stabilization and GFAP staining (glial cell activation marker) were increased and co-localized in optic nerves of ocular hypertensive eyes. Additionally, pro-inflammatory cytokines (e.g. TNF-a, and IL-1β) were up-regulated in ocular hypertensive animals and their levels were reduced significantly (P<0.05) in a HIF-1a inhibitor (e.g., KC7F2) treated animals.

Conclusions: Our data suggest that glial cell activation, HIF-1a stabilization, and their co-localization occur within optic nerves in an early stage of glaucoma development. Glial cell-induced pro-inflammatory cytokines could be regulated by transcriptional factor like HIF-1a and that can be a potential therapeutic target for the glaucoma therapy.

Poster #33 Use of ultrasound biomicroscopy to improve assessment of outflow facility by tonography

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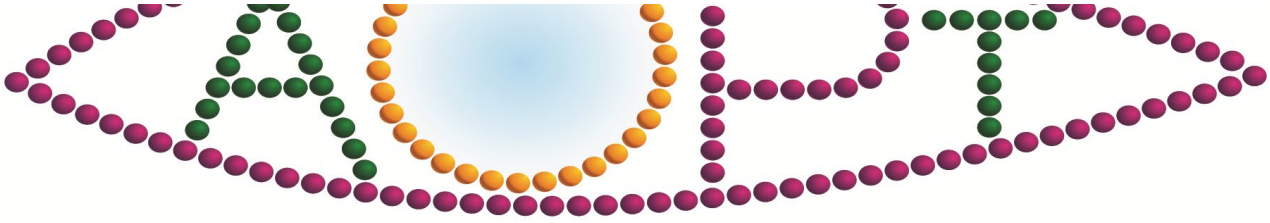
Purpose: Accurate assessment of outflow facility (C) is crucial for understanding glaucoma and treatment potential. C, is the ratio of a change in aqueous flow with change in intraocular pressure (IOP). The Friedenwald tables provide an estimate of aqueous flow change during the two-minute tonography procedure. This proof of concept experiment modifies the technique by utilizing ultrasound biomicroscopy (UBM) to measure individualized changes in aqueous flow.

Methods: Ten sedated rabbits were placed in lateral decubitus position. Tonography was performed for 2-minutes using a pneumatonometer with a 10-gram weight applied to the probe shaft and placed on the cornea. Serial images of the anterior chamber (AC) from nasal to temporal edges were captured with UBM before and after probe placement. Both eyes were measured. Five observers identified the posterior corneal and anterior iris surfaces as the borders of the AC in serial images. Algorithm-based reconstruction of the surfaces were used to determine AC volume and volume change during two minutes. Two-tailed t-tests compared IOP and AC volume, before and after tonography.

Results: The AC volume was 219.5±25.7 µL (mean±SD) before tonography and 211.8±22.3 µL after tonography (change of 7.7 µL, $p=0.03$). The aqueous flow was 3.6±4.3 µL/min. The IOP was 26.2±3.59 mmHg before tonography and 22.4±3.89 mmHg after tonography (change of 3.82 mmHg, $p<0.001$). C was 1.54±1.77 µL/mmHg.

Conclusions: UBM shows potential for measuring real-time AC volume changes during tonography. Automation, better border identification and faster image collection should make tonography a valuable tool for patient care and research purposes.



Poster #34 Activation of sigma 1 receptor (Sig1R) regulates NRF2 activity in cone photoreceptor cells

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Purpose: Sig1R is a novel target for treatment retinal degenerative diseases, but the mechanism is uncertain. We recently reported remarkable rescue of cone photoreceptor cell function when (+)-pentazocine ((+)-PTZ)), a high affinity Sig1R ligand was administered to *rd10* mice, a model of retinitis pigmentosa. *Rd10* degeneration is accompanied by significantly increased oxidative stress. In (+)-PTZ-treated *rd10* mice, levels of NRF2, a master regulator of the antioxidant response, were normalized and retinal oxidative stress decreased significantly, leading us to explore the role of Sig1R in modulating NRF2 activity and expression.

Methods: Sig1R was activated in 661W cone cells by exposure to (+)-PTZ [0-100μM] followed by assessment of: (A) oxidative stress (induced by tBHP) using CellROX; (B) viability (MTT assay); (C) NRF2 activation; (D) NRF2-KEAP1 binding; (E) NRF2 expression at the gene/protein level. The consequences on NRF2 expression when Sig1R was silenced using siRNA were investigated.

