

# PATHOBIOLOGY



*Mechanisms of Disease*

# 2026

## Meeting Program

May 16–19 | Fort Myers, FL

# ASIP

American Society for Investigative Pathobiology

Annual Meeting of the American Society for Investigative Pathobiology

UNIVERSITY OF MIAMI MILLER SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

# Bold Research. Powerful Discoveries. Next Gen Scientists.

## OUR DEPARTMENT

Our faculty are committed to discovery and educating the next generation of scientists and physicians within a highly collegial, interactive Department. We have 23 primary faculty and 26 secondary faculty with appointments across the Miller School of Medicine who enrich our research and teaching. We are dedicated to fostering creativity and learning. Our research investigates immune mechanisms, host pathogen infections, autoimmunity, cancer, cardiovascular disease and aging.

Our faculty has secondary appointments with the Sylvester Comprehensive Cancer Center, the Diabetes Research Institute, the Center for AIDS Research, and Interdisciplinary Stem Cell Institute. Our department supports translation to patient benefits with our Industry partnerships. We also educate Next Gen Scientists campus-wide, including a Coral Gables undergraduate major with research opportunities.

Immune mechanisms

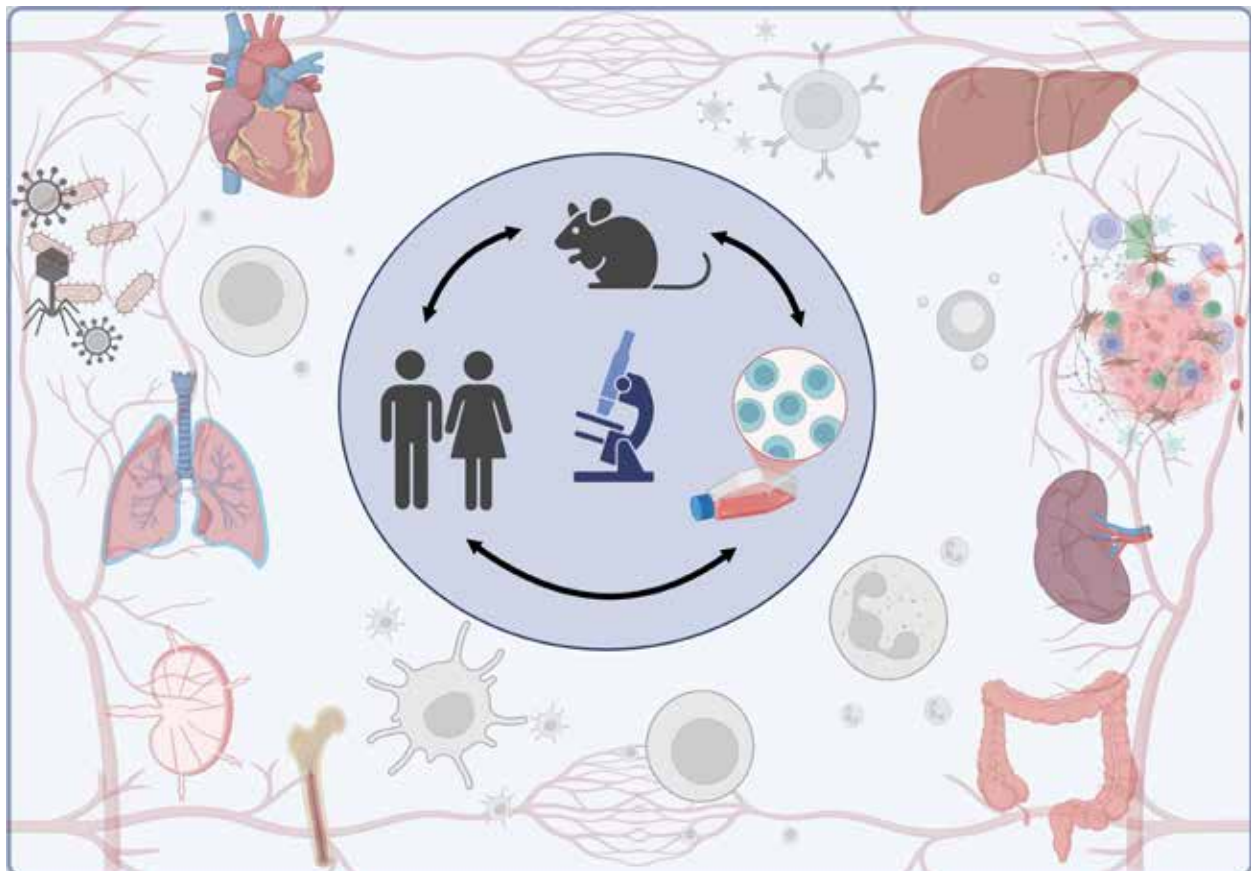
Host pathogen infections

Autoimmunity

Cancer

Cardiovascular disease

Aging



VISIT US: [www.micro.med.miami.edu](http://www.micro.med.miami.edu)

## Table of Contents

Program Committee.....	5
ASIP Council.....	6
Awardees.....	7–9
PathFinder Meeting Mentors.....	9
General Information.....	10–13
Maps.....	14–15
Exhibitors & Sponsors.....	17
Scientific Program.....	19
Friday.....	19
Saturday.....	19
Sunday.....	30
Monday.....	36
Tuesday.....	42
Abstracts.....	48
Oral Presentations.....	48
Poster Presentations...	78

# The American Journal of Pathology

- The most frequently cited pathology journal
- Over 34,000 citations per year
- Over 2.5 million downloads per year
- Discounted charges for ASIP Regular Members
- Flat-rate publication fee
- Average time to first decision: 38 days



Editor-in-Chief  
Martha B. Furie, PhD

Official Journal of the American Society for Investigative Pathology [ajp.amjpathol.org](http://ajp.amjpathol.org)

## The American Journal of **PATHOLOGY**

Discoveries in Basic and Translational Pathobiology

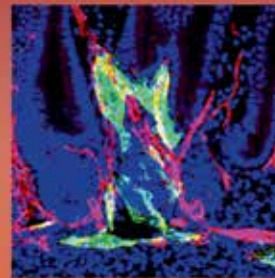
March 2026 // Volume 196 // Number 3

Inside:

**Review:** Neutrophils, Inflammation and Immune Dysregulation in Psoriasis: Mechanistic Pathways and Emerging Interventions

**Review:** Emerging Mechanistic Roles of STING Signaling in Kidney Diseases  
Interpreting Deep Learning-Based Prediction of the BRAF-V600E Mutation Using Diagnostic Whole Slide Images in Skin Cutaneous Melanoma

Mechanistic Insights into Glucocorticoid-Induced Ocular Hypertension Using Differences in Mouse Strain Responsiveness



Sporadic Intestinal Inflammatory Lethality With Lymphatic Defects in *Angptl4*<sup>fl/fl</sup> Mice

## 2026 Program Committee



**Andrei Ivanov, PhD, Chair**  
Cleveland Clinic Foundation



**Christi Kolarcik, PhD**  
University of Pittsburgh



**Kari Nejak-Bowen, PhD**  
University of Pittsburgh



**Wendy Mars, PhD**  
University of Pittsburgh



**D. Hunter Best, PhD**  
University of Utah



**Jayshree Mishra, PhD**  
Texas A&M University



**Sanjukta Chakraborty, PhD**  
Texas A&M University Health  
Science Center



**Maryknoll Palisoc Linscott, MD, PhD**  
Penn State College of Medicine



**Yabing Chen, PhD**  
Oregon Health and Science University



**Ramon Bossardi Ramos, PhD**  
Albany Medical College



**Piyali Dasgupta, PhD**  
Marshall University



**Douglas Stairs, PhD**  
Penn State College of Medicine



**Wen-Xing Ding, PhD**  
University of Kansas



**James R. Stone, MD, PhD**  
Massachusetts General Hospital



**Andrew Duncan, PhD**  
University of Pittsburgh



**Ronen Sumagin, PhD**  
Northwestern University



**Bethany Hannafon, PhD**  
The University of Oklahoma



**Michael Thompson, MD, PhD**  
Washington University School of  
Medicine



**Dennis Jones, PhD**  
Boston University



**Menglu Yang, MD, PhD**  
Schepens Eye Research Institute of  
Massachusetts Eye and Ear



**Bilon Khambu, PhD**  
Tulane University



**Xiao-Ming Yin, MD, PhD**  
Tulane University School of Medicine

## ASIP 2025–2026 Council



### **President**

Pilar Alcaide, PhD  
University of Miami



### **President-Elect**

Jonathon Homeister, MD, PhD  
University of North Carolina



### **Vice-President**

David Williams, MD, PhD  
University of North Carolina



### **Past-President**

Satdarshan (Paul) Singh Monga, MD  
University of Pittsburgh



### **Secretary-Treasurer**

John Hanna, MD, PhD  
Harvard Medical School, Brigham and  
Women's Hospital



### **Councilor At-Large**

Charleen Chu, MD, PhD  
University of Pittsburgh



### **Councilor At-Large**

Kelsey Dillehay McKillip, PhD  
University of Cincinnati College of  
Medicine



### **Early-in-Career Councilor At-Large**

Bethany Hannafon, PhD  
University of Oklahoma Health Sciences  
Center



### **Early-in-Career Councilor At-Large**

Traci Parry, PhD  
University of North Carolina at  
Greensboro



### **Committee for Career Development Chair**

Veronica Contreras-Shannon, PhD  
St. Mary's University



### **Education Committee Chair**

Julie Randolph-Habecker, PhD  
Pacific Northwest University of Health  
Sciences



### **Committee for Equal Representation and Opportunity Chair**

Cecelia C. Yates, PhD  
University of Pittsburgh



### **Program Committee Chair**

Andrei Ivanov, PhD  
Cleveland Clinic Foundation



### **Program Committee Chair-Elect**

Ronen Sumagin, PhD  
Northwestern University



### **Publications Committee Chair**

Heather Francis, PhD  
Indiana University



### **Publications Committee Chair-Elect**

Xiao-Ming Yin, MD, PhD  
Tulane University



### **Research and Science Policy Committee Chair**

Elaine Bearer, MD, PhD  
University of New Mexico



### **President's Circle Chair**

Robinna Lorenz, MD, PhD  
Genentech



### **Editor-in-Chief of *The American Journal of Pathology***

Martha Furie, PhD  
Stony Brook University



### **FASEB Board Representative**

Richard Mitchell, MD, PhD  
Brigham & Women's Hospital



### **FASEB Science Policy Committee**

Sharon DeMorrow, PhD  
The University of Texas at Austin

# 2026 Awardees

## Meritorious Awards

**Gold-Headed Cane Award**  
Mark L. Tykocinski, MD  
Thomas Jefferson University



**Rous-Whipple Award**  
Anna Mae Diehl, MD  
Duke University School of Medicine



**Outstanding Investigator Award**  
John W. Hanna, MD, PhD  
Brigham and Women's Hospital/Dana  
Farber Cancer Institute



**Robbins Distinguished Educator Award**  
Michael B. Prystowsky, MD, PhD  
Montefiore Medical Center



**Frieda Robscheit-Robbins Award for  
Exceptional Achievement in the  
Advancement of Women in Experimental  
Pathology**  
Patricia A. D'Amore, PhD  
Harvard Medical School, Massachusetts  
Eye & Ear



**Excellence in Mentoring Award**  
Richard Mitchell, MD, PhD  
Brigham and Women's Hospital, Harvard  
Medical School



**Cotran Early Career Investigator Award**  
Dennis Jones, PhD  
Boston University Chobanian & Avedisian  
School of Medicine



**Marilyn G. Farquhar Early Career Award  
for Exceptional Achievement in the  
Advancement of Women in Experimental  
Pathology**  
Melinda A. Engevik, PhD  
Medical University of South Carolina



**Young Scientist Leadership Award**  
Vik Meadows, PhD  
University of Pittsburgh School of  
Medicine



## Junior Faculty Scholar Awards

**Fred Sanfilippo-ASIP Visiting Lectureship  
Award Recipients**  
Tapasree Roy Sarkar, PhD  
Texas A&M University



Goran Micevic, MD, PhD  
Yale School of Medicine



Daisy Y. Shu, PhD  
University of New South Wales



Amy C. Engevik, PhD  
Medical University of South Carolina



Stephania Libreros, PhD  
Yale School of Medicine



**Dani and Erik Zander Junior Faculty  
Scholar Award**

Michele Alves, PhD  
Florida International University



Ramon Bossardi Ramos, PhD  
Albany Medical College



**George K. Michalopoulos Junior Faculty  
Scholar Award**

Kristen Engevik, PhD  
Medical University of South Carolina



Kevin Van der Jeught, PhD  
University of Miami



**Monga Family Junior Faculty Scholar  
Award**

Jessica Fortin, BPharm, BSc, MSc, DVM, PhD  
Purdue University



Goran Micevic, MD, PhD  
Yale School of Medicine



# 2026 Awardees

## Trainee Merit Awards

### ASIP Experimental Pathologist-in-Graduate Training Award (EPIGT)

Alyssa Gutierrez, BS  
Medical University of South Carolina



### ASIP Experimental Pathologist-in-Graduate Training Merit Award (EPIGT)

Lucien Garo, III, BA  
Boston University Chobanian & Avedisian School of Medicine



Tyler Yasaka, BS  
University of Pittsburgh



### ASIP Experimental Pathologist-in-Training Award (EPIT)

Louisa Tichy, PhD  
Wake Forest University School of Medicine



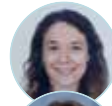
### ASIP Experimental Pathologist-in-Training Merit Award (EPIT)

Xing-Sheng Ren, PhD  
Northwestern University



### A.D. Sobel Trainee Scholar Award

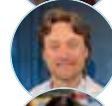
Cristina Bauset, B.Pharm, Med, PhD  
University College, Dublin



Zoe Libramento, MS  
University of North Carolina at Greensboro



Joseph Williams, BS  
Case Western Reserve University



Anna Tingler, BS  
Medical University of South Carolina



## Trainee Scholar Awards

### ASIP Trainee Scholar Award

Serena Artone, PhD  
Case Western Reserve University



Yuanyuan Li, PhD  
Tulane University



Ana Pettijohn, BS  
Medical University of South Carolina



Cian Ohlendieck, BSc  
University College Dublin



### GALL Trainee Scholar Award for Excellence in Cardiovascular Research

Ramona Emig, PhD  
University of Miami



### Gotlieb Undergraduate Education in Pathobiology Scholar Award

Avraham Levi  
FIU Herbert Wertheim College of Medicine



Keira Smith  
Beth Israel Deaconess Medical Center

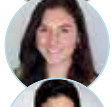


### Histochemical Society Sponsored Trainee Travel Award

Sandy Huynh  
Boston Children's Hospital



Camille Longabardi, BS  
Beth Israel Deaconess Medical Center



Abdulrahman Nakshabandi, BDS  
Harvard School of Dental Medicine Boston Children's Hospital



Alexandra Tomasevich, BA  
Medical University of South Carolina



Arturo Valenzuela Padilla, BSc  
Center for Research and Advanced Studies of the National Polytechnic Institute



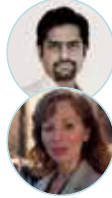
## 2026 Awardees

### Trainee Scholar Awards (cont.)

**Marion and Lawrence (Larry) Muller Trainee Scholar Award for Excellence in Inflammation Research**

Harsh Dongre, PhD  
Boston Children's Hospital

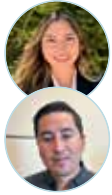
Michelle Thayer, MA  
Iowa State University



**Marion and Lawrence (Larry) Muller Trainee Scholar Award for Excellence in Neurodegenerative Research**

Emily Silva  
Florida International University

Isaac Vargas Rodriguez, PhD  
Florida International University



**Monga Family Trainee Scholar Award for Excellence in Cardiovascular Research**

Maria Zambrano, BS  
Tufts University

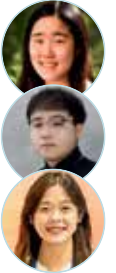


**Monga Family Trainee Scholar Award for Excellence in Liver Pathobiology Research**

Hongkun Lu  
Virginia Commonwealth University

Yubo Wang, M.Med  
The University of Texas at Austin

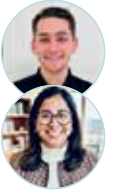
Chen Zhang  
University of Kansas Medical Center



**Monga Family Trainee Scholar Award for Excellence in Neoplasia Research**

Cole Hladik, PhD  
University of Oklahoma

Neha Rana, PhD  
Beth Israel Deaconess Medical Center

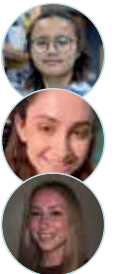


**ASIP Summer Research Opportunity Program in Pathology (SROPP)**

Alison Sham  
Boston Children's Hospital

Abigail Smith  
University of North Carolina at Greensboro

Celleste Wohlfarth  
University of North Carolina Greensboro



## 2026 PathFinder Meeting Mentors

**Michele Alves, PhD** (Florida International University)

**Diane Bielenberg, PhD** (Boston Children's)

**Fran Carrillo-Salinas, PhD** (MIT)

**Ian Cartwright, PhD** (University of Colorado Anschutz)

**Harsh Dongre, PhD** (Boston Children's)

**Amy Engevik, PhD** (Medical University of South Carolina)

**Mindy Engevik, PhD** (Medical University of South Carolina)

**Cole Hladik, PhD** (University of Oklahoma)

**Jonathon Homeister, MD, PhD** (University of North Carolina)

**Christi Kolarcik, PhD** (University of Pittsburgh)

**Zoe Libramento, MS** (UNC Greensboro)

**Vik Meadows, PhD** (University of Pittsburgh)

**Traci Parry, PhD** (UNC Greensboro)

**Ronen Sumagin, PhD** (Northwestern)

**Louisa Tichy, PhD** (Wake Forest University)

**Anna Tingler, BS** (Medical University of South Carolina)

**David Williams, MD, PhD** (University of North Carolina)

**Tyler Yasaka, BS** (University of Pittsburgh)

**Maria Zambrano, BS** (Tufts University)

## General Information

Welcome to Pathobiology 2026: Mechanisms of Disease! All scientific sessions, Career Development, Poster Blitzes, and Education Sessions will be held in the Caloosa Sound Convention Center & Amphitheater (directly adjacent to the Luminary Hotel & Co.). The Women in Pathology Networking Event will be held at the Luminary Hotel Pool Deck & Lounge I (4<sup>th</sup> Floor) and the Society-Wide President's Reception and Networking Event will take place at Baystreet Yard (2136 Bay Street, Fort Myers, FL 33901)

Below you will find information that will be helpful while on-site during the meeting. If you have any questions, make your way to the registration desk located in the Galleria. Volunteer badge pick-up and directional assistance will also be provided by the Lee County Visitor & Convention Bureau (VCB).

### Code of Conduct

The Planning Committee for Pathobiology 2026 is committed to providing a friendly, safe, and welcoming environment for all, regardless of gender, sexual orientation, disability, race, ethnicity, religion, national origin, or other protected characteristics. We expect all attendees, speakers, and organizers to help us ensure a safe and positive conference experience for everyone and to abide by the Code of Conduct while attending Pathobiology 2026 in all venues, including ancillary events and official and unofficial social gatherings.

- Exercise consideration and respect in your speech and actions
- Refrain from demeaning, discriminatory or harassing behavior and speech

### What to Do If You Witness or Experience Conduct That Violates the Code

If you experience or witness discrimination or harassment, you are encouraged to immediately inform the alleged violator that their comments or behavior are unwelcome. Individuals may be unaware that their conduct is offensive and are often willing to correct their behavior if so informed.

However, please note that you are not required to directly address or confront a person you believe is discriminating against or harassing you or another person. If you do not feel comfortable addressing the alleged violator, or if the alleged violator continues the behavior after being advised that such conduct is unwelcome, you should report the incident.

If you wish to report discrimination or harassment you have witnessed or experienced, you may do so by contacting:

William B. Coleman, PhD  
Executive Officer  
[wbc Coleman@asip.org](mailto:wbc Coleman@asip.org)  
Cell Phone: (919) 818-6198

Lisa McFadden, CMM  
Director of Scientific Meetings, Membership & Education  
[Lmcfadden@asip.org](mailto:Lmcfadden@asip.org)  
Cell Phone: (202) 498-0197

You are also encouraged to write down as many relevant details as you can recall (e.g., names, dates, times, behavior or statements made, etc.), which can be helpful in filling out a report later if needed.

The meeting organizers will maintain your confidentiality except where doing so would compromise another person's rights or the organizer's ability to conduct a thorough investigation. In such cases, the organizers will limit disclosure only to that information necessary to ensure proper investigation and compliance with procedures. No retaliation will be taken or tolerated against anyone who makes a good faith report of discrimination or harassment.

### **Registration Hours**

The meeting registration desk is located in the Galleria of Caloosa Sound Convention Center & Amphitheater. Name badges can be picked up during the hours noted below.

- Saturday, May 16 7:00 AM–5:00 PM
- Sunday, May 17 7:00 AM–5:00 PM
- Monday, May 18 7:00 AM–5:00 PM
- Tuesday, May 19 7:00 AM–5:00 PM

### **Registration**

Registration fees include the main conference scientific sessions, access to the online meeting program, poster sessions, social events, and the meals included below. Registration fees exclude hotel costs.

### **Catering**

Included in registration fees are the following catered events:

- Breakfast on Saturday, Sunday, Monday, and Tuesday
- Lunch on Saturday, Sunday, Monday, and Tuesday
- Evening Reception on Saturday, Sunday, and Monday

### **Internet Access**

Internet access is provided in the guest rooms for those staying onsite at Luminary Hotel & Co. within the meeting block. Complimentary Wi-Fi access is also provided by the ASIP in the meeting spaces.

### **Business Meeting**

All ASIP members are encouraged and invited to attend the ASIP Business Meeting and Meritorious Awards Presentations on Monday, May 18 from 5:30–7:00 PM in Caloosa Salons D/E. If you are not currently a member, membership applications are available in the ASIP Networking Lounge (Salon C). Non-members are welcome at the business meeting to learn more about the ASIP community.

### **Women in Pathology Networking Event**

Meeting attendees are invited to celebrate the ASIP community with this Women in Pathology-sponsored event on Saturday, May 16, from 6:30–8:30 PM (Luminary Hotel Pool Deck and Lounge, 4<sup>th</sup> Floor). Take a break from the science and bring your outgoing, collegial, and team-building self—join us for an evening of treats, drinks, and games! Women in Pathology invites you to shake things up a bit and connect with your colleagues in a night of conversation, networking, and fun competition, playing games outside your everyday routine.

### **PathoMingle Social Event**

All Trainee and first-time meeting attendees are welcome to attend this social event on Friday, May 15 from 6:00–10:00 PM at the Beacon Social Drinkery (Luminary Hotel, 12th Floor)

### **Poster Blitzes**

Each poster blitz features a short (3-minute) presentations based upon selected posters and presented by a young investigator. The objective is to highlight a subset of posters to be presented in a standard poster session to generate interest. Each short presentation will focus on the objectives of the study, the

major results, and the conclusions. Details of the studies will be available during the scheduled poster sessions.

### **Poster Sessions**

Poster boards will be set up in Caloosa Salon C. All posters will be on display throughout the entire meeting and all attendees are encouraged to view the posters.

- Poster Session I (**ODD** Numbered Posters)  
Sunday, May 17  
5:00–6:30 PM
- Poster Session II (**EVEN** Numbered Posters)  
Monday, May 18  
4:00–5:30 PM

The organizers are not responsible for any materials posted. Push pins will be provided. Set-up and breakdown for poster boards is as follows:

### **Poster Set-up**

Sunday, May 17 at 7:00 AM

### **Poster Dismantle**

Tuesday, May 19 at 8:00 AM

*Please note: If your poster is not removed by the designated day and time, it will be thrown away when the poster boards are dismantled.*

### **Society-Wide President's Reception and Networking Event**

Monday, May 18, from 7:00–9:00 PM

Bay Street Yard (2136 Bay Street, Fort Myers, FL 33901)

### **Exhibits**

Please take time to visit the exhibit displays in Salon C during the breaks and poster sessions. See the exhibitor listing for detailed information regarding our sponsoring companies.

### **Exhibits Schedule:**

- Saturday, May 16      10:00 AM–2:00 PM; 4:30–5:30 PM
- Sunday, May 17      5:00–8:30 PM
- Monday, May 18      12:30–5:30 PM
- Tuesday, May 19      10:00 AM–2:00 PM

### **Guest Badges**

Guest badges are available for a spouse, family member, or significant other accompanying a paid registrant and can only be used on the day specified.

Guest badges will be available for pick-up in Salon C during registration and badge pick-up hours. Please note that guest badge is not replaceable if lost.

### **Special Needs**

Registrants with special needs are invited to contact Lisa McFadden ([Lmcfadden@asip.org](mailto:Lmcfadden@asip.org)) for assistance.

### **Liability**

Neither the host venue nor the organizers can be held responsible for any personal injury, loss, damage to private property or additional expense incurred as a result of delays or changes in air, rail, sea, road

or other services. All participants are encouraged to make their own arrangements for health and travel insurance.

### **Photography and Recording Policy**

Pathobiology 2026 is committed to honoring the rights of copyright owners and to respectful sharing of scientific research and data. Attendees are expected to adhere to this policy.

### **Health and Safety Guidelines**

Travel and the gathering of people in a public place incur the risk of communicable diseases, including influenza and COVID-19. All meeting attendees should take personal responsibility to keep themselves, other attendees, exhibitors, vendors, and staff safe, prior to and during the meeting.

Mask wearing is optional throughout the meeting, and masks will be available at the Registration Desk. You are encouraged to practice health and safety habits that make you most comfortable.

Regardless of vaccination status, if you have symptoms of COVID-19, have tested positive, or are sick prior to traveling to the meeting, please do not attend the meeting. Contact Lisa McFadden ([Lmcfadden@asip.org](mailto:Lmcfadden@asip.org)) to discuss your participation as a presenter/chair/poster presenter so that other arrangements can be made.

Thank you in advance for your understanding and cooperation!

# ASIP Women in Pathology Month

Each year, ASIP Women in Pathology highlight the history of women in the ASIP for their service through leadership, recognition of their exceptional accomplishments as scientists, activities, and events as a community equipping women scientists with strategies to overcome real-life issues.

On each weekday of May, ASIP will highlight one woman in the Society. Learn more and check out the features at [asip.org/women-in-pathology](http://asip.org/women-in-pathology) or scan the QR code.



# Pathobiology 2026 Meeting Maps

## Caloosa Sound Convention Center



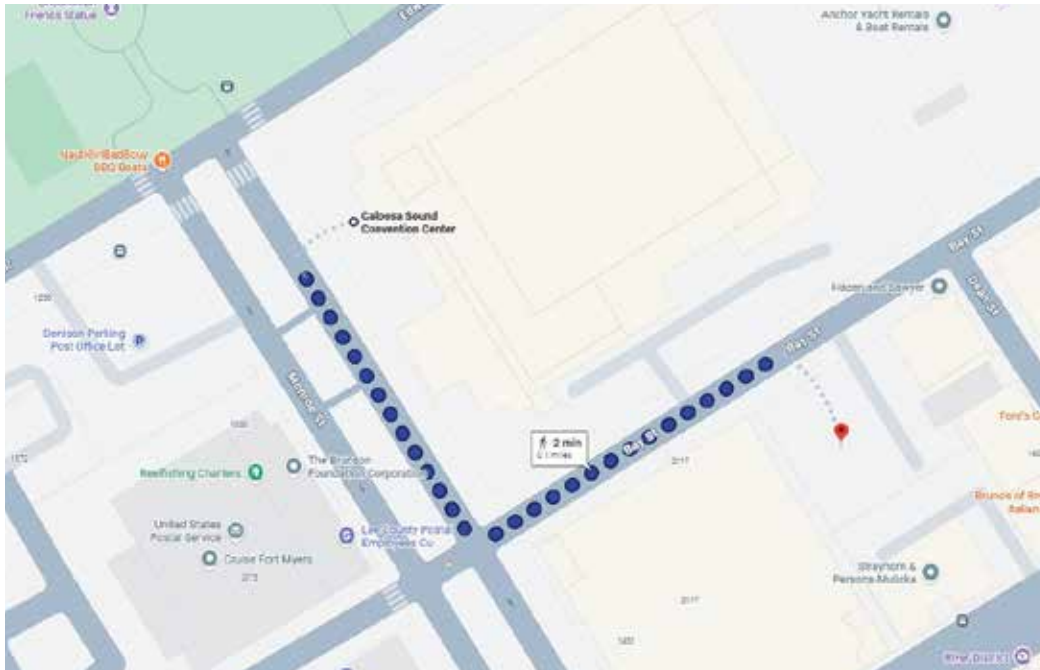
## Luminary Hotel Fourth Floor



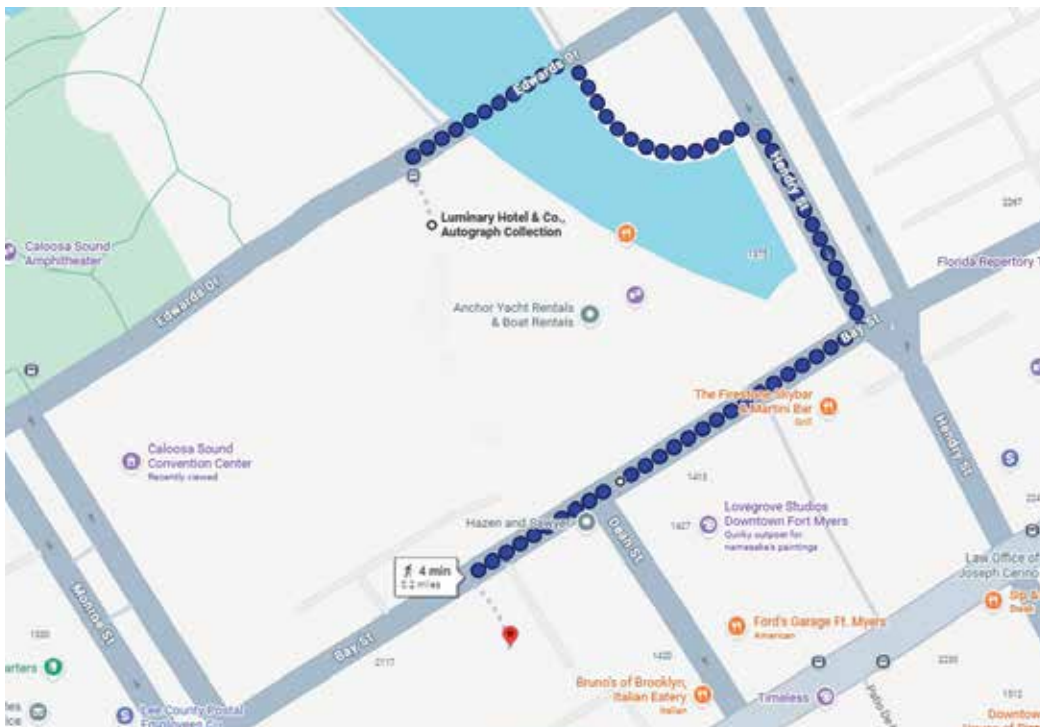
# Pathobiology 2026 Meeting Maps

The Society-Wide President's Reception and Networking Event is on Monday, May 18, from 7:00–9:00 PM at Bay Street Yard (2136 Bay Street, Fort Myers, FL 33901). Walking directions from the Caloosa Convention Center and The Luminary Hotel are below.

## Caloosa Sound Convention Center to Bay Street Yard



## The Luminary Hotel to Bay Street Yard



# 2026 ASIP Virtual Events



**Young Investigator Spotlight Seminar**  
June 25 | 1 PM ET

*The Double-Edge Sword of Antibiotics in Cystic Fibrosis*

**Anna Tingler, BS**  
Medical University of South Carolina



**Young Investigator Spotlight Seminar**  
July 16 | 1 PM ET

*Repairing Children's Livers: Exploring Modifiers of Disease Severity in Pediatric Cholestatic Diseases*

**Laura Molina, MD, PhD**  
University of Pittsburgh



**Young Investigator Spotlight Seminar**  
August 20 | 1 PM ET

*Neuropilin-2 Driven T-Cell Suppression: Implications in Early Tumor Development*

**Harsh Dongre, PhD**  
Boston Children's Hospital



**Young Investigator Spotlight Seminar**  
September 17 | 1 PM ET

*Investigative Dermatopathology for Cutaneous Wound Repair and Regeneration*

**Anthony Sheets, MD, PhD**  
Brigham and Women's Hospital



**PISA: Young Investigators Virtual Meeting**  
October 27-29 | 2:30-6 PM ET Daily

Co-Chairs:

- **Anna Tingler, BS** (Medical University of South Carolina)
- **Cole Hladik, PhD** (University of Oklahoma Health Campus)

Abstract Submission Deadline is September 15



**Young Investigator Spotlight Seminar**  
November 19 | 1 PM ET

*Immune Checkpoint Inhibitor Toxicity: Muscle Wasting and Pathological Signaling*

**Louisa Tichy, PhD**  
Wake Forest University

Learn more and register at [ASIP.org/meetings/virtual-events](https://ASIP.org/meetings/virtual-events) or scan the QR code



# 2026 Exhibitors, Sponsors & Academic Partners

Thank you to our exhibitors, sponsors, and academic partners for making this meeting possible!

## The American Journal of Pathology (AJP)

ajp.amjpathol.org  
Booth 1



## American Society for Investigative Pathobiology (ASIP)

asip.org  
Booth 1



## American Society for Matrix Biology (ASMB)

asmb.net



## Case Western Reserve University Department of Pathology

case.edu/medicine/pathology



## Earlier.org

earlier.org



## Elsevier

elsevier.com  
Booth 2



## The Federation of American Societies for Experimental Biology (FASEB)

faseb.org  
Booth 3



## The Histochemical Society (HCS)

histochemicalsociety.org



## Houston Methodist

houstonmethodist.org



## Lecturio

lecturio.com  
Booth 4



## Pittsburgh Liver Research Center

livercenter.pitt.edu



## Pfizer

pfizer.com



## Stony Brook Medicine Department of Pathology

renaissance.stonybrookmedicine.edu/pathology



## University of Colorado Anschutz Medical Campus Department of Pathology

medschool.cuanschutz.edu/pathology



## University of Miami Miller School of Medicine

med.miami.edu



## University of New Mexico School of Medicine

hsc.unm.edu/medicine



## UNC Greensboro Department of Kinesiology

kin.uncg.edu



## UNC School of Medicine Pathology and Lab Medicine

med.unc.edu



## University of Pittsburgh Department of Pathology

path.pitt.edu



## University of Pittsburgh Organ Pathobiology and Therapeutics Institute

upddi.pitt.edu



Funding for this conference was made possible (in part) by 1R13CA314160-01 from the National Cancer Institute. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does the mention by trade names, commercial practices, organizations imply endorsement by the U.S. Government.