Results: We found that (+)-PTZ-mediated activation of Sig1R significantly attenuated tBHP-induced decrease in cell viability and increase in oxidative stress. (+)-PTZ-treated 661W cells showed an increase in NRF2-ARE binding activity, however (+)-PTZ did not directly inhibit KEAP1-NRF2 binding. (+)-PTZ-treatment led to an increase in NRF2 expression, both in whole lysates and nuclear extracts. Significantly decreased NRF2 levels and increased oxidative stress were observed in Sig1R-siRNA-661W cells.

Conclusion: (+)-PTZ does not directly inhibit KEAP1-NRF2 binding, however, its activation of Sig1R profoundly influences NRF2 expression and activity. Based on our findings, we hypothesize that a novel mechanism by which Sig1R activation mediates retinal neuroprotection is by modulating NRF2.

Poster #35 Decoding the anti-cataractogenic mechanism of grapes via a systemic pharmacology approach

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Introduction: Our previous study has shown that grapes could protect against *in vivo* ultraviolet B (UV-B) radiation-induced cataract. To better understand their mechanisms of action in cataract prevention, this follow-up study was designed to identify the molecular targets of grapes in the lens by using a systemic pharmacology approach.

Methods: As recommended by the California Table Grape Commission (CTGC), we selected four compounds including resveratrol, catechin, quercetin, and anthocyanins as the major phytoconstituents of grapes for target prediction. All genes that can be regulated by grapes were obtained from NCBI (www.pubmed.gov) and TCMSP (http://lsp.nwu.edu.cn/tcmsp.php). Genes that are associated with cataracts were collected from GeneCards (www.GeneCards.org). The comparison between grape-related targets and cataract-associated genes was conducted using Cytoscape 3.2.1 with ClueGo plugin. Gene Ontology (GO) enrichment analysis of grape-regulated genes was conducted using Database for Annotation, Visualization, and Integrated Discovery (www.david.ncifcrf.gov).

Results: A total of 332 targets that are regulated by grapes were identified and visualized by protein network. Subsequently, 147 GO functional pathways were clustered, including anti-apoptotic, anti-inflammatory, PI3K-Akt signaling, ATP binding, and FOXO pathways. Among these protein targets, X-linked inhibitor of apoptosis (XIAP), heat shock protein (HSP) 90, and prostaglandin-endoperoxide synthase (PTGS) were correlated with all of our selected phytoconstituents. Comparison between grape targets and cataract disease genes showed that 13 grape targets overlapped with cataract associated genes, including PTGS2, HSP90AA1, HSP90AA2P, mitogen-activated protein kinase 1 (MAPK1), MAPK14, MAPK3, amyloid precursor protein (APP), glycogen synthase kinase 3B (GSK3B), protein kinase a (PRKCA), protein kinase C delta (PRKCD), B-cell lymphoma 2 (BCL2), BCL2L1, and K-ras (KRAS).

Conclusions: The anticataractogenesis effects of grapes may encompass more than direct scavenging of free radicals but also activating the anti-apoptotic pathway.

Poster #36 Treatment of Pseudomonas aeruginosa infectious keratitis with photodynamic antimicrobial therapy (PDAT): Riboflavin and Rose Bengal

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Purpose: Infectious keratitis is a potentially blinding disease affecting patients worldwide. Photodynamic antimicrobial therapy (PDAT) is a novel, promising treatment for infectious keratitis. This study compares the clinical outcomes of two patients successfully treated with PDAT using two different photosensitizers: riboflavin and rose bengal.

Methods: Two patients presented with *Pseudomonas aeruginosa* keratitis spreading from limbus-to-limbus. Despite antibiotic management, both patients worsened in the subsequent two weeks and were treated with PDAT. Patient 1 received PDAT with 0.1% riboflavin and UV-A (365 nm) irradiation. Patient 2 received PDAT with 0.1% rose bengal and green (518 nm) irradiation. Irradiation energy was 5.4 J/cm^2 for both cases. After PDAT, a dry-preserved amniotic membrane and a bandage contact lens were placed. Topical antibiotics were continued throughout management.

Results: Patient 1: At presentation, slit-lamp exam revealed rapid progressive melting. Corneal thinning worsened causing an early descemetocele and PDAT was performed. One week after PDAT, the cornea had re-epithelialized and the ulcer shrank. After 6 months, the infection was cleared and an optical keratoplasty was performed.