# 2026 ASIP Meritorious Award Lectures



## **Gold-Headed Cane Award Lecture**

Sunday, May 17, from 6:30–7:30 PM  
Caloosa Salon D

***Cells and Networks in Flux: Rethinking Ontogenesis and Pathogenesis***

**Mark Tykocinski, MD**  
Thomas Jefferson University



## **Rous-Whipple Award Lecture**

Saturday, May 16, from 5:30–6:30 PM  
Caloosa Salon D

***Pros and Cons of Living Longer***

**Anna Mae Diehl, MD**  
Duke University School of Medicine



## **Outstanding Investigator Award Lecture**

Sunday, May 17, from 2:00–2:45 PM  
Caloosa Salon E

***Magical Moments in Protease Biology: Proteasome Autocatalytic Activation and PI3I-Mediated Inhibition***

**John Hanna, MD, PhD**  
Harvard Medical School, Brigham and Women's Hospital



## **Cotran Early Career Investigator Award Lecture**

Saturday, May 16, from 2:00–2:45 PM  
Caloosa Salon D

***The Lymphatic System in Disease Progression***

**Dennis Jones, PhD**  
Boston University



## **Young Scientist Leadership Award Lecture**

Tuesday, May 19, from 2:00–2:45 PM  
Caloosa Salon D

***Gut Feelings: How Bile Duct Integrity Regulates the Gut-Liver Axis***

**Vik Meadows, PhD**  
University of Pittsburgh

# Scientific Meeting Program

FRIDAY, MAY 15, 2026

## ASIP Council Meeting *(By Invitation Only)*

1:00-5:00 PM

**Location: Heitman Room (Luminary Hotel, 4<sup>th</sup> Floor)** ▪ Council Meeting

**Location: Hendry Room (Luminary Hotel, 4<sup>th</sup> Floor)** ▪ Council Strategy Sessions

## ASIP Council Dinner *(By Invitation Only)*

6:00-9:00 PM

**Location: Oxbow Bar & Grill (2<sup>nd</sup> Floor)**

1300 Hendry Street

Fort Myers, FL 3390

(239) 314-3856

## PathoMingle Social Event

6:00-10:00 PM

**Location: Beacon Social Drinkery (Luminary Hotel, 12<sup>th</sup> Floor)**

Join us for the annual PathoMingle Dinner sponsored by the ASIP!

All trainees and first-time attendees will meet at the Beacon Social Drinkery (Luminary Hotel, 12th Floor) at 6:00 PM for a time of networking, socializing, and becoming acquainted with your peers before the start of Pathobiology 2026. We are so excited to meet y'all and build a community.

Please contact Vik Meadows ([vem72@pitt.edu](mailto:vem72@pitt.edu)) and Michele Alves ([malves@fiu.edu](mailto:malves@fiu.edu)) with any questions.

SATURDAY, MAY 16, 2026

## Badge Pick-Up

7:00 AM-5:00 PM

**Location: Galleria**

## Continental Breakfast

7:00-8:00 AM

**Location: Galleria**

## Session 001 - Pathobiology of Alcohol-Associated Liver Disease

8:00-9:45 AM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Liver Pathobiology Scientific Interest Group*

Chair: Laura Nagy, PhD ▪ Cleveland Clinic

Co-Chair: Chen Zhang ▪ University of Kansas

**Session Overview:** Alcohol-Associated Liver Disease (ALD) is a major global health issue with complex causes. This session will showcase recent findings on ALD's pathogenic mechanisms, focusing on molecular, cellular, and immunological pathways and their interactions with genetic, environmental, and microbial factors. Experts will highlight advances in multi-omics, models, and imaging that aid in understanding ALD's development and present insights into future therapies and the molecular basis of ALD.

- **Chair - Welcome and Introductions**

- 8:00-8:25 AM

***From Inflammation to Fibrosis: Morphogen-Driven Reprogramming in Alcohol-Associated Liver Disease***

Rebecca McCullough, PhD ▪ University of Colorado Anschutz Campus

# PISA

PATHOBIOLOGY FOR INVESTIGATORS, STUDENTS & ACADEMICIANS



**2026 PISA Co-Chair**  
**Anna Tingler, BS**  
Medical University of  
South Carolina



**2026 PISA Co-Chair**  
**Cole Hladik, PhD**  
University of Oklahoma  
Health Sciences Center

**2026 Young Investigators Virtual Meeting**

**SAVE THE DATE: October 27-29**

**Abstract Deadline is September 15**

**ASIP**  
American Society for Investigative Pathology

- 8:25-8:50 AM  
***Alcohol-Induced Inflammasome Activation in Alcohol-Associated Hepatitis and MetALD***  
Gyongyi Szabo, MD, PhD ▪ Harvard Medical School, Beth Israel Deaconess Medical Center
- 8:50-9:15 AM  
***Alcohol and Lipid Traffic Don't Mix***  
Carol Casey, PhD ▪ University of Nebraska Medical Center, Omaha VA Medical Center
- 9:15-9:40 AM  
***Inflammation and Cell Death in Alcohol-Associated Liver Disease***  
Laura Nagy, PhD ▪ Northern Ohio Alcohol Center, Cleveland Clinic

### Session 002 - Secretory Cells: Guardians of Epithelial Homeostasis and Mucosal Inflammation

8:00-9:45 AM

**Session Room: Caloosa Salon E**

*Sponsored by the ASIP Mucosal Pathobiology Scientific Interest Group*

Chair: Andrei Ivanov, PhD ▪ Cleveland Clinic Foundation

Co-Chair: Amy Engevik, PhD ▪ Medical University of South Carolina

**Session Overview:** This session will focus on specific types of epithelial cells which functions involve secretion of mucin and antimicrobial substances. These cells play key roles in establishing acellular barriers in different mucosal tissues and protecting epithelial layers from microbial attachment and invasion. The symposium will discuss these conventional functions of secretory epithelial cells along with recently discovered unconventional functions that include sampling of luminal antigen and training of mucosal immune system. Functional abnormalities of secretory cells in chronic inflammatory diseases such as inflammatory bowel diseases and asthma will also be discussed.

- **Chair - Welcome and Introductions**
- 8:00-8:35 AM  
***Roles of Paneth Cells in Regulating Gut Homeostasis and Mucosal Inflammation***  
Nan Gao, PhD ▪ Rutgers University
- 8:35-9:10 AM  
***Mucins and Their Roles in Asthma***  
Christopher Evans, PhD ▪ University Colorado Anschutz Medical Campus
- 9:10-9:45 AM  
***Roles of Intestinal Goblet Cells in Luminal Antigen Uptake and Presentation***  
Rodney Newberry, MD ▪ Washington University School of Medicine
- 9:45-10:00 AM  
***ABSTRACT 001 - Co-Operation Between Gasdermin (GSDM) Family Members, GSDMB and GSDMD Regulate Goblet Cell Function During Homeostasis and is Dysregulated During Inflammatory Bowel Disease (IBD)***  
Joseph Williams ▪ Case Western Reserve University School of Medicine

### Coffee Break

10:00-10:25 AM

**Location: Galleria**

### Session 003 - Exosome-Mediated Tissue Repair

10:30 AM-12:15 PM

**Session Room: Caloosa Salon D**

Chair: Andy Duncan, PhD ▪ University of Pittsburgh

Co-Chair: Menglu Yang, MD, PhD ▪ Harvard Medical School

**Session Overview:** Extracellular vesicles (EVs), including exosomes and matrix-bound vesicles, are central to intercellular communication and hold significant promise in tissue repair, regeneration, and disease diagnostics. This session will highlight recent advances in the use of EVs as mediators of tissue healing, while also emphasizing cutting-edge technologies for their isolation and analysis. Talks will feature novel approaches that improve the recovery, characterization, and functional assessment of EVs, with applications ranging from mechanistic studies in preclinical models to biomarker discovery. By bridging regenerative biology with technological innovation, this session will provide a comprehensive view of how EV research is transforming both basic science and translational strategies.

- **Chair - Welcome and Introductions**
- 10:30-10:55 AM  
***Advanced Extracellular Vesicle Technologies for Liquid Biopsy-Based Cancer Diagnostics and Therapeutics***  
Yong Zeng, PhD ▪ University of Florida
- 10:55-11:20 AM  
***Regenerative Extracellular Vesicles for Advanced Hearing Tissue Repair***  
Mei He, PhD ▪ University of Florida
- 11:20-11:45 AM  
***Matrix Bound Nanovesicles: Advancing the Next Generation of ECM-Based Nanotechnology***  
George Hussey, PhD ▪ University of Pittsburgh School of Medicine
- 11:45 AM-12:00 PM  
***ABSTRACT 002 - Inhibition of Inflammation in the Pulmonary Circulation by a Cell-Permeant Peptide Targeting the Lateral Border Recycling Compartment (LBRC)***  
Jonathan Sutkowski ▪ Northwestern University Feinberg School of Medicine
- 12:00-12:15 PM  
***ABSTRACT 003 - Matrix-Bound Nanovesicles as Modulators in Alcohol-Related Liver Disease***  
Yekaterina Krutshenko, PhD ▪ University of Pittsburgh School of Medicine

#### **Session 004 - Pathogen Strategies to Invade, Persist, and Damage the Gut**

10:30 AM-12:15 PM

**Session Room: Caloosa Salon E**

*Co-Sponsored by the ASIP Infectious Disease Scientific Interest Group and the Mucosal Pathobiology Scientific Interest Group*

Chair: Melinda Engevik, PhD ▪ Medical University of South Carolina

Co-Chair: Anna Tingler ▪ Medical University of South Carolina

**Session Overview:** This session will highlight cutting-edge research on how enteric bacterial pathogens, including *Escherichia coli*, *Salmonella enterica*, and *Clostridioides difficile* interact with the intestinal epithelium and immune system to drive infection and disease. Talks will explore the virulence strategies these organisms use to breach the mucus barrier, hijack host signaling pathways, and manipulate immune responses. The session will also cover host factors that determine susceptibility and recovery, such as epithelial transport, inflammation, and microbiome disruption. Emphasis will be placed on shared and divergent mechanisms of pathogenesis, including toxin production, epithelial remodeling, and chronic inflammatory sequelae.

- **Chair - Welcome and Introductions**
- 10:30-10:55 AM  
***Evolving the Gut Microbiome: Dietary Impact on Colonization and Infection by Pathobionts***  
Aaron Hecht, MD, PhD ▪ Mayo Clinic College of Medicine
- 10:55-11:20 AM  
***Host and Microbiota Factors That Shape Clostridioides Difficile Infection***  
Melinda Engevik, PhD ▪ Medical University of South Carolina

- 11:20-11:45 AM  
***Small RNA Promotes Negative Feedback Regulation of General Stress Response Upon Sensing of Cell Envelope Damage in Salmonella***  
Hubert Salvail, PhD ▪ University of Central Florida
- 11:45 AM-12:00 PM  
***ABSTRACT 004 - Linking Multi-Barrier Permeability with Cognitive Dysfunction in Borrelia Infection-Associated Chronic Illness***  
Francisco Carrillo-Salinas, PhD ▪ Massachusetts Institute of Technology
- 12:00-12:15 PM  
***ABSTRACT 005 - IL6 Role in Brain-Spleen Axis in Neonatal Immune Activation in Response to Sepsis***  
Emily Silva ▪ Florida International University

### Session 005 - Implementing Human Research Approaches in Alignment with Evolving NIH Policies

Lunch and Science Policy Session

12:45-1:45 PM

**Session Room: Caloosa Salon B**

*Sponsored by the ASIP Research and Science Policy Committee*

Co-Chair: Elaine Bearer, MD, PhD ▪ University of New Mexico

Co-Chair: Rajanikanth Vadigepalli, PhD ▪ University of New Mexico

**Session Overview:** The NIH's recent animal model policy shifts away from funding animal-based research *exclusively*. The modified policy requires new funding opportunities to also support human-focused or new approach methodologies like organoids, in vitro models, ex vivo models, human data, or AI. The objective of the new policy is to accelerate discoveries by prioritizing models most relevant to human health. However, animal studies can still be funded if scientifically appropriate and justified. This recent policy change promotes broader model consideration, not restriction, with peer reviewers assessing model suitability, and continues to uphold existing ethical guidelines for animal welfare. This session will aid investigators in navigating this transition. In moderated discussions, we will brainstorm together to unpack the practicalities of accessing human tissue and data repositories, the financial hurdles of retooling a laboratory, and the nuances of justifying animal model suitability in the current peer review climate. We aim to highlight actionable insights on how to leverage institutional resources and diverse funding streams to align pathology research with these evolving NIH policies and priorities.

- **Chair - Welcome and Introductions**
- 12:45-12:50 PM  
***Session Overview***  
Rajanikanth Vadigepalli, PhD ▪ University of New Mexico

### Table Discussions:

#### ***Table 1 - Best Practices in Accessing Research Resources and Repositories***

Moderator: Kelsey McKillip, PhD

- Data Repositories
  - NIH-controlled/supported data repositories
  - HHS Open Data/State data repositories
  - Institutional data repositories
- Biospecimen Repositories
  - Institutional biospecimen resources/repositories
  - NIH-controlled/supported biospecimen resources
  - Commercial biospecimen resources
  - Leveraging archived diagnostic tissue for research

### Discussion questions:

- Have you leveraged human biospecimen and data repositories for your research, and if so, what types of biospecimens and/or data have proven most valuable to your research?
- When your institutional biobank does not meet your specific needs, what other resources have you leveraged and/or what "boots-on-the-ground" methods have you used to establish reliable tissue procurement pipelines?
- How have you obtained clinical metadata (e.g., patient history, treatment response) associated with a sample and ensured it is robust enough for high-impact human research?
- What are the most common administrative bottlenecks you have faced with external repositories, and how have you worked with your tech transfer office to overcome them?

### **Table 2 - Institutional Support and Other Funding Avenues**

Moderator: Rajanikanth Vadigepalli, PhD

- Internal Institutional Support
  - Seed grants and bridge funding for model transitions
  - Intramural core facility subsidies
  - Start-up package alignment with NIH policy shifts
- Alternative Federal & State Funding
  - Administrative supplements for human-centric research
  - State-level biotechnology and health research grants
- Non-Federal Funding Streams
  - Philanthropic foundations and patient advocacy groups
  - Industry partnerships and sponsored research agreements
  - Crowdfunding and private donor cultivation for "human-first" models

### Discussion questions:

- What specific pilot funding models has your institution implemented to help labs transition from animal-heavy research to human-focused New Approach Methodologies (NAMs)?
- How are you and your institutional leadership engaging non-federal partners (philanthropy or industry) specifically interested in human-first predictive models?
- What existing mechanisms such as T32 or administrative supplements have you successfully used to support the retraining of staff in these new techniques?

### **Table 3 - Study Section Experience and Justification of Animal Models**

Moderator: Sharon DeMorrow, PhD

- Grant Narrative Strategies
  - Framing Scientific Rigor when human models are unavailable
  - Justifying the animal-to-human bridge in the Approach section
- Reviewer Sentiment & Policy Interpretation
  - Feedback trends on Model Suitability
  - Addressing the New Approach Methodologies (NAMs) in Specific Aims
- Responding to Critiques
  - Effective rebuttals for "lack of human relevance"
  - Integrating AI/*in silico* data as a secondary model validation

### Guiding questions:

- Since the policy shift, how have you successfully framed the necessity of an animal model in your Specific Aims to satisfy reviewers?
- For those who have recently served on or submitted to study sections, what are the emerging "red flags" reviewers cite regarding model suitability?
- How can we better inform reviewers about the current *limitations* of NAMs (e.g., lack of systemic immune response) so they don't unfairly penalize animal-based studies?
- If a reviewer critiques a model as "not human-relevant," what types of preliminary human data or AI-modeling evidence have you found most effective in a rebuttal?

#### **Table 4 - Policies on Human Subject Research**

Moderator: Elaine Bearer, MD, PhD

- Regulatory Navigation
  - Streamlining IRB approvals for bio-computational research:  
<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-26-032.html>
  - Managing multi-center Clinical Trial requirements (Single IRB)
- Ethics and Inclusion
  - Addressing the new NIH Primary/Secondary Data Sharing mandates:  
<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-26-046.html>
  - Innovative strategies for diverse cohort recruitment
- Data Privacy and Policy
  - De-identification standards in the age of high-resolution imaging
  - Informed consent for secondary use and long-term data banking

#### **Guiding questions:**

- What best practices has your institution adopted to streamline the IRB approval process for multi-site studies or complex human data sharing?
- What innovative recruitment strategies are you seeing that successfully meet the new NIH requirements for diversity and inclusion in clinical cohorts?
- As we move toward more AI-driven analysis, how are you navigating the ethical gray areas of de-identifying complex genomic or imaging data?
- How can we better incentivize clinicians to partner with basic scientists to facilitate the flow of samples and data from the bedside to the lab?

#### **Table 5 - Policies on In Vitro and Ex Vivo Human Cell and Tissue Models**

Moderator: William Muller, MD, PhD

New NIH restrictions on fetal tissue

- In vitro models with human cells
  - Appropriate sources
  - Appropriate uses
  - Validation of cell lines
- Ex vivo experiments with human tissues
  - IRB issues
  - Appropriate uses
  - Pros and cons of autopsy tissue
- Use of human data bases

#### **Guiding questions:**

- What are the pros and cons of primary human cells vs. established cell lines vs. ex vivo human tissue in the study of pathobiology? How does it depend on the question being asked?
- In the absence of a gold standard, what criteria should we use to validate that an in vitro model, organoid or organ-on-chip model is a faithful representation of human physiology or pathology?
- How can we move toward a consensus on New Approach Methodologies (NAMs) so that results are comparable across different labs and institutions?

#### **Session 006 - Beyond the Data: The Lived Experience of Scientific Discovery**

Lunch and Equal Representation and Opportunity Session

12:45-1:45 PM

**Session Room: Caloosa Salon E**

*Sponsored by the ASIP Committee for Equal Representation and Opportunity*

Chair: Diane Bielenberg, PhD ▪ Boston Children's Hospital

Co-Chair: Kojo Elenitoba-Johnson, MD ▪ Memorial Sloan Kettering Cancer Center

**Session Overview:** This session reintroduces ASIP's Committee for Equal Opportunity and Representation mission by reflecting on how formative influences shape scientific inquiry, innovation, leadership, and professional growth. Members' stories will illuminate the often-unseen human elements that subtly influence how questions are posed, collaborations are built, and discoveries are made. The session emphasizes a core principle central to both ASIP's and CERO's mission: investing in intellectual breadth and professional belonging is essential.

- 12:45-12:48 PM  
**Chair - Welcome and Introductions**
- 12:48-12:52 PM  
***Reflections from the CERO Chair***  
Cecelia Yates, PhD ▪ University of Pittsburgh
- 12:52-12:56 PM  
Speaker 1 - Robinna Lorenz, MD, PhD ▪ Genentech, Inc.
- 12:56-1:00 PM  
Speaker 2 - Andrew Duncan, PhD ▪ University of Pittsburgh
- 1:00-1:04 PM  
Speaker 3 - Michele Alves, PhD ▪ Florida International University
- 1:04-1:08 PM  
Speaker 4 - Ramon Ramos, PhD ▪ Albany Medical College
- 1:08-1:12 PM  
Speaker 5 - Vik Meadows, PhD ▪ University of Pittsburgh
- 1:12-1:16 PM  
Speaker 6 - Nakisha Rutledge, PhD ▪ Temprian Oncology, Inc.
- 1:16-1:20 pm  
Speaker 7 - Jonathon Homeister, MD, PhD ▪ University of North Carolina
- 1:20-1:24 pm  
Speaker 8 - Diane Bielenberg, PhD ▪ Boston Children's Hospital
- 1:24-1:28 pm  
Speaker 9 - Kojo Elenitoba-Johnson, MD ▪ Memorial Sloan Kettering Cancer Center
- 1:28-1:32 PM  
**Co-Chair - Closing Remarks**

#### **Session 007 - Cotran Early Career Investigator Award Lecture**

2:00-2:45 PM

#### **Session Room: Caloosa Salon D**

- 2:00-2:05 PM  
***Introduction***  
Melinda Engevik, PhD ▪ Medical University of South Carolina
- 2:05-2:45 PM  
***The Lymphatic System in Disease Progression***  
Dennis Jones, PhD ▪ Boston University

**Lecture Overview:** My laboratory is dedicated to elucidating the fundamental mechanisms that drive lymphatic metastasis and dysregulate lymphatic vessel function. Through our research, we have demonstrated that lymph node metastases grow independently of angiogenesis and are unresponsive to conventional anti-angiogenic therapies. Rather, we discovered that solid tumors remodel blood vessels within lymph nodes, which impairs lymphocyte infiltration and compromises immune surveillance. Building on these findings, we are actively investigating strategies to enhance T-cell

infiltration and effector function within breast cancers at all tumor sites, with the goal of improving immunotherapeutic responses in patients with metastatic cancer. Additionally, we are investigating the impact of bacterial skin infections on the autonomous contraction of lymphatic vessels and lymphatic flow. Our studies demonstrate that such infections cause persistent disruptions in lymphatic function, which may increase susceptibility to repeated infections and the development of lymphedema. We are currently focused on uncovering the molecular pathways underlying these effects and developing therapeutic interventions aimed at restoring lymphatic vessel function, strengthening local immune defenses, and normalizing tissue fluid homeostasis following bacterial infection.

### **Session 008 - Emerging Technologies and Artificial Intelligence in Unraveling Cancer Heterogeneity**

3:00-4:45 PM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Neoplasia, Tumor Microenvironment, and Metastasis Scientific Interest Group*

Chair: Sanjukta Chakraborty, PhD ▪ Texas A&M University

Co-Chair: Piyali Dasgupta, PhD ▪ Marshall University

**Session Overview:** Cancer is a profoundly heterogeneous disease, and its progression is shaped by dynamic genetic, epigenetic, spatial, and microenvironmental factors. Cancer heterogeneity, both between tumors and also within a single tumor, represents a major clinical challenge in understanding disease progression, therapeutic resistance, and patient stratification. This session will highlight how emerging technologies, particularly artificial intelligence (AI) and spatial transcriptomics, are advancing our ability to decode this complexity. Recent breakthroughs in AI-driven analysis of multi-modal data are enabling the extraction of hidden patterns from histopathology slides, radiology scans, and single-cell datasets. Deep learning models can now accurately predict molecular subtypes, immune phenotypes, and treatment response directly from digital pathology images, offering new avenues for integrative cancer diagnostics and prognosis. In parallel, spatial transcriptomics technologies are providing unprecedented resolution into the spatial organization of cells and the molecular signaling networks within the tumor microenvironment. These platforms enable researchers to simultaneously capture gene expression and spatial context, revealing how interactions between cancer cells, stromal components, and immune infiltrates shape disease behavior, enabling the ability to target individual cells. Speakers in this session will present cutting-edge applications that combine these tools such as, AI-guided spatial transcriptomic analysis to map clonal architecture, tumor-immune niches, and therapy-induced remodeling. The integration of these technologies will not only fundamentally enhance the mechanistic understanding of tumor ecosystems but also pave the way for targeted precision oncology strategies grounded in the spatial and molecular realities of individual tumors.

- **Chair - Welcome and Introductions**

- 3:00-3:25 PM

- ***Decoding Cancer Using Large Language Models to Map Gene-Drug Interactions***

- Chris (Yu-Chiao) Chiu, PhD ▪ University of Pittsburgh School of Medicine

- 3:25-3:50 PM

- ***Precision Oncology in the Era of Clinicogenomics: Tackling Cancer Heterogeneity***

- Kenneth Ramos, MD, PhD ▪ Texas A&M Health

- 3:50-4:15 PM

- ***Artificial Intelligence Driven Radiogenomics and Radiomics to Decode Cancer Heterogeneity and Treatment Response***

- Issam El Naqa, PhD ▪ Moffit Cancer Center

- 4:15-4:30 PM

- ***ABSTRACT 006 - Spatially Informed Histomolecular Subtyping of Hepatocellular Carcinoma: Clinical, Molecular, and Therapeutic Implications***

- Tyler Yasaka ▪ University of Pittsburgh School of Medicine

- 4:30-4:45 PM  
***ABSTRACT 007 - Spatial Transcriptomics Reveals Obesity-Associated Remodeling of Epithelial and Immune Compartments in Invasive Breast Cancer***  
Cole Hladik ▪ University of Oklahoma Health Campus

### **Session 009 - Pathobiology for Basic Scientists Course – Deciphering the Molecular Events Involved in Neuroinflammation, Neurodegeneration, and Neurological Pain**

3:00-4:45 PM

**Session Room: Caloosa Salon E**

*Sponsored by the ASIP Neuropathology Scientific Interest Group*

Chair: Jessica Fortin, DVM, PhD ▪ Purdue University

Co-Chair: Christi Kolarcik, PhD ▪ University of Pittsburgh

Co-Chair: Michele Alves, PhD ▪ Florida International University

**Session Overview:** This session will feature speakers to cover cutting edge topics in the field of neuroscience. Experts of the field will review fundamental notions and showcase recent advancements in 1) neurodegenerative proteinopathies and neuroinflammation, 2) mitophagy, 3) neuronal circuits and cognitive processes, 4) pain perception and modulation. This session provides opportunities for interactions between experts in the field, experimental pathology researchers, and trainee. The Neuropathology Scientific Interest Group is committed to engage members at all levels with common interests in advancing their knowledge in neuroscience, neuropathology, neurotoxicology, and neuropsychological disorders.

- **Chair - Welcome and Introductions**
- 3:00-3:25 PM  
***Inflammation and Protein Aggregation in Neurodegenerative Diseases***  
Jessica Fortin, DVM, PhD ▪ Purdue University
- 3:25-3:50 PM  
***Mitophagy at the Center of Alzheimer's Disease***  
George Perry, PhD ▪ University of Texas at San Antonio
- 3:50-4:15 PM  
***Swine Models of Alzheimer's Disease***  
Timothy Allen, PhD ▪ Florida International University
- 4:15-4:40 PM  
***A Primer of Peripheral Pathways in Chronic Pain***  
LaTasha Crawford, VMD, PhD ▪ University of Wisconsin

### **Session 010 - Minisymposia: Pathobiology of the Barrier and Stromal Tissue Compartments in Inflammation**

3:00-5:00 PM

**Session Room: Caloosa Salon B**

Co-Chair: Kevin Van der Jeught, PhD ▪ University of Miami Miller School of Medicine

Co-Chair: Susana Lechuga, PhD ▪ Cleveland Clinic

- **Chair - Welcome and Introductions**
- 3:00-3:15 PM  
***ABSTRACT 008 - High-Fat Diet Elicits a Type 2 Immune Circuit Linking Mast Cells to Gastric Metaplasia***  
Charulekha Packirisamy ▪ Medical University of South Carolina
- 3:15-3:30 PM  
***ABSTRACT 009 - Protein O-GlcNAcylation Controls Abnormal Myofibroblast Differentiation in Localized Scleroderma***  
Yan Wang, MD, PhD ▪ Cleveland Clinic

- 3:30-3:45 PM  
**ABSTRACT 010 - Impacts of Germline CYP2E1 Deletion on the Small Intestinal Epithelium**  
Alexandra Tomasevich ▪ Medical University of South Carolina
- 3:45-4:00 PM  
**ABSTRACT 011 - Endothelial JunB Deletion Improves Survival and Preserves Glomerular Filtration Rate (GFR) in Acute Sepsis**  
Ramon Bossardi Ramos, PhD ▪ Albany Medical College
- 4:00-4:15 PM  
**ABSTRACT 012 - Pulmonary Fibroblasts as Mediators of Immune Regulation and Tissue Remodeling During Aspergillus Fumigatus Challenge**  
Jose Guirao-Abad, PhD ▪ The Ohio State University
- 4:15-4:30 PM  
**ABSTRACT 013 - Loss of Arpin, an Endogenous Arp2/3 Complex Inhibitor, Causes Intestinal Epithelial Barrier Dysfunction and More Severe Colitis**  
Michael Schnoor, PhD ▪ Nacional Polytechnic Institute
- 4:30-4:45 PM  
**ABSTRACT 014 - The Impact of Mitochondrial Haplotype on Inflammation and Fibrosis In Novel OKC-HETB/W Rats**  
Ramasamy Selvarani, PhD ▪ University of Oklahoma
- 4:45-5:00 PM  
**ABSTRACT 015 - The Rho Guanine Nucleotide Exchange Factor 16 is a Novel Regulator of the Intestinal Epithelial Barrier and Repair**  
Susana Lechuga, PhD ▪ Cleveland Clinic

#### Afternoon Break

4:45-5:15 PM

**Location: Galleria**

Wine and beer will be served

#### Session 011 - Rous-Whipple Award Lecture

5:30-6:30 PM

**Session Room: Caloosa Salon D**

- 5:30-5:35 PM  
**Introduction**  
Satdarshan Paul Singh Monga, MD ▪ University of Pittsburgh
- 5:35-6:30 PM  
**Pros and Cons of Living Longer**  
Anna Mae Diehl, MD ▪ Duke University School of Medicine

**Lecture Summary:** In this lecture, Dr. Diehl will discuss determinants of hepatic biological aging and the role of hepatocyte senescence in the pathogenesis of cirrhosis and liver cancer.

#### Women in Pathology Networking Event - Connect, Communicate, and Collaborate

6:30-8:30 PM

**Location: Luminary Hotel Pool Deck and Lounge (4<sup>th</sup> Floor)**

Join us for an evening celebrating the ASIP community with this Women in Pathology-sponsored event! Take a break from the science and bring your outgoing, collegial, and team-building self – join us for an evening of treats, drinks, and games! Women in Pathology invites you to shake things up a bit and connect with your colleagues in a night of conversation, networking, and fun competition, playing games outside your everyday routine. Everyone is welcome!

**Badge Pick-Up**

7:00 AM-5:00 PM

**Location: Galleria**

**Continental Breakfast**

7:00-8:00 AM

**Location: Galleria**

**ASIP President's Circle Breakfast (By Invitation Only)**

7:00-8:00 AM

**Location: Salon B**

**Session 012 - Specialized Pro-Resolving Mediators in Action: New Insights into Inflammation Resolution and Beyond**

8:00-9:45 AM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Inflammation and Immunopathology Scientific Interest Group*

Chair: Ramon Bossardi Ramos, PhD ▪ Albany Medical College

Co-Chair: Gabrielle Fredman, PhD ▪ Albany Medical College

**Session Overview:** This session will spotlight new advances in the biology of specialized pro-resolving mediators, highlighting their dynamic roles not only in resolving inflammation but also in orchestrating tissue repair, immune reprogramming, and disease resolution across diverse pathophysiological contexts.

- **Chair - Welcome and Introductions**

- 8:00-8:25 AM

- ***Resolvin Biosynthesis and Functions in the Acute Inflammatory Response***

- Charles Serhan, PhD, DSc ▪ Harvard Medical School, Harvard University

- 8:25-8:50 AM

- ***Inflammation-Resolution Circuits as Key Determinants of Skeletal Muscle Plasticity in Health and Disease***

- James Markworth, PhD ▪ Purdue University

- 8:50-9:15 AM

- ***Inflammation-Resolution Signaling in Cardiometabolic Disease and Heart Failure***

- Ganesh Halade, PhD ▪ University of South Florida College of Medicine

- 9:15-9:40 AM

- ***Targeting Resolution Programs to Modulate Hematopoiesis in Aging and Disease***

- Katherine MacNamara, PhD ▪ Albany Medical College

**Session 013 - Drug Development and the Role of Investigative Pathology**

8:00-9:45 AM

**Session Room: Caloosa Salon E**

*Sponsored by the ASIP Pathology in Biotech and Industry Scientific Interest Group*

Chair: Cary Austin, MD, PhD ▪ Genentech, Inc.

Co-Chair: Sripad Ram, PhD ▪ Pfizer, Inc.

**Session Overview:** This session will explore recent scientific and technological advances involving the application of investigative pathology in drug research and development. Presentations from pathology thought leaders across a broad set of industry settings will provide attendees with an in-depth perspective on how pathology as a discipline is making an impact in tissue-based research and the discovery and development of new drugs to address unmet medical needs, and will highlight some of the pathology activities underway in the Biopharma industry.

- **Chair - Welcome and Introductions**
- 8:00-8:25 AM  
***Deep B-Cell Depletion with Obinutuzumab in Lupus Nephritis: Exploratory Multiplex IF Insights from the Phase 3 REGENCY Trial***  
Cary Austin, MD, PhD ▪ Genentech Inc.
- 8:25-8:50 AM  
***A Multimodal Approach to Characterizing the Tumor Microenvironment in Pre-Clinical Treatment Models***  
James Ziai, MD ▪ Genentech Inc.
- 8:50-9:15 AM  
***Spatial Transcriptomics Reveals the Role of mmp12 in the Pathogenesis of Autoimmune Arteritis***  
Shinichi Onishi, DVM ▪ Chugai Pharmaceutical Co.
- 9:15-9:40 AM  
***Use of Non-Traditional Toxicology Species Models to Support Safety Characterization***  
Elizabeth Clark, DVM, PhD, DACVP ▪ Boehringer Ingelheim Pharmaceuticals, Inc.

### Coffee Break

10:00-10:25 AM

**Location: Galleria**

### Session 014 - Ocular Cell Regeneration and Replacement

10:30 AM-12:15 PM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Ocular Pathobiology Scientific Interest Group*

Chair: Patricia D'Amore, PhD ▪ Schepens Eye Research Institute and Harvard Medical School

Co-Chair: Menglu Yang, MD, PhD ▪ Schepens Eye Research Institute and Harvard Medical School

**Session Overview:** This session will highlight cutting-edge advances in regenerative therapies aimed at restoring vision through the replacement and repair of ocular cells and tissues. The featured talks will span innovations in retinal pigment epithelium (RPE) transplantation, neuroprotection-based regeneration strategies, and the development of bioengineered corneas to address global tissue shortages. By integrating cellular, molecular, and bioengineering approaches, this session aims to spark interdisciplinary dialogue and chart future directions for clinical translation in ocular regenerative medicine.

- **Chair - Welcome and Introductions**
- 10:30-11:00 AM  
***RPE Transplantation: Bench to Bedside Progress***  
Kapil Bharti, PhD ▪ National Eye Institute, National Institutes of Health
- 11:00-11:30 AM  
***Advancing Vision Restoration: Strategies from Neuroprotection to Regeneration***  
Dong Feng Chen, PhD ▪ Schepens Eye Research Institute, Mass Eye and Ear, Harvard Medical School
- 11:30 AM-12:00 PM  
***Bioengineered Cornea to Overcome Donor Corneal Shortage***  
Jingjing You, PhD ▪ The University of Sydney

### Session 015 - Pathobiology of Obesity

10:30 AM-12:15 PM

**Session Room: Caloosa Salon E**

*Sponsored by the ASIP Società Italiana di Patologia e Medicina Traslazionale*

Chair: Max Corsi Romanelli, MD, PhD ▪ University of Milan

Co-Chair: Michael Thompson, MD, PhD ▪ Washington University St. Louis

**Session Overview:** Obesity rates continue to increase around the world and have emerged as a significant modifiable risk factor for various cancers. The pathobiological mechanisms linking obesity to cancer are multifaceted and create a pro-tumorigenic environment that promotes cancer initiation and progression. Understanding these complex interactions including molecular, cellular, and microbial mediators is crucial for developing targeted prevention and treatment strategies. This research session aims to delve into the molecular and cellular pathways that are associated with obesity and provide a potential connection between obesity and cancer.