Patient 2: At presentation, slit-lamp exam revealed severe corneal thinning and an inferior microperforation that was glued. With worsening clinical picture, patient received PDAT. Two weeks after PDAT, corneal melting halted, the cornea had re-epithelialized, and conjunctiva was quiet. After 7 months, the infection was cleared and an optical keratoplasty was performed.

Conclusions: PDAT successfully treated two patients with *Pseudomonas aeruginosa* keratitis in which medical therapy alone was not working. PDAT may be considered in the management of patients with infectious keratitis.

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Poster #37 Efficacy and Safety of SRG003 in Rhesus Monkeys (macaca mulatta) with Spontaneous Primary Optic Atrophy

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Purpose: SRG003 is an active ingredient of a medicinal herb with neuroregenerative properties. Synthetic SRG003 exhibits neuroprotective, neurite outgrowth and neurotrophic effects in neuronal models and positively impacts neurogenesis signaling pathways. Its effect was evaluated in two male Rhesus monkeys with spontaneous unilateral primary optic atrophy.

Methods: RNFL thickness were measured by OCT during a 4-month screening period followed by a 5-month treatment period. Neither monkey has diabetes, multiple sclerosis or glaucoma. They were treated orally with ascending doses of 5, 15, 30, 50, 75 mg/kg for a total of 5 months.

Results: RNFL thickness in both diseased eyes (OD) were stable during the screening period. SRG003 showed efficacy from 2-4 weeks. One monkey had a cumulative increase in RNFL thickness of 20-21 µm in nasal regions (N: 24 to 45 µm; NI: 85 to 105 µm); the other monkey had a cumulative increase of 14 µm (N: 15 to 29 µm; NS: 3 to 17 µm). No change was observed in both healthy eyes (OS). Both monkeys maintained normal body weight, food intake and daily activities. IOP, SL, or FP, blood routine or biochemistry did not change.

Conclusion: Increased RNFL thickness indicates an ability of SRG003 to reverse neurodegenerative processes in the retina. Decreased RNFL thickness in nasal subfields may predict cognitive decline (Ko et al, JAMA 2018). These *in vivo* results in monkeys converge with *in vitro* data to support a neuroregenerative potential for SRG003 and encourages its further research and development.

Poster #38 Ziv-aflibercept Efficacy in Better Regulating Neovascular AMD (ZEBRA) Trial

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Purpose: To determine if ziv-aflibercept is a safe, effective alternative to currently available anti-VEGF medications in eyes with neovascular age-related macular degeneration (nAMD).

Methods: This is a prospective, randomized, IRB-approved study. Inclusion criteria were active nAMD, prior treatment with aflibercept, ranibizumab, or bevacizumab, and BCVA≤20/250. Exclusion criteria included active intraocular inflammation, recent vitreous hemorrhage, and uncontrolled glaucoma. The treatment group received 1.25 mg/0.05mL intravitreal ziv-aflibercept, while the control group continued their existing anti-VEGF regimen. Primary outcomes were best corrected visual acuity (BCVA) and central foveal thickness (CFT).

Results: Of the 52 patients enrolled, 26 patients have been enrolled for at least nine months. Mean baseline BCVA in the control and treatment groups was 1.51±0.37 logMAR (Snellen equivalent: CF 6 ft) and 1.72±0.38 logMAR (Snellen equivalent: CF 5 ft) respectively, and mean change in BCVA was 0.17 logMAR and 0.07 logMAR respectively (p=0.45). Baseline CFT in the control and treatment groups was 246±62 µm and 240±95 µm respectively, and mean change in CFT was 40 µm and 21 µm respectively (p=0.49).

Ziv-aflibercept did not demonstrate any retinal toxicity, adverse events, vision loss, or significant change in mean arterial blood pressure during the study (p=0.35).

Conclusions: In this study, ziv-aflibercept was safe, well-tolerated, and effective in treating nAMD. Compared to aflibercept, ziv-aflibercept is non-inferior with respect to anatomy, function, and complication rate. Ziv-aflibercept may represent an important addition to current anti-VEGF treatment options, especially as a cost-effective alternative to aflibercept and as second-line therapy for eyes resistant to bevacizumab.

Poster #39 OKYO-0101, an agonist of G-protein coupled receptor (GPCR), ameliorates inflammation in an experimental model of dry eye disease in mice

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Purpose: The purpose of the study was to evaluate the ability of OKYO-0101, an agonist of Chemerin receptor, a member of GPCR family, to inhibit eye inflammation in a mouse model of scopolamine-induced dry eye.

Methods: Female C57Bl/6J mice were administered with subcutaneous injections of scopolamine (4 times a day for 5 Days) and housed in chambers with low humidity and constant airflow creating desiccating stress (DS) to induce acute dry eye disease (DED). Mice were randomized into 4 separate arms (n = 10) as follows. Arm 1: No treatment; Arm 2: Positive control; Arm 3: Vehicle and Arm 4: Test group. On Days 1-4, Arms 2-4 received bilateral topical administration (twice daily) of 0.1% Cyclosporine A (CsA-MiDROPS™), Vehicle, and OKYO-0101, respectively. Corneal permeability was assessed by Oregon Green Dextran (OGD) staining at baseline and at end of the studies. Eye tissues were collected and processed for histological quantification of conjunctival goblet cell (GC) density, and quantification of $CD4^+$ T-cells. Ocular tolerance is currently being evaluated in rabbits.

Results: DED-induced corneal permeability was reduced significantly by OKYO-0101 compared to vehicle group ($p \leq 0.001$). Potency of OKYO-0101 to reduce corneal permeably was comparable to cyclosporine, an active ingredient of Restasis® (Allergan). In addition, OKYO-0101 normalized DED-induced loss of GC density ($p \leq 0.001$) and reduced DED-induced enhancement of $CD4^+$ T-cells ($p \leq 0.05$), which are biomarkers of inflammation.

Conclusions: Topically instilled OKYO-0101 reduced symptoms of DED considerably in mice, suggesting its anti-inflammatory properties and it may **have** therapeutic potential for dry eye.

Poster #40 Extended Release of Protein to the Ocular Surface

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Purpose: To examine an extended release system that would expand treatment options for biomolecules in ophthalmology.

Methods: The formulations consisted of either 0.25% Insulin in PBS or 0.22% Insulin in PROLOC® gel. Fresh rabbit corneas or dialysis membranes were placed on spherical Franz Diffusion Cells. Solution cleared from the pre-corneal layer was analyzed via HPLC to evaluate drug retention and release profile for each formulation.

Results: The insulin solution formulation delivered a large amount of insulin that was quickly cleared from the ocular surface while the PROLOC® gel formulation delivered insulin at a sustained rate throughout the 5-hour study.

The PROLOC® formulation reduced the total amount of insulin cleared from the ocular surface compared to the solution formulation. PROLOC® gel was visible at the end of the study. Analysis confirmed insulin was still present in the gel on the cornea after 5 hours. No insulin remained in the cells dosed with solution.

Conclusions: The PROLOC® gel formulation continued to deliver drug at a steady rate throughout the 5-hour period studied while the solution formulation was rapidly removed from the ocular surface. PROLOC® has previously been shown to provide sustained release of an antibiotic from an ocular mini tablet. This study indicates in a gel form it is suitable for ocular delivery of a protein. Since PROLOC® can be supplied as a dry ocular mini tablet or as a partially hydrated gel it has the potential to provide both product stability and constant ocular drug delivery.

Poster #41 Lipid Metabolism Signaling and Phosphatase Inhibitors in Reducing Neuronal Apoptosis in Diabetic Retinopathy