- **Chair - Welcome and Introductions**
- 10:30-10:55 AM  
***Epicardial Adipose Tissue Pathophysiology and Clinical Applications in Contemporary Medicine***  
Ron Varghese, MD ▪ University of Miami, Miller School of Medicine
- 10:55-11:20 AM  
***Dysbiosis and Obesity: Are Postbiotics Next in Line for the Prevention and Management of Obesity?***  
Luigina Romani, MD, PhD ▪ University of Perugia
- 11:20-11:45 AM  
***Obesity and Breast Cancer: Molecular Insights and Perspectives***  
Sebastiano Andò, MD ▪ University of Calabria
- 11:45 AM-12:10 AM  
***Obesity Facilitated Colon Cancer Progression is Mediated by Increased Lipid Droplets Dynamics***  
Suzana Savkovic, PhD ▪ Tulane University School of Medicine

#### **Session 016 - Minisymposia: Immune Cells and Inflammatory Signaling in Disease States**

10:30 AM-12:15 PM

**Session Room: Caloosa Salon B**

Co-Chair: Louisa Tichy, PhD ▪ Wake Forest University School of Medicine

Co-Chair: William Muller, MD, PhD ▪ Northwestern University

- **Chair - Welcome and Introductions**
- 10:30-10:45 AM  
***ABSTRACT 016 - The Role of Neutrophils and Myeloperoxidase in Cancer Cachexia***  
Louisa Tichy, PhD ▪ Wake Forest University School of Medicine
- 10:45-11:00 AM  
***ABSTRACT 017 - MicroRNA-29a Reprograms CD8 T Cell Differentiation by Rewiring Key Memory- and Exhaustion-Driving Epigenetic Circuits***  
Erietta Stelekati, PhD ▪ University of Miami
- 11:00-11:15 AM  
***ABSTRACT 018 - TWEAK-Induced Trained Immunity is Associated with Alterations in Activation of Molecular Signaling Pathways and Expression of IBD-Relevant Inflammatory Fibroblasts Markers***  
Cristina Bauset, PhD ▪ University College Dublin
- 11:15-11:30 AM  
***ABSTRACT 019 - TNFSF14/LIGHT Responses in Intestinal and Oesophageal Fibroblasts are Differentially Modulated by Hydroxylase-Inhibitors***  
Cian Ohlendieck ▪ University College Dublin
- 11:30-11:45 AM  
***ABSTRACT 020 - A Novel Role for PECAM in the Regulation of Leukocyte Transendothelial Migration***  
William Muller, MD, PhD ▪ Northwestern University, Feinberg School of Medicine

- 11:45 AM-12:00 PM  
**ABSTRACT 021 - Proton-Activated Chloride Channel 1 (PAC1) is Essential for Macrophage Defense in Bacterial Sepsis**  
Lucien Garo, III ▪ Boston University Chobanian & Avedisian School of Medicine
- 12:00-12:15 PM  
**ABSTRACT 022 - The Protein Tyrosine Phosphatase CD45 Regulates PMN Transepithelial, Antimicrobial Function and Colonic Mucosal Repair**  
Jennifer Brazil, PhD ▪ University of Michigan
- 12:15-12:30 PM  
**ABSTRACT 023 - Reprogramming CD8 T Cell Metabolic Fitness Using MicroRNA-29a to Enhance CAR T Cell Function**  
Natasha Khatwani, PhD ▪ University of Miami, Miller School of Medicine

### Session 017 - Lunch and Poster Blitzes

12:45-1:45 PM

**Session Room: Caloosa Salon B**

Co-Chair: Zoe Libramento ▪ University of North Carolina at Greensboro

Co-Chair: Maria Antonia Zambrano ▪ Tufts University

**Session Description:** Each poster blitz features short (3-minute) presentations based upon selected posters and presented by a young investigator. The objective is to highlight a subset of posters to be presented in a standard poster session to generate interest. Each short presentation will focus on the objectives of the study, the major results, and the conclusions. Details of the studies will be available during the regular poster session.

- **Chair - Welcome and Introductions**
- 12:45-12:48 PM  
**ABSTRACT 051 - Capsaicin Suppresses the Growth of Human Endometrioid Ovarian Cell Carcinoma Via the Calcium Signaling Pathway**  
Piyali Dasgupta ▪ Marshall University
- 12:48-12:51 PM  
**ABSTRACT 059 - Immunologically Active Cardiac Fibroblasts Express MHC-II in Humans and Promotes Doxorubicin Cardiotoxicity in Mice**  
Maria Zambrano ▪ Tufts University
- 12:51-12:54 PM  
**ABSTRACT 061 - Low Volume Aerobic Exercise Modulates Proteolysis in Cachectic Hearts of Male Mice**  
Zoe Libramento ▪ University of North Carolina at Greensboro
- 12:54-12:57 PM  
**ABSTRACT 065 - Burn Wound Bacteria Remodel Local Oxygen Landscapes to Support Anaerobic Niches and Drive Inflammation**  
Subhomitra Ghoshal ▪ Medical University of South Carolina
- 12:57-1:00 PM  
**ABSTRACT 071 - MUC1 and MUC4 Hypomethylation and Elevation in Uterine Corpus Endometrial Carcinoma Correlates with Poor Prognosis**  
Anna Tingler ▪ Medical University of South Carolina
- 1:00-1:03 PM  
**ABSTRACT 073 - Reduced PAI-1 Activity Promotes Longevity and Stress Resilience: Insights from a Drosophila Spn42Dd Model**  
Michelle Thayer ▪ Iowa State University

- 1:03-1:06 PM  
**ABSTRACT 077 - Eicosanoid Regulation of Cancer Cachexia**  
Neha Rana, PhD ▪ Beth Israel Deaconess Medical Center, Harvard Medical School
- 1:06-1:09 PM  
**ABSTRACT 095 - Mapping the Lesion Landscape of White Matter Hyperintensities Using Deep Learning**  
Dana Julian Austin ▪ University of Pittsburgh School of Medicine
- 1:09-1:12 PM  
**ABSTRACT 103 - Maternal Obesogenic Diet Exposure Programs Early Luminal Bile Acids and Gut Immune Development**  
Holly Hinrichs ▪ Washington University School of Medicine
- 1:12-1:15 PM  
**ABSTRACT 105 - Effect of the DPP-4 Inhibitor Linagliptin on Heart Proteome in Mice Fed Normal and High Fat Diets: Gender-Related Metabolic Responses**  
Massimiliano Corsi Romanelli, MD, PhD ▪ Università degli Studi di Milano

### Session 018 - Outstanding Investigator Award Lecture

2:00-2:45 PM

#### Session Room: Caloosa Salon E

- 2:00-2:05 PM  
**Introduction**  
Mel Feany, MD, PhD ▪ Harvard Medical School, Brigham and Women's Hospital
- 2:05-2:45 PM  
**Magical Moments in Protease Biology: Proteasome Autocatalytic Activation and PI31-Mediated Inhibition**  
John Hanna, MD, PhD ▪ Harvard Medical School, Brigham and Women's Hospital, Dana Farber Cancer Institute

**Lecture Overview:** The proteasome is the molecular machine responsible for most intracellular protein degradation. Like other large multi-subunit complexes, the proteasome is too complicated to assemble spontaneously. Instead, it is built through an ordered multistep pathway orchestrated by dedicated chaperone proteins. Here I will discuss our efforts to understand the detailed mechanisms of proteasome assembly, including the determination of several high-resolution cryo-EM structures of assembly intermediates. Inherited mutations in proteasome genes are the basis for an emerging family of diseases known as proteasomopathies. I will also discuss how some of these mutations impair proteasome assembly, leading to defects in proteasome structure and function.

### Session 019 - Robert E. Stowell Memorial President's Symposium – Novel Insights in Cardiac Pathobiology

3:00-4:45 PM

#### Session Room: Caloosa Salon D

Chair: Pilar Alcaide, PhD ▪ University of Miami Miller School of Medicine

**Session Overview:** Heart failure is the leading cause of human mortality, and despite an active research community many of the underlying mechanisms are unknown. Open questions surround molecular triggers and the cellular response of the heart to insults that result in cardiac remodeling. Pathological hallmarks of cardiac remodeling include a set of cellular changes in the heart that include cardiomyocyte hypertrophy, fibrosis, inflammation and capillary rarefaction, and the molecular signals that govern these adaptations remain incompletely understood. The goal of this symposium is to bring together new findings related to cardiac remodeling, and understand their implication in cardiac dysfunction and heart failure.

- **Chair - Welcome and Introductions**
- 3:00-3:25 PM  
**Myeloid-Specific Regulation by RhoA in Response to Cardiac Injury**  
Maria Kontaridis, PhD ▪ Masonic Medical Research Institute

- 3:25-3:50 PM  
***Mitochondrial Quality Control in the Heart***  
Asa Gustaffson, PhD ▪ University of California at San Diego
- 3:50-4:15 PM  
***Unraveling the Secrets of PHLPP Protein Turnover in the Heart with Stress and Aging***  
Nicole Purcell, PhD ▪ Huntington Medical Research Institutes
- 4:15-4:40 PM  
***Extracellular Vesicles in the Pathogenesis of Heart Failure in Patients with Chronic Reno-Cardiac Disease***  
Susmita Sahoo, PhD ▪ Icahn School of Medicine at Mount Sinai

#### Session 020 - Poster Session I (ODD Numbered Posters)

5:00-6:30 PM

**Session Room: Caloosa Salon C**

Beer and wine will be served

#### Poster Categories

Cancer Pathobiology ▪ Cardiac and Vascular Pathobiology ▪ Cell Death and Tissue Repair ▪ Computational Pathobiology  
Gene Regulation in Disease ▪ Inflammation and Immunopathology ▪ Liver Pathobiology ▪ Mucosal Pathobiology  
Neuropathology ▪ Nutrition and Obesity ▪ Toxicologic Pathology ▪ AI-Based Image Analysis

#### Session 021 - Gold-Headed Cane Award Lecture

6:30-7:30 PM

**Session Room: Caloosa Salon D**

- 6:30-6:35 PM  
***Introduction***  
Pilar Alcaide, PhD ▪ University of Miami Miller School of Medicine
- 6:35-7:30 PM  
***Cells and Networks in Flux: Rethinking Ontogenesis and Pathogenesis***  
Mark Tykocinski, MD ▪ Sidney Kimmel Medical College, Thomas Jefferson University

**Lecture Overview:** This lecture will explore advances in fusion protein therapeutics, with a particular focus on the subclass of chimeric, surface molecule-targeting proteins that are multifunctional, impact intercellular crosstalk, and in so doing, rewire cellular networks. Fusion proteins pioneered by our laboratory over the years mediate trans signal conversion, trans signal redirection, and cis loop-back signaling. A paradigmatic fusion protein – SIRP $\alpha$  (counter-receptor for CD47) fused to 4-1BBL – coordinately activates more than one immune effector via trans signal redirection, thereby showcasing multifunctionality through chimerization. This fusion protein has already shown considerable promise as a cancer immunotherapeutic in Phase 2 clinical trials for advanced colorectal and non-small cell lung cancers. A series of other network-rewiring fusion proteins are in development, with natural killer and mast cells as prime targets. Stepping beyond experimental and clinical trial data in hand, this lecture will further propose a generalized framework for rethinking cellular ontogeny that borrows and applies principles from information, network and complexity theories. In the spirit of experimental pathology, this conceptual framework weaves together manifold, often disconnected, observational and experimental threads from the literature. A more dynamic view of cellular networks and pathogenic mechanisms emerges, which, significantly, can inform alternative designs for next-generation fusion proteins geared to pathogenic cell targeting and cellular network rewiring.

#### Scientific Interest Group Networking Event and Trainee Scholar Awards Presentation

7:30-9:30 PM

**Session Room: Caloosa Salon C**

**Badge Pick-Up**

7:00 AM-5:00 PM

**Location: Galleria**

**Continental Breakfast**

7:00-8:00 AM

**Location: Galleria**

**ASIP Fred Sanfilippo Fellow's Breakfast (By Invitation Only)**

7:00-8:00 AM

**Location: Heitman Room (Luminary Hotel, 4th Floor)**

**Session 022 - Spatial Tools to Study Liver Pathobiology**

8:00-9:45 AM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Liver Pathobiology Scientific Interest Group*

Chair: Anita Saraf, MD, PhD ▪ University of Pittsburgh School of Medicine

Co-Chair: Tyler Yasaka ▪ University of Pittsburgh School of Medicine

**Session Overview:** Traditional approaches often obscure the intricate, spatially resolved changes fundamental to liver pathogenesis. This session will explore how cutting-edge spatial 'omics technologies, including spatial transcriptomics and spatial proteomics, are informing our understanding of liver diseases. We'll highlight their power to decipher cellular heterogeneity, microenvironmental cues, and cell-cell interactions to help resolve complexities in disease pathogenesis. This session will showcase how these tools are revealing critical insights into the precise localization of injury, inflammation, and fibrosis in liver cancer, cholestasis, and fatty liver diseases.

- **Chair - Welcome and Introductions**
- 8:00-8:25 AM  
***Fontan Associated Liver Disease, and Metabolic Reprogramming of Hepatocytes***  
Anita Saraf, MD, PhD ▪ University of Pittsburgh School of Medicine
- 8:25-8:50 AM  
***Uncovering Mechanisms of Epithelial-Mesenchymal Crosstalk for Rescuing Intrahepatic Bile Duct Paucity***  
Stacey Huppert, PhD ▪ University of Cincinnati College of Medicine, Cincinnati Children's Hospital Medical Center
- 8:50-9:15 AM  
***Resolving the Spatial Organization of Cellular Communities in Liver Cancer***  
Lichun Ma, PhD ▪ National Cancer Institute, National Institutes of Health
- 9:15-9:30 AM  
***ABSTRACT 024 - 'Lobular' Infers Liver Zonation and Reveals Perturbed Zonation in Progressive Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)***  
Tyler Yasaka ▪ University of Pittsburgh School of Medicine
- 9:30-9:45 AM  
***ABSTRACT 025 - The Significance of miR-22-Galectin-1 Axis in HCC Development and Treatment***  
Yu-Jui Yvonne Wan, PhD ▪ University of California, Davis

**Session 023 - Minisymposia: Cancer Pathobiology**

8:00-9:45 AM

**Session Room: Caloosa Salon E**

Co-Chair: Goran Micevic, MD, PhD ▪ Yale University

Co-Chair: Jayshree Mishra ▪ Texas A&M University

- **Chair - Welcome and Introductions**
- 8:00-8:15 AM  
**ABSTRACT 026 - PRMT5 Represses TH1 Transcriptional Programs and T-bet<sup>+</sup> T Cell Differentiation Through H3R8 Methylation to Promote Immune Evasion in Melanoma**  
Goran Micevic, MD, PhD ▪ Yale University
- 8:15-8:30 AM  
**ABSTRACT 027 - Stage-Dependent Effects of the SEMA3F/NRP2 Axis in Oral Carcinogenesis**  
Joud Omari, DDS, DMSc ▪ Harvard School of Dental Medicine
- 8:30-8:45 AM  
**ABSTRACT 028 - Tumoral IL-33/ST2 Signaling Drives Immune Escape Through Reduced Antigen Presentation**  
Kevin Van der Jeught, PhD ▪ University of Miami Miller School of Medicine
- 8:45-9:00 AM  
**ABSTRACT 029 - Comprehensive Preclinical Evaluation of Natural Compounds with Cytotoxic, Anti-Invasive, and Anti-Angiogenic Activity in TNBC Brain Metastasis**  
Jayshree Mishra, PhD ▪ Texas A&M University Lerma Rangel School of Pharmacy
- 9:00-9:15 AM  
**ABSTRACT 030 - Secreted PTEN-Long Downregulates PI3K Signaling and PD-L1 and Promotes Anti-Tumor Antigen-Presenting Cell Functions to Cause Regressions of Mouse Tumors**  
Jia Xu, PhD ▪ Tisch Cancer Institute at Mount Sinai, Icahn School of Medicine at Mount Sinai
- 9:15-9:30 AM  
**ABSTRACT 031 - StarD10 Phosphorylation Promotes ErbB2-Mediated Alcohol-induced Breast Cancer Progression**  
Manisha Dagar, PhD ▪ Cedars-Sinai Medical Center
- 9:30-9:45 AM  
**ABSTRACT 032 - Stromal Modulation of Tertiary Lymphoid Structures in BRCA-Mutated High Grade Serous Ovarian Cancer**  
Swathi Suresh ▪ University of Pittsburgh School of Medicine
- 9:45-10:00 AM  
**ABSTRACT 033 - NRP2 Drives Tumor Progression, Angiogenesis, and Lymphangiogenesis in OSCC**  
Abdulrahman Nakshabandi ▪ Boston Children's Hospital

#### Coffee Break

10:00-10:25 AM

Location: Galleria

#### Session 024 - The Polyploidy Paradox: Whole Genome Duplication in Homeostasis, Regeneration, and Cancer

10:30 AM-12:15 PM

Session Room: Caloosa Salon D

Chair: Andy Duncan, PhD ▪ University of Pittsburgh

Co-Chair: Evan Delgado, PhD ▪ Medical University of South Carolina

**Session Overview:** Polyploidy, the presence of whole genome duplications, is a fundamental biological phenomenon observed in both normal and diseased tissues. While polyploidy is essential for the function of organs such as the liver and heart, it also plays complex roles in cancer and tissue regeneration. This session will explore the polyploidy paradox—the dual nature of polyploidy as both beneficial and deleterious. Presentations will examine polyploidy across multiple organ systems and cancer, shedding light on its mechanistic underpinnings and implications for therapy.

- **Chair - Welcome and Introductions**

- 10:30-10:55 AM  
***Variation in Cardiomyocyte Ploidy Unveils Genetic Drivers of Ventricular Dilation and Impaired Contractility***  
Michaela Patterson, PhD ▪ Medical College of Wisconsin
- 10:55-11:20 AM  
***The Adaptive Endocycle Drives Whole Genome Doubling in Lethal Cancer***  
Sarah Amend, PhD ▪ The Brady Urological Institute, Johns Hopkins University
- 11:20-11:45 AM  
***Ploidy Dynamics in Liver Injury and Regeneration***  
Andrew Duncan, PhD ▪ University of Pittsburgh School of Medicine
- 11:45 AM-12:00 PM  
***ABSTRACT 034 - SpatioScope: Complementing Interpretable Machine Learning with Explainable Deep Learning to Visualize Morpho-Molecular Signals in Triple Negative Breast Cancer***  
Shrey Sukhadia ▪ Dartmouth Health
- 12:00-12:15 PM  
***ABSTRACT 035 - Functional Impact of Gasdermin B on Epithelial Wound Healing in IBD***  
Serena Artone, PhD ▪ Case Western Reserve University School of Medicine

### Session 025 - Deciphering Epithelial-Immune Crosstalk Using Organoid Models

10:30 AM-12:15 PM

**Session Room: Caloosa Salon E**

Chair: Ronen Sumagin, PhD ▪ Northwestern University

Co-Chair: Mario Manresa, PhD ▪ University College Dublin

**Session Overview:** This session will highlight cutting-edge advances in modeling immune epithelial interactions using human intestinal organoid co-cultures. Speakers will present innovative systems that integrate primary immune cells such as macrophages, neutrophils, and lymphocytes with human intestinal organoid to recapitulate the complex cellular dynamics of the gut mucosa. Attendees will gain insight into translational applications of these platforms and mechanistic studies of inflammatory diseases of the gut.

- **Chair - Welcome and Introductions**
- 10:30-10:55 AM  
***Modeling Inflammatory Bowel Disease Using Multicellular Organoids***  
Brooke Druliner, PhD ▪ Mayo Clinic
- 10:55-11:20 AM  
***Impact of the Microenvironment on Innate Immune Responses to Enteropathogens Using Human Enteroid Co-Culture Models***  
Nicholas Zachos, PhD ▪ Vanderbilt University Medical Center
- 11:20-11:45 AM  
***Defining Immune Contributions to Epithelial Plasticity in Inflammatory Bowel Disease***  
Kathryn Hamilton, PhD ▪ Children's Hospital of Philadelphia, University of Pennsylvania
- 11:45 AM-12:10 PM  
***Beyond Immunity – T-Cell Roles in Intestinal Health***  
Liza Konnikova, MD, PhD ▪ Yale School of Medicine, Yale University

### Session 026 - Educating the Educators – 21<sup>st</sup> Century Strategies in Undergraduate and Graduate Education

*Career Development/Education Session*

*Sponsored by the ASIP Education Committee*

10:30 AM-12:15 PM

**Session Room: Caloosa Salon B**

Co-Chair: Richard Mitchell, MD, PhD ▪ Brigham and Women's Hospital, Harvard Medical School

Co-Chair: Julie Randolph-Habecker, PhD ▪ Pacific Northwest University

**Session Overview:** The undergraduate and graduate education landscape continues to evolve at an ever-accelerating pace. A new generation of learners – born with smartphones in their cribs and raised on social media – eschew the traditional textbook and lecture-based curricula the majority of their Faculty know (and sometimes loved). From 'flipped classrooms' to case-based collaborative learning, asynchronous coursework and Zoom break-out rooms, newer concepts on adult learning are driving a re-evaluation of teaching approaches. Fortunately, there is also a burgeoning array of resources that can help deliver more interactive content, spaced repetition, and better individual assessments. The trick is to identify the best materials to effectively reach our students, and also to become comfortable (and innovative) with the new techniques. The ASIP Education Committee and the Special Interest Group for Novel Education and Teaching (SIGNET) will host a full symposium/workshop to understand the new technologies and explore how these can be used in 'flipped' classroom instruction. To better appreciate the value-added, we will showcase digital active-learning and adaptive educational platforms that support interactive, competency-aligned, and learner-centered instruction. Participants will view demonstrations and engage with platforms including Lecturio, H5P, and ScholarRx to see how these multimedia-rich, interactive modules (videos, quizzes, cases, flashcards, animations) move beyond passive reading or lecture.

### Session 027 - Lunch and Poster Blitzes

12:45-1:45 PM

**Session Room: Caloosa Salon B**

Co-Chair: Glaucia Maria de Mendonça Fernandes, PhD ▪ Florida International University

Co-Chair: Anthony Sheets ▪ Brigham and Women's Hospital

**Session Description:** Each poster blitz features short (3-minute) presentations based upon selected posters and presented by a young investigator. The objective is to highlight a subset of posters to be presented in a standard poster session to generate interest. Each short presentation will focus on the objectives of the study, the major results, and the conclusions. Details of the studies will be available during the regular poster session.

- **Chair - Welcome and Introductions**

- 12:45-12:48 PM

- ***ABSTRACT 054 - Alterations in Purinergic Signaling in Colorectal Cancer Impact Proliferation and Apoptosis***

- Ana Pettijohn ▪ Medical University of South Carolina

- 12:48-12:51 PM

- ***ABSTRACT 058 - Doxorubicin Imprints a Pro-fibrotic Program in CD8<sup>+</sup> T-cells that Promotes Mechanical Remodeling of Cardiac Extracellular Matrix***

- Ramona Emig ▪ University of Miami Miller School of Medicine

- 12:51-12:54 PM

- ***ABSTRACT 060 - Pentadecanoic Acid (C15:0) Reprograms Mice Cardiac Inflammation-Resolution Pathways Altering Hepato-Cardiac Lipid Remodeling Under Metabolic Stress***

- Vasundhara Kain ▪ University of South Florida

- 12:54-12:57 PM

- ***ABSTRACT 066 - Dermal Fibroblastic Responses to Pro-Fibrotic Stimuli in Human iPSC-Derived Skin Organoids***

- Anthony Sheets ▪ Brigham and Women's Hospital

- 12:57-1:00 PM

- ***ABSTRACT 070 - From Breath to Call: Neuromodulatory Role of IL-6 in Neonatal Brainstem Function. Ultrasonic Vocalization in Neonates***

- Isaac Rodriguez ▪ Florida International University Herbert Wertheim College of Medicine

- 1:00-1:03 PM  
**ABSTRACT 072 - Optimized Intraoperative Tissue Preservation Reveals circRNA Signatures Associated with WHO Grade 2 Meningiomas**  
Glauca Maria de Mendonça Fernandes, PhD ▪ Florida International University
- 1:03-1:06 PM  
**ABSTRACT 086 - Transforming Growth Factor Beta 1 Signaling in Neurons Induces Type C Hepatic Encephalopathy**  
Matthew McMillin ▪ Baylor College of Medicine
- 1:06-1:09 PM  
**ABSTRACT 094 - Bioinformatic Insight Into Gut-Brain Communication Modeling Type-2 Diabetes-Linked Alzheimer's Disease**  
Narendra Kumar ▪ Texas A&M University Health Science Center
- 1:09-1:12 PM  
**ABSTRACT 096 - Spatiotemporal Mapping of Motor Circuit-Level Changes in a Mouse Model of Amyotrophic Lateral Sclerosis**  
Christi Kolarcik ▪ University of Pittsburgh School of Medicine

### Session 028 - Endothelial Functions and Metabolism in Health and Disease

2:00-3:45 PM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Cardiac and Vascular Pathobiology Scientific Interest Group*

Chair: Michael Schnoor, PhD ▪ Nacional Polytechnic Institute

Co-Chair: Maria Zambrano ▪ Tufts University

**Session Overview:** This session will examine the multifaceted roles of endothelial cells in regulating vascular function and their contributions to disease processes. Talks will focus on how endothelial dysfunction plays a key role in pathologies like shock and metabolic disorders. The session will highlight how the dynamic connection between endothelial cells and inflammation influences immune responses, tissue injury, and metabolic regulation. Attendees will gain a broader understanding of endothelial metabolism and its emerging relevance as a therapeutic target in systemic disease.

- **Chair - Welcome and Introductions**
- 2:00-2:35 PM  
**Endothelial Mechanisms of Multiorgan Dysfunction During Shock**  
Alejandro Adam, PhD ▪ Albany Medical College
- 2:35-3:10 PM  
**Endothelial Cell Dysfunction During Inflammation and Metabolic Diseases**  
Jerry Breslin, PhD ▪ University of South Florida
- 3:10-3:25 PM  
**ABSTRACT 036 - Inflamed Intestinal Endothelial Cells Establish Self-Regulatory Niches with Vessel-Associated Macrophages to Promote Tissue Damage**  
Xingsheng Ren, PhD ▪ Northwestern University
- 3:25-3:40 PM  
**ABSTRACT 037 - Neutrophil Dysfunction in Sepsis: Linking Cell Behavior and Metabolism**  
Stephania Libreros, PhD ▪ Yale University

### Session 029 - Mapping the Future of Cancer Prevention: Pre-Cancers, Atlas Insights, and Interception Strategies

2:00-3:45 PM

**Session Room: Caloosa Salon E**

*Sponsored by the ASIP Breast Cancer Scientific Interest Group*

Chair: Dennis Jones, PhD ▪ Boston University

Co-Chair: Bethany Hannafon, PhD ▪ University of Oklahoma

Co-Chair: Maryknoll Linscott, MD, PhD ▪ Penn State College of Medicine

**Session Overview:** This session examines recent advancements in cancer interception by exploring the biology of precancerous lesions, insights from comprehensive atlas projects, and emerging strategies to halt cancer progression before it becomes invasive. The session highlights recent findings on the molecular and evolutionary landscape of pre-breast cancer lesions, particularly hormone receptor-positive subtypes, and discusses the role of pathogenic genetic variants in predisposing individuals to early-stage disease such as ductal carcinoma in situ. Building on these biological insights, the session also explores innovative immunological interventions, including vaccine-based strategies, aimed at mobilizing the immune system to intercept cancer development. Attendees will gain an understanding of novel approaches to cancer interception that target early-stage lesions.

- **Chair - Welcome and Introductions**
- 2:00-2:25 PM  
***Clinical Classification of BRCA2 Variants Using Saturation Genome Editing***  
Shyam Shyran, PhD ▪ National Cancer Institute
- 2:25-2:50 PM  
***Molecular Alterations Associated with Airway Injury and Lung Squamous Precancerous Lesions to Inform Early Lung Cancer Intervention***  
Sarah Mazzilli, PhD ▪ Chobanian & Avedisian School of Medicine
- 2:50-3:15 PM  
***Intercepting Breast Cancer Through Vaccination***  
Brian Czerniecki, MD, PhD ▪ Moffit Cancer Center
- 3:15-3:30 PM  
***ABSTRACT 038 - Neuropilin-2 Driven T-Cell Suppression – Implications for Tumor Development***  
Harsh Dongre, PhD ▪ Boston Children’s Hospital
- 3:30-3:45 PM  
***ABSTRACT 039 - Targeting Inflammation-Induced Tumor Dormancy Escape via Eicosanoids***  
Lily Ceraso ▪ Beth Israel Deaconess Medical Center, Harvard Medical School

### Session 030 - Poster Session II (EVEN Numbered Posters)

4:00-5:30 PM

**Session Room: Caloosa Salon C**

Beer and wine will be served

### Poster Categories

Cancer Pathobiology ▪ Cardiac and Vascular Pathobiology ▪ Cell Death and Tissue Repair ▪ Computational Pathobiology  
Gene Regulation in Disease ▪ Inflammation and Immunopathology ▪ Liver Pathobiology ▪ Mucosal Pathobiology  
Neuropathology ▪ Nutrition and Obesity ▪ Toxicologic Pathology ▪ AI-Based Image Analysis

### ASIP Business Meeting and Meritorious Awards Presentations

5:30-7:00 PM

**Session Room: Caloosa Salons D/E**

Chair: Pilar Alcaide, PhD ▪ University of Miami Miller School of Medicine

### Society-Wide President’s Reception and Networking Event

7:00-9:00 PM

**Location: Baystreet Yard** (2136 Bay Street, Fort Myers, FL 33901)

**Badge Pick-Up**

7:00 AM-5:00 PM

**Location: Galleria**

**Continental Breakfast**

7:00-8:00 AM

**Location: Galleria**

**Session 031 - Spatial Awareness – Defining Cell Function Based on Tissue Location**

8:00-10:00 AM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Mucosal Pathobiology Scientific Interest Group and the ASIP Inflammation and Immunopathology Scientific Interest Group*

Chair: Ronen Sumagin, PhD ▪ Northwestern University

Co-Chair: Evan Delgado, PhD ▪ Medical University of South Carolina

**Session Overview:** This session will explore how emerging spatial transcriptomics and imaging platforms are enhancing our understanding of cell function within the context of intact tissue architecture. Speakers will showcase how spatially resolved technologies are being leveraged to map gene expression, cellular interactions, and microenvironmental cues at high resolution across diverse tissues and cell types.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM  
***Translational and Clinical Integration of Spatial Biology for Irritable Bowel Disease***  
Parambir Dulai, MD ▪ Northwestern University
- 8:30-9:00 AM  
***Neuroimmune Interactions in Gastric Motility***  
Madhusudan Grover MBBS ▪ Mayo Clinic
- 9:00-9:30 AM  
***Spatial Microniches of IL-2 Direct T-Helper Cell Pathways that Drive Allergic Asthma***  
Amanda Poholek, PhD ▪ University of Pittsburgh School of Medicine
- 9:30-10:00 AM  
***Mapping the Lesion Landscape of White Matter Hyperintensities Using Deep Learning***  
Dana Julian Austin, PhD ▪ University of Pittsburgh School of Medicine

**Session 032 - Cardiovascular Disease and Aging: Novel Mechanisms and Therapeutic Potential**

8:00-9:45 AM

**Session Room: Caloosa Salon E**

*Sponsored by the ASIP Cardiac and Vascular Pathobiology Scientific Interest Group*

Chair: Yabing Chen, PhD ▪ Oregon Health and Science University

Co-Chair: Rajeev Malhotra, MD ▪ Massachusetts General Hospital, Harvard Medical School

**Session Description:** This session will bring together leading basic science researchers and physician-scientists, providing new insights and perspectives on the pathogenesis of cardiovascular disease and aging. Cardiovascular disease remains the leading cause of death, which was increased with aging. Management of cardiovascular disease and aging have continued to be a very hot area in the field of cardiovascular biology and disease. Trending topics will be discussed in this forum include genetic and environments regulation and therapeutic strategies, which provides an opportunity to exchange on cutting edge research in the field of cardiovascular pathology as well as clinical management.

- **Chair - Welcome and Introductions**

- 8:00-8:25 AM  
***Molecular and Genetic Regulators of Vascular and Valvular Calcification***  
Rajeev Malhotra, MD ▪ Massachusetts General Hospital, Harvard Medical School
- 8:25-8:50 AM  
***Pan-Vascular Pathology View on Cardiovascular Disease and Aging***  
Yabing Chen, PhD ▪ Oregon Health and Science University
- 8:50-9:15 AM  
***Prmt1-Mediated Platelet Hyperactivation and Thromboinflammation***  
Xinyang Zhao, PhD ▪ University of Kansas Medical Center
- 9:15-9:40 AM  
***Non-Alzheimer's Pathologic Contributions to Dementia in the Oldest Old People***  
Randy Woltjer, MD, PhD ▪ Oregon Health and Science University School of Medicine

10:00-10:25 AM

**Coffee Break**

**Location: Galleria**

**Session 033 - Immune Memory and Cellular Cross-Talk at the Mucosal Surfaces**

10:30 AM-12:15 PM

**Session Room: Caloosa Salon D**

*Sponsored by the ASIP Mucosal Pathobiology Scientific Interest Group*

Chair: Sean Colgan, PhD ▪ University of Colorado School of Medicine

Co-Chair: Jennifer Brazil, PhD ▪ University of Michigan School of Medicine

**Session Overview:** This session will focus on cell crosstalk at the mucosal surface. The speakers will focus on innate immunity and how cells communicate in response to changes in the local immune environment. Aspects of this ongoing research will include molecular mechanisms of innate immune cell interaction with the mucosal epithelial cells, imprinting of molecular cues onto mucosal tissue during active disease processes as well as innate immune memory, also known as "trained immunity," referring to the ability of the innate immune system to develop a heightened or altered response to a secondary challenge after an initial exposure, even in the absence of specific antigen recognition.

- **Chair - Welcome and Introductions**
- 10:30-10:55 AM  
***Training the Innate Immune System: TLR Pathways as Tools to Boost Infection Resistance***  
Julia Bohannon, PhD ▪ Vanderbilt University Medical Center
- 10:55-11:20 AM  
***Mechanisms of Neutrophil-Epithelial Interactions***  
Charles Parkos, MD, PhD ▪ University of Michigan School of Medicine
- 11:20-11:45 AM  
***Impact of Neutrophil-Derived Mediators on the Inflammatory Microenvironment***  
Ian Cartwright, PhD ▪ University of Colorado Anschutz Medical Campus
- 11:45 AM-12:00 PM  
***ABSTRACT 040 - Intestinal Epithelial Cell-Derived Gasdermin C Regulates IL-33 Subcellular Trafficking During Chronic Intestinal Inflammation***  
Kaylynn Vidmar ▪ Case Western Reserve University School of Medicine
- 12:00-12:15 PM  
***ABSTRACT 041 - TWEAK Represses Homeostatic Signaling and Disrupts Intestinal Fibroblast-Epithelial Communication***  
Bella Raphael ▪ University College Dublin

### Session 034 - Micro/Nanoplastics and Pathology: Seeing is Believing

10:30 AM-12:15 PM

**Session Room: Caloosa Salon E**

Chair: Elaine Bearer, MD, PhD ▪ University of New Mexico

**Session Overview:** Micro/nanoplastics have been recently found by chemical analysis in postmortem human brains. Where are they located, how did they get there, and what do they do to neural functioning? In this symposium we will explore most recent findings in human and studies in preclinical models to begin addressing these questions.

- **Chair - Welcome and Introductions**
- 10:30-10:55 AM  
***Micro/Nanoplastics: Visualization and Potential Roles in Pathogenesis***  
Elaine Bearer, MD, PhD ▪ University of New Mexico School of Medicine
- 10:55-11:20 AM  
***Mouse Models of Micro/Nanoplastic Uptake and Distribution***  
Jaime Ross, PhD ▪ University of Rhode Island
- 11:20-11:45 AM  
***Imagining Microplastics by Polarizing Wave Microscopy in Human Tissue and Effect on Liver Pathology***  
Rama Gullapalli, MD, PhD ▪ University of New Mexico School of Medicine
- 11:45 AM-12:10 PM  
***Micro/Nanoplastics and Parkinson's Disease***  
Andrew West, PhD ▪ Duke University School of Medicine

### Session 035 - Collaboration in Action: Building Connections That Drive Science Forward

12:45-1:45 PM

**Session Room: Caloosa Salon E**

Lunch and Career Development/Education Session

*Sponsored by the ASIP Career Development Committee*

Chair: Onur Kanisicak, PhD ▪ The Ohio State University

Co-Chair: Menglu Yang, MD, PhD ▪ Schepens Eye Research Institute, Harvard University

#### **Panelist:**

Patricia D'Amore, PhD • Schepens Eye Research Institute and Harvard Medical School

George Perry, PhD • The University of Texas at San Antonio

Maria Zambrano, BS • Tufts University

Onur Kanisicak, PhD • The Ohio State University

**Session Overview:** In research, collaboration shouldn't be a buzzword – it's a skill that requires intention, practice, planning, and a commitment to build partnerships grounded in fairness and mutual respect. This interactive session will highlight real examples of successful scientific collaborations and explore how researchers can move beyond idealistic goals to adopt practical strategies for building strong, inclusive, and lasting partnerships. Designed for scientists, clinicians, and trainees at all levels, this session will highlight common pitfalls and proven approaches for setting up collaborations that are fair, transparent, and mutually beneficial. Participants will gain insight into how to establish shared expectations, navigate authorship and credit, protect ideas, and ensure open communication from the start. One unique focus of this session will be *"the power of the social trainee"* – how graduate students and postdocs can act as catalysts in forming connections between mentors and external collaborators, thus expanding networks and sparking new opportunities.