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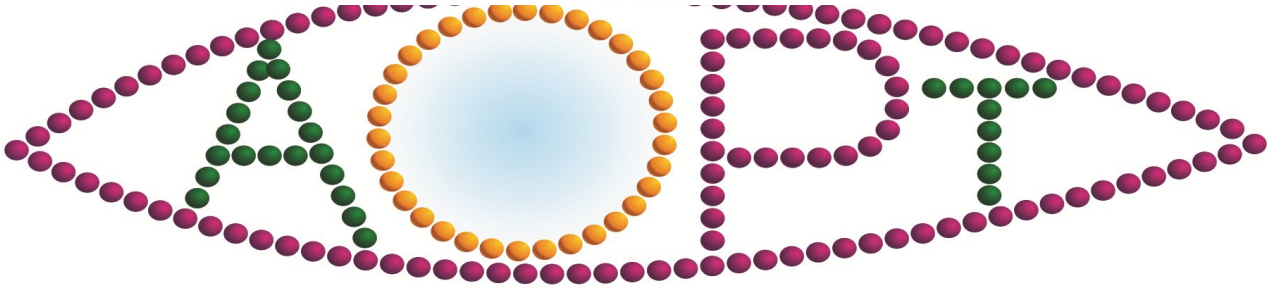
Purpose: Diabetic retinopathy (DR) is a visual complication of diabetes mellitus (DM). Retinal neurodegeneration precedes retinal vascular abnormalities used as indicators of DR. An important feature of retinal neurodegeneration is apoptosis of the retinal ganglion cells (RGCs). Previous studies with db/db mice (mimicking type 2 DM) indicate a lipid metabolism-regulated signaling pathway involving lipoprotein receptor-related protein 1 (LRP-1), suggesting that apoEdp (dimerized LRP-1-binding apolipoprotein E-derived protein) stimulates LRP-1 and triggers the PI3k/AKT pathway, lowering RGC apoptosis. Two phosphatases, protein phosphatase 2A (PP2A) and protein phosphatase 2B (PP2B), contribute to apoptosis antagonizing PI3k/AKT signaling. Current therapies have not addressed the key issues of early retinal neurodegeneration induced by hyperglycemia. We aim to determine the mechanisms of neuroprotection via the LRP-1 signaling pathway.

Methods: Intravitreal apoEdp treatment of diabetic db/db mice. Retinal protein extracts were used for western blot analysis. Retinas from 6-8-week-old neonatal C57BL/6 mice were used to prepare primary RGC cultures. ApopTag/TUNEL assays were used to quantify RGC apoptosis. Effects of varying concentrations of PP2A inhibitor endothall, PP2B inhibitor cyclosporine A, and apoEdp on RGCs were studied via immunofluorescence.

Results: Intravitreal apoEdp treatment induces (1) LRP-1 activation, (2) PP2A deactivation, (3) and reduces RGC loss in db/db mice retinas. *In vitro* results suggest that LRP-1 stimulation via apoEdp results in reduced glutamate-induced excitotoxicity and that cyclosporine A and endothall can reduce apoptosis of



Poster #42 Marijuana, Cannabinoids and Retina

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Purpose: There are currently ten states that have legalized recreational marijuana and thirty-three for medicinal purposes. There are increasing numbers using cannabis. We utilized Frequency Doubling Technology perimetry to assess the functional visual field and retinal function, of an opportunistically dosed group of non-chronic young adult marijuana users. Cannabinoids have been shown to enhance extreme peripheral vision, decrease glare recovery and impair perception of low spatial frequency contrast. Cannabinoid receptors have also been identified in multiple layers of the retina, the lateral geniculate nucleus, primary visual cortex, visual association cortex as well as other regions involved in vision processing. It is important to understand how cannabis affects these structures and possible resultant impairments.

Methods: Through a National Institutes of Health – National Institutes on Drug Abuse funded, Institutional Review Board approved protocol, we included visual screening at baseline and dosed sessions as part of a broader study related to impaired driving. Participants used their own marijuana without qualitative or quantitative dosing characteristics. However, saliva samples were taken during baseline and dosed testing to verify the presence or absence of marijuana and other drugs. Blood was also taken during dosed sessions to determine cannabinoids; 11-Hydroxy Delta-9 THC, Cannabidiol, Cannabigerol, Cannabinol; Delta-9 Carboxy THC and Delta-9 THC.

Results: Current trends reveal reduced function in the left superior quadrant, however this initial data is based on a small number of participants.

Conclusions: With more participants we predict we will be able to determine a retinal regional predilection for cannabinoids and potential brain lateralization.

Poster #43 Glucocorticoid-induced cell derived matrices modulate human trabecular meshwork cell behavior via integrins/caveolin-1-Rho GTPase axis

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Purpose: Trabecular meshwork (TM) extracellular matrix (ECM) remodeling is an important causal risk factor for ocular hypertension. However, partly due to inadequate mechanistic understanding and inappropriate ocular hypertensive models, limited therapeutics directly target the TM. Here, using glucocorticoid-induced cell derived matrices, we demonstrate how a remodeled matrix modulates TM cell behavior via integrins/caveolin-1-Rho GTPase axis.

Methods: Primary human TM (hTM) cells (n=5 donors) were cultured for 4 weeks in the absence/presence of dexamethasone to obtain vehicle control (VehM) and glucocorticoid-induced (GIM) matrices respectively. Subsequently, a fresh batch of hTM cells from the same donor was seeded on these matrices in growth media containing 1% FBS without further treatments for up to 7 days. Changes in protein expression and cellular biomechanics were quantified at 4 timepoints.