Panelists and speakers will offer examples from diverse fields and stages of training, offering perspectives on:

- Best practices for initiating and maintaining equitable collaborations
- Navigating collaborations across institutions, disciplines, and career stages
- The evolving role of trainees in shaping collaborative ecosystems
- Recognizing and addressing power dynamics in team science

This is not just a talk about collaboration – this is a session to learn how to *do* collaboration better.

### Session 036 - Young Scientist Leadership Award Lecture

2:00-2:45 PM

Session Room: Caloosa Salon D

- 2:00-2:05 PM

#### **Introduction**

Satdarshan Paul Singh Monga, MD ▪ University of Pittsburgh

- 2:05-2:45 PM

#### **Gut Feelings: How Bile Duct Integrity Regulates the Gut-Liver Axis**

Vik Meadows, PhD ▪ University of Pittsburgh School of Medicine

**Lecture Overview:** Cholangiopathies are rare chronic liver diseases marked by cholangiocyte damage, bile acid dysregulation, and liver inflammation and scarring. The heterogeneity of these diseases and their complex causes, such as in primary sclerosing cholangitis (PSC), make treatment challenging. The biliary epithelium is essential for maintaining bile flow and composition, and the degree of biliary injury significantly impacts intestinal health and the gut–liver axis. Up to 70% of PSC patients also have inflammatory bowel disease. Understanding the mechanisms of the gut–liver axis in cholangiopathies is crucial for identifying biomarkers that can predict patient outcomes. Here, I share my work from the Monga lab, where we explore how loss of adherens junctions in cholangiocytes affects both liver and gut health. Using inducible dual knockout of  $\beta$ - and  $\gamma$ -catenin proteins in mice, we demonstrate that loss of biliary integrity causes cholestasis, disrupts bile acid circulation, and results in the buildup of bile acids in the liver. These changes are also seen in the intestine, with disrupted epithelial cell differentiation, increased inflammatory markers, and growth of harmful microbial taxa. Notably, the severity of gut inflammation and microbiome imbalance aligns with the extent of bile duct adherens junction loss, indicating a direct link between bile duct structure and intestinal health. We also provide evidence that hepatocytes can transdifferentiate into cholangiocyte-like cells during recovery, suggesting a potential repair process that may help restore gut–liver communication. Overall, this work emphasizes bile duct integrity as a key factor in regulating the gut–liver axis and suggests that biliary injury could contribute to intestinal comorbidities through altered bile acid signaling and subsequent microbial imbalance. These findings have important implications for understanding cholestatic liver diseases and could guide the development of fecal biomarkers and microbiome-based strategies for patient stratification and treatment.

### Session 037 - Breast Cancer Workshop: Circulating Biomarkers in Breast Cancer – Liquid Biopsy for Detection, Monitoring Therapy, and Recurrence

3:00-4:45 PM

Session Room: Caloosa Salon D

*Sponsored by the ASIP Breast Cancer Scientific Interest Group*

Chair: Bethany Hannafon, PhD ▪ University of Oklahoma

Co-Chair: Dennis Jones, PhD ▪ Boston University

Co-Chair: Maryknoll Linscott, MD, PhD ▪ Penn State College of Medicine

**Session Overview:** This workshop highlights recent advances in circulating biomarkers and their growing role in breast cancer management. Presentations will focus on the development and application of liquid biopsy approaches for early detection, real-time monitoring of treatment response, and identification of recurrence. The session will emphasize the clinical potential of non-invasive biomarker strategies to improve patient outcomes and support personalized medicine.

- **Chair - Welcome and Introductions**

- 3:00-3:10 PM

#### **Principles and Promise of ctDNA in Clinical Care**

Dennis Jones, PhD ▪ Boston University

- 3:00-3:40 PM

#### **Clinical Applications of Circulating Tumor DNA (ctDNA) Testing Across the Breast Cancer Spectrum**

Jamie McKenzie, MD ▪ Natera Inc.

- 3:40-4:10 PM  
***Circulating Tumor DNA to Monitor Disease Progression and Recurrence***  
Muhammed Murtaza, MBBS, PhD ▪ University of Wisconsin-Madison
- 4:10-4:40 PM  
***Developing a Liquid Biopsy Biomarkers for Diagnosis and Treatment of Malignant Effusions From Metastatic Breast Cancer***  
Mark Magbanua, PhD ▪ University of California at San Francisco

### Session 038 - Minisymposium: Liver Pathobiology

3:00-4:45 PM

Session Room: Caloosa Salon E

Co-Chair: Kari Nejak-Bowen, PhD ▪ University of Pittsburgh

Co-Chair: Michael Thompson, MD, PhD ▪ Washington University School of Medicine

- **Chair - Welcome and Introductions**
- 3:00-3:15 PM  
***ABSTRACT 042 - Histopathology AI and Machine Learning for Morphological Analysis of Ductular Reaction and Predicting Hepatic venous Pressure Gradient in Alcoholic Hepatitis***  
Ankita Srivastava, PhD ▪ Thomas Jefferson University
- 3:15-3:30 PM  
***ABSTRACT 043 - Widespread Bacterial Bile Acid Conjugation and Its Implications for Gut Health***  
Selene Shore, PhD ▪ Medical University of South Carolina
- 3:30-3:45 PM  
***ABSTRACT 044 - Early Bile Acid Supplementation in Mice Ameliorates Developmentally Programmed Liver Disease***  
Holly Hinrichs ▪ Washington University School of Medicine
- 3:45-4:00 PM  
***ABSTRACT 045 - Transcriptional Regulation of UBC9 Reprograms NF- $\kappa$ B Signaling in Alcohol-Associated Liver Disease***  
Swati Chandla, PhD ▪ Cedars-Sinai Medical Center
- 4:00-4:15 PM  
***ABSTRACT 046 - Integrated Cross-Species Analysis of Circular and Linear RNA Landscapes in Alcohol-Associated Liver Disease***  
Hongkun Lu ▪ Virginia Commonwealth University, Richmond Veterans Affairs Medical Center
- 4:15-4:30 PM  
***ABSTRACT 047 - Complete Loss of Biliary  $\beta$ - and  $\gamma$ -Catenin Induces Cholestatic Liver Damage and Intestinal Inflammation in Mice***  
Vik Meadows, PhD ▪ University of Pittsburgh School of Medicine
- 4:30-4:45 PM  
***ABSTRACT 048 - Loss of Hepatic Lipid Transporter Protein VPS13D Promotes Alcohol-Associated Steatohepatitis in Mice***  
Chen Zhang ▪ University of Kansas Medical Center

# SLAM 2026

## Summer Liver Academy Meeting

June 14–18  
Cape Coral, FL

### There is still time to submit an abstract for SLAM 2026! Last-chance abstract deadline is May 18

The **Summer Liver Academy Meeting (SLAM)** is back for 2026! Formerly the biennial FASEB Liver Biology Conference, SLAM was hosted for the first time in 2024 under the direction of the American Society for Investigative Pathology (ASIP). After a very successful 2024 meeting, we are excited to continue the mission of our prior liver meetings: cutting-edge science, an interactive format, and opportunities for trainees—all within an inviting resort environment.

Sessions will cover topics such as:

- Liver development
- Homeostasis, injury, and repair
- Plasticity and transdifferentiation
- Liver stem cell biology and organoids
- Non-parenchymal cell biology
- MASH and fibrosis
- Liver cancer
- Novel technologies
- Metabolism and microbiome

The meeting will be returning to Cape Coral—on the west coast of Florida—with convenient access from major airports in the US and Europe.

#### Upcoming Deadlines

- **May 18: Last-chance abstract, registration, and housing**

#### SLAM 2026 Organizing Committee

Kari Nejak-Bowen, MBA, PhD (University of Pittsburgh)

Georg Halder, PhD (KU Leuven)

Robert E. Schwartz, MD, PhD (Weill Cornell Medical College)



Learn more at [slam26.asip.org](https://slam26.asip.org)



# Pathobiology 2026 Abstracts

## Session 002 - Secretory Cells: Guardians of Epithelial Homeostasis and Mucosal Inflammation

### Abstract 001

#### Co-Operation Between Gasdermin (GSDM) Family Members, GSDMB and GSDMD Regulate Goblet Cell Function During Homeostasis and is Dysregulated During Inflammatory Bowel Disease (IBD)

Joseph J. Williams<sup>1</sup>, Kaylynn J. Vidmar<sup>1</sup>, Serena Artone<sup>1,3</sup>, Giuseppe Privitera<sup>1</sup>, E. Ricky Chan<sup>2</sup>, and Theresa T. Pizarro<sup>1</sup>

<sup>1</sup>Department of Pathology and <sup>2</sup>Institute for Computational Biology, Case Western Reserve University School of Medicine, Cleveland, OH, <sup>3</sup>Department of Physical and Chemical Science, University of L'Aquila, Italy

**Background:** Gasdermins (GSDMs) are a family of structurally-related proteins known for their role in pyroptosis, and whose dysregulation has been reported in inflammatory bowel disease (IBD). Our group reported increased GSDMB in colonocytes/crypt top colonocytes of ulcerative colitis (UC) patients; however, a significant increase in goblet cells (GCs) was also observed, but its function(s) in GCs are unknown. GCs are specialized intestinal epithelial cells (IECs) that produce and secrete mucins and antimicrobial peptides (AMPs) that are critical to maintain gut homeostasis. The depletion of GCs and the mucus barrier is a hallmark feature of IBD. Taken together, the aim of the present study is to determine the role of GSDMB in GCs and how its function may be dysregulated in the setting of chronic intestinal inflammation, such as that observed during IBD. **Methods:** scRNA-Seq data from colonic mucosae of healthy controls and inflamed/non-inflamed UC patients was used to investigate *GSDMB*-expressing cells and co-expression of other GSDMs. Based on these findings, further analysis of *GSDMB/D* positive GCs was done by confocal imaging, Western blot (WB), and Duolink<sup>®</sup> assay in UC-derived organoids and the GC line, LS174T. We queried preliminary bulk RNAseq data of LS174T and HT29+methotrexate (MTX), the latter taking on a GC-like phenotype, as well as healthy and diseased patient samples from publicly available datasets to determine the regulation of GC-associated molecules and expression of *GSDMB* transcripts encoding different protein isoforms. Furthermore, we examined specific GC-associated molecules in the above cell lines, including mucins and AMPs, by gene expression and WB. Finally, mucin secretion was analyzed *in vivo* using transgenic *GSDMB*-expressing mice. **Results:** scRNA-Seq showed increased *GSDMB/D* expression in GCs from inflamed vs. non-inflamed areas of colonic mucosa. Co-localization and upregulation of *GSDMB/D* was observed in UC-derived organoids. Also, GSDMB-dependent GSDMD regulation was seen in WT vs. *GSDMB*<sup>-/-</sup> IECs. Preliminary data indicate differential expression of isoform-specific *GSDMB* in HT29+MTX (mainly GSDMB-416 and -407) vs. LS174T (mainly GSDMB-403) cells. In fact, genes related to defense molecules, including *LYZ*, were differentially regulated in WT vs. *GSDMB*<sup>-/-</sup> LS174T cells, while genes such as *REG4*, were differentially regulated in WT vs. *GSDMB*<sup>-/-</sup> HT29 cells. *GSDMB*-411<sup>IEC-Tg</sup> vs. control mice produced and released copious amounts of mucus, with robust accumulation, which is recapitulated in *ex vivo* organoids. **Conclusions:** These data indicate GSDMB/GSDMD upregulation and co-operation in GCs and a potential functional relevance of GC-expressing GSDMB and its varying isoform(s), specifically regarding defense molecule synthesis/secretion. These implications could provide insights into the role of GSDMB in IBD pathophysiology. **Acknowledgements:** NIH: R01 DK125293 and P01 AI141350, Project 4 (TTP).

## Session 003 – Exosome-Mediated Tissue Repair

### Abstract 002

#### Inhibition of Inflammation in the Pulmonary Circulation by a Cell-Permeant Peptide Targeting the Lateral Border Recycling Compartment (LBRC)

Jonathan Sutkowski<sup>1</sup>, Maureen Haynes<sup>2</sup>, David P. Sullivan<sup>1</sup>, and William A. Muller<sup>1</sup>

<sup>1</sup>Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, <sup>2</sup>Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO

**Background:** Transendothelial migration (TEM) of leukocytes is a pivotal step in the inflammatory response. We have previously shown TEM to be dependent on PECAM and CD99 in four different models of inflammation: acid aspiration (modelling aspiration pneumonia), Gram-positive and Gram-negative pneumonia, and ischemia/reperfusion injury (IRI) (doi: 10.1152/ajplung.00069.2025). The lateral border recycling compartment (LBRC) is a system downstream of PECAM and CD99 signaling that shuttles endothelial membrane to the leukocyte-endothelial interface during TEM. We developed a cell-permeant peptide (Tat-KLC1c) that selectively blocks this recycling and have used it to inhibit TEM in a variety of inflammatory disease models including post-myocardial-infarction IRI. However, whether this peptide would work in the pulmonary circulation, where TEM occurs across capillaries rather than venules, was not known. We set out

to test its efficacy in a simple model of acid-induced injury prior to testing its effect on more complex models such as primary graft dysfunction. **Methods:** Mice received a retroorbital injection of 100  $\mu$ L of the anti-TEM peptide (Tat-KLC1c) at 20  $\mu$ M or the same dose of a scrambled version of the peptide (Scr), which has no effect on TEM. Mice were then anesthetized and 50  $\mu$ L of 0.1 N HCl was introduced above the vocal cords. At 24 hours post-injury, mice were sacrificed and bronchoalveolar lavages were collected and analyzed by flow cytometry to count total CD45+ cells, neutrophils, and monocyte-lineage cells. **Results:** In an experiment with FVB mice (n = 9), mice in the KLC1c group had a 52.6% reduction in CD45+ cells when compared with the Scr (control) group (Student's t-test; p = 0.031). A follow-up experiment in C57BL/6 mice (n = 24) showed a comparable effect, with a significant decrease in monocyte-lineage and total CD45+ cell counts (Wilcoxon rank-sum test; p = 0.027, 0.101, and 0.024 for total CD45+, neutrophil, and monocyte-derived cells respectively). **Conclusions:** Our initial experiments with the acid-injury model are promising regarding an effect of Tat-KLC1c blocking inflammation in the pulmonary vasculature. Lung primary graft dysfunction, a syndrome which leads to severe hypoxemia shortly after lung transplant in up to half of lung transplant recipients, is primarily due to IRI. In future experiments, we plan to observe the peptide's effect in a unique model of PGD developed at our institution involving allogenic murine lung transplantation. **Acknowledgements:** This work was funded by R35HL155652 to WAM.

### Abstract 003

#### Matrix-Bound Nanovesicles as Modulators in Alcohol-Related Liver Disease

Yekatarina Krutshenko<sup>1,2</sup>, Bashar Al Matour<sup>2</sup>, Jiang Li<sup>1</sup>, Jia-Jun Liu<sup>3,4,8</sup>, Lorien Walker<sup>1</sup>, Sam Taborski<sup>1</sup>, Michael Merchant<sup>5</sup>, Silvia Liu<sup>3,4,8</sup>, George Hussey<sup>6,7</sup>, Stephen Badylak<sup>2,6</sup>, Gavin Arteel<sup>1,8</sup>, and Melanie J Scott<sup>2,8</sup>

<sup>1</sup>Department of Medicine, <sup>2</sup>Department of Surgery, <sup>3</sup>Department of Pharmacology and Chemical Biology, <sup>4</sup>Organ Pathobiology and Therapeutics Institute, University of Pittsburgh, Pittsburgh, PA, <sup>5</sup>Department of Medicine, University of Louisville, Louisville, KY, <sup>6</sup>Department of Pathology, <sup>7</sup>McGowan Institute for Regenerative Medicine, <sup>8</sup>Pittsburgh Liver Research Center (PLRC), University of Pittsburgh, Pittsburgh, PA

**Background:** Alcohol-related liver disease (ALD) is driven by metabolic stress, inflammation, and progressive extracellular matrix (ECM) remodeling, yet effective targeted therapies remain limited. Matrix-bound nanovesicles (MBVs) are ECM-embedded vesicles enriched in bioactive proteins and microRNAs that regulate cell-matrix signaling and tissue repair in multiple injury settings. However, the contribution of MBVs to ALD pathogenesis has not been yet examined. This study investigates how binge and chronic ethanol exposure alters hepatic MBV cargo, and assesses whether exogenously provided healthy MBVs modulate alcohol-induced liver injury. **Methods:** Using the NIAAA chronic-plus-binge ethanol model, hepatic MBVs were isolated and analyzed by LC-MS/MS proteomics, followed by pathway enrichment analysis to define ethanol-induced MBV cargo remodeling. To evaluate therapeutic potential, MBVs derived from healthy porcine urinary bladder matrix were administered to ethanol-exposed mice. Liver injury and treatment responses were assessed by histopathology and bulk RNA sequencing. **Results:** Chronic ethanol exposure induced marked remodeling of hepatic MBV cargo, with enrichment of proteins associated with metabolic stress responses and detoxification pathways. Administration of healthy MBVs attenuated ethanol-induced steatosis, lipid peroxidation, and fibrogenic gene expression, while partially normalizing transcriptional programs related to oxidative stress, inflammation, and metabolic regulation. MBV effects were context-dependent, promoting modest ECM-associated signaling changes in healthy liver while dampening pathological responses in ethanol-injured tissue. **Conclusions:** These findings identify hepatic MBVs as dynamic components of the ECM microenvironment in ALD. MBVs appear to modulate hepatic homeostasis in a context-dependent manner rather than acting as direct immunosuppressive agents, supporting their potential utility as both biomarkers of ECM remodeling and therapeutic modulators in alcohol-related liver disease.

### Session 004 – Pathogen Strategies to Invade, Persist, and Damage the Gut

#### Abstract 004

#### Multi-Barrier Breakdown as a Foundational Mechanism for Cognitive Dysfunction in Post-Treatment Lyme Disease

Francisco Carrillo-Salinas, Yuri Kim, Sangmita Singh, Brandon Lee, Qingying Feng, Jade Kuan, Marina Dixon, Grace Loeser, Guido Pisani, Paige S. Hansen Colburn, and Michal Caspi Tal

Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

**Background:** Infection-Associated Chronic Illnesses (IACI), particularly Post-Treatment Lyme Disease (PTLD), represent a significant public health burden. Despite appropriate antibiotic therapy, approximately 10% of patients experience persistent debilitating symptoms, including severe cognitive dysfunction ("brain fog"). Clinical progress is currently

impeded by a lack of objective, mechanism-based biomarkers, forcing reliance on inadequate symptom-based diagnostic criteria. Our central hypothesis posits that systemic barrier breakdown is a foundational mechanism in IACI, driving the systemic inflammation and neurocognitive dysfunction observed in these patients. **Methods:** We employed a translational approach combining human clinical data with an animal model. First, we analyzed preliminary data from the MAESTRO study, assessing PTLD patients via cognitive tasks targeting processing speed and oculomotor control. Concurrently, we quantified plasma biomarkers indicative of neural injury, astrogliosis, and multi-barrier disruption. These findings were validated using a C3H mouse model of Lyme disease, where we utilized an Evan's blue dye assay to visualize gut barrier permeability and conducted behavioral assessments to monitor activity levels. **Results:** Human subjects with PTLD exhibited measurable cognitive deficits that correlated with elevated plasma concentrations of biomarkers for neural injury (NfL), astrogliosis (GFAP), and barrier disruption (occludin, sCD14). This correlation was corroborated by our animal model, where infected C3H mice demonstrated severe gut barrier compromise. Furthermore, infected mice displayed a distinct "frozen," immobile behavioral phenotype, providing a robust biological correlation for the profound malaise and functional decline reported by human patients. **Conclusion:** These results implicate barrier dysfunction as a key driver of pathology in IACI. Consequently, therapeutic strategies focused on restoring barrier integrity may possess broad clinical utility and warrant investigation for their potential to alleviate debilitating symptoms, particularly neurocognitive decline. **Acknowledgements:** Emily and Malcolm Fairbairn donor advised fund, Massachusetts life sciences, NIH NIAID R01 AI 178713-01.

### Abstract 005

#### IL6 Role in Brain-Spleen Axis in Neonatal Immune Activation in Response to Sepsis

Emily Silva, Isaac Vargas Rodriguez, Jose J. Otero, and Michele J. Alves

Department of Cellular and Molecular Medicine, FIU Herbert Wertheim College of Medicine, Florida International University, Miami, FL

**Background:** Neonatal sepsis accounts for approximately 1.3 million cases yearly leading to high mortality rates. During neonatal immune activation, the brain-spleen axis is a key network regulating neuroimmune response. Spleen function as a central hub for innate and adaptive immunity while brainstem nuclei are the primary afferent relay. Interleukin 6 (IL-6) act as an early marker for inflammation and sepsis, reshaping innate response and autonomic control of splenic immune function, yet IL6 role in neonatal immune activation still not fully understood. Our study aims to investigate the role of IL6 in the brainstem-spleen axis in neonatal immune activation. **Methods:** Postnatal Day 5 (PD5) *Il6<sup>tm1Kopf</sup>/J* (IL6<sup>KO</sup>) and C57BL/6J (IL6<sup>+/+</sup>) mice were *i.p.* administered with either saline or LPS. Following euthanasia brainstem and spleen tissue was collected for further analysis. Protein from spleen tissue was utilized for ELISA assays were performed using spleen. Total RNA was extracted from spleen and brainstem tissue, and RT-PCR was performed to evaluate gene expression levels. All analysis were performed using R Studio. **Results:** To investigate whether IL6-dependent inflammatory effects mediate central and peripheral responses, we promoted neonatal immune activation using LPS. The chemokines, including IL6, TNF $\alpha$ , IL1b, IL10, *Chrna7*, CD11b, Ly6a, were used to delineate the acute (3h) and early-adaptive phase (24h) LPS-response. Our results indicate that neonatal immune activation by LPS leads to an increase of IL6 protein levels at 3h, highlighting the importance of this cytokine in the neonatal acute phase response. Splenic IL1b protein levels were found increased acutely and in the early-adaptive phase both IL6<sup>+/+</sup> and IL6<sup>KO</sup>. Gene expression levels of IL6 were also enhanced at 3h in the spleen and brainstem. Ablation of IL6 attenuated TNF $\alpha$  and IL1b gene expression in both spleen and brainstem at 3h related with IL6<sup>+/+</sup>. Although, at the early-adaptive phase, IL6<sup>+/+</sup> and IL6<sup>KO</sup> displayed similar splenic TNF $\alpha$  and IL1b mRNA levels compared to control. The acetylcholine receptor (*Chrna7*) showed no significant differences. In addition, Ly6a and CD11b, as markers for immune cell and early-stress response, exhibited a robust response at 24h, which was amplified by IL-6 loss. IL10, an anti-inflammatory cytokine, was acutely attenuated in the IL6<sup>KO</sup> compared to IL6<sup>+/+</sup>. However, IL6<sup>KO</sup> displayed elevated IL10 expression during the early-adaptive phase. **Conclusions:** Loss of IL-6 attenuates neonatal immune responses towards a compensatory regulatory phenotype integrating brainstem-spleen signaling. During the early-adaptive phase, IL-6 ablation appears to favor IL10- and Ly6a-mediated regulation, suggesting a shift from pro-inflammatory resolution to protective immunosuppression. **Acknowledgements:** This work was supported by: NIH/NHLBI R01HL132355 for JJO and FIU Start-up for MJA.

**Abstract 006**

**Spatially Informed Histomolecular Subtyping of Hepatocellular Carcinoma: Clinical, Molecular, and Therapeutic Implications**

Tyler M. Yasaka<sup>1,2,3,4,5</sup>, Junyan Tao<sup>1,2,4</sup>, Chang Kyung Kim<sup>1,2,3,4,5</sup>, Po-Yuan Chen<sup>5</sup>, Satdarshan P. Monga<sup>1,2,4,5,6\*</sup>, and Yu-Chiao Chiu<sup>4,5,6</sup>

<sup>1</sup>Organ Pathobiology and Therapeutics Institute, <sup>2</sup>Department of Pharmacology and Chemical Biology, <sup>3</sup>Medical Scientist Training Program, <sup>4</sup>Pittsburgh Liver Research Center, <sup>5</sup>University of Pittsburgh Medical Center Hillman Cancer Center, <sup>6</sup>Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

**Background:** Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality with rising global incidence. Although molecular classification systems have been developed to capture HCC heterogeneity, their clinical utility remains limited. With the increasing use of tissue biopsies in targeted therapy trials, there is an opportunity to advance integrated molecular and histologic approaches for improved HCC stratification. **Methods:** Publicly available spatial transcriptomics data with paired hematoxylin and eosin (H&E) images from 10 HCC slides were used to train machine learning models to predict gene expression signatures within spatial transcriptomics spots from corresponding H&E tiles. Models were applied to independent H&E whole-slide images from The Cancer Genome Atlas (TCGA;  $n=340$ ). The outputs were integrated into 3 clusters, referred to as Spatially Informed Histo-Molecular Subtypes (SIHMS; A, B, and C). SIHMS were then assessed for survival differences and clinical correlates, followed by somatic mutation analysis and gene set enrichment analysis (GSEA) of associated bulk RNA sequencing data. Clinical findings from TCGA were validated on an independent in-house cohort (P1;  $n=48$ ). Finally, a murine model expressing gain-of-function *CTNNB1* and *NFE2L2*, representing the SIHMS A subtype, was treated with a small molecule inhibitor of PPAR-alpha to investigate a potential therapeutic vulnerability. **Results:** Spatial signature prediction models achieved holdout AUROCs of 0.92-0.94. In TCGA, subclasses predicted overall survival (A vs B,  $p<0.0001$ ; A vs C,  $p<0.0001$ ), disease-free interval (A vs B,  $p<0.001$ ; A vs C,  $p<0.0001$ ), and progression-free interval (A vs B,  $p<0.01$ ; A vs C,  $p<0.0001$ ). Each cluster associated with distinct clinical features (e.g. cluster A with early pathologic stage and HBV etiology, and cluster B with late stage), mutations, and enriched pathways (e.g. SIHMS A with fatty acid beta-oxidation). In the P1 cohort, overall survival trends were maintained (A vs B,  $p=0.121$ , A vs C,  $p=0.005$ ). Pharmacologic targeting of PPAR-alpha (a transcriptional regulator of hepatic beta-oxidation) in *CTNNB1-NFE2L2* mice resulted in decreased tumor burden as measured by liver weight ( $p=0.042$ ) and glutamine synthetase mRNA expression ( $p=0.024$ ). **Conclusions:** Using a spatially informed approach to predict transcriptomic signatures from H&E whole slide images, we developed a histology-based stratification with improved prognostic power compared to existing HCC subtypes. The associated clinical and molecular features suggest that these subtypes exhibit not only distinct phenotypes, but also potentially unique pathogenesis and targetable vulnerabilities. **Acknowledgements:** This work was supported by NIH grants 1R01CA251155 to SPM, R00CA248944 & R35GM154967 to YCC, T32EB001026 & 1F30CA298277 to TMY, University of Pittsburgh Liver Research Center (PLRC award to YCC, part of P30 DK120531 to SPM).

**Abstract 007**

**Spatial Transcriptomics Reveals Obesity-Associated Remodeling of Epithelial and Immune Compartments in Invasive Breast Cancer**

Cole Hladik<sup>1</sup>, Malika Sekhri<sup>3</sup>, Sugantha Priya Elayapillai<sup>2,4</sup>, Alexander Filatenkov<sup>3,4</sup>, Elizabeth A. Wellberg<sup>3,4,5</sup>, and Bethany N. Hannafon<sup>1,2,3,4</sup>

<sup>1</sup>Department of Cell Biology, <sup>2</sup>Department of Obstetrics and Gynecology, <sup>3</sup>Department of Pathology, <sup>4</sup>Stephenson Cancer Center, <sup>5</sup>Harold Hamm Diabetes Center, University of Oklahoma Health Campus, Oklahoma City, OK

**Background:** Breast cancer remains the most frequently diagnosed malignancy among women worldwide, with disease progression governed by both tumor-intrinsic programs and systemic host factors, including obesity. Obesity is associated with an increased risk of progression from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC); however, existing clinical and pathological metrics lack sufficient resolution to predict invasive transition. Although circulating factors, extracellular matrix remodeling, and microenvironmental reprogramming have been implicated in obesity-associated tumor progression, conventional molecular profiling approaches fail to preserve the spatial context necessary to resolve these interactions. **Methods:** We employed digital spatial transcriptomics (DSP) on human DCIS and IDC specimens stratified by body mass index (BMI; non-obese  $<30$  kg/m<sup>2</sup>, obese  $\geq 30$  kg/m<sup>2</sup>) to interrogate cytokeratin

(CK)+ epithelial and CD45+ immune compartments within intact tissue architecture. In silico deconvolution of CD45+ transcriptomes was performed to delineate obesity-associated immune alterations, and a gene-level Immune Remodeling Index (IRI) was developed to quantify lineage-specific immune functional states. Complementary immunohistochemistry (IHC) was performed in an independent cohort to assess macrophage polarization (CD86 for M1 and CD206 for M2). **Results:** DSP revealed that transcriptional programs distinguishing IDC from DCIS are strongly modulated by obesity status. In non-obese CK+ IDC epithelia, an upregulation of proliferative and metabolic transcripts and concurrent enrichment of MYC- and E2F-driven transcriptional programs, G2/M checkpoint signaling, and oxidative and fatty acid metabolism were characterized. In contrast, obese IDC specimens exhibited a stress-adaptive invasive phenotype marked by enrichment of TNF $\alpha$ /NF- $\kappa$ B signaling, reactive oxygen species response pathways, and cholesterol homeostasis. Immune remodeling analyses demonstrated preferential activation of M2 macrophages and depletion of B cell populations in obese IDC, as reflected by elevated IRI scores. These findings were corroborated by IHC-based analyses of macrophage polarization. **Conclusions:** These data demonstrate that obesity alters the molecular and immunologic programs associated with breast cancer invasion. Obesity is associated with a distinct stress-adaptive invasive phenotype in invasive CK+ epithelial cells, coupled to an immunosuppressive microenvironment characterized by M2 macrophage enrichment and B-cell loss. Collectively, these findings underscore the importance of incorporating metabolic health into precision risk stratification and biological modeling of breast cancer progression.

**Acknowledgements:** This project was supported by a Team Science Grant from the Stephenson Cancer Center/Harold Hamm Diabetes Center.

## Session 010 – Minisymposia – Pathobiology of the Barrier and Stromal Tissue Compartments in Inflammation

### Abstract 008

#### High-Fat Diet Elicits a Type 2 Immune Circuit Linking Mast Cells to Gastric Metaplasia

Charulekha Packirisamy<sup>1</sup>, Makenna Grozis<sup>1</sup>, Annika Matthiesen<sup>2</sup>, Pooja Pradeep<sup>2</sup>, Janet Boggs<sup>2</sup>, Sarah A. Dooley<sup>1</sup>, Rachel Edens<sup>1</sup>, Piper McKee<sup>1</sup>, Advije Ergul<sup>2</sup>, Catrina Robinson<sup>3</sup>, and Amy C. Engevik<sup>1</sup>

<sup>1</sup>Department of Regenerative Medicine and Cell Biology, <sup>2</sup>Department of Pathology and Laboratory Medicine,

<sup>3</sup>Department of Neurology, Medical University of South Carolina, Charleston, SC

**Background:** Western diets rich in fats and low in fiber are increasingly common and are linked to inflammation, metabolic disease, and cancer. Gastric cancer risk is ~2 fold higher in people consuming a Western diet compared to those eating a balanced diet. While the effects of a high-fat diet (HFD) are well studied in the intestine, they remain underappreciated in the stomach. Our preliminary data show that wild-type mice fed a long-term HFD develop gastric metaplasia marked by parietal cell loss and increased expression of metaplastic markers CD44v9 and AQP5. Long-term HFD also increased tuft cells and immune infiltration. We hypothesize that HFD damages the gastric epithelium and triggers release of the alarmin IL-33, which recruits ST2 (IL-33 receptor)-expressing immune cells that produce cytokines such as IL-13 to drive metaplasia and tuft cell hyperplasia. Over time, sustained epithelial injury and chronic inflammation may predispose the gastric mucosa to malignant transformation. **Methods:** C57BL/6J mice were fed either a control diet (10% kcal fat) or a HFD (45% kcal fat) for 12 or 26 weeks. Gastric tissue was analyzed for epithelial (chief cells, G-cells, tuft cells) remodeling, fibroblast and extracellular matrix changes, immune infiltration (ILC2s, macrophages, mast cell and subsets) and cytokine expression (IL-33, IL-13). To connect these findings to humans, we analyzed human gastric single cell RNA-seq datasets for the IL-33 receptor, ST2, expression. We also used a gastric organoid and immortalized stomach mesenchymal cell (ISMC) co-culture model to test whether palmitic acid (PA), a major component of HFD, directly impacts parietal cell survival and inflammatory signaling. **Results:** After 12 weeks of HFD, mice showed early accumulation of ILC2s and expansion of tuft cells before histologic metaplasia developed. By 26 weeks, mice displayed robust gastric metaplasia with extensive chief cell loss, altered fibroblast populations and matrix proteins, increased gastrin-expressing G-cells, and marked tuft cell hyperplasia. These cellular changes were accompanied by upregulation of IL-33 and recruitment of ST2-expressing immune cells, including mast cells, ILC2s, and macrophages- findings supported by human transcriptomic data. Mast cells emerged as the dominant IL-13 producing immune population, with MCPT1<sup>+</sup> intraepithelial mast cells being significantly increased after 26 weeks of HFD. *In vitro*, organoids co-cultured with ISMCs showed increased size and proliferation when treated with PA. **Conclusion:** Chronic HFD consumption triggers a type 2 immune cascade in the stomach. Early increases in ILC2s and tuft cells progress to tuft cell hyperplasia and IL-33 driven recruitment of immune cells. These immune populations produce IL-13, which further promotes and sustains metaplasia. Together, our data suggests that HFD alone is sufficient to drive gastric metaplasia. **Acknowledgements:** Amy Engevik's startup funds (MUSC).

## Abstract 009

### Protein O-GlcNAcylation Controls Abnormal Myofibroblast Differentiation in Localized Scleroderma

Yan Wang<sup>1</sup>, Ankita Prasad<sup>1</sup>, Gaurav Chauhan<sup>1</sup>, Jennifer Ko<sup>2,3</sup>, Mellisa Piliang<sup>2,3</sup>, Natasha Zachara<sup>4</sup>, Edward V. Maytin<sup>3,5</sup>, Vincent C. Hascall<sup>5</sup>, and Florian Rieder<sup>1,6</sup>

<sup>1</sup>Department of Inflammation and Immunity, <sup>2</sup>Department of Pathology, <sup>3</sup>Department of Dermatology, Dermatology and Plastic Surgery Institute, Cleveland Clinic, Cleveland, OH, <sup>4</sup>Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, <sup>5</sup>Department of Biomedical Engineering, <sup>6</sup>Department of Gastroenterology, Hepatology & Nutrition, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH

**Background:** Localized Scleroderma (LS) is a chronic autoimmune disease characterized by abnormal fibrosis in the skin and underlying tissues, resulting in pain, disfigurement and reduced mobility. The pathogenesis of LS is poorly understood and the efficacy of current therapeutics is unsatisfactory. Fibroblast hyperactivation is the primary cellular basis for LS pathology. Protein O-GlcNAcylation (O-GlcNAc) is a posttranslational modification that a sugar molecule,  $\beta$ -N-acetyl-glucosamine (GlcNAc), is covalently added to proteins by O-GlcNAc transferase (OGT), and removed by O-GlcNAcase (OGA). Abnormal O-GlcNAc is associated with many chronic diseases. However, whether it is involved in LS pathology remains unknown. **Methods:** Immunohistochemical staining of O-GlcNAc with human skin tissues from twelve LS patients and thirteen healthy controls (HC) was carried out to assess the association between O-GlcNAc and LS lesions. To investigate the role of O-GlcNAc in regulating fibroblast function, the gene expression of either OGT or OGA in primary mouse skin fibroblasts was inhibited by RNA interference (RNAi). Markers for myofibroblast differentiation were assessed at both protein and mRNA levels. Transcriptomic analyses were performed to identify the candidate genes and signaling pathways mediating the changes in the RNAi-treated fibroblasts. OGT or OGA inhibitors were given to mice to test the effects on bleomycin-induced skin fibrosis. **Results:** We found that the levels of O-GlcNAc were significantly higher in both epidermis and dermis of LS tissues than those of HCs; inhibition of O-GlcNAc by OGT RNAi: (a) significantly suppressed the gene expression of  $\alpha$ -smooth muscle actin and collagen-I, and impaired the stress fiber formation; (b) decreased the gene expression of SRF (serum response factor), the key transcription factor regulating contractile gene expression, and disrupts its binding with its cofactor, MRTF (Myocardin-related Transcription Factor); (c) the expression of genes with established profibrotic function such as IGFBP-5 (Insulin-like Growth Factor Binding Protein-5) was significantly downregulated as revealed by transcriptomic analysis. Further Gene Set Enrichment Analyses showed that profibrotic pathways including TGF- $\beta$  receptor and Wnt signaling were downregulated in OGT-RNAi fibroblasts. Forced expression of SRF but not IGFBP-5 in fibroblasts rescued the phenotype caused by OGT RNAi. OGA inhibition did not yield opposite effects to OGT inhibition. Lastly, pharmacological inhibition of OGT significantly alleviated the bleomycin-induced skin fibrosis in female WT mice. **Conclusion:** These findings suggest a crucial role of dysregulated O-GlcNAc in the pathology of skin fibrosis by promoting myofibroblast activation; in addition, reducing O-GlcNAc levels could be a novel promising therapeutic strategy in treating LS. **Acknowledgement:** Supported by NIAMS R21 and National Scleroderma Foundation New Investigator Award to YW.

## Abstract 010

### Impacts of Germline CYP2E1 Deletion on the Small Intestinal Epithelium

Alexandra Tomasevich<sup>1</sup>, Reagan Roberts<sup>1</sup>, Melinda A. Engevik<sup>2</sup>, Amy C. Engevik<sup>2</sup>, and Jessica Hartman<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, <sup>2</sup>Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

**Background:** Cytochrome P450 2E1 (CYP2E1) is a monooxygenase enzyme that metabolizes fatty acids, ketones, drugs such as ethanol and acetaminophen, and environmental pollutants including benzene and trichloroethylene. CYP2E1 activity is frequently linked to liver and intestinal pathology through the production of reactive oxygen species (ROS) and toxic metabolites such as acetaldehyde, N-acetyl-p-benzoquinone imine (NAPQI), and methylglyoxal. Although most studies focus on hepatic CYP2E1, the enzyme is also expressed throughout the small intestine. Induction of CYP2E1 by ethanol, fructose, or high-fat diet increases oxidative stress, disrupts tight junctions, and impairs intestinal barrier function via ROS and reactive metabolites. As a result, CYP2E1 is widely regarded as detrimental, and CYP2E1 inhibitors have been proposed to mitigate alcohol- and acetaminophen-induced injury. However, CYP2E1 is highly conserved in mammals with no known loss-of-function mutations, suggesting an underappreciated endogenous role. This study investigates how loss of CYP2E1 impacts the gut to better define its physiological function. **Methods:** Mice with germline deletion of CYP2E1 (129/Sv-Cyp2e1tm1Gonz/J, CYP2E1-KO) and wild-type controls (129S1/SvImJ, WT) were used. RNA-

seq was performed on ileum samples from 14-week-old mice (M-WT, F-WT, M-KO, F-KO; n=4/group). Small intestine length was measured at sacrifice, and histological analyses including H&E and Picrosirius Red (PSR) staining were used to assess tissue architecture and collagen deposition. **Results:** CYP2E1-KO mice of both sexes exhibited a modest but significant increase in small intestine length compared to WT. RNA-seq revealed substantially more differentially expressed genes in female CYP2E1-KO mice (957 DEGs, padj<0.05) than in males (137 DEGs) compared to WT within each sex. Gene Set Enrichment Analysis identified 53 significantly enriched GO biological processes in females, compared to seven pathways when sexes were combined and none in males alone. PSR staining demonstrated a two-fold increase in collagen deposition in the female CYP2E1-KO ileum (p<0.01), which was not observed in males. These findings were supported by RNA-seq enrichment of extracellular matrix pathways in females. **Conclusions:** Loss of CYP2E1 disproportionately affects females, driving transcriptional remodeling of extracellular matrix and nutrient absorption pathways that coincide with increased intestinal length and collagen deposition. Ongoing studies will define the mechanisms underlying these changes and determine how CYP2E1 status influences barrier function and inflammation. **Acknowledgements:** The authors thank Drs. Zengdun Shi and Don Rockey for assistance with Picrosirius Red staining and analysis. This work was supported by NIGMS (R35GM150843 to JHH) and NIDDK (Pilot and Feasibility Award and Advanced Imaging Core, MUSC Digestive Disease Research Core Center, NIH P30DK123704).

### Abstract 011

#### Endothelial JunB Deletion Improves Survival and Preserves Glomerular Filtration Rate (GFR) in Acute Sepsis

Daniel Gonsales Spindola, Samantha Clark, Andrew Seeman, Nina Martino, Alejandro Pablo Adam, Ramon Bossardi Ramos

Department of Molecular and Cellular Physiology, Albany Medical College, Albany, NY

**Background:** Sepsis-associated acute kidney injury (AKI) frequently fails to fully resolve and transitions into acute kidney disease, a vulnerable recovery window marked by incomplete restoration of renal function and heightened sensitivity to subsequent inflammatory stress. This state increases risk for hypertension and chronic kidney disease (CKD) progression. Renal endothelial cells (ECs) are central coordinators of microvascular integrity and immune instruction during acute sepsis and early recovery. JunB, an inducible AP-1 transcription factor activated by inflammatory cytokines and pattern-recognition signaling, is positioned to translate the septic inflammatory surge into sustained endothelial activation programs. We aimed to determine whether endothelial JunB is a causal determinant of acute sepsis outcomes, survival and early GFR decline, and whether it programs the acute EC transcriptomic response to CLP. **Methods:** Wild-type (WT) and inducible endothelial-specific JunB knockout mice (JunB<sup>IEKO</sup>) underwent cecal ligation and puncture (CLP) or sham surgery. Renal function was assessed during acute sepsis by measuring glomerular filtration rate (GFR). At day 3 post-CLP, renal ECs were isolated by CD31-based magnetic enrichment and profiled by low-input RNA-seq. Differential expression analyses compared CLP vs sham within genotype and identified JunB-dependent gene modules. **Results:** JunB deletion improved survival after CLP (77.3% JunB<sup>IEKO</sup> vs 55.5% WT) and preserved early renal function: by day 2 post-CLP, WT mice showed a marked drop in GFR, whereas JunB<sup>IEKO</sup> mice maintained GFR at near-sham (baseline) levels. At day 3, WT renal ECs showed a focused CLP response (202 upregulated and 149 downregulated genes; adj. P < 0.05, |logFC| > 1), consistent with acute endothelial activation. In contrast, endothelial JunB deletion produced a much broader CLP-driven transcriptional shift in JunB<sup>IEKO</sup> ECs (915 upregulated and 333 downregulated genes; adj. P < 0.05, |logFC| > 1), indicating extensive transcriptomic reprogramming in the absence of JunB. Within this rewired response, JunB loss was associated with reduced expression of key endothelial activation and immune-instruction pathways, including adhesion/trafficking cues (Vcam1, Jam3, Ccl21 family), interferon-stimulated genes (Ifit1/3, Isg15, Oas1a), and antigen-presentation/MHC-II genes (H2-Ab1, H2-Eb1, H2-Aa, H2-DMa), consistent with blunted IFN/MHC-II-linked immune instruction during sepsis priming. **Conclusions:** Endothelial JunB is required for an acute renal EC priming program after CLP that aligns with early AKD physiology and survival outcomes. These findings position JunB as a mechanistic lever linking sepsis-driven endothelial activation to persistent renal vulnerability and motivate ongoing chromatin studies to test whether JunB-dependent transcriptional modules predict memory-associated regulatory loci.

## Abstract 012

### Pulmonary Fibroblasts as Mediators of Immune Regulation and Tissue Remodeling During *Aspergillus Fumigatus* Challenge

José P. Guirao-Abad<sup>1</sup>, Daniel A. Kasprovic<sup>1</sup>, Dongseong Seo<sup>1</sup>, Mustafa Ozdemir<sup>2</sup>, Shannon M. Shearer<sup>2</sup>, Bo-Yao Wen<sup>1</sup>, Jonathan Bowden<sup>2</sup>, Michael Tranter<sup>1</sup>, David S. Askew<sup>2</sup>, and Onur Kanisicak<sup>1</sup>

<sup>1</sup>The Ohio State University Wexner Medical Center, Columbus, OH, <sup>2</sup>University of Cincinnati College of Medicine, Cincinnati, OH

**Background:** *Aspergillus fumigatus* is the principal etiologic agent of invasive aspergillosis, a rapidly progressive and frequently lethal pulmonary disease initiated by inhalation of airborne conidia. In immunocompetent hosts, inhaled conidia are efficiently eliminated by innate immune defenses. When clearance fails, fungal germination and the release of hydrolytic enzymes and secondary metabolites compromise lung tissue integrity, resulting in severe pulmonary damage. Although fibroblasts are central regulators of tissue homeostasis and repair, their functional contribution to host defense during *A. fumigatus* challenge remains poorly defined. **Methods:** Using expression of the matricellular protein periostin (Postn) as a readout of fibroblast activation, we combined inducible lineage tracing and targeted fibroblast ablation in Postn-Cre mouse models to drive either reporter expression or diphtheria toxin subunit A-mediated cell ablation. This strategy enabled *in vivo* tracking and functional interrogation of activated pulmonary fibroblasts following oropharyngeal inoculation with *A. fumigatus*. Additionally, single-cell RNA sequencing was used to resolve fibroblast heterogeneity and transcriptional dynamics during early fungal clearance and subsequent resolution. **Results:** We identified the emergence of Postn-expressing fibroblasts in immunocompetent hosts, localized to inflammation foci containing fungal elements across alveolar, peribronchial, and adventitial regions. Bulk RNA-seq analysis of Postn+ fibroblasts revealed enrichment of extracellular matrix remodeling and tissue repair pathways. To define the functional significance of this fibroblast response in a model of invasive aspergillosis, we induced ablation of Postn-expressing fibroblasts. Loss of Postn+ fibroblasts exacerbated disease severity, characterized by dysregulated immune cell recruitment, tissue injury, and severe pulmonary hemorrhage. Strikingly, unbiased single-cell RNA sequencing of the stromal compartment in immunocompetent hosts at day 1 post-challenge identified additional activated fibroblast subsets independent of Postn expression, exhibiting transcriptional programs enriched for immunomodulatory mediators, cytokine-responsive pathways, and enhanced matrix production. By day 7 post-challenge, activated fibroblasts transitioned toward a semi-quiescent state, coinciding with resolution of inflammation. **Conclusions:** Our findings identify pulmonary fibroblasts as key regulators of antifungal host defense. Their capacity to acquire immunomodulatory properties and limit both fungal- and immune-driven tissue injury challenges the view of fibroblasts as merely passive structural cells and positions them as active modulators of host-pathogen interactions. Modulating fibroblast activation states may represent a novel therapeutic strategy to improve outcomes in invasive aspergillosis, particularly in immunosuppressed populations.

## Abstract 013

### Loss of Arpin, an Endogenous Arp2/3 Complex Inhibitor, Causes Intestinal Epithelial Barrier Dysfunction and More Severe Colitis

Karina B. Hernández-Almaraz, Hilda Vargas-Robles, and Michael Schnoor

Department of Biomedicine, Center of Research and Advanced Studies of the Nacional Polytechnic Institute, Mexico City, Mexico

**Background:** Ulcerative colitis is an inflammatory bowel disease characterized by chronic mucosal inflammation that disrupts epithelial integrity, promotes dysbiosis, and drives leukocyte recruitment to the lamina propria. Epithelial barrier function critically depends on the actin cytoskeleton, which stabilizes tight and adherens junctions. The Arp2/3 complex, a key regulator of branched actin polymerization, contributes to junction stability. However, whether endogenous Arp2/3 inhibitors such as arpin regulate epithelial barrier function during colitis remains unknown. Therefore, this project investigated the role of arpin in epithelial barrier integrity during experimental colitis. **Methods:** We analyzed colons from wild-type (WT) C57BL/6 and arpin knockout (KO) mice generated by CRISPR/Cas9. **Results:** Arpin was expressed throughout the WT colon and absent in KO mice, without affecting levels of Arp2/3 or its major regulators. Arpin-KO mice displayed altered tight junction organization and increased basal epithelial permeability, but did not develop spontaneous colitis. However, during DSS-induced colitis, arpin-KO mice developed earlier and more severe disease signs. Histological analysis revealed crypt destruction, strong edema and leukocyte infiltration. During

colitis, in arpin KO mice, E-cadherin, and ZO-1 levels were reduced, whereas PICK1, another Arp2/3 inhibitor, and claudin-2 were increased, and epithelial permeability was further enhanced. Immunophenotyping showed increased CD4<sup>+</sup>-T and B lymphocytes in the lamina propria of arpin-KO mice, accompanied by reduced neutrophil infiltration into the mucosa and lumen. In arpin-depleted colon epithelial cells, we observed disrupted junction architecture, increased permeability, and enhanced actin stress fiber formation. Importantly, ROCK1/2 inhibition reduced both permeability and stress fibers, indicating that arpin deficiency promotes barrier dysfunction via excessive actomyosin contractility. Finally, we found that mesalamine increased arpin protein levels and rescued TNF $\alpha$ /IFN $\gamma$ -induced barrier dysfunction in colon epithelial cells. **Conclusions:** Together, these data identify arpin as a critical regulator of epithelial barrier integrity and suggest that mesalamine exerts part of its therapeutic effect by restoring arpin-dependent control of epithelial permeability in ulcerative colitis.

#### **Abstract 014**

##### **The Impact of Mitochondrial Haplotype on Inflammation and Fibrosis in Novel OKC-HET<sup>B/W</sup> Rats**

Ramasamy Selvarani<sup>1</sup>, Hoang Van Michelle Nguyen<sup>1</sup>, and Arlan Richardson<sup>1,2</sup>

<sup>1</sup>Department of Biochemistry and Physiology, University of Oklahoma, Oklahoma City, OK, <sup>2</sup>VA Oklahoma Health Care System, Oklahoma City, OK

**Background:** Non-resolving chronic inflammation is a key contributor to aging and plays a significant role in the development of many age-associated diseases, including chronic liver diseases such as metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, and liver cancer. However, limited information is available on how the host environment such as the mitochondrial haplotype (mt-haplotype) influences inflammation, fibrogenesis, and liver cancer. While obesity and Western diet-associated metabolic stress are central to liver pathology and cancer development, this study uniquely and directly tests the impact of mitochondrial haplotypes on key factors involved in diet-induced liver diseases. **Methods:** We developed a novel rat model (OKC-HET) by crossbreeding four inbred rat strains—Brown Norway (BN), Fischer 344 (F344), Lewis (LEW), and Wistar Kyoto (WKY)—in a heterogeneous nuclear background resulting in two mitochondrial haplotypes (OKC-HET<sup>B</sup> and OKC-HET<sup>W</sup>), which differ by 94 nucleotides. These rats were fed a Western diet (WD) for 6 months starting at 3 months. Liver samples were collected from chow-diet fed and WD fed male OKC-HET<sup>B</sup> and OKC-HET<sup>W</sup> rats. **Results:** We observed elevated inflammation (e.g., TNF $\alpha$ , IL-1 $\beta$ , IL-6), fat accumulation, and fibrosis (Col3 $\alpha$ , TGF $\beta$ , Col $\alpha$ 1 transcript levels and accumulation of collagen fibers as shown by trichrome, PSR staining) were observed in the WD fed male rats of both B- and W-haplotypes compared to their chow-fed counterparts. Importantly, these phenotypes were increased significantly in W-haplotype compared to the B-haplotype. **Conclusions:** As fibrosis is a recognized risk factor for cancer, our data suggest that mitochondrial haplotype modulates western diet-induced liver inflammation and fibrosis, potentially contributing to the development of liver cancer in response to western diet exposure. **Acknowledgements:** U.S. Department of Veterans Affairs, 16005238, 11K6BX005238, Arlan Richardson.

#### **Abstract 015**

##### **The Rho Guanine Nucleotide Exchange Factor 16 is a Novel Regulator of the Intestinal Epithelial Barrier and Repair**

Susana Lechuga, Amanda Daulagala, Armando Marino-Melendez, and Andrei I. Ivanov

Department of Inflammation and Immunity, Cleveland Clinic Research Institute, Cleveland Clinic Foundation, Cleveland, OH

**Background:** Establishment of the intestinal epithelial barrier is a critical feature of healthy gut, and it is regulated by the assembly of the apical junctional complex consisting of tight junctions (TJs) and adherens junctions (AJs). Both TJs and AJs are coupled to the underlying perijunctional actomyosin belt, which are tightly regulated by different members of the Rho family of small GTPases. Rho GTPases are known to be activated by Guanine Nucleotide Exchange Factors (GEFs), however the roles of these GEFs in regulating intestinal epithelial barrier integrity and repair remain poorly understood. Here we focused on the Rho Guanine Nucleotide Exchange Factor 16 (ArhGEF16), a multifunctional activator of RhoG, Rac1 and Cdc42 GTPases and investigated its roles in the regulation of barrier permeability and repair in human intestinal epithelial cells (IEC). **Methods:** ArhGEF16 functions were investigated by generating stable CRISPR/Cas9-mediated knock-out of this protein in human colonic DLD1 cells. Epithelial barrier properties were determined by measuring transepithelial electrical resistance (TEER) and transepithelial flux of FITC-dextran. Integrity of epithelial junctions and the perijunctional actomyosin cytoskeleton was determined by immunofluorescence and

confocal microscopy. Collective and individual cell migration were measured by using wound healing and Boyden Chamber migration assay, respectively. **Results:** ArhGEF16 was found to be enriched at apical junctions in IEC and in healthy human colonic mucosa. Such junctional localization was increased in inflamed colonic epithelium of Crohn's Disease but was markedly decreased in ulcerative colitis patients. CRISPR/Cas9 mediated knock-out of ArhGEF16 in DLD1 cells significantly enhanced TEER and decreased FITC-dextran flux thereby indicating strengthening of the paracellular barrier. In addition, loss of ArhGEF16 accelerated AJ and TJ reassembly during extracellular calcium switch. The underlying mechanism of barrier enhancement in ArhGEF16-depleted IECs involved activation of the perijunctional actomyosin belt manifested by the enhanced recruitment of phosphorylated myosin light chains and Rho-associated kinase. To mimic peristalsis, periodic apical pressure was applied to IEC monolayers. Apical pressure resulted in TJ disassembly; however, the loss of ArhGEF16 protected TJs from disruption caused by apical pressure. In addition to strengthening IEC barrier integrity, loss of ArhGEF16 significantly decreased both collective epithelial and individual cell migration, due to decreased cell-matrix adhesions and diminished assembly of focal adhesions. **Conclusions:** Our findings identified ArhGEF16 as a novel dual regulator of the intestinal epithelial barrier integrity and repair. ArhGEF16 could contribute to altered intestinal epithelial permeability and mucosal repair in IBD patients. **Acknowledgements:** Supported by Leona M. and Harry B. Helmsley Charitable Trust grant to A.I.I.

## Session 016 – Minisymposia – Immune Cells and Inflammatory Signaling in Disease States

### Abstract 016

#### The Role of Neutrophils and Myeloperoxidase in Cancer Cachexia

Louisa Tichy, Pavitra B. Desai, Mariam K. Diab, Tyler M. Parrish, DeQuentin O. Overton, and Brandon N. VanderVeen  
Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC

**Background:** Immune cell-driven pathways continue to emerge as key regulators of cancer cachexia, the unintentional muscle wasting that occurs with cancer. However, the immunology of cachexia beyond circulating cytokine changes remains largely unexplored. Previous research has shown that circulating and tissue infiltrating neutrophils are upregulated in cancer patients with cachexia. While neutrophils use myeloperoxidase (MPO) for their antimicrobial functions, the impact of MPO and neutrophils on cancer cachexia remains unknown. Therefore, the purpose of this study was to determine the role of neutrophil-induced MPO release in cancer-mediated skeletal muscle wasting in the preclinical colon-26 adenocarcinoma (C26) model and cachectic pancreatic cancer patients. **Methods:** CD2F1 male mice were implanted subcutaneously with  $10^6$  C26 (cachectic), CT26 (weight stable) tumor cells or heat-killed C26 cell injections. Body weight was recorded for 14 days post injection and cachexia indices were assessed at study endpoint (Day 14). Skeletal muscle immune cells were assessed using high-dimensional flow cytometry. RNAseq, immunofluorescent staining, ELISA and Western Blot were used to analyze MPO expressions in murine skeletal muscle, immune cells and serum. Additionally, human samples from weight stable and cachectic pancreatic cancer patients were analyzed for MPO serum levels via ELISA. **Results:** Cachectic mice experienced significant loss of body weight (-10%,  $p=0.017$ ) and skeletal muscle mass (-14%,  $p=0.0035$ ) compared to non-tumor and CT26 mice. Isolated immune cells of cachectic skeletal muscle showed significantly more neutrophils compared to non-tumor controls, accompanied by greater MPO RNA (+1798%) expression. In cachectic mice, skeletal muscle protein levels of MPO were increased by 1.5-fold compared to non-tumor controls. Additionally, serum MPO levels of cachectic mice were 4.5-fold ( $p=0.01$ ) higher compared to non-tumor controls and weight stable CT26 mice. Human serum samples of cachectic cancer patients also showed elevated MPO levels compared to weight stable patients (+16%,  $p=0.1$ ). **Conclusions:** These data highlight the role of neutrophils and neutrophil-released MPO in a preclinical cancer cachexia model and cachectic human cancer patients. Neutrophils, and specifically MPO, may represent a promising therapeutic target to overcome the detrimental effects of muscle wasting and weakness associated with cancer cachexia. **Acknowledgements:** R00CA276891.

### Abstract 017

#### MicroRNA-29a Reprograms CD8 T Cell Differentiation by Rewiring Key Memory- and Exhaustion-Driving Epigenetic Circuits

Xuebing Leng<sup>1,2\*</sup>, Lance A. Buchness<sup>1,2\*</sup>, Christine I. Rafie<sup>2,3</sup>, Panagiotis I. Vlantis<sup>4,2</sup>, Svetlana Ristin<sup>1,2</sup>, Miguel A. Gallardo<sup>1,2</sup>, Supipi L. Auwardt<sup>1,2</sup>, Natasha K. Khatwani<sup>2,3</sup>, Yuande Tan<sup>2</sup>, Yuguang Ban<sup>2,5</sup>, Corneliu M. Sologon<sup>2</sup>, Benjamin B. Currall<sup>2</sup>, Sion L. Williams<sup>2,6</sup>, Kevin Van der Jeught<sup>1,2</sup>, Jashodeep Datta<sup>2,7</sup>, Alejandro Villarino<sup>1,2</sup>, Aristeidis G. Telonis<sup>2,8#</sup>, and Erietta Stelekati<sup>1,2#</sup>

<sup>1</sup>Department of Microbiology and Immunology, <sup>2</sup>Sylvester Comprehensive Cancer Center, <sup>3</sup>Cancer Biology Program, <sup>4</sup>Department of Biochemistry and Molecular Biology, University of Miami, Miami, FL, <sup>5</sup>Department of Public Health Sciences, <sup>6</sup>Department of Neurology, <sup>7</sup>Department of Surgery, Miller School of Medicine, University of Miami, Miami, FL, <sup>8</sup>Human Genetics and Genomics Program, University of Miami, Miami, FL  
\*These authors contributed equally; #Co-corresponding authors.

**Background:** CD8 T cells mediate protective immune responses. However, persisting antigens such as chronic viruses or tumors redirect CD8 T cell differentiation to a sub-optimal state called exhaustion. Exhausted T cells (T<sub>EX</sub>) lose their ability to persist long-term and initiate functional memory responses. Checkpoint inhibitor blockade temporarily restores effector functions, but reinvigoration is not long-lasting. Therefore, finding effective means leading to durable T cell responses is essential to improve disease control. **Methods:** Here, we used the prototypical mouse model to induce CD8 T cell exhaustion by using a viral infection with a chronic strain of Lymphocytic Choriomeningitis Virus (LCMV). **Results:** Here, we provide the mechanistic evidence how microRNA-29a (miR-29a) attenuates exhaustion and promotes progenitor-like T<sub>EX</sub> differentiation. We demonstrate that miR-29a epigenetically re-directs T<sub>EX</sub> differentiation leading to highly persisting progenitor T<sub>EX</sub>. These reprogrammed miR-29a T cells are more sensitive to PD-L1 checkpoint blockade. T cell state analysis indicates that ectopic expression of miR-29a with aPD-L1 promotes T cell stemness and enhances effector responses. **Conclusions:** Together, our findings suggest that miR-29a can be leveraged to overcome current barriers to immune checkpoint blockade. **Acknowledgements:** Research reported herein was performed in part at the Onco-Genomics Shared Resource (RRID: SCR\_022502) and at the Flow Cytometry Shared Resource (RRID: SCRO22501) at Sylvester Comprehensive Cancer Center, which is supported by the National Cancer Institute (NCI; P30CA240139). This study was supported by NIH/NIAID 1R01AI183292-01(E.S.), NIH/NIAID R21AI178184 (E.S.), ACS-IRG (E.S.), B+ Foundation (ES). C.I.R. is supported by NIH 5F31CA294908-02.

## Abstract 018

### TWEAK-Induced Trained Immunity is Associated with Alterations in Activation of Molecular Signalling Pathways and Expression of IBD-Relevant Inflammatory Fibroblasts Markers

Cristina Bauset<sup>1</sup>, Emma Doyle<sup>1</sup>, Ciaran Kennedy<sup>1,2</sup>, Chinmayi Pednekar<sup>3</sup>, Cian Ohlendieck<sup>1</sup>, Sean T. Martin<sup>4</sup>, Glen Doherty<sup>4,5</sup>, Eric Conway<sup>1</sup>, Alex von Kriegsheim<sup>3</sup>, and Mario C. Manresa<sup>1</sup>

<sup>1</sup>School of Biomolecular and Biomedical Sciences, <sup>2</sup>Diabetes Complications Research Centre, School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland, <sup>3</sup>Cancer Research UK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK, <sup>4</sup>Centre for Colorectal Disease, St Vincent's University Hospital, Elm Park, Dublin, Ireland, <sup>5</sup>School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland

**Background:** Inflammatory fibroblasts identified in severe and therapy-resistant Inflammatory Bowel Disease (IBD) patients acquire immunomodulatory properties by mechanisms that remain poorly understood. We previously observed that TWEAK-mediated training enhances intestinal fibroblasts responses to secondary stimulation, which is associated with epigenetic reprogramming and consistent with the acquisition of trained immunity. Here we explore the alterations in molecular signaling that drive TWEAK-mediated training and its effects on IBD-associated fibroblast transcriptomes. **Methods:** Human primary colonic fibroblasts isolated from healthy or IBD surgical resections were unstimulated or stimulated with TWEAK and/or treated with IL-1 $\alpha$  either alone or sequentially, allowing a 2-day stimuli-free period in between. Bulk RNA-sequencing, Cleavage Under Targets & Tagmentation (CUT&Tag), mass spectrometry and Western-Blot were used for analysis. **Results:** Transcriptome alignment studies between fibroblasts stimulated with TWEAK, rested and re-stimulated with IL-1 $\alpha$  or IL-1 $\alpha$  only, and single-cell RNAseq of IBD stroma identified a cohort of genes that: 1) Are enhanced by trained immunity; B) Are overexpressed in inflammatory IBD stromal populations; and C) Accumulate histone modifications at promoters. These genes included *CHI3L1*, *MME*, *CTSK*, *CD82* or *PRRX1* among others. Proteomics and WB analysis unveiled differences in protein expression and rewired activation of molecular signaling pathways, such as ERK, JNK and STAT1, after TWEAK-training and IL-1 $\alpha$  re-stimulation. Ongoing experiments suggest that IBD fibroblasts display similar alterations in MAPK and STAT1 activity upon IL-1 $\alpha$  stimulation, whereas in situ analysis of IBD biopsies confirms increased expression of markers sensitive to training like *CHI3L1*. **Conclusions:** Our studies identify the expression of markers sensitive to trained immunity in the IBD stroma. At the molecular level, we find a rewiring of molecular signalling cascades as a potential novel mechanism associated with the acquisition and recall of this immune training in fibroblasts. **Acknowledgements:** Project funded by Research Ireland (GOIPD/2025/1089) /Science Foundation Ireland / Irish Research Council Pathway Award (21/PATH-S/9621) and UCD SBBS-SPARK.

## Abstract 019

### TNFSF14/LIGHT Responses in Intestinal and Oesophageal Fibroblasts are Differentially Modulated by Hydroxylase-Inhibitors

Cian M. Ohlendieck<sup>1,2</sup>, Carlos Matellan<sup>1,3</sup>, Cristina Bauset<sup>1,2</sup>, Mary Nwaezeigwe<sup>4</sup>, Emer Flynn<sup>1,2</sup>, Kevin Okamoto<sup>5</sup>, Desmond Winter<sup>3,4</sup>, Colm Collins<sup>1,2</sup>, Katie Doogan<sup>2</sup>, Seán T. Martin<sup>4</sup>, Glen Doherty<sup>3,4</sup>, Seema S. Aceves<sup>5,6</sup> and Mario C. Manresa<sup>1,2\*</sup>

<sup>1</sup>Conway Institute of Biomolecular and Biomedical Research, <sup>2</sup>School of Biomolecular and Biomedical Sciences, <sup>3</sup>School of Medicine, University College Dublin, Belfield, Dublin, Ireland, <sup>4</sup>Centre for Colorectal Disease, St Vincent's University Hospital and School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland, <sup>5</sup>Department of Pediatrics, School of Medicine, University of California San Diego, La Jolla, CA, <sup>6</sup>Rady Children's Hospital, San Diego, CA

**Background:** Fibroblasts have emerged as inflammatory entities in ulcerative colitis (UC) and eosinophilic oesophagitis (EoE), positioning these cells as attractive therapeutic targets. Previous studies have shown that hydroxylase-inhibitors elicit anti-inflammatory responses, but their effects on fibroblasts during inflammation remain unknown. Here we test the effects of hydroxylase-inhibitors on LIGHT-driven inflammation in intestinal and oesophageal fibroblasts. **Methods:** Human endoscopic biopsies were obtained from paired inflamed/non-inflamed areas of active UC patients. Primary human colonic and oesophageal fibroblasts from healthy donors were pre-treated with hydroxylase-inhibitors and/or treated with LIGHT. Cellular responses and molecular signaling activation were analyzed using flow-cytometry, qRT-PCR, ELISA, immunoblotting and RNA-sequencing (RNAseq). **Results:** LIGHT induced inflammatory gene/protein expression in intestinal fibroblasts predominantly via LT $\beta$ R, which was more highly expressed than HVEM in UC biopsies and colonic fibroblasts. A comparative analysis of the response to LIGHT between colonic and oesophageal fibroblasts revealed highly unique gene expression profiles. Pre-exposure to hydroxylase-inhibitors had a selective inhibitory effect on the expression of several LIGHT-mediated inflammatory factors in colonic fibroblasts and oesophageal fibroblasts. Mechanistic studies revealed differential molecular targets as LIGHT-driven non-canonical NF- $\kappa$ B activity was targeted by hydroxylase-inhibitors in oesophageal fibroblasts. Surprisingly, NF- $\kappa$ B signaling was unaffected in colonic fibroblasts, where DMOG abrogated LIGHT induced p38 phosphorylation. **Conclusion:** We established a previously unrecognized LIGHT-driven inflammatory response in intestinal fibroblasts via LT $\beta$ R and characterized differential effects and signaling-pathways for LIGHT in intestinal and oesophageal fibroblasts. Finally, we identified potential therapeutic effects of hydroxylase-inhibitors via targeting of distinct pathways in these cells. **Acknowledgements:** This work was supported by an SFI/IRC Pathway award to MCM (21/PATH-S/9621). CB and CM were supported by IRC GOI fellowships (GOIPD/2025/1089; GOIPD/2023/1118).

## Abstract 020

### A Novel Role for PECAM in the Regulation of Leukocyte Transendothelial Migration

Edward J. Dominguez<sup>1,2</sup>, Annette M. Gonzalez<sup>1</sup>, and William A. Muller<sup>1</sup>

<sup>1</sup>Department of Pathology, Northwestern University, Feinberg School of Medicine, Chicago IL, and <sup>2</sup>University of Illinois Urbana-Champaign, Urbana-Champaign, IL

**Background:** Mechanical traction between platelet/endothelial cell adhesion molecule (PECAM, PECAM-1, CD31) on a transmigrating leukocyte and PECAM at the endothelial cell border initiates calcium signaling that is critical for leukocyte diapedesis at areas of inflammation. The PECAM-initiated signaling pathway mediates transendothelial migration (TEM) by promoting the movement of a storage compartment of lateral border membrane, the lateral border recycling compartment (LBRC) to the site of TEM. Previously, we demonstrated that kinesin light chain 1, variant 1 (KLC1c) regulates TEM by controlling the targeted delivery of the (LBRC) along microtubules to the site of TEM. Unpublished studies in mice and pigs *in vivo* demonstrate that a Tat fusion peptide containing the C-terminal 10 amino acids of KLC1c blocks TEM and inflammatory disease by 50-80%. However, the molecular mechanisms mediating these effects remain unclear, particularly the identity of KLC1c's binding partners. The goal of this study was to identify and characterize KLC1c-interacting proteins in endothelial cells and to elucidate the molecular pathways through which KLC1c moves the LBRC and facilitates TEM. **Methods:** To identify potential binding partners, we performed an unbiased proteomics screen using FLAGAPEX2-tagged KLC1c expressed in endothelial cells to label vicinal proteins. Biotinylated proteins were affinity-purified and analyzed via mass spectrometry. In parallel, we conducted mass spectrometry on proteins co-immunoprecipitated with KLC1c. We utilized high-performance computing and AlphaFold-Multimer to model potential protein interactions between KLC1c and candidate binding partners. **Results:** Across both datasets, we identified a

convergent set of top candidate interactors, many of which are associated with cytoskeletal dynamics, membrane trafficking, and scaffolding – hallmarks of kinesin light chain function. AlphaFold Multimer modeling revealed PECAM as a direct binding partner for KLC1c. Notably, the PECAM ITIM1 motif docked onto the 6TPR domain on KLC1c, a region typically associated with cargo and adaptor binding. Co-immunoprecipitation experiments in endothelial cells expressing Flag-tagged KLC1c confirmed this interaction and as PECAM was pulled down using anti-FLAG magnetic beads.