Results: Compared to hTM cells on VehM, αV and β5 integrins were overexpressed ($p<0.05$) in cells on GIM at all timepoints. While β1 integrin was increased ($p<0.05$) at a single timepoint (1d), β3 integrin was repressed at all timepoints. Phosphorylated focal adhesion and integrin linked kinases were upregulated at 5d. Caveolin-1 (Cav1) was upregulated at 1d, decreased at 3d, and overexpressed from 5d to 7d in cells on GIM. This trend was mostly mirrored by Rho GTPases, Rac1/2/3, Cdc42 and RhoA with Rac1/2/3 having more sustained effects. Concurrently, cells on GIM were stiffer ($p<0.05$) at all timepoints than VehM.

Conclusions: Specific integrins may serve as adhesion molecules in dysregulated cell-ECM interaction. However, close correlation between Cav1 and Rho GTPases, may implicate caveolae as critical mechanosensors.

Support: NIH T35 student Vision Research Support Grant (2018), Bright Focus National Glaucoma Research Award (VKR), and NIH/NEI grant 1 R01 EY026048-01A1 (VKR/JAV).

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43	Late Breaking (Travel Awardee)	Yemanyi	Glucocorticoid-induced cell derived matrices modulate human trabecular meshwork cell behavior via integrins/caveolin-1-Rho GTPase axis	153

AOPT-2019 Program Guide

March 7: Registration (15:00-17:15, Iberville Ballroom)

SESSION 1 (17:20 - 18:50, Queen Anne Ballroom) NOLA Ophthalmic Research (Moderators: Nicolas G. Bazan and Partha Bhattacharjee)

1. LRP-1 targeted retinal neuroprotection in diabetic db/db mice (Partha Bhattacharjee)
2. Antisense and Gene Therapy Rescues Hearing, Balance and Vision in Usher syndrome (Katelyn Robillard)
3. Molecular organization of lipids in the human macula and retinal periphery William Gordon)
4. Mechanisms by which ciliary neurotrophic factor (CNTF) protects rods and cones (Minghao Jin)
5. Neuroprotection by novel lipid mediators: significance in retinal degenerations(Nicolas G. Bazan)

Welcome Reception (18:50-20:00, Iberville Ballroom)

March 8: SESSION 2 (8:30 - 10:00, Queen Anne Ballroom) Therapeutic Modalities in Ophthalmology (Moderators: Ashwath Jayagopal and Dan Stamer)

1. AAV-mediated gene therapy for long-term effective intraocular pressure (IOP) control in a canine open-angle glaucoma (OAG) model (Andras Komaromy)
2. Bimatoprost Sustained-Release Implants for Glaucoma Therapy (Mike Robinson)
3. Activation of PPARa, a Potential therapeutic strategy for Age-Related Macular Degeneration (Jian-xing Ma)
4. Development of Luxturna™ (voretigene neparvovec-rzyl): Gene Therapy for RPE65 Biallelic Mutation Associated Inherited Retinal Disease (Dan Chung)
5. Small molecule ligand targeting of locked nucleic acids to enable corneal delivery (Kameron V. Kilchrist)

Break & Exhibits (10:00-10:20)

SESSION 3 (10:20 - 11:50) Inflammation in Retinal Degenerative Diseases: Immune Therapy (Moderators: Heping Xu and Florian Sennlaub)

1. HTRA1 inactivates thrombospondin-1 mediated subretinal immune-suppression (Florian Sennlaub)
2. An immune target for neuroprotection in glaucoma (Dongfeng Chen)
3. Role and regulation of the innate inflammatory system in diabetic retinopathy (Patrice E. Fort)
4. Monocyte-Derived Macrophages in Diabetic Retinopathy (Xavier Guillonneau)
5. Immune suppression as an alternative approach to control retinal angiogenesis (Heping Xu)

LUNCH SYMPOSIUM (11:50 - 13:00, March 8, Orleans A room and room TBD) Maximizing collaboration between academia and industry (Panelists academia: Tom Yorio, Carol Toris, Iok-Hou Pang, Achim Krauss, Ashwath Jayagopal)

SESSION 4 (13:00 - 14:30) Gene Therapy Approaches in Treating Eye Diseases (Moderators: Alfred Lewin, Stephen Tsang and Jijing Pang)

1. Precision Genome Surgery for Imprecision Medicine(Stephen Tsang)
2. Knocking down blindness: a gene therapy for autosomal dominant retinitis pigmentosa (William A. Beltran)
3. Targeting the PERK arm of the Unfolded Protein Response in Retinal Degeneration (Marina Gorbatyuk)
4. Gene therapy for Leber's Hereditary Optic Neuropathy (Jiajia Yuan)

Break & Exhibits (14:30-14:50)

SESSION 5 (14:50 - 16:20) YOUNG INVESTIGATOR (TRAVEL AWARDEE) SESSION (Moderators: Malinda Fitzgerald and Monica Jablonski)

1. AAV delivery of modified erythropoietin (EPO) therapy delays retinal degeneration in a mouse model of geographic atrophy (Manas Biswal)
2. Degradable fibrin scaffolds for induced pluripotent stem cell (iPSC)-retinal pigment epithelium (RPE) Transplantation Using a Pig Model (Jarel Gandhi)
3. Pregabalin Microemulsion Once Daily Eye Drops for Management of Glaucoma (Mohamed M Ibrahim)
4. Vasoregulators mediate distal vessel lumen diameters and outflow facility in human anterior segments (Fiona McDonnell)
5. A CRISPR-based inducible system for VEGF repression for AMD (Bo Yu)
6. TRPV4 -dependent calcium influx regulates strain-induced neurodegenerative pathways in retinal ganglion cells (Monika Lakk)

AOPT GENERAL BUSINESS MEETING (16:20 - 17:20, Queen Anne Ballroom) for AOPT members

POSTER SESSION (17:30 - 19:00, Iberville Ballroom) (Moderators: Maria Reinoso and Juana Gallar)

March 9: SESSION 6 (8:30 - 10:00) New Approaches for Treating Age-related Macular Degeneration (AMD) (Moderators: Cathy Bowes Rickman and James Handa)

1. The Role of Mitochondrial Dysfunction in the Pathogenesis of Dry Age-Related Macular Degeneration: From Concept to Clinic for the Mitochondria-directed Drug, Elamipretide (Scott Cousins)
2. TFEB (transcription factor, EB) as a potential therapeutic target for AMD (Debasish Sinha)
3. The Conundrum of Targeting the Complement Pathway to Treat AMD – Lessons from Animal Models (Catherine Bowes Rickman)
4. A roadmap to find treatment for dry AMD (James Handa)
5. Targeting soft drusen in age-related macular degeneration (AMD): rationale and pre-clinical studies of an apolipoprotein mimetic peptide (Christine Curcio)

Break & Exhibits (10:00-10:20)

SESSION 7 (10:20 - 11:50 March 9) Latest Development in Neuroprotection for Glaucoma (Moderators: Raghu Krishnamoorthy and Rebecca Sappington)

1. Synaptic disassembly and rewiring of the adult retina in a mouse model of glaucoma (Luca Della-Santina)
2. Higher Reliance on Glycolysis Limits Responsiveness in Degenerating Glaucomatous Optic Nerve (Denise Inman)
3. Interleukin-6 in Retinal Health and Disease(Rebecca Sappington)
4. Microvascular dysfunction and role of pericytes in glaucoma (Luis Alarcon-Martinez)
5. Alpha B crystallins in glaucoma neuroprotection (Dorota Stankowska)
6. A simple chronic ocular hypertensive murine model of glaucoma – opportunities for neuroprotection studies (Chenying Guo)

LUNCH-N-LEARN ROUNDTABLE (11:50 - 13:00) Orleans A room and room TBD How to setup and run a new lab in academic setting? (Panel Leaders: Carol Toris, Christine Wildsoet and Vivian Lee)

SESSION 8 (13:00 - 14:30) Drug Discovery and Development of Novel Ocular Therapeutics (Moderators: Carol Toris and Iok-Hou Pang)

1. Clusterin regulates intraocular pressure by modulating extracellular matrix in trabecular meshwork outflow pathway (Padmanabhan Pattabiraman)
2. Ligandomics for retinal angiogenesis drug discovery (Wei Li)
3. Metabolomics for ocular drug discovery (Sanjoy Bhattacharya)
4. Epigenetic Modulators as Novel Therapeutics: Translational Perspectives on 20 Years of Preclinical Success (Jeffery Gidday)
5. 21st Century Ocular Pharmacology and Therapeutics: Viral Vectors as Drugs (Carl Romano)
6. Lessons learned from drugs that fail (Stephen Poor)