**Conclusions:** We have identified the cytoplasmic tail of PECAM as a candidate binding partner for KLC1c. Concurrently, we plan to validate this interaction using recombinant proteins via fluorescence polarization to quantify binding kinetics and affinities. These studies will provide mechanistic insight into KLC1c's role in TEM and identify potential targets for anti-inflammatory therapy. **Acknowledgements:** We thank ASIP for the SROP fellowship to EJD; Allan J.R. Ferrari (Rocklin lab) for his guidance with AlphaFold, as well as Peter A. Faul at the NU Proteomics Facility for his input. Funded by NIH R35HL15565 to WAM.

## Abstract 021

### Proton-Activated Chloride Channel 1 (PACC1) is Essential for Macrophage Defense in Bacterial Sepsis

Lucien P Garo<sup>1</sup>, Kevin Brueck<sup>1,2</sup>, Sarah Walachowski<sup>1,2</sup>, Archana Jayaraman<sup>1</sup>, Marcel Strueve<sup>2</sup>, Shuang Xu<sup>1</sup>, Hulbert Yang<sup>1</sup>, Matthew Helmkamp<sup>1</sup>, Seung Hoan Choi<sup>3</sup>, Christoph Reinhardt<sup>2,4,5</sup>, and Markus Bosmann<sup>1,2</sup>

<sup>1</sup>Pulmonary Center, Department of Medicine, Boston University Chobanian & Avedisian School of Medicine, Boston, MA,

<sup>2</sup>Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany,

<sup>3</sup>Department of Biostatistics, Boston University School of Public Health, Boston, MA, <sup>4</sup>German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Mainz, Germany, <sup>5</sup>Research Center for Immunotherapy (FZI),

University Medical Center of the Johannes Gutenberg-University Mainz, Germany

**Background:** Bacterial sepsis is a serious clinical problem responsible for millions of deaths annually. Tissue-resident macrophages play a critical role during infection through the phagocytosis of invading bacteria for elimination within the acidifying phagolysosome. We hypothesized that a recently discovered acid-sensitive chloride channel, PACC1, is important for the macrophage phagolysosome and innate immunity during sepsis. **Methods:** Human *PACC1* expression was analyzed from public RNA sequencing data, and *PACC1* variants from genome-wide association studies. In wildtype (WT) mice, *Pacc1* was measured in immune cells by RNA and single-nucleus sequencing, RNA PrimeFlow, and qRT-PCR. *Pacc1* knockout ( $^{-/-}$ ) and myeloid cell-targeted *Pacc1*-floxed ( $^{fl/fl}$ ) mice (*Pacc1* $^{fl/fl}$  x LysM-Cre) were engineered to interrogate PACC1. To assess phagocytosis, bacterial killing, and inflammatory responses, *Pacc1* $^{-/-}$  bone marrow-derived macrophages (BMDMs) were incubated with acid-insensitive and acid-sensitive inactivated *E. coli* BioParticles, live *E. coli*, and lipopolysaccharide (LPS). Phagocytosis *in vivo* was studied by intraperitoneal (i.p.) injection of *BioParticles*. *Pacc1* $^{-/-}$  mice were inoculated i.p. with *E. coli* to study bacterial sepsis, intranasally with pneumococcus to study bacterial pneumonia, and i.p. with LPS to study endotoxemia. Immunologic outcomes were determined by flow cytometry, ELISA, Luminex, and RNA sequencing; and clinical outcomes by survival and bacterial burdens. **Results:** Human *PACC1* was enriched in macrophages and other mononuclear phagocytes while human *PACC1* loss-of-function variants were associated with risk of sepsis. Murine *Pacc1* expression was also enriched in macrophages and was responsive to inflammatory stimuli. *Pacc1* $^{-/-}$  macrophages showed indistinguishable phagocytic uptake of acid-insensitive *E. coli* BioParticles vs. WT macrophages, while acid-sensitive BioParticles revealed impaired development of the acidifying phagolysosome. Transcriptomics of *E. coli*-challenged *Pacc1* $^{-/-}$  macrophages exhibited dysregulated innate immune and phagolysosomal responses. *Pacc1* $^{-/-}$  mice with sepsis and pneumonia displayed increased mortality, bacterial burdens, local myeloid cell infiltration, and inflammatory markers. In contrast, *Pacc1* $^{-/-}$  survival was indistinguishable from WT during endotoxemia, a model which does not depend on phagocytosis. *LysM-Cre/Pacc1* $^{fl/fl}$  mice recapitulated the high sepsis mortality of global *Pacc1* $^{-/-}$  mice, supporting a predominant role for PACC1 in phagocytic macrophages and other myeloid cells *in vivo*. **Conclusions:** *Pacc1* is highly expressed in macrophages, where it controls the acidifying phagolysosome, inflammation, and bacterial clearance. *Pacc1* deletion negatively impacts infection outcomes. In summary, PACC1 in macrophages is essential for host defense during sepsis. **Acknowledgements:** NIH 1R01HL141513, 1R01HL139641, 1R01HL166588 to MB; F31HL176388-01A1 to LG; 5T32HL007035-50 supported LG.

## Abstract 022

### The Protein Tyrosine Phosphatase CD45 Regulates PMN Transepithelial, Antimicrobial Function and Colonic Mucosal Repair

Jael Miranda, Dylan J. Fink, Zachary S. Wilson, Asma Nusrat, Charles A. Parkos, and Jennifer C. Brazil  
Department of Pathology, University of Michigan, Ann Arbor, MI

**Background:** Polymorphonuclear neutrophils (PMNs) serve as frontline defenders against injury and infection, eliminating pathogens and initiating key mucosal tissue repair processes. However, excessive PMN transepithelial migration (TEpM) contributes to chronic mucosal inflammatory disorders, including inflammatory bowel disease. PMN pro-inflammatory and pro-repair functions are regulated by incompletely understood interactions between surface receptors (including  $\beta 2$  integrins) and intracellular signaling cascades orchestrated by kinases and phosphatases. The protein tyrosine phosphatase receptor type C (PTPRC), better known as CD45, is expressed by all nucleated hematopoietic cells but has almost exclusively been studied in the context of T and B cell adaptive immune function. Despite its abundant expression on neutrophils, very little is known about how CD45 regulates neutrophil function during mucosal inflammation and repair. **Methods and Results:** Here, we determined how CD45 regulates PMN trafficking and effector functions in the gut. Using a surgical colonic loop model, it was determined that pharmacologic inhibition of CD45 significantly reduced PMN colonic TEpM *in vivo*. Furthermore, decreased intestinal PMN trafficking was observed in transgenic mice with PMN-specific deletion of CD45 (*MRP8-Cre;Cd45<sup>fl/fl</sup>*). Pharmacologic inhibition of CD45 also reduced TEpM of human PMN across inverted monolayers of primary colonoid derived intestinal epithelial cells. Beyond limiting TEpM, CD45 depletion (in *MRP8-Cre;Cd45<sup>fl/fl</sup>* mice or in PMN like lentiCRISPR CD45KO HL60 cells) impaired key antimicrobial functions, including degranulation and phagocytosis, indicating broader effects on PMN effector activity. Importantly, recovery from dextran sodium sulfate (DSS)-induced colitis and biopsy-induced colonic wounding was delayed in *MRP8-Cre;Cd45<sup>fl/fl</sup>* mice, linking altered PMN function to defective mucosal healing. Mechanistically, CD45 depletion or inhibition reduced surface expression and activation of the  $\beta 2$  integrin CD11b/CD18 in PMN. In support of CD45 mediated regulation of CD11b/CD18 in PMN, increased CD11b/CD18 surface expression was observed in PMN isolated from CD45<sup>E613R</sup> mice that have a mutation in the CD45 inhibitory wedge resulting in constitutive CD45 activation. Finally robust inactivation of the Src family kinase member Lyn at Tyr507 was observed in PMN from *MRP8-Cre;Cd45<sup>fl/fl</sup>* mice and in CD45KO HL60 cells identifying CD45 as a key regulator of Lyn kinase activity in PMN. **Conclusions:** Together, data highlight a novel CD45-CD11b-Lyn signaling axis that regulates PMN trafficking and effector functions in the intestine and identify CD45 as a promising target for modulating PMN function to promote mucosal tissue repair in IBD and other mucosal disorders.

## Abstract 023

### Reprogramming CD8 T Cell Metabolic Fitness Using MicroRNA-29a to Enhance CAR T Cell Immunotherapy

Natasha K. Khatwani<sup>1</sup>, Xuebing Leng<sup>1</sup>, Venu V.G. Saralamma<sup>1</sup>, Aristeidis G. Telonis<sup>1</sup>, Durga P. Gannamed<sup>2</sup>, Lance Buchness<sup>1</sup>, Christine I. Rafie<sup>1</sup>, Supipi Auwardt<sup>1</sup>, Austin Newsam<sup>1</sup>, Caitlin Hopkins<sup>3</sup>, Oriana T. Pumar<sup>1</sup>, Dionysios C. Watson<sup>1</sup>, Pinar Atilla<sup>1</sup>, Despina Kolonias<sup>2</sup>, Joseph A. Fraietta<sup>3</sup>, Damien J. Green<sup>1</sup>, Jay Y. Spiegel<sup>2</sup>, David B. Lombard<sup>1</sup>, Jonathan H. Schatz<sup>1</sup>, and Erietta Stelekati<sup>1</sup>

<sup>1</sup>University of Miami Miller School of Medicine, Miami, FL, <sup>2</sup>Sylvester Comprehensive Cancer Center, Miami, FL,

<sup>3</sup>University of Pennsylvania Pearlman School of Medicine, Philadelphia, PA

**Background:** CAR T immunotherapy has transformed cancer treatment; however, limited persistence and progressive T cell exhaustion remain major barriers to durable clinical responses. In a mouse model of chronic lymphocytic choriomeningitis virus (LCMV) infection, we previously demonstrated that microRNA-29a overexpression (miR-29aOE) attenuates T cell exhaustion and promotes long term persistence of stem-like, antigen-specific CD8 T cells while preserving effector function. We hypothesized that miR-29a enhances T cell persistence through metabolic regulation of T cell differentiation and further posited that incorporation of miR-29a into CAR T cell expansion protocol could precondition CAR T cells for enhanced metabolic fitness, persistence and anti-tumor efficacy. **Methods:** Transcriptomic profiling was performed by RNA sequencing to define gene expression and metabolic programs in miR-29aOE versus control CD8 T cells in chronic disease. Mitochondrial content and morphology were assessed by confocal microscopy, electron microscopy and flow cytometry. Cellular metabolic fitness was evaluated by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using a Seahorse Flux Analyzer. miR-29a was incorporated into second-generation CAR T cell constructs, which were evaluated in murine lymphoma models for metabolic capacity, in

vivo persistence, tumor burden, and overall survival following adoptive transfer. **Results:** miR-29aOE CD8 T cells exhibited enrichment of transcriptional programs associated with favorable CAR T cell clinical responses and enhanced metabolic pathways, including fatty acid oxidation and oxidative phosphorylation. In contrast, miR-29a-deficient T cells demonstrated reduced mitochondrial content and diminished spare respiratory capacity, indicating impaired metabolic fitness. Furthermore, CAR T cells engineered to overexpress miR-29a displayed superior metabolic capacity prior to infusion, enhanced post-infusion persistence, reduced tumor burden and increased accumulation of tumor-infiltrating CAR T cells in vivo. **Conclusions:** These findings identify miR-29a as a critical regulator of T cell metabolic fitness and persistence. Incorporation of miR-29a into CAR T cell engineering strategies enhances CAR T durability and anti-tumor efficacy, establishing miR-29a as a promising molecular modality to improve the therapeutic performance of CAR T cell immunotherapy.

#### Abstract 024

##### **'Lobular' Infers Liver Zonation and Reveals Perturbed Zonation in Progressive Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)**

Tyler M. Yasaka<sup>1,2,3,4,5</sup>, Hiroki Inada<sup>1,2,4</sup>, Nicholas Khoo<sup>1,2,4</sup>, Yu-Chiao Chiu<sup>4,5,6</sup>, and Satdarshan P. Monga<sup>1,2,4,5,6</sup>

<sup>1</sup>Organ Pathobiology and Therapeutics Institute, <sup>2</sup>Department of Pharmacology and Chemical Biology, <sup>3</sup>Medical Scientist Training Program, <sup>4</sup>Pittsburgh Liver Research Center, <sup>5</sup>University of Pittsburgh Medical Center Hillman Cancer Center, <sup>6</sup>Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

**Background:** Liver zonation serves as the coordinate system of the hepatic lobule, from the portal triad (zone 1) to the central vein (zone 3), upon which gene expression and consequent functions are delineated. While inextricably linked to homeostasis, regeneration, and disease, the characterization of zonation using both single markers and systematic methods remains inconsistent, limiting the generalization of findings beyond individual studies. Additionally, despite plethora of multi-omic studies in MASLD, the state of liver zonation in this disease remains poorly characterized.

**Methods:** An R package (*lobular*) was implemented utilizing an iterative alignment process to infer the zonation of genes and cells across the transcriptome in user-provided data. By applying this tool to public MASLD datasets, zonation was assessed across control and MASLD livers at various fibrosis stages using single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics (ST). *Lobular* was then applied to snRNA-seq and ST data from both wild-type (WT) and *Lrat-Cre/Wls<sup>fllox/fllox</sup>* (HSC-Wls-KO) mouse livers at baseline, and findings were validated using immunohistochemistry (IHC). Following a three-month Western diet (WD) challenge, mice were assessed by comparing change in body weight alongside Oil Red O staining. **Results:** In the ST MASLD dataset, which was normalized to F0 MASLD liver, zone 3 proportion tended to decrease from F0 (31.5%) to F4 (7.2%). Similarly, in the snRNA-seq MASLD dataset which was normalized to non-steatotic control livers, zone 3 proportion decreased from 33.2% in controls to 0% in F3. In mice, HSC-Wls-KO demonstrated a marked increase in proportion of zone 3 cells compared to WT as assessed by both snRNA-seq and ST and confirmed by IHC staining of zonation markers. While the body weight of WT mice increased an average of 8.5 g on WD, HSC-Wls-KO mice lost an average of 2.4 g body weight ( $p=0.007$ ). WT mice displayed gross and microscopic steatotic changes typical of WD while HSC-Wls-KO mice were comparable to baseline. **Conclusions:** Systematic and robust inference of liver zonation across multiple transcriptomic studies is enabled by the *lobular* R package, a novel bioinformatic tool for basic and translational investigations. Application of *lobular* to MASLD datasets revealed a previously unidentified and progressive loss of hepatocyte zone 3 gene expression. Additionally, we found that a murine model enriched for zone 3 was resistant to WD-induced steatosis and weight gain, implying a functional role for zone 3 in preventing the development of steatosis. **Acknowledgements:** This work was supported by NIH grants 2R01DK062277, 5R01DK103775, 5R01CA251155 and 5R01CA250227 to SPM, R00CA248944 & R35GM154967 to YCC, T32EB001026 & 1F30CA298277 to TMY, University of Pittsburgh Liver Research Center (PLRC award to YCC, part of P30 DK120531 to SPM).

#### Abstract 025

##### **The Significance of miR-22-Galectin-1 Axis in HCC Development and Treatment**

Yu-Jui Yvonne Wan, Ying Hu, and Tahereh Setayesh

Department of Pathology and Laboratory Medicine, University of California Davis, Sacramento, CA

**Background:** miR-22 is a tumor suppressor whose expression level is induced by nutrients, including bile acids (FXR), retinoic acid (RAR $\beta$ ), and vitamin D3 (VDR), via nuclear receptor activation. miR-22 is also induced by short-chain fatty

acids produced by bacterial fermentation of fibers. In hepatocellular carcinoma (HCC) and colorectal cancer (CRC), miR-22 expression is reduced, underscoring the role of miR-22 signaling in the diet-gut-liver axis. While miR-22 acts as a tumor suppressor of HCC and CRC, galectin-1 (Gal-1) is a biomarker of those cancers whose expression is directly silenced by miR-22. Gal-1 acts as a significant immune inhibitor, especially in cancer. The goal of our project is to establish the significance of miR-22-galectin-1 signaling in HCC development and treatment. **Methods:** HCC was produced using FVB/N mice with hydrodynamic delivery of oncogenes, pT3-EF1 $\alpha$ -HA-myr-AKT plus pT/Caggs-Nras-v12 or pT3-EF1 $\alpha$ -N90- $\beta$ -catenin, along with Sleeping Beauty transposase for stable expression of the transgenes. Metabolically dysregulated-associated steatohepatitis (MASH)-HCC were developed using the same method, except that the mice were fed a Western diet from weaning through the entire experiment. The roles of miR-22 and/or Gal-1 were studied using AAV-mediated overexpression or siRNA-mediated knockdown, and interventions were delivered either before tumor initiation (e.g., oncogene injection) or after HCC formation. Outcome measures included tumor load, histology, liver function panels, and biomarkers. Bulk and spatial transcriptomics were performed to identify key pathways affected. **Results:** miR-22 reduction and Gal-1 overexpression are both essential for carcinogenesis, as miR-22 treatment and Gal-1 silencing prior to oncogene injection can prevent tumorigenesis. After HCC was formed, both miR-22 treatment and Gal-1 siRNA treatment extended the overall survival time of HCC mice. The treatment effects were demonstrated in different HCC models, including  $\beta$ -catenin-positive HCC and MASH-HCC. In addition, the anti-HCC effects of miR-22 were dependent on Gal-1, as Gal-1 overexpression abolished the effects of miR-22 treatment on HCC. Furthermore, miR-22-high and Gal-1-low HCC patients had the best survival outcomes. Transcriptomic analysis identified common pathways affected by miR-22 treatment and Gal-1 silence using siRNA. Those pathways included metabolism, the complement and clotting cascades, and the immune pathway. Rho GTPase and cell-matrix signaling were affected and predominantly detected at the tumor margin, suggesting a role for the miR-22-Gal-1 axis in matrix remodeling and cancer cell mobility. **Conclusion:** The miR-22-Gal-1 axis can serve as an HCC diagnostic biomarker and a treatment target. It plays a vital role in regulating innate and adaptive immune responses, thereby improving liver metabolic function. **Acknowledgements:** NIH, NCI, R01CA222490.

## Session 023 – Minisymposia – Cancer Pathobiology

### Abstract 026

#### PRMT5 Represses TH1 Transcriptional Programs and T-bet<sup>+</sup> T Cell Differentiation Through H3R8 Methylation to Promote Immune Evasion in Melanoma

Goran Micevic, Simon Milette, Simon F. Roy, Veronica T. Brooks, Koonam Park, Marcus W. Bosenberg, and Richard A. Flavell

Department of Pathology, Yale School of Medicine, New Haven, CT

**Background:** Melanoma remains a leading cause of cancer-related mortality, and despite the success of immune checkpoint inhibitors (ICI), many patients fail to achieve durable responses. Impaired effector and memory T cell differentiation has emerged as a key determinant of therapeutic resistance, yet the epigenetic mechanisms regulating these processes remain incompletely defined. Protein arginine methyltransferase 5 (PRMT5) is a histone methyltransferase frequently overexpressed in human melanoma and has been implicated in tumor progression in select genetic contexts, such as MTAP deletion. However, its role in regulating antitumor T cell programs and tumor-immune interactions is not well understood. **Methods:** We used genetic and pharmacologic inhibition of PRMT5 in murine melanoma models, including a transgenic OT-1 system to enable tumor antigen-specific T cell tracking. Flow cytometry, transcriptomic profiling, and Cut&Run chromatin mapping were used to assess T cell differentiation, cytokine expression, and histone modification at regulatory loci. Tumor growth was evaluated in the presence and absence of PD-1 blockade. Primary human melanoma samples from the Yale SPORE program were studied for PRMT5 expression, associated gene programs, and clinical outcomes. **Results:** PRMT5 inhibition enhanced expansion and effector differentiation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, with increased representation of T-bet<sup>+</sup> subsets producing IFN- $\gamma$  and TNF- $\alpha$ . In tumor-specific OT-1 T cells, PRMT5 blockade promoted sustained clonal proliferation and acquisition of TH1 phenotypes. Cut&Run analysis demonstrated loss of H3R8me2 at key TH1-related loci, including *Tbx21* and *Ifng*, consistent with direct epigenetic de-repression of effector gene networks. In tumor cells, PRMT5 inhibition activated cytosolic DNA sensing and cGAS/STING-dependent type I interferon signaling, enhancing tumor immunogenicity. In ICI-resistant melanoma models (YUMM1.7, B16), combined PRMT5 inhibition and PD-1 blockade significantly improved tumor control in a CD4<sup>+</sup> T cell- and type I IFN-dependent manner. Human melanoma samples with high PRMT5 expression showed repression of TH1 gene signatures and inferior survival outcomes, supporting PRMT5 as a potential biomarker of

immune resistance. **Conclusions:** PRMT5 acts as an important epigenetic regulator of antitumor immunity in melanoma by repressing TH1 transcriptional programs and limiting T cell effector differentiation through H3R8 methylation. Concurrently, PRMT5 suppresses tumor-intrinsic cGAS/STING-mediated interferon signaling, enforcing immune evasion. Targeting PRMT5 restores T-bet-driven effector programs and sensitizes resistant melanomas to checkpoint blockade, supporting its potential as both a biomarker and therapeutic target in melanoma immunotherapy. Since clinical-grade PRMT5 inhibitors are in use for MTAP-deficient cancers, this suggests an opportunity to therapeutically target PRMT5 in immunotherapy-resistant melanoma.

## Abstract 027

### Stage-Dependent Effects of the SEMA3F/NRP2 Axis in Oral Carcinogenesis

Joud Y. Omari<sup>1,6</sup>, Asma Almazayad<sup>1,6</sup>, Yao Gao<sup>1,4</sup>, Sausan Alfaris<sup>1,6</sup>, Abdulrahman Nakshabandi<sup>1,6</sup>, Dakshnapriya Balasubramanian<sup>1,4</sup>, Harsh N. Dongre<sup>1,4</sup>, David M. Briscoe<sup>2,5</sup>, Rosalyn M. Adam<sup>3,4</sup>, and Diane R. Bielenberg<sup>1,4</sup>

<sup>1</sup>Vascular Biology Program, <sup>2</sup>Transplant Research Program, and <sup>3</sup>Department of Urology Research, Boston Children's Hospital, Boston, MA, <sup>4</sup>Department of Surgery and <sup>5</sup>Department of Pediatrics, Harvard Medical School, Boston, MA, <sup>6</sup>Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA

**Background:** Semaphorin-3F (SEMA3F) is a secreted protein that binds the Neuropilin-2 (NRP2) receptor and exerts a suppressive and repulsive effect on NRP2-expressing immune and endothelial cells. Our previous studies have demonstrated that SEMA3F suppresses tumor angiogenesis and exerts immunomodulatory effects by inhibiting T-cell activation and proliferation. Although *SEMA3F* was originally proposed as a tumor suppressor in lung cancer, its role in the tumor microenvironment remains poorly defined. Our recent work demonstrates downregulation of SEMA3F and upregulation of NRP2 expression in epithelial cells during the progressive stages of oral carcinogenesis. The present study evaluates the effects of the SEMA3F-NRP2 axis on tumor initiation, progression, and host immune surveillance during oral carcinogenesis. **Methods:** Oral cancer initiation and progression were evaluated histologically in transgenic mice with inducible, keratinocyte-specific deletion of *Sema3F* either before or after exposure to the carcinogen, 4-nitroquinoline-1-oxide, compared to control mice. To isolate immune-specific effects, syngeneic oral carcinoma xenografts were implanted into wildtype and *Nrp2*-knockout (KO) mice. Antigen-induced hypersensitivity assays were performed in *Sema3F*-KO, *K14-Sema3F-iKO*, *Nrp2*-KO, and *CD4-Nrp2*-KO mice to evaluate immune activation and T-cell migration. **Results:** Deletion of *Sema3F* in the epithelium prior to oral carcinogen exposure markedly restricted carcinogenesis with only 4/23 mice progressing to carcinoma in situ (CIS) and no progression to invasive oral squamous cell carcinoma (OSCC). In contrast, 25/30 control mice developed CIS or OSCC. However, epithelial *Sema3F* knockout after tumor initiation resulted in increased tumor burden and metastatic spread compared to controls, indicating stage-dependent effects of SEMA3F signaling. OSCC xenografts in *Nrp2*-deficient mice demonstrated significantly increased infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T- cells compared to controls. Similarly, in antigen-hypersensitivity assays, mice lacking SEMA3F or NRP2 exhibited significantly increased ear swelling and CD4<sup>+</sup> lymphocyte recruitment, compared to controls. **Conclusion:** These findings demonstrate that the SEMA3F/NRP2 axis functions as a stage-dependent regulator of epithelial-immune and vascular interactions during oral carcinogenesis. Disruption of the axis at early stages of carcinogenesis enhances antitumor immune surveillance, characterized by increased T-cell recruitment and prevention of malignant progression. In contrast, disruption of SEMA3F/NRP2 signaling following tumor initiation promotes tumor growth and metastatic dissemination, potentially driven by enhanced tumor-associated angiogenesis and vascular invasion. Together, this data suggests that the SEMA3F/NRP2 axis exerts a stage-dependent immunomodulatory and angio-regulatory effect with important implications for the timing and design of therapeutic strategies in OSCC.

## Abstract 028

### Tumoral IL-33/ST2 Signaling Drives Immune Escape Through Reduced Antigen Presentation

Alyssa Mauri Cornista<sup>1</sup>, Tao Yu<sup>2,3</sup>, Zhuolong Zhou<sup>2,3</sup>, Nikša Roki<sup>1</sup>, Alberto Sigler<sup>1</sup>, David Suissa<sup>1</sup>, Haniyeh Eyvani<sup>2</sup>, George Earl Sandusky<sup>4</sup>, Rimpi Khurana<sup>5</sup>, Yan Guo<sup>5,6</sup>, Molly Dalzell<sup>1</sup>, Shyamananda Singh Mayengbam<sup>7</sup>, Prasenjit Dey<sup>7</sup>, Christine Rafie<sup>1</sup>, Erietta Stelekati<sup>1,5</sup>, Jashodeep Datta<sup>5,8</sup>, Saratchandra Singh Khumukcham<sup>9,10</sup>, Jatin Roper<sup>9,10</sup>, Nivedh Paluvoi<sup>8</sup>, Sandro Satta<sup>5,11</sup>, Daniel Bilbao Cortes<sup>5,11</sup>, Alejandro Villarino<sup>1,5</sup>, and Kevin Van der Jeught<sup>1,5</sup>

<sup>1</sup>Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, <sup>2</sup>Indiana University School of Medicine, Department of Medical and Molecular Genetics, <sup>3</sup>Melvin and Bren Simon Comprehensive Cancer Center, <sup>4</sup>Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, <sup>5</sup>Sylvester Comprehensive Cancer Center, <sup>6</sup>Department of Public Health and Sciences, University of Miami, Miami, FL,

<sup>7</sup>Department of Immunology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, <sup>8</sup>Division of Surgical Oncology, Dewitt Daughtry Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, <sup>9</sup>Division of Gastroenterology, Department of Medicine,<sup>10</sup>Department of Pharmacology and Cancer Biology, Duke University, Durham, NC, <sup>11</sup>Department of Pathology and Laboratory Medicine, University of Miami Miller School of Medicine, Miami, FL

**Background:** Immune checkpoint blockade (ICB) therapy delivers promising clinical results in colorectal cancer (CRC), especially in microsatellite instability high (MSI-H) patients; however, the majority (up to 85%) of CRC patients have a microsatellite stable (MSS) tumor phenotype, which are poor responders to ICB. Therefore, there is a need to identify novel checkpoints in the tumor microenvironment, such as ST2 (Stimulation 2, IL33 receptor) and uncover their mechanism of action. **Methods:** Multiplexing was done to define ST2 (Stimulation 2, interleukin-33 receptor) expression in human CRC tissue samples. The inhibitory effects of ST2 were assessed using both patient derived organoids co-cultured with autologous T cells as well as murine subcutaneous and orthotopically transplanted models in WT, TCRbeta<sup>KO</sup>, and ST2<sup>KO</sup> mice. Endoscopy-guided injection of tumor cells is used to study microenvironmental changes. We performed single-cell RNA sequencing on orthotopically implanted CRISPR/Cas9-edited tumor cells to identify the mechanism of action. Sequencing results were validated using an array of *in vitro* experiments. Advanced spectral flow cytometry was used to study tumoral ST2 expression and its effect on T cells. **Results:** Our data show that tumor cells have ST2 expression, both in murine and clinical samples. Particularly, we demonstrated ST2 to be prominently expressed on MSS tumors. Activation of tumoral IL-33/ST2 signaling protects tumor cells from T-cell-mediated killing. Functional studies showed reduced antigen presentation, driven by reduced immunoproteasome activity, and leading to the observed T-cell killing escape. Removing tumoral ST2 signaling using CRISPR/Cas9-gene editing leads to significantly reduced tumor growth and increased infiltration of T cells, with a reduced terminally exhausted profile. **Conclusions:** Our findings suggest that tumoral ST2 signaling drives immune escape through a reduced antigen presentation and could serve as checkpoint target for CRC immunotherapy. **Acknowledgements:** This work is supported by the NIH grant R00 CA248846, and through the Sylvester Comprehensive Cancer Center (SCCC) PG014702, PG013213 and PG014459, University of Miami Department of Microbiology and Immunology PG013597 to K.V.d.J. Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number T32AI162624 to A.M.C. Research reported in this study was performed in part at the Sylvester Cytometry and Imaging Shared Resource (CISR; SCR\_022501), Onco-Genomics Shared Resource (OGSR; SCR\_022502), Cancer Modeling Shared Resource (CMSR; SCR\_022891) and Biostatistics and Bioinformatics Shared Resource (BBSR; SCR\_022890). These shared resources of the SCCC at the University of Miami Miller School of Medicine are supported by the National Cancer Institute (NCI) Cancer Center Support Grant (CCSG) P30-CA240139.

## Abstract 029

### Comprehensive Preclinical Evaluation of Natural Compounds with Cytotoxic, Anti-Invasive, and Anti-Angiogenic Activity in TNBC Brain Metastasis

Jayshree Mishra<sup>1</sup>, Mimansha Saha<sup>1</sup>, Narendra Kumar<sup>1</sup>, Priyam Kumar<sup>2</sup>, and Soaham Kumar<sup>3</sup>

<sup>1</sup>Pharmaceutical Sciences, Texas A&M College of Pharmacy, Kingsville, TX, <sup>2</sup>University of Pennsylvania, Philadelphia, PA,

<sup>3</sup>Veteran Memorial High School, Corpus Christi, TX

**Background:** Triple-negative breast cancer (TNBC) brain metastases remain a major clinical challenge due to aggressive tumor behavior, limited therapeutic options, and poor blood–brain barrier (BBB) penetration of current agents. Here, we designed and developed Jak3 kinase domain targeted natural compound data base and performed a comprehensive multi-assay evaluation of a panel of ten natural compounds (NC1–NC10) to identify candidates with cytotoxic, anti-invasive, anti-angiogenic, anti-migratory, and BBB-permeable properties relevant to TNBC brain metastasis. **Methods:** Cell viability assays revealed dose-dependent cytotoxicity across most compounds, with NC8 and NC1 demonstrating the greatest potency, followed by NC9. *In vitro* kinase assays using MDA-MB-BRM cells identified differential functional profiles, with NC7, NC8, and NC10 maintaining robust activity across concentrations, suggesting distinct or complementary mechanisms of action. Invasion assays highlighted NC1, NC3, and NC4 as the strongest suppressors of invasive potential, while wound-healing assays identified NC6 and NC8 as the most effective inhibitors of cell migration. Long-term clonogenic assays further demonstrated that NC1, NC3, and NC6 significantly reduced colony formation, indicating sustained anti-proliferative effects. Angiogenesis assays revealed substantial anti-angiogenic activity, particularly in direct treatment settings, with NC1 consistently emerging as one of the most potent inhibitors of

endothelial tube formation. Integrated IC50 analyses based on both percent inhibition and absorbance changes revealed distinct potency and selectivity patterns across assays. Importantly, several lead compounds, including NC1, NC3, NC6, NC7, and NC8, are predicted to cross the BBB, with NC1 and NC8 combining strong cytotoxicity, multi-assay efficacy, and BBB permeability. **Results and Conclusion:** Collectively, these findings identify NC1, NC3, NC6, and NC8 as the most consistent and promising candidates, exhibiting multi-functional anti-tumor activity and translational relevance for TNBC brain metastasis. This integrated preclinical assessment provides a strong foundation for further mechanistic studies and in vivo validation of these compounds as potential therapeutic leads for metastatic breast cancer involving the brain.

**Acknowledgement:** This work is supported by the funding from TAMU seedling and Advancing the Discovery to Market grant (JM and NK) from Texas A&M University.

### Abstract 030

#### Secreted PTEN-Long Downregulates PI3K Signaling and PD-L1 and Promotes Anti-Tumor Antigen-Presenting Cell Functions to Cause Regressions of Mouse Tumors

Jia Xu<sup>1,2†</sup>, Tiphaine C. Martin<sup>1†</sup>, Daniel Lozano-Ojalvo<sup>3</sup>, Andrew Baik<sup>4</sup>, Bruno Giotti<sup>1,5</sup>, Ashikur Rahaman<sup>1</sup>, Andrew Wolfe<sup>6</sup>, Natalie Sully<sup>1</sup>, Royce W. Zhou<sup>1</sup>, Zhengxiang He<sup>7</sup>, Kaitlyn Bosch<sup>1</sup>, Madhuri Kalathur<sup>1</sup>, Elias Stratikopoulos<sup>1,4</sup>, Shen Yao<sup>1</sup>, Ruifang Qiao<sup>1</sup>, Sergio A. Lira<sup>7</sup>, Emily J. Gallagher<sup>8</sup>, Joshua Brody<sup>1</sup>, Jordi Ochando<sup>1</sup>, Alexander Tsankov<sup>1,5</sup>, Katherine Cygnar<sup>4</sup>, Aris N. Economides<sup>4</sup>, and Ramon Parsons<sup>1</sup>

<sup>1</sup>Tisch Cancer Institute at Mount Sinai, Icahn School of Medicine at Mount Sinai, New York, NY, <sup>2</sup>Department of Genetics, Department of Pathology, O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, <sup>3</sup>Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, NY, <sup>4</sup>Regeneron Pharmaceuticals, Inc., Tarrytown, NY, <sup>5</sup>Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, <sup>6</sup>Department of Immunology & Immunotherapy, Icahn School of Medicine at Mount Sinai, New York, NY, <sup>7</sup>Department of Biological Sciences, Hunter College of the City University of New York, New York, NY, <sup>8</sup>Division of Endocrinology, Diabetes and Bone Disease, Icahn School of Medicine at Mount Sinai, New York, NY. †Co-first authors.

**Background:** PTEN is well known as a tumor suppressor that inhibits the PI3K/AKT pathway. Loss of PTEN has been observed in ~30-40% of breast cancers and confers resistance to Trastuzumab-based therapy. PTEN-Long (PTEN-L) was recently identified as a novel secreted form of PTEN. PTEN-L has been detected in human tissues and serum, and its expression is reduced in multiple cancer types. However, the role of its secreted isoform PTEN-Long (PTEN-L) is not fully understood. **Methods:** We developed tools to study PTEN-L independent of PTEN using cancer cell lines engineered to express PTEN-L, a mouse engineered to overexpress Pten-L, and Adeno-Associated Virus (AAV)-Pten-L treated xenograft models. AAV-Pten-L was engineered to be expressed in the liver, where it was efficiently secreted into the blood via a potent liver-specific signal peptide. **Results:** Beyond its known ability to inhibit PI3K/AKT signaling, we showed that Pten-L entered tumor cells and macrophages, altering interferon- $\gamma$  and TGF- $\beta$  signaling and immune cell composition, thereby triggering rapid regression of small mouse tumors. Pten-L treatment activated immune cells in the tumor microenvironment, including macrophages, T cells, and NK cells. These changes were associated with enhanced MHC-II and CD80 expression in macrophages and reduced PD-L1 expression in tumor cells and macrophages. Depletion experiments using anti-CD8 or anti-CD80 antibodies revealed that CD8+ T cells and antigen-presenting macrophages were required for AAV-Pten-L-induced tumor regression. **Conclusions:** These results demonstrate the capacity of secreted Pten-L to stimulate the innate immune system and to attenuate PD-L1 expression in the tumor, likely orchestrating, together, an adaptive immune response to inhibit the growth of established tumors. **Acknowledgements:** This research was supported by the NCI at the NIH (NCI R35CA220491 PI: Parsons). J.X. is supported by a T32 postdoc fellow training grant (T32CA078207) and a Leo and Julia Forchheimer Foundation Postdoc Fellowship. K.B. is supported by the Ruth L. Kirschstein National Research Service Award for Individual Predoctoral Fellows (F31 CA183268).

### Abstract 031

#### StarD10 Phosphorylation Promotes ErbB2-Mediated Alcohol-Induced Breast Cancer Progression

Manisha Dagar, Andrea Floris, Swati Chandla, Youngyi Lim, Joshua Martinez, Komal Ramani, and Maria Lauda Tomasi Karsh Division of Gastroenterology and Hepatology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA

**Background:** Breast cancer remains the second most common cancer among women worldwide. Excessive alcohol consumption significantly increases breast cancer risk, with even moderate drinking (one drink per day) raising the risk

by about 10% compared to non-drinkers. STAR-related lipid transfer domain-containing protein 10 (StarD10), a phosphoprotein overexpressed in 35–40% of primary human breast cancers, interacts with the ErbB2 signaling pathway to promote tumor growth. Our previous studies showed that ethanol exposure causes StarD10 dephosphorylation and enhances ErbB2 expression, leading to increased malignancy and aggressiveness. However, the mechanistic role of StarD10 as a subcellular lipid transporter in regulating ErbB2-driven signaling is still not well understood. This study investigates how ethanol affects StarD10 activity and the ErbB2 signaling pathway using three-dimensional breast cancer organoid models. **Methods:** Lipid overlay assays and co-immunostaining were performed to assess StarD10's lipid-binding specificity in breast cancer cells (MCF-7, SKBR-3, and BT-474) and three-dimensional breast cancer organoid models (PDxO). Additionally, organoids were CRISPR gene-edited to validate the functional outcome of pre-identified StarD10 phospho-sites. Activation of the ErbB2-regulated AKT-mTOR pathway was analyzed by Western blot. Cell viability and migration were analyzed in CRISPR gene-edited breast cancer cells. **Results:** A significant decrease in StarD10 phosphorylation was observed in breast cancer cells (MCF-7, SKBR-3, BT-474) and breast cancer organoids (PDxO) in the presence of ethanol. Lipid overlay assay showed that StarD10 interacts with multiple phosphoinositides, including PI(3)P, PI(4)P, PI(5)P, PI(3,4)P<sub>2</sub>, PI(4,5)P<sub>2</sub>, and PI(3,4,5)P<sub>3</sub>. Co-immunostaining further demonstrated an ethanol-induced increase in the interaction between StarD10 and PIP2/PIP3. Inhibition of PP2A activity by CRISPR-mediated gene editing prevented ethanol-induced StarD10 dephosphorylation and the ErbB2-mediated AKT-mTOR pathway, indicating that these phosphatases act as positive regulators of StarD10 function in the organoid model. Moreover, PP2A gene editing significantly reduced cell viability and migration compared to ethanol-treated cells, suggesting a critical role for PP2A-mediated dephosphorylation of StarD10 in promoting ethanol-induced breast cancer cell aggressiveness. **Conclusions:** Our findings show that ethanol promotes breast cancer progression by causing dephosphorylation of StarD10 at the T288 residue. Using organoid models, we demonstrate that PP2A phosphatase positively regulates StarD10 activity. Targeting the phosphorylation pathway of StarD10 may serve as a potential therapeutic strategy to reduce alcohol-related breast cancer progression.

#### Abstract 032

##### **Stromal Modulation of Tertiary Lymphoid Structures in BRCA-Mutated High Grade Serous Ovarian Cancer**

Swathi Suresh<sup>1</sup>, Erika Lampert<sup>2</sup>, Grace Gorecki<sup>3</sup>, Ian P. MacFawn<sup>4</sup>, Huda Atiya<sup>5</sup>, Tullia C. Bruno<sup>6</sup>, and Lan Coffman<sup>5,7,8</sup>

<sup>1</sup>Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, <sup>2</sup>Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh PA, <sup>3</sup>Internal Medicine, Allegheny Health Network, Pittsburgh, PA, <sup>4</sup>Department of Biology, Grove City College, Grove City, PA, <sup>5</sup>Division of Hematology/Oncology, Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, <sup>6</sup>Department of Immunology, University of Pittsburgh, Pittsburgh, PA, <sup>7</sup>Department of Pathology, University of Pittsburgh, Pittsburgh, PA, <sup>8</sup>Magee-Women Research Institute, Pittsburgh, PA

**Background:** High-grade serous ovarian cancer (HGSOC) is the most lethal subtype of ovarian cancer, with more than 70% of patients presenting with metastatic disease at the time of diagnosis. While germline BRCA1/2 mutations carry a 30-40-fold higher risk of HGSOC, paradoxically, patients with BRCA mutations have improved responses to treatment and overall survival benefit compared to BRCA-wildtype patients. This study investigates how BRCA status influences the tumor microenvironment (TME), with a particular focus on stromal remodeling that supports anti-tumor immunity. Our lab has previously demonstrated that ovarian cancer cells epigenetically reprogram their resident tissue mesenchymal stromal/stem cells (MSCs) to develop a cancer-supportive phenotype. These cancer-associated mesenchymal stem cells (CA-MSCs) acquire pro-tumorigenic properties and express high levels of WT1, a transcription factor implicated in immune evasion. **Methods and Results:** Analysis of our mRNA sequencing data revealed a positive correlation between WT1 and CD200, an immunomodulatory protein known to suppress immune activation, in CA-MSCs. With the help of multispectral flow analysis, we found that elevated CD200 expression in CA-MSCs impairs their differentiation into follicular dendritic cells (fDCs), a specialized stromal cell type essential for the formation and maintenance of tertiary lymphoid structures (TLS). These are ectopic lymphoid aggregates within the TME that coordinate interactions among immune cell populations, including B cells and T cells, and are associated with enhanced anti-tumor immunity and improved responses to immunotherapy. To determine whether BRCA status influences this stromal differentiation axis, we isolated CA-MSCs from primary HGSOC tumors with known germline BRCA status and assessed CD200 expression using qPCR and flow cytometry. CA-MSCs derived from germline BRCA-mutant tumors exhibited significantly reduced CD200 expression compared to those from BRCA-wildtype tumors. Functionally, MSCs with lower CD200 expression demonstrated an enhanced capacity to differentiate into fDCs in vitro, as assessed by multispectral flow cytometry using

canonical fDC markers. Differentiation was further validated through functional assays such as ELISA to assess CXCL13 secretion, a key chemokine produced by fDCs, and the efficiency of B-cell binding. MSCs that differentiated to fDCs with reduced CD200 levels showed increased CXCL13 production and significantly greater B-cell binding capacity.

**Conclusions:** Collectively, these findings suggest that BRCA mutations may downregulate the WT1-CD200 axis in CA-MSCs, promoting stromal reprogramming that supports TLS formation and antitumor immune responses. Overall, our findings reveal a novel mechanism that highlights the WT1-CD200 axis in HGSOEC stroma as a potential target for enhancing TLS formation and anti-tumor immune responses.

### Abstract 033

#### **NRP2 Drives Tumor Progression, Angiogenesis, and Lymphangiogenesis in OSCC**

Abdulrahman Z. Nakshabandi,<sup>1,3</sup> Harsh N. Dongre,<sup>1,2</sup> Joud Y. Omari,<sup>1,3</sup> Asma Almazayad<sup>1,3</sup>, Sandy Huyhn<sup>1</sup>, Yao Gao<sup>1,2</sup>, Daniela E. Costea<sup>4</sup>, and Diane R. Bielenberg<sup>1,2</sup>

<sup>1</sup>Vascular Biology Program, Boston Children's Hospital, Boston, MA, <sup>2</sup>Harvard Medical School, Boston, MA, <sup>3</sup>Department of Oral Medicine Infection and Immunity, Harvard School of Dental Medicine, Boston, MA, <sup>4</sup>The Gade Laboratory for Pathology and Centre for Cancer Biomarkers CCBIO, Department of Clinical Medicine, Faculty of Medicine, University of Bergen, Norway

**Background:** Human oral squamous cell carcinoma (OSCC) presents with regional or distant metastasis in approximately 67% of cases at diagnosis. Tumor angiogenesis and lymphangiogenesis are prognostic factors for OSCC progression. Neuropilin-2 (Nrp2) is a transmembrane co-receptor crucial for both VEGF-A and VEGF-C signaling which promotes neovascularization. Previous work from our laboratory has demonstrated the importance of Nrp2 expression during 4NQO-induced oral carcinogenesis with 82-90% tumor incidence in control mice reduced to 22% tumor incidence in mice lacking Nrp2 in keratinocytes. Herein, we evaluated the necessity for Nrp2 in the vascular compartments critical for tumor angiogenesis and lymphangiogenesis in OSCC. **Methods:** Human OSCC (n=208) tissue microarrays were stained for NRP2, CD31 (blood vessels), and podoplanin (lymphatic vessels) by immunohistochemistry. Vessel density and NRP2 expression were analyzed in relation to clinicopathologic parameters including tumor stage, HPV status, and survival outcomes. Additionally, syngeneic mouse OSCC cells were orthotopically injected into either constitutive *Nrp2*-deficient mice or tamoxifen-inducible *Nrp2*-knockout mice (*R26-Cre<sup>ERT2</sup>;Nrp2<sup>fl/fl</sup>*) and compared to control mice to assess the effects on tumor vascularization and metastasis. **Results:** In human OSCC tissue microarrays, NRP2 was expressed in tumor cells, tumor-associated blood vessels, and tumor-associated lymphatic vessels. Preliminary analysis suggests a relationship between NRP2 expression, vascular density, and tumor stage. In orthotopic syngeneic isografts, mouse OSCC tumors were smaller in size with fewer tumor-associated vessels in *Nrp2*-deficient mice compared to controls. **Conclusions:** NRP2 is expressed in three important cell types within the tumor microenvironment – tumor cells, vascular endothelium, and lymphatic endothelium. Knocking out Nrp2 expression in any of these three target cells resulted in diminished tumorigenicity and progression. Overall, these findings support NRP2 as a potential therapeutic target for inhibiting tumor burden and dissemination in OSCC. **Acknowledgements:** This project was supported by the Kingdom of Saudi Arabia's Custodian of the Two Holy Mosques Scholarship Program and the Vascular Biology Program at Boston Children's Hospital (DRB).

### Abstract 034

#### **SpatioScope: Complementing Interpretable Machine Learning with Explainable Deep Learning to Visualize Morpho-Molecular Signals in Triple Negative Breast Cancer**

Vibha R. Rao<sup>1</sup>, Madhumala K. Sadanandappa<sup>1</sup>, Candice C. Black<sup>1,2</sup>, Scott M. Palisoul<sup>1</sup>, Adrienne A. Workman<sup>1,2</sup>, Todd A. MacKenzie<sup>2</sup>, Mary D. Chamberlin<sup>2</sup>, Louis J. Vaickus<sup>1,2</sup>, George J. Zanazzi<sup>1,2</sup>, and Shrey S. Sukhadia<sup>1,2</sup>

<sup>1</sup>Dartmouth Health, Lebanon, NH, <sup>2</sup>Geisel School of Medicine, Dartmouth College, Hanover, NH

**Background:** Triple-negative breast cancer (TNBC) is a clinically aggressive and heterogeneous disease lacking established molecular targets. Although bulk transcriptomic profiling informs TNBC biology, it averages signals across diverse cellular and spatial regions, obscuring subregion-specific transcriptional programs linked to tumor behavior. While spatial transcriptomic technologies preserve tissue context, they are costly and challenging for routine clinical use. There is a need for computational approaches that extend spatial molecular insight into standard histopathology workflows using hematoxylin and eosin (H&E)-stained slides. To address this gap, we developed SpatioScope as an integrative framework to infer spatial gene/protein (biomarker) expression from H&E morphology while preserving

interpretability. **Methods:** SpatioScope was evaluated using a GeoMx Digital Spatial Profiler dataset comprising two formalin-fixed paraffin-embedded TNBC tissue microarrays (104 tissues), from which 272 pathologist-annotated regions of interest (ROIs) were selected based on H&E and immunofluorescence and profiled for spatial gene/protein expression. SpatioScope exploits two complementary modeling approaches: (i) an interpretable machine learning (ML) using quantitative morphologic features from each ROI, to predict biomarker expression and (ii) an explanatory deep learning (DL) that applies weakly supervised multiple-instance learning to generate spatial expression maps highlighting regions predictive of expression. Outputs from both approaches were analyzed for concordance. **Results:** The explanatory-DL approach achieved strong performance across biologically relevant biomarkers, with area under curve (AUC)=[0.79, 0.97], while the interpretable-ML approach achieved AUC values up to 0.82. Complementary interpretations emerged:(i) the ML approach identified nuclear intensity, skewness, shape irregularity, and architectural organization as dominant morphologic drivers, explaining why specific molecular states were predicted, and (ii)the DL-derived maps localized predictive signals to biologically relevant tissue regions, including tumor-invasive fronts and immune-enriched compartments, indicating where these morphologic cues manifest within the tissue. Across biomarkers, morphologic drivers aligned with spatial attention patterns, indicating that quantitative tissue architecture and spatial localization act complementarily to predict morpho-molecular signals. **Conclusion:** Together, feature-based and spatially localized modeling reinforce the principle that tissue morphology and molecular phenotype are coupled through quantifiable spatial patterns reflecting tumor heterogeneity. By explaining both why morphologic features drive biomarker expression and where these signals arise, SpatioScope provides biologically grounded insight into tumor heterogeneity from routine H&E slides for researchers, pathologists, and clinicians without costly molecular assays.

### Abstract 035

#### Functional Impact of Gasdermin B on Epithelial Wound Healing in IBD

Serena Artone<sup>1,2</sup>, Joseph J. Williams<sup>1</sup>, Kaylynn J. Vidmar<sup>1</sup>, Michelle Pan<sup>1,3</sup>, Katarzyna Bulek<sup>1</sup>, Derek W. Abbott<sup>1,3</sup>, E. Ricky Chan<sup>4</sup>, Benedetta Cinque<sup>5</sup>, Tsan Sam Xiao<sup>1</sup>, and Theresa T. Pizarro<sup>1</sup>

<sup>1</sup>Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH, <sup>2</sup>Department of Physical and Chemical Science, University of L'Aquila, L'Aquila, Italy, <sup>3</sup>Department of Immunology and Genomic Medicine, National Jewish Hospital, Denver, CO, <sup>4</sup>Case Comprehensive Cancer Center, Cleveland, OH, <sup>5</sup>Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

**Background.** Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disorder of the gastrointestinal tract (GI) characterized by defective mucosal repair and epithelial barrier dysfunction. Gasdermin B (*GSDMB*), an IBD-linked gene at the 17q21 locus, is expressed in the GI epithelium as multiple splice variants (encoding different protein isoforms), and harbors two IBD-associated single-nucleotide polymorphisms (SNPs) occurring in linkage disequilibrium. Although GSDMs are classically known to induce pyroptosis, we previously showed that *GSDMB* promotes epithelial restitution; however, how specific *GSDMB* isoforms and genetic variants contribute to this process remains unclear. Post-translational regulation of GSDMs, particularly through S-palmitoylation, has emerged as a key modulator of localization and function, yet the post-translational regulation and functional consequences of *GSDMB* isoforms remain unexplored. **Methods.** To define *GSDMB* isoforms relevant to IBD, we analyzed bulk RNA deep sequencing datasets from intestinal biopsies of Crohn's disease (CD) and ulcerative colitis (UC) patients. Bulk RNA sequencing was also performed on HT-29 IECs treated with methotrexate (MTX) or vehicle to assess *GSDMB* isoform expression. For functional analyses, *GSDMB*<sup>-/-</sup> HT-29 cells were reconstituted with individual *GSDMB* isoforms, carrying either wild-type (WT) or mutant (IBD-associated) SNPs, with expression confirmed by immunoblotting. Wound healing assays were performed on these cells (+/- MTX) to evaluate isoform-specific epithelial function. To assess *GSDMB* S-palmitoylation, acyl-biotin exchange (ABE) assays were performed in HT-29 cells expressing individual WT *GSDMB* isoforms, followed by immunoblotting. **Results.** Analysis of publicly available bulk RNA-seq datasets from intestinal biopsies of CD and UC patients identified *GSDMB*-416 and *GSDMB*-407 isoforms as the predominant protein-coding transcripts, and *GSDMB*-403, to a lesser extent. Consistently, bulk RNA-seq analysis performed in HT-29 cells revealed preferential expression of *GSDMB*-416 and *GSDMB*-407, with lower, but detectable expression of *GSDMB*-403. Using isoform-specific HT-29 cells, functional wound-healing assays demonstrated that expression of *GSDMB*-407, but not *GSDMB*-403 or *GSDMB*-416, significantly enhanced epithelial gap closure, whereas the IBD-SNPs attenuated this effect. Preliminary biochemical analyses further indicate that *GSDMB* isoforms are differentially S-palmitoylated, with *GSDMB*-416 and *GSDMB*-407 exhibiting stronger palmitoylation signals compared to *GSDMB*-403, identifying isoform-dependent differences in lipid modification. **Conclusions.** These findings suggest that *GSDMB* isoforms differentially regulate epithelial restitution and repair.

Additionally, GSDMB isoforms are differentially S-palmitoylated, potentially contributing to specific GSDMB-dependent functions of IECs in IBD. **Acknowledgements.** NIH: R01 DK125293, P01 AI141350, Project 4 (TTP).

## Session 028 – Endothelial Functions and Metabolism in Health and Disease

### Abstract 036

#### Inflamed Intestinal Endothelial Cells Establish Self-Regulatory Niches with Vessel-associated Macrophages to Promote Tissue Damage

Xingsheng Ren, Laura D. Manzanares, and Ronen Sumagin

Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL

**Background:** Macrophages play essential roles in both initiation and resolution of inflammation. A specialized subset of tissue macrophages, vessel-associated macrophages (VAMs) spatially localizes to the blood vessels, where they regulate vascular permeability and neutrophil (PMN) transendothelial migration (TEM). While VAMs have been identified in human inflammatory bowel disease (IBD), the mechanisms governing their recruitment and specialization within inflamed tissues remain unexplored. **Methods and Results:** In this study, we define a novel endothelial-macrophage signaling axis that promotes VAM recruitment during intestinal inflammation. Using whole-mount confocal imaging in CX3CR1-EGFP reporter mice, we identify robust expansion of VAMs across multiple murine models of intestinal inflammation. We found that the classical T-cell marker CD4 represented the majority of VAMs which exhibited an inflammatory phenotype enriched for TNF $\alpha$  expression, consistent with its role in vascular activation. Transcriptomic profiling and flow cytometric analyses revealed that inflamed intestinal endothelial cells (ECs) upregulate IL-16, the cognate ligand for CD4. In vitro, recombinant IL-16 and conditioned media from inflamed murine endothelial cells induced robust migration of activated bone marrow-derived macrophages in transwell assays. CRISPR-mediated deletion of IL-16 in ECs significantly reduced macrophage migratory responses, establishing IL-16 as a key EC-derived chemoattractant. Integrative cross-species analyses further identified a conserved CD4<sup>+</sup> VAM-like macrophage program in human IBD tissue with strong predicted signaling to activated endothelium. **Conclusions:** Together, these findings establish CD4 as a defining feature of inflammatory VAMs and identify endothelial IL-16 as a novel regulator of macrophage recruitment to the vascular niche. This work reveals a previously unrecognized mechanism by which inflamed endothelium actively shapes macrophage positioning and vascular inflammation during intestinal disease.

### Abstract 037

#### Neutrophil Dysfunction in Sepsis: Cell Behavior Dynamics and Metabolism

Stephania Libreros

Department of Pathology and Vascular Biology Program, Yale University, New Haven, CT

**Background:** Sepsis is a leading cause of mortality in the United States and a major driver of hospital readmission, with annual costs exceeding \$38 billion. It is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, characterized by rapid innate immune activation and surges of cytokines, chemokines, and lipid mediators (LMs) that amplify systemic inflammation. Neutrophils are central to this response: early recruitment and antimicrobial activity can be protective, yet in sepsis neutrophil programs become maladaptive, with dysregulated chemotaxis and migration, impaired targeting of infection, and excessive tissue infiltration that fuels organ injury and immune dysfunction. There are no FDA-approved targeted therapies for sepsis, highlighting an unmet need to define mechanistic drivers of failed resolution. Since LMs are rapid, potent regulators of neutrophil trafficking and immunometabolism, we asked whether LM signaling drives neutrophil heterogeneity by shaping distinct neutrophil behavioral and metabolic programs across the sepsis progression. **Methods:** Neutrophils were isolated from peripheral blood from healthy donors and patient cohorts including ICU non-infectious controls, sepsis, and septic shock. Cells were imaged by high-speed confocal microscopy to quantify 4D morphodynamics. Single cells were segmented and tracked, and >60 morpho-kinetic features were extracted to define a behavioral landscape and classify neutrophils into five behavioral states (B1–B5). Neutrophils were profiled under inflammatory and LM stimulation conditions. In parallel, targeted LC–MS/MS quantified lipidome features, focusing on acylcarnitines and cholesterol esters. **Results:** Behavioral analysis resolved five distinct neutrophil states spanning sessile/spherical, small, migratory/amoeboid, small flat/oblate, and large/oscillatory phenotypes. LTB<sub>4</sub> preferentially expanded migratory states, consistent with LM-dependent regulation of neutrophil swarming and this was counter regulated by Resolvins. In patient samples, neutrophils have distinct behavioral profiles that represented immune paralysis increasing severity from infection to septic shock.

Metabololipidomics demonstrated concurrent lipid remodeling in severe disease, including reduced acylcarnitines and increased cholesterol esters, consistent with impaired mitochondrial fatty-acid utilization and increased neutral lipid storage. Behavioral heterogeneity co-occurred with metabolic remodeling, supporting linked regulation of neutrophil trafficking phenotypes and immunometabolic state in sepsis. **Conclusion:** These data support a model in which variability in LM signaling contributes to patient-specific of neutrophil behavioral states coupled to distinct metabolic programs, providing a translational framework to stratify sepsis heterogeneity and advance resolution-directed biomarkers and therapies.

## Session 029 – Mapping the Future of Cancer Prevention: Pre-Cancers, Atlas Insights, and Interception Strategies

### Abstract 038

#### Neuropilin-2 Driven T-Cell Suppression- Implications for Tumor Development

Harsh Nitin Dongre<sup>1,2</sup>, Joud Omari<sup>1</sup>, Abdulrahman Nakshabandi<sup>1</sup>, Asma Almazayad<sup>1</sup>, Sandy Huynh<sup>1</sup>, Yao Gao<sup>1</sup>, and Diane R. Bielenberg<sup>1</sup>

<sup>1</sup>Vascular Biology Program, Boston Children's Hospital, Department of Surgery, Harvard Medical School, Boston, MA,

<sup>2</sup>The Gade Laboratory for Pathology and Centre for Cancer Biomarkers CCBio, Department of Clinical Medicine, Faculty of Medicine, University of Bergen, Norway

**Background:** Neuropilin-2 (NRP2) is a transmembrane receptor involved in axonal guidance and vascular development. NRP2 has recently emerged as an immunoregulatory molecule capable of influencing inflammation and allograft rejection, yet its role in tumor immunity is unknown. Previous data from our laboratory demonstrated increased immunosurveillance of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in syngeneic oral squamous cell carcinomas (OSCC) implanted into the tongue of global *Nrp2*-deficient mice compared to *Nrp2*-intact wildtype littermates. However, the specific role of NRP2 in modulating T-cell function remains unclear. The goal of this study is to investigate how *Nrp2* expression influences T-cell activation, differentiation, and effector responses within the tumor microenvironment. **Methods:** Murine splenic and circulating T-cells from *R26cre<sup>ERT2</sup>;Nrp2<sup>fl/fl</sup>* (iNrp2-KO) mice and *Nrp2<sup>fl/fl</sup>* (control) littermates were isolated and characterized using multicolor flow cytometry to identify cytotoxic T cells (CD8<sup>+</sup>), regulatory T-cells (FoxP3<sup>+</sup>), activated T-cells (CD69<sup>+</sup>), effector memory T-cells (CD62L<sup>-</sup> CD27<sup>-</sup>), short-lived effector cells (CD127<sup>lo</sup> KLRG1<sup>high</sup>), memory precursor cells (CD127<sup>+</sup> KLRG1<sup>-</sup>), and immunological checkpoint markers (PD-1<sup>+</sup>, CTLA4<sup>+</sup>, TIM-3<sup>+</sup>, LAG-3<sup>+</sup>) in addition to CD4<sup>+</sup> T-cells. Orthotopic injection of OSCC cell line (4MOSC2) was performed in syngeneic control and iNrp2-KO mice, and resulting tumors were stained by immunofluorescence to quantify infiltrated CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. Further, quantitative PCR was performed on isolated T-cells to profile transcriptional changes associated with the NRP2 signaling axis. **Results:** *Nrp2* was expressed in murine CD4<sup>+</sup> T-cell subsets, including FoxP3<sup>+</sup> Tregs and FoxP3<sup>-</sup> Teff cells, with increased transcript levels following T-cell activation. Deletion of *Nrp2* in mouse CD4<sup>+</sup> T-cells enhanced effector function, leading to significantly increased IFN $\gamma$  production upon activation. *Nrp2*-deficient CD4<sup>+</sup> T-cells showed elevated IFN $\gamma$  production upon stimulation, consistent with a heightened effector profile. Checkpoint molecules including PD-1, CTLA-4, TIM-3, and LAG-3 were also modulated in iNrp2-KO T-cells compared to controls, indicating altered activation and exhaustion dynamics. In vivo, tumor growth was markedly reduced in iNrp2-KO mice compared to controls. Histological analysis revealed a striking increase in tumor-infiltrating lymphocytes with abundant infiltration of both CD4<sup>+</sup> and CD8<sup>+</sup> T-cells in iNrp2-KO tumors compared to controls. **Conclusions:** These findings identify NRP2 as a key modulator of the tumor immune microenvironment and suggest that targeting NRP2 may strengthen endogenous antitumor immunosurveillance and improve cancer immunotherapy outcomes. **Acknowledgements:** The authors acknowledge funding support from the Norwegian Research Council, Norway (2025/ 357542) (HND) and the Vascular Biology Program at Boston Children's Hospital, USA (DRB).

### Abstract 039

#### Targeting Inflammation-Induced Tumor Dormancy Escape Via Eicosanoids

Lily M. Ceraso<sup>1,2</sup>, Jianjun Deng<sup>1-3</sup>, Camille Longabardi<sup>1,2</sup>, Molly Gilligan<sup>1,2</sup>, Neha Rana<sup>1,2</sup>, Katherine M. Quinlivan<sup>1,2</sup>, Sung Hee Hwang<sup>4</sup>, Bruce D. Hammock<sup>4</sup>, Haixia Yang<sup>1,2,5</sup>, and Dipak Panigrahy<sup>1,2</sup>

<sup>1</sup>Center for Vascular Biology Research, <sup>2</sup>Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, <sup>3</sup>State Key Laboratory of Vegetable Biobreeding, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China, <sup>4</sup>Department of Entomology and Nematology, and UC Davis Comprehensive Cancer Center, University of California, Davis, CA, <sup>5</sup>College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China

**Background:** Chronic unresolved inflammation is a critical driver of tumor progression and tumor dormancy escape. Over 70% of cancer cases can be traced to specific external stress risk factors including toxic exposure to chemical carcinogens. Pre-cancerous lesions such as pancreatic cysts typically remain dormant throughout a lifetime. Altered stromal stress mechanisms in the tumor microenvironment can promote carcinogenesis via tumor dormancy escape. Chronic inflammation can trigger the transition of pre-cancerous lesions to invasive cancer to reduce tumor latency. While tumor dormancy escape can be induced by external stressors such as chronic inflammation, the mechanisms remain poorly characterized. We hypothesize that stabilization of endogenous pro-resolving epoxy-fatty acids via dual COX-2/sEH inhibition may be a promising new strategy to prevent tumor dormancy escape via inflammation resolution. **Methods:** To interrogate the tumor growth activity of carcinogen-generated cell debris, we developed a mouse debris-stimulated tumor model applicable to many cancer types in which debris generated *in vitro* can stimulate the growth of grafted tumors from a subthreshold inoculum of tumor cells, mimicking tumor dormancy. RT-qPCR, western blot analysis, and cytokine arrays of murine spleen and tumors were performed for pro-inflammatory eicosanoids, cytokines, and pro-resolving lipid mediators. **Results:** Exposure to carcinogens perchloroethylene (PCE) or trichloroethylene (TCE) induces pro-inflammatory eicosanoid enzymes soluble epoxide hydrolase (sEH) and cyclooxygenase-2 (COX-2). Debris generated due to exposure to carcinogens (e.g., PCE or TCE) stimulates tumor dormancy escape via a pro-inflammatory cytokine storm. PCE- or TCE-generated apoptotic cell debris up-regulates the pro-inflammatory transcription factor NF- $\kappa$ B, eicosanoid enzymes 5-LOX and 12-LOX, and stimulates tumor angiogenesis. Environmental or occupational carcinogen exposure overexpresses sEH, which degrades host-protective anti-inflammatory and pro-resolving epoxy-fatty acid lipid mediators in the tumor microenvironment. Additionally, debris generated by PCE, TCE, toluene, or hexavalent chromium from endothelial or macrophage cells induces a pro-inflammatory, pro-angiogenic macrophage-derived cytokine storm. The dual COX-2/sEH inhibitor PTUPB counter-regulates this debris-induced cytokine storm leading to inhibition of tumor growth. **Conclusions:** Targeting eicosanoids via inhibition of sEH and COX-2 simultaneously represents a novel strategy to prevent tumor dormancy escape induced by carcinogen-induced inflammation. Therefore, the stabilization of endogenous pro-resolving epoxy-fatty acids via dual COX-2/sEH inhibition may be a promising new strategy to prevent tumor dormancy escape via inflammation resolution. **Acknowledgements:** This work was supported by RIVER Grant R35 ES030443-01 (B.D.H.); Credit Union Kids at Heart (DP); Carter Joseph Buckley Pediatric Brain Tumor Fund (D.P.).

### Session 033 – Immune Memory and Cellular Cross-Talk at the Mucosal Surfaces

#### Abstract 040

#### Intestinal Epithelial Cell-Derived Gasdermin C Regulates IL-33 Subcellular Trafficking During Chronic Intestinal Inflammation

Kaylynn J. Vidmar<sup>1</sup>, Joseph J. Williams<sup>1</sup>, Serena Artone<sup>1,2</sup>, Stefania De Santis<sup>1</sup>, Carlo De Salvo<sup>1</sup>, and Theresa T. Pizarro<sup>1</sup>  
<sup>1</sup>Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH, <sup>2</sup>Department of Physical and Chemical Science, University of L'Aquila, L'Aquila, Italy

**Background:** Inflammatory bowel disease (IBD) is a chronic, multifactorial disorder of the GI tract, in which intestinal epithelial cell (IEC) dysregulation is implicated in disease pathogenesis; however, their mechanistic contributions remain unknown. Increasing evidence indicates the importance of gasdermin (GSDM) proteins in the pathophysiology of GI-related diseases, including IBD, specifically for their function(s) in IECs. Gasdermin C (GSDMC) is highly expressed in IECs and upregulated in a context-dependent manner; however, its precise contribution to chronic intestinal inflammation has not been investigated. In fact, *in vivo* GSDMC function has primarily been studied during intestinal helminth infection, wherein GSDMC is upregulated and implicated in the extracellular release of IEC-derived IL-33, leading to mounting of Th2 immune responses towards worm expulsion. Notably, nuclear sequestration is recognized as a mode of regulation of IL-33 secretion, although the mechanism(s) of its movement throughout the cell is unknown. The aim of this study is to determine the potential role of GSDMC and its contribution to IL-33 nuclear translocation during chronic intestinal inflammation. **Methods:** To investigate the expression of *Gsdmc1-4* in ileitis-prone SAMP mice as disease progresses, we performed bulk RNA sequencing on ileal tissues as well as Western blot on ileal-derived IECs. Immunofluorescent (IF) staining/confocal imaging was performed on inflamed vs. non-inflamed areas of SAMP ilea to investigate the cellular localization of GSDMC and IL-33. Subcellular fractionation was also performed on ileal-derived IECs to validate protein translocation. **Results:** Our preliminary data shows inherent and robust upregulation of *Gsdmc2-4* prior to the onset of inflammation in ilea of SAMP compared to age-matched control AKR (4-wk-old) mice, which

further increases when disease is established (20-wk-old). Additionally, we observe an IEC-subtype specific alteration in *Gsdmc2-4*, particularly in enterocytes and intestinal stem cells (ISCs) vs. AKR. Not only is GSDMC expression altered as disease increases, but so is its activation (i.e., cleavage). IF imaging shows co-expression of GSDMC and IL-33 in ileal IECs from highly inflamed areas, with scant staining in areas of low inflammation in SAMP, and none in non-inflamed AKRs. Notably, GSDMC accumulates at the nuclear membrane, and IL-33 in the nucleus, of IECs. Likewise, subcellular fractionation from 20-wk old SAMP indicated increased expression and nuclear localization of full length and cleaved GSDMC compared to 4-wk SAMP and age-matched AKR mice. **Conclusions:** GSDMC is increased and plays a critical regulatory role in the bi-directional translocation of IL-33 into/out of the nucleus during chronic intestinal inflammation, such as that observed in IBD. **Acknowledgements:** NIH: R01 DK125293, P01 AI141350, Project 4 (TTP); T32 AI089474 (Immunology Training Program-Predocctoral to KJV).

#### Abstract 041

##### **TWEAK Represses Homeostatic Signaling and Disrupts Intestinal Fibroblast-Epithelial Communication**

Bella Raphael<sup>1</sup>, Carlos Matellan<sup>1,2</sup> Mary Nwaezeigwe<sup>3</sup>, Glen Doherty<sup>3</sup>, Seán T. Martin<sup>3</sup>, and Mario C. Manresa<sup>1</sup>

<sup>1</sup>School of Biomolecular and Biomedical Science, <sup>2</sup>School of Medicine, University College Dublin, Dublin, Ireland, <sup>3</sup>Centre for Colorectal Disease, St Vincent's University Hospital, Dublin, Ireland

**Background:** Intestinal fibroblasts produce WNT, BMP and Notch mediators that contribute to epithelial homeostasis. Impairment of these signals results in loss of barrier integrity, a critical factor in Inflammatory bowel disease (IBD). However, the mediators and mechanism that control the interaction between fibroblasts and epithelial cells in homeostasis and IBD remain unknown. Here we investigate how the cytokine TNFSF12/TWEAK dysregulates fibroblast-epithelial crosstalk in the colon by impairing a homeostatic signaling network. **Methods:** Primary human colonic fibroblast were stimulated with TWEAK and the expression of factors of the WNT/ $\beta$ -catenin pathway were analyzed by qPCR and western blot. Mass spectrometry was used to analyze the TWEAK repressed proteome in fibroblasts. Wound scratch assay was performed in fibroblast monocultures and in fibroblast-colonic epithelial co-cultures. Transepithelial electrical resistance was used to assess epithelial barrier function in co-culture. Human colonic biopsies from ulcerative colitis were obtained from St. Vincent's hospital and analyzed by immunofluorescence. **Results:** TWEAK represses the expression of WNT ligands and regulators RSPO2, AXIN2 and TCF21 at gene and/or protein level, and induced WNT inhibitors like DKK3, in colonic fibroblasts. TWEAK delayed fibroblast wound closure and altered the composition of the fibroblast matrisome at 24h. Importantly, TWEAK-treated fibroblasts adversely affect epithelial regeneration, whereas TWEAK did not exert direct effects on this parameter in colonic epithelium. Additionally, supernatants from TWEAK-exposed fibroblasts caused a marked decrease in transepithelial-electrical resistance (TEER), indicating compromised epithelial barrier integrity. Inflamed human ulcerative colitis biopsies displayed reduced RSPO2 expression compared to matched non-involved biopsies, linking WNT suppression to disrupted epithelial homeostasis in the inflamed colon. To elucidate the mechanism underlying TWEAK mediated suppression of WNT factors, restoration of WNT signaling with RSPO3 was assessed and was found to significantly reduce TWEAK induced inflammatory responses but did not rescue the TWEAK mediated loss of WNT factors. **Conclusion:** TWEAK specifically alters the expression of WNT targets in colonic fibroblasts, and this impairment of WNT signals in stromal cells disrupts epithelial regeneration and barrier integrity. **Acknowledgements:** This work was supported by Ad astra, UCD awarded to MCM. CM was supported by IRC GOI fellowship (GOIPD/2023/1118).