Break & Exhibits (14:30-14:50)

SESSION 9 (14:50 - 16:20) Advances in Ophthalmic Drug Delivery (Moderators: Chris Wildsoet and David Waterbury)

1. Specific drug targeting to enhance treatment efficacy (Ilva Rupenthal, PhD)
2. Can Drug Delivery Enhance the Efficacy of Ocular Therapeutics (Heather Sheardown)
3. Suprachoroidal drug delivery to the eye (Uday B. Kompella)
4. Novel topical formulation for glaucoma (Monica Jablonski)
5. A new nanomedicine method for treating corneal graft rejection (Qingguo Xu)

KEYNOTE ADDRESS (17:00 - 18:20, Queen Anne Ballroom) (Moderator: Thomas Yorio) BETWEEN A ROCK AND A HARDENED MESHWORK: THE DISCOVERY AND DEVELOPMENT OF RHOPRESSA (Casey Kopczynski)

Banquet Dinner (19:00-21:30), Royal Ball Room

March 10: SESSION 10 (8:30 - 10:00) Novel Therapies for Corneal Diseases (Moderators: Claudio Bucolo and Filippo Drago)

1. Novel lipid mediators and neurotrophins targeting cornea nerve integrity (Haydee Bazan)
2. Cell therapy and gene therapy in eye diseases (Graziella Pellegrini)
3. Old and new cation channel blockers to treat ocular discomfort and pain (Juana Gallar)
4. Neuropathic corneal pain: approaches for management (Pedram Hamrah)
5. Xanthohumol protects corneal epithelial cells against oxidative stress in vitro (Simon Kaja)
6. The miR-29b Mimic Remlarsen as an Anti-Fibrotic Therapeutic in the Eye (Corrie, Gallant-Behm)

Break & Exhibits (10:00-10:20)

SESSION 11 (10:20 - 11:50) Hot Topics from Abstract Submission (Moderators: Ze Zhang and Rajashekhar Gangaraju)

1. Exploration of the secretome of adipose stem cells for the design of retinal therapeutics (Rajashekhar Gangaraju)
2. Novel topically delivered small molecule with IOP lowering and neuroprotective activity (Suchismita Acharya)
3. Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts (Peter Kador)
4. More than just a reactive oxygen species scavenger: grapes prevent UV-B radiation-induced cataract by upregulating anti-apoptotic protein XIAP (HongLi Catherine Wu)
5. The Endothelin Receptor Antagonist Macitentan Attenuates Neurodegeneration in a Rodent Model of Glaucoma and Ameliorates Endothelin-Mediated Vasoconstriction (Raghu Krishnamoorthy)
6. Protection of kaempferol on oxidative stress-induced retinal pigment epithelial cell damage (Donglei Zhang)

AOPT Board Meeting (12:00-13:00, Bienville C)

SESSION 12 (13:00 - 14:30) Disruptive Technologies: Ophthalmic Tools and Methods that Have Changed the Ways We See the Eye (Moderators: Cheryl Rowe-Rendleman and Randolph Glickman)

1. Audacious Goals Initiative: Status and Impact (Steven Becker)
2. Photoacoustic Imaging and Sensing: a New Way to See the Eye (Randolph Glickman)
3. A Platform to Take on the Entire Progressive Retinal Degeneration Disease Continuum (Francois Binette)
4. Early stage detection of Glaucoma by monitoring nanostructure and function of RGC layer using Multifractal OCT (Subrata Batabyal)
5. Preclinical Evaluation of ADVM-022, a Novel Gene Therapy Approach to Treating Wet Age-Related Macular Degeneration (Claire M. Gelfman)

Panel Discussion (moderated by Cheryl Rowe-Rendleman)

Break & Exhibits (14:30-14:50)

SESSION 13 (14:50 - 16:20)What Every Ophthalmologist Needs to Know About the FDA (Moderators: (Bing Cai and Jayne Weiss)

1. Advancing Technology Challenges in Ophthalmic Drug Approvals (Wiley A Chambers)
2. Generic Drugs and Their Role in Bringing Next Generation Products: An FDA Perspective (Markham Luke)
3. Generic Ophthalmic Drug Products, Physical Characteristics and Bioequivalence (Darby Kozak)
4. How FDA Ensures Quality of Ophthalmic Drug Products (Patricia Onyimba)