#### Session 038 – Minisymposium – Liver Pathobiology

##### Abstract 042

##### **Histopathology AI and Machine Learning for Morphological Analysis of Ductular Reaction and Predicting Hepatic Venous Pressure Gradient in Alcoholic Hepatitis**

Ankita Srivastava<sup>1</sup>, Meritxell Ventura Cots<sup>2</sup>, Ramon Bataller<sup>3</sup>, and Rajanikanth Vadigepalli<sup>4</sup>

<sup>1</sup>Daniel Baugh Institute for Functional Genomics and Computational Biology, Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia, PA, <sup>2</sup>Liver Unit, Hospital Universitari Vall d'Hebron, Vall d'Hebron Institute of Research, Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>3</sup>Hospital Clinic of Barcelona, University of Barcelona, Barcelona, Spain, <sup>4</sup>Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM

**Background:** Alcoholic Hepatitis (AH) is a clinical syndrome characterized by acute-onset jaundice and liver enzyme abnormalities in the setting of long-term heavy alcohol use with high short-term mortality and limited therapies. AH is a frequent complication of alcohol-associated liver disease (ALD) that accounts for ~40% of liver transplantations. AH is characterized by hepatocellular insufficiency, immune activation, and aberrant proliferation of a biliary phenotype (cholangiocyte-like cells), referred to as the ductular reaction (DR). DR is both a hallmark of AH and a contributor to fibrotic remodeling and portal hypertension. Hepatic venous pressure gradient (HVPG) is the gold-standard measure of portal hypertension and a crucial prognostic indicator in cirrhosis; however, its role in AH is less defined. Since portal hypertension is a key consequence of AH, and DR has a characteristic morphology, we hypothesized that quantifiable morphological features of DR, extracted from histopathology images of the liver needle biopsies, could be used to predict HVPG levels in AH patients. **Methods:** We analyzed Cytokeratin 7 (CK7-DAB) stained whole slide images from 53 AH patients (fibrosis F1-F4) for general CK7+ objects, and 41 AH patients (F3-F4 fibrosis) for specific elongated and circular DR. We developed image analysis workflows, including AI-based U-Net segmentation for refined DR morphologies, to extract numerous shape and size parameters. Feature selection was based on correlation with HVPG. Tabular Prior-data Fitted Network (TabPFN) models, augmented with linear corrections, were used to predict HVPG. Models were evaluated using 80-20 train-test splits over 10 runs and compared to randomized data. **Results:** We identified CK7+ cellular objects with larger than 100  $\mu\text{m}^2$  area and collated data on 43602 CK7+ cellular objects ( $751.76 \pm 509.41$ ; mean  $\pm$  s.d. per image) across 53 images. For all CK7+ objects, the proportion of CK7-DAB area correlated with HVPG ( $r = 0.48$ ), with poor prediction performance in model testing. When focusing on elongated and circular DR, the proportion of CK7-DAB area ( $r = 0.46$ ) and elongated DR count ( $r = 0.31$ ) also correlated with HVPG. This refined approach significantly improved prediction performance in testing ( $p\text{-value} = 0.0068$ ), outperforming the general CK7+ model. **Conclusions:** Our machine learning approach demonstrates the potential to quantitatively relate the histopathological aspects of AH liver tissue to physiological variables such as HVPG. These findings establish a link between histological remodeling and physiological dysfunction, opening new avenues for risk stratification and therapeutic decision-making in AH. **Funding:** National Institute of Alcoholism and Alcohol Abuse: R01 AA018873.

### Abstract 043

#### Widespread Bacterial Bile Acid Conjugation and Its Implications for Gut Health

Selene F.H. Shore<sup>1</sup>, Sigmund J. Haidacher<sup>2,3</sup>, Ben Ahiadu<sup>4</sup>, Alexey V. Melnik<sup>4</sup>, Thomas D. Horvath<sup>2,3,5,6\*</sup>, and Melinda A. Engevik<sup>1\*</sup>

<sup>1</sup>Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC, <sup>2</sup>Texas Children's Research Institute (TCRI), Houston, TX, <sup>3</sup>Baylor College of Medicine, Houston, TX, <sup>4</sup>BileOmix, Farmington, CT, <sup>5</sup>Texas Medical Center, Houston, TX, <sup>6</sup>University of Houston, Houston, TX. \*Co-senior authors.

**Background:** Bile acids facilitate intestinal lipid absorption and function in signaling. In the liver, primary bile acids are synthesized from cholesterol and conjugated to glycine or taurine prior to secretion. In the intestine, microbes deconjugate and chemically modify bile acids through reactions such as dehydroxylation, generating secondary bile acids. Recently, microbial communities have been shown to conjugate bile acids to amino acids and bioactive amines, producing an expanded pool of microbially conjugated bile acids. While this capacity has been identified in select isolates, the breadth of microbes capable of bile acid modification and the functional consequences of these noncanonical bile acids is poorly understood. **Methods:** Publicly available genomic datasets were surveyed for genes associated with bile acid modification to assess the distribution of bile acid modification potential. We also evaluated bile acid tolerance and conjugation capacity in *Limosilactobacillus reuteri* ATCC 6475. Minimum inhibitory concentrations were determined media supplemented with individual primary (cholic acid, chenodeoxycholic acid - CDCA) or secondary bile acids (deoxycholic acid, ursodeoxycholic acid). Bile acid conjugation was assessed using targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) with authenticated standards. Functional consequences were evaluated using bioinformatic modeling of farnesoid X receptor (FXR) binding with MolModa and validated experimentally using FXR activation assays. **Results:** Genomic analysis revealed bile acid modification potential across multiple bacterial taxa. *L. reuteri* exhibited tolerance to several bile acids *in vitro*. Metabolomic profiling demonstrated that *L. reuteri* synthesized diverse amino acid-conjugated bile acids from chenodeoxycholic, deoxycholic, and ursodeoxycholic acids, with high levels of conjugates containing aspartic acid, glutamine, glutamate, histidine, lysine, and threonine. Computational modeling predicted altered bile acid-FXR interactions, which was supported by FXR activation assays showing that *L. reuteri* CDCA conjugation exerts an overall inhibitory effect on FXR activation. **Conclusions:** These findings demonstrate that a single commensal bacterium can markedly expand bile acid chemical

diversity through the production of multiple noncanonical conjugated bile acids. Such structural modifications significantly alter bile acid signaling properties, including FXR activation, advancing our understanding of microbial bile acid metabolism and its impact on host signaling. **Acknowledgements:** We would like to give thanks to our funding sources, including to NRSA (1F32DE035388-01), NIDCR (5T32DE017551-15), and the Medical University of South Carolina. LC-MS/MS equipment was funded through the NIH (1S10ODO36416) and the Virginia and L.E. Simmons Family Foundation. Standards were purchased using Texas Medical Center Digestive Diseases Center funds (5P30DK056338).

#### **Abstract 044**

##### **Early Bile Acid Supplementation in Mice Ameliorates Developmentally Programmed Liver Disease**

Holly Hinrichs<sup>1</sup>, Fareeha Siddique<sup>1</sup>, Francisco Victorino<sup>2</sup>, Monica Young<sup>1</sup>, Tarin M. Bigley<sup>2</sup>, and Michael D. Thompson<sup>1</sup>,  
<sup>1</sup>Division of Endocrinology and Diabetes, <sup>2</sup>Division of Rheumatology and Immunology, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO

**Background:** Maternal obesogenic diet exposure (MODE) promotes worse cholestatic liver injury and alters bile acid homeostasis in offspring. One mechanism of transmission is via vertical transfer of an altered microbiome to offspring which affects the development of critical gut immune cell populations including regulatory T cells. Bile acids (BA) may be a critical intermediate in this process as we have previously shown that MODE decreases secondary bile acid levels in the gut lumen and liver of early offspring. Furthermore, supplementation with a secondary bile acid, ursodeoxycholic acid (UDCA), in the perinatal period resolves deficiencies in gut immune cell populations in MODE offspring. We hypothesize that early shifts in offspring bile acid metabolism will also affect hepatic immune cell populations and that early secondary bile acid supplementation will ameliorate developmental programming of worse cholestatic liver injury.

**Methods:** Beginning at four weeks of age, female mice were fed either chow (CON) or high fat, fructose, cholesterol (MODE) diet for 6 weeks before being bred with lean males. A subset of MODE offspring was gavaged with UDCA daily between 2 and 3 weeks of age. Liver was collected from offspring at 3 and 4 weeks of age. BA homeostasis was assessed through measurement of BAs via mass spectrometry on liver of 3-week-old offspring. Flow cytometry was performed on liver at 4 weeks of age to assess changes in immune cell populations. A cohort of offspring were weaned to a regular chow diet and at 10 weeks of age placed on DDC diet for 2 weeks to induce cholestatic liver injury. Histological, serum, and qPCR analyses were performed following DDC diet exposure. **Results:** BA profiling on 3-week-old liver identified an increase in abundance of tauro- and glycine- conjugated primary bile acids and a decrease in abundance of the secondary bile acid UDCA in MODE offspring. Hepatic CD4 lymphocytes,  $\gamma\delta$ TCR lymphocytes, and regulatory T cells were decreased in MODE offspring liver. After DDC diet feeding, MODE offspring exhibit increased ductular reaction, inflammation, fibrosis, and bile infarcts. UDCA supplementation early in life in MODE offspring ameliorates worsening of cholestatic liver disease by MODE. **Conclusions:** MODE shifts offspring bile acid metabolism including reductions in secondary bile acids with associated decreases in critical immune cell populations. While MODE worsens offspring cholestatic liver disease, early bile acid supplementation may serve to mitigate this developmental programming event.

#### **Abstract 045**

##### **Transcriptional Regulation of UBC9 Reprograms NF- $\kappa$ B Signaling in Alcohol-Associated Liver Disease**

Swati Chandla, Youngyi Lim, Manisha Dagar, Joshua Martinez, Komal Ramani, and Maria Lauda Tomasi  
Karsh Division of Gastroenterology and Hepatology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA

**Background:** Alcohol abuse activates liver-resident macrophages (Kupffer cells, KCs) by increasing gut-derived endotoxin translocation. Activated KCs secrete pro-inflammatory cytokines that drive hepatocyte lipogenesis via NF- $\kappa$ B. Ubiquitin-conjugating enzyme 9 (UBC9), the sole E2 for SUMOylation, regulates transcription by modifying nuclear factors. We previously showed that alcohol induces SRC kinase-mediated phosphorylation of UBC9 at Y68 in KCs, promoting hepatocyte lipogenic gene expression. Here, we examine how UBC9 phosphorylation alters its interactome and inflammatory signaling in alcohol-associated liver disease (ALD). **Methods:** RAW 264.7 cells, THP1 cells, the NIAAA mouse model, in vivo CRISPR/Cas9-mediated UBC9 Y68 editing, phosphomimetic peptides, Western blotting, co-immunoprecipitation, RT-PCR, promoter activity assays, ChIP, single-cell RNA sequencing, and immunofluorescence were used. **Results:** LPS stimulation of RAW 264.7 and THP1 cells reduced UBC9 mRNA expression and promoter activity, lowering total UBC9 protein levels. Despite this reduction, LPS induced phosphorylation of the remaining UBC9 pool, so that phosphorylated UBC9 accounted for approximately 95% of detectable UBC9 protein. Cycloheximide (CHX) chase assays showed that UBC9 has a half-life of ~9 hours, and blocking global protein synthesis reduced total UBC9 without

enriching phosphorylated UBC9, indicating that phosphorylation is not a passive consequence of protein loss. Notably, LPS failed to further reduce total UBC9 levels in CHX-treated cells, suggesting that LPS-mediated UBC9 downregulation requires active, translational-dependent processes. Proteasome inhibition with MG132 prevented the LPS-induced reduction in total UBC9 protein, supporting a role for proteasome-dependent degradation in regulating UBC9 protein abundance. Functionally, a UBC9 Y68 phosphomimetic peptide enhanced pro-inflammatory cytokine expression. Mass spectrometry-based interactome analysis revealed that phosphorylated UBC9 preferentially associates with NF- $\kappa$ B p65 and related inflammatory signaling components. These interactions were validated by co-immunoprecipitation and immunoblotting. In vivo, CRISPR/Cas9-mediated mutation of UBC9 Y68 to phenylalanine (UBC9 Y68F) impaired nuclear translocation of NF- $\kappa$ B p65 and reduced expression of SREBF1 and CEBP $\beta$  by diminishing p65 occupancy at their promoters. **Conclusion:** These findings indicate that UBC9 functions as a regulatory switch, whereby reduced mRNA levels and kinase-mediated phosphorylation reprogram SUMOylation signaling toward NF- $\kappa$ B activation in Kupffer cells, driving inflammatory and lipogenic responses in alcohol-associated liver disease. Targeting upstream mechanisms linking UBC9 mRNA loss to its phosphorylation may provide novel therapeutic strategies for ALD. **Acknowledgment:** This work was supported by NIH/NIAAA grants R01AA029723 (Role of phospho UBC9 in alcohol-associated liver disease).

#### Abstract 046

##### Integrated Cross-Species Analysis of Circular and Linear RNA Landscapes in Alcohol-Associated Liver Disease

Hongkun Joy Lu, Nan Wu, Yun-Ling Tai, Grayson Way, Hui Li, Meiyi Song, Chen Chen, Sareh Bayatpour, Derrick Zhao, Lianyong Su, Xuan Wang, Emily Gurley, Phillip B. Hylemon, and Huiping Zhou  
Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University and Richmond Veterans Affairs Medical Center, Richmond, VA

**Background:** Alcohol-associated liver disease (ALD) is a major cause of liver-related death worldwide, yet patients still lack effective treatments and reliable non-invasive diagnostic tools. Developing preclinical models that truly reflect human ALD has also remained challenging. Chronic alcohol use disrupts liver metabolism, damages cellular organelles, and drives inflammation, but the molecular mechanisms linking these processes remain poorly understood. Clinically, liver injury is most often monitored using blood levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST); however, these markers provide limited insight into disease mechanisms and progression. Circular RNAs (circRNAs) are highly stable, covalently closed non-coding RNAs that have emerged as important regulators in metabolic and inflammatory diseases. Despite this, their role in ALD and their potential as diagnostic biomarkers have not been systematically explored. **Methods:** We performed integrated transcriptomic analyses of liver tissue from patients with ALD (n=8) and healthy controls, alongside C57BL/6J mice exposed to the NIAAA chronic-plus-binge alcohol model (n=6) or calorie-matched control diets. Total liver RNA was isolated using TRIzol and analyzed by bulk RNA sequencing or circRNA microarray. Differential gene expression and pathway enrichment analyses were conducted using DESeq2 and Gene Ontology, incorporating newly generated data and publicly available ALD datasets (GSE155907, GSE143318). CircRNA datasets were annotated and integrated across species using circInteractome and circBase. **Results:** In human ALD livers, pathway analysis revealed marked suppression of alcohol metabolism, fatty acid metabolism, and xenobiotic detoxification pathways. Similar metabolic impairments were observed in alcohol-fed mice, with strong downregulation of alcohol and steroid metabolic processes. In both species, linear RNA profiles showed heightened immune activation and evidence of oxidative stress. CircRNA profiling identified 8,003 significantly dysregulated circRNAs in human ALD and 614 in alcohol-fed mice. These circRNAs originated from 4,727 human and 513 murine host genes, respectively, with 279 shared across species, suggesting a conserved circRNA landscape in ALD. Notably, circRNAs derived from GPT2 (ALT) and GOT1/2 (AST) were consistently dysregulated in humans and mice, highlighting their potential as stable biologically relevant markers of liver injury. **Conclusion:** By integrating circular and linear RNA profiles across humans and mice, we identify conserved circRNA host genes linked to ALD pathogenesis. These findings support circRNAs, particularly those derived from clinically relevant transaminase genes, as promising translational candidates for non-invasive diagnostics and future mechanistic studies of ALD progression. **Acknowledgement:** This study was supported by VA Merit Award 5 I01 BX005730; Research Career Scientist Award IK6BX004477; NIAAA 5R01 AA030180.

## Abstract 047

### Complete Loss of Biliary $\beta$ - and $\gamma$ -Catenin Induces Cholestatic Liver Damage and Intestinal Inflammation in Mice

Vik Meadows<sup>1,2,3</sup>, Joanna Kim<sup>1,2,3,4</sup>, Zachary Cannova<sup>1</sup>, Matthew Carson<sup>1,2,3</sup>, Elena Provencal<sup>1</sup>, Minakshi Poddar<sup>1,2,3</sup>, Sucha Singh<sup>1,2,3</sup>, and Satdarshan P Monga<sup>1,2,3,4</sup>

<sup>1</sup>Organ Pathobiology and Therapeutics Institute, <sup>2</sup>Department of Pharmacology and Chemical Biology, <sup>3</sup>Pittsburgh Liver Research Center, <sup>4</sup>Division of Gastroenterology and Hepatology, Department of Medicine, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, PA

**Background:** Loss of  $\beta$ -catenin in adherens junctions is compensated by  $\gamma$ -catenin, a homologous desmosomal protein. Dual loss of  $\beta$ - and  $\gamma$ -catenin in hepatocytes and cholangiocytes leads to progressive intrahepatic cholestasis and mortality. Loss of these catenins in hepatocytes alone leads to cholemia, but without notable mortality. In this study we define the impact of dual catenin loss in cholangiocytes on hepatic and intestinal health. **Methods:** We utilized *Ctnnb1* fl/fl; *Jup* fl/fl mice with *Opn-iCreERT2*<sup>+/-</sup> to delete both  $\beta$ -catenin (*Ctnnb1*) and  $\gamma$ -catenin (*Jup*) from cholangiocytes. Littermate control with no *Opn-iCreERT2*<sup>+/+</sup> were used as controls. All mice received four doses of tamoxifen (100 mg/kg in corn oil) intraperitoneally over seven days. Male and female mice were used in this study. Plasma, liver, ileum, colon, feces, and cecum content were collected from all mice. **Results:** DKO mice exhibit jaundice, over 20% body weight loss by 2 weeks, and elevated serum liver enzymes. Hepatic total bile acids increased DKO mice compared to controls. Histological analysis indicated extensive bile infarcts, immune cell infiltration, stellate cell activation, and severe portal fibrosis in DKO4 mice, along with increased ductular reaction. DKO mice displayed increased portal Sox9-positive cells, some also HNF4-positive, indicating activation of the biliary program in hepatocytes. DKO mice show reduced fecal TBA levels and circulating secondary bile acids compared to control mice. DKO mice displayed abnormal ileal epithelial cell differentiation and increased fecal lipocalin2 compared to control. DKO mice display increased hydrophobicity of bile acid species in the liver and serum compared to control mice. **Conclusions:** Dual loss of  $\beta$ - and  $\gamma$ -catenin in cholangiocytes causes severe intrahepatic cholestatic injury in mice, disrupting ileal epithelial cell populations. Severe bile duct loss in DKO4 and DKO2 mice results in decreased fecal TBA, suggesting that bile duct health may influence gut function through disrupted BA circulation. Understanding the role of cholangiocytes in the gut-liver axis may assist in patient stratification based on fecal biomarkers and microbial signatures.

## Abstract 048

### Loss of Hepatic Lipid Transporter Protein VPS13D Promotes Alcohol-Associated Steatohepatitis in Mice

Chen Zhang, Hong-Min Ni, and Wen-Xing Ding

Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, Kansas City, KS

**Background:** Alcohol-associated liver disease (ALD) remains a major cause of liver-related illness and death worldwide without effective treatments. Vacuolar Protein Sorting 13 Homolog D (VPS13D) is a lipid transporter that is found at organelle contact sites, particularly between the endoplasmic reticulum (ER) and mitochondria, as well as between mitochondria and lipid droplets. This positioning is essential for lipid transfer and communication between organelles. The aim of this study was to investigate the role of VPS13D in regulating mitochondrial-ER contact and its impact on lipid metabolism in the context of ALD. **Methods:** *Vps13d* Flox/Flox mice were crossed with albumin Cre mice to generate liver-specific *Vps13d* knockout (L-*Vps13d* KO) mice. These mice were subjected to the Gao-binge alcohol model and an acute binge model. Biochemical, histological, transcriptomic, and metabolomic analyses were conducted on blood and liver tissues collected from these mice. **Results:** Both mRNA and protein levels of VPS13D were elevated, alongside notable histopathological features such as megamitochondria, steatosis, and Mallory-Denk bodies in liver samples of human alcohol-associated hepatitis (AH). L-*Vps13d* KO mice fed with Gao-binge alcohol had decreased hepatic levels of alcohol dehydrogenase 1 (ADH1) and associated with increased serum ethanol levels compared with matched wildtype (WT) mice. Increased serum ethanol levels were also confirmed in L-*Vps13d* KO mice after acute ethanol gavage. Gao-binge alcohol-fed L-*Vps13d* KO mice had significantly increased serum ALT levels, hepatic triglyceride and infiltrated inflammatory cells associated with increased lipid peroxidation and DNA damage. Mechanistically, loss of hepatic VPS13D led to increased accumulation of megamitochondria and mega-lipid droplets associated with decreased mitochondria-ER contacts. Furthermore, cytokine array analysis revealed that Gao-binge alcohol-fed L-*Vps13d* KO mice exhibited increased serum cytokines and chemokines as well as EGF growth factors and the receptor for advanced glycation end products (RAGE), which are associated with severe adipose tissue atrophy. VLDL secretion was impaired in Gao-binge alcohol-fed L-*Vps13d* KO mice, which was also consistent with decreased expression of VLDL secretion-related

genes in RNAseq analysis. Targeted metabolomics showed defective long-chain fatty acid metabolism, which was associated with impaired peroxisome biogenesis at the mRNA and protein levels. **Conclusion:** Loss of VPS13D promotes ALD by impairing ethanol clearance, VLDL secretion, and peroxisomal metabolism, resulting in severe steatosis, liver injury, and inflammation in mice. Targeting VPS13D-mediated organelle contact may be a promising strategy to protect against ALD.

## Poster Presentations

### Poster Session – Cancer Pathobiology

#### Poster Board 1

##### Abstract 049

#### Detection of Elevated Anti-Ectopically Phosphorylated PDGFRA Antibodies in Serum to Predict Hepatocellular Carcinoma

Muhamuda Kader<sup>1</sup>, Yan-Ping Yu<sup>1</sup>, Silvia Liu<sup>2</sup>, David Geller<sup>3</sup>, and Jian-Hua Luo<sup>1</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup>Department of Pharmacology, <sup>3</sup>Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA

**Background:** Hepatocellular carcinoma (HCC) is one of the most lethal malignancies for humans. The diagnosis of early-stage HCC remains challenging. Serum biomarkers for HCC are important components to screen for the disease.

**Methods:** In this study, we analyzed 195 serum samples from individuals with HCC or without HCC but with a variety of non-malignant liver conditions. **Results:** We detected significantly elevated anti-ectopically phosphorylated PDGFRA (anti-epPDGFRA) antibody levels in the serum samples of HCC patients in comparison with those from non-HCC individuals. When the samples were randomly split, a logistic regression model based on the pretreatment anti-epPDGFRA levels produced a prediction rate of 85.3% at the training cohort, 65.7% in the testing cohort and 74.8% in the combined training and testing cohorts. The elevation of anti-epPDGFRA antibodies persisted even after the HCC were surgically removed. The post-treatment anti-epPDGFRA antibodies produced similar accuracy of the prediction. When serum AFP and MAN2A1-FER fusion gene status were incorporated into the anti-epPDGFRA prediction model, it improved the accuracy of the model to above 90% accuracy consistently. **Conclusion:** These results suggest that detection of anti-epPDGFRA holds promise to be a cost-effective and efficient approach to screen HCC.

#### Poster Board 2

##### Abstract 050

#### Characterizing Neuropilin Receptor Status and Vascular Density in Squamous Cell Carcinomas of Various Grade

Alison Sham<sup>1</sup>, Lili Wang<sup>1</sup>, Abdulrahman Nakshabandi<sup>1,2</sup>, Joud Omari<sup>1,2</sup>, Sandy Huynh<sup>1</sup>, Harsh N. Dongre<sup>1,3</sup>, and Diane R. Bielenberg<sup>1,3</sup>

<sup>1</sup>Vascular Biology Program, Boston Children's Hospital, Boston, MA, <sup>2</sup>Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA, <sup>3</sup>Department of Surgery, Harvard Medical School, Boston, MA

**Background:** Cutaneous squamous cell carcinomas (SCC) are the second most common skin cancer and associated with ultraviolet radiation. SCCs often originate from precancerous actinic keratosis lesions. When caught early, SCCs are highly curable but can progress and even metastasize if neglected or undetected. Our laboratory previously found a correlation between SCC grade and Neuropilin-1 receptor expression in human patient biopsies. Neuropilin (NRP) proteins, NRP1 and NRP2, are unique transmembrane receptors that bind both pro-angiogenic ligands such as vascular endothelial growth factor (VEGF) and anti-angiogenic ligands such as Semaphorin 3 (SEMA3) family members. VEGF/NRP interaction promotes angiogenesis and tumor growth, whereas SEMA3/NRP signaling inhibits neovascularization and tumor progression. Building on our previous research, the present study investigates the link between NRP expression in the tumor cells and tumor-associated endothelium with tumor vascular density in SCC models of varying tumor grade.

**Methods:** Growth rates and differentiation status were compared in vitro for three human SCC cell lines (A431, DJM, and SCC13). Additionally, three SCC cell lines were injected subcutaneously into immunocompromised mice (n = 5 mice per group). After 5-9 weeks, mice were euthanized and necropsied. Excised tumors were embedded in OCT or formalin-fixed and paraffin-embedded. Immunohistochemistry was used to examine expression of cytokeratins, CD31 (blood vessel marker), Ki67 (proliferation marker), and NRP1/2 in all tumor specimens. **Results:** Undifferentiated A431 cells

grew rapidly in vitro and in vivo. Histologically, A431 tumors exhibited necrotic tumor cores with high vascular density only on the proliferating tumor leading edges. Moderately differentiated DJM cells proliferate at a similar rate to A431 cells in vitro and in vivo and contained adequate microvessel density throughout the tumor stromal compartments and lacked necrosis. SCC13 cells are highly differentiated and consequently grew significantly slower than either A431 or DJM cells in vitro and in vivo. SCC13 tumors contained numerous keratin pearls and minimal microvessel density. NRP1 expression in the tumor cells correlated with tumor grade with high expression in SCC13 and low in A431. **Conclusions:** High NRP1 expression in cutaneous SCC tumor cells does not correlate with high microvessel density in xenograft models. This data is in contrast to our previous studies in oral squamous cell carcinoma and suggests that the tumor microenvironment may play a role in neovascularization and metastasis. **Acknowledgements:** This work was supported by the American Society for Investigative Pathology Summer Research Opportunities Program in Pathology (AS) and the Vascular Biology Program at Boston Children's Hospital (DRB).

### Poster Board 3

#### Abstract 051

#### **Capsaicin Suppresses the Growth of Human Endometrioid Ovarian Cell Carcinoma Via the Calcium Signaling Pathway**

Piyali Dasgupta<sup>1</sup>, Ramillie Acevedo Reyes<sup>2</sup>, Gladielys Torres Rivera<sup>2</sup>, Kushal J. Modi<sup>1</sup>, Sarah L. Miles<sup>1</sup>, and Yi Charlie Chen<sup>3</sup>

<sup>1</sup>Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV,

<sup>2</sup>University Ana G. Méndez, Carolina, Puerto Rico, <sup>3</sup>Department of Biology and Environmental Science, Bluefield State University, Bluefield, WV

**Background:** Endometrioid ovarian cancer (EOC), a distinct subtype of epithelial ovarian cancer, is associated with endometriosis and Lynch syndrome, and is often accompanied by synchronous endometrial carcinoma. It accounts for about 15-20% of all ovarian cancers. Due to a lack of detection methods for early stage EOC, patients are often diagnosed with advanced disease and have poor survival outcomes. Another challenge with EOC therapy is the propensity of the tumor to become resistant to chemotherapy. All these factors underline for the need for novel therapies in human EOC patients. The primary objective of this research project was to test the growth-suppressive activity of capsaicin (the spicy ingredient in chili peppers) in human endometrioid ovarian cancer. **Methods:** MTT assays were performed to evaluate the growth-inhibitory activity of capsaicin in human EOC cells. The anti-tumor activity of capsaicin in human EOC tumors was also observed using chicken chorioallantoic membrane (CAM) models. The signaling pathways underlying the growth-hindering properties of capsaicin were investigated using highly specific chemical inhibitors to known signaling pathways. **Results:** Capsaicin decreased the viability of human EOC cell lines in a concentration-dependent and time-dependent manner. MTT assays revealed that capsaicin did not impact the viability of normal human lung cells, liver cells or kidney cells. Capsaicin decreased the growth rate of human EOC tumors xenografted on chicken CAM. The growth-inhibitory effects of capsaicin were mediated by decrease of intracellular calcium levels in human EOC cells. **Conclusions:** Capsaicin may be a promising nutrition-based therapy for human EOC. **Acknowledgements:** Funding for our study was supported by a NIH R15-AREA Grant (2R15CA161491-03) and a Center for Natural Products pilot grant from the WV-INBRE Grant Program (P20GM103434, PI: Gary Rankin).

### Poster Board 4

#### Abstract 052

#### **Characterization of Modified Monobenzone as Targeted Therapies for Metastatic Melanoma**

Nakisha S. Rutledge<sup>1</sup>, Sofia Vujevich<sup>2</sup>, Shitong Yang<sup>2</sup>, Eddie Dao Zhou<sup>2</sup>, Cheryl Hai-Yang Tang<sup>2</sup>, Ari Balal<sup>2</sup>, SonBinh Nguyen<sup>2</sup>, and Caroline Le Poole<sup>2</sup>

<sup>1</sup>Temprian Oncology Inc., Chicago, IL, <sup>2</sup>Department of Dermatology, Northwestern University, Chicago, IL

**Background:** Melanoma is the deadliest form of skin cancer, accounting for the majority of skin cancer-related deaths. While early-stage melanoma has a five-year survival rate exceeding 99%, this falls to ~35% once metastasis occurs. Despite advances in targeted and immunotherapeutic approaches, metastatic melanoma remains difficult to fully eradicate. Targeting the melanogenic pathway offers a novel strategy, as melanin biosynthesis occurs exclusively in melanocytes and melanoma cells via oxidation of L-tyrosine by tyrosinase and TRP-1 within melanosomes. **Methods:** Monobenzyl ether of hydroquinone (MBEH) is a phenolic compound historically used as a topical depigmenting agent that causes melanocyte destruction via tyrosinase-dependent toxic quinone generation and subsequent immune activation. MBEH has demonstrated anti-tumor effects in vivo when applied topically to tumors, including >4-fold tumor

volume reduction and increased CD8+ T cell infiltration in B16F10-challenged mice. To mitigate the severe toxicity and poor solubility of MBEH and to enhance therapeutic selectivity, we synthesized a lipophilic derivative and encapsulated it within ~100–150 nm liposomal nanoparticles (Tonc-LNP). **Results:** Treatment with TOnc-LNP resulted in pronounced, concentration-dependent killing of B16-F10 murine cutaneous melanoma cells and primary human uveal melanoma cells in vitro, reducing viable cell populations by more than 90% within 24 hours. In contrast, unencapsulated MBEH exerted only limited growth-inhibitory effects. Time-resolved live-cell imaging revealed extensive nanoparticle-induced membrane disruption, culminating in widespread cell death and lysis. In a murine pulmonary metastasis model using immunodeficient mice, systemic administration of TOnc-LNPs markedly reduced metastatic burden, with treated animals exhibiting over 50% fewer grossly visible lung metastases and more than a 40% reduction in gp100-positive tumor coverage relative to control mice, indicating substantial decreases in both metastatic nodule number and tumor-occupied lung area. Consistent with these findings, in an immunocompetent murine pulmonary metastasis model, TOnc-LNP treatment resulted in a greater than 70% reduction in metastatic lung nodules compared with controls.

**Conclusions:** These results demonstrate that nanoparticle-mediated delivery significantly enhances the therapeutic efficacy of phenolic prodrugs, achieving potent suppression of metastatic melanoma. Collectively, these findings support the further development of lipid nanoparticle formulations of modified monobenzone as a promising systemic strategy for melanoma therapy, with potential translational relevance for improving clinical outcomes in patients with metastatic disease.

## Poster Board 5

### Abstract 053

#### Identifying the Molecular Mechanism of Bilateral Diffuse Uveal Melanocytic Proliferation

Sarah L. Miles,<sup>1</sup> and Jose S. Pulido<sup>2</sup>

<sup>1</sup>Marshall University Joan C. Edwards School of Medicine, Huntington WV, <sup>2</sup>Wills Eye Institute, Philadelphia PA

**Background:** Bilateral diffuse uveal melanocytic proliferation (BDUMP) represents a rare paraneoplastic ocular syndrome characterized by profound bilateral vision loss caused by benign pigmented and non-pigmented uveal melanocytic tumors, exudative retinal detachment and rapid cataract formation. Correct diagnosis is often delayed as ocular symptoms frequently appear prior to the discovery of a primary malignancy. Our laboratory identified the presence of Cultured Melanocyte Elongation and Proliferation (CMEP), a serum borne factor in patients with BDUMP syndrome. However, the molecular identity and melanocyte selective mechanism of CMEP remain unknown. The lack of a clinical molecular diagnostic marker or molecular target for therapy has greatly hampered the clinical management of this syndrome. **Methods:** Normal neonatal human melanocytes (HEMn-LP) and keratinocytes (HEKn) were grown under standard culture conditions. HEMn-LP were seeded in 35mm cell culture dishes and treated using Media 254 containing PSA, with HMGS supplement at a 1:1 (v/v) dilution of HMGS to patient serum, maintaining 1% of media volume. HEKn cells were grown in Media 154 with HKGS supplement and treated with the same method. Non-patient controls were treated standard HMGS or HKGS supplementation. PathScan® Antibody Arrays (Cell Signaling) were used following manufacturers protocol to evaluate CMEP-induced changes in signaling mechanisms/pathways in HEMn-LP (and HEK) cells in response to BDUMP patient serum. Antibody array results were validated by western blot analysis. Inhibition of CMEP induced signaling was evaluated using chemical cMET inhibitor PF-04217903 mesylate. **Results:** Cell signaling activation analysis in HEMn-LP and HEKn cells revealed a CMEP-dependent activation of the hepatocyte growth factor (cMET) receptor and downstream PI3K/AKT pathway signaling. Chemical inhibition of cMET abrogated CMEP-stimulated activation in HEMn-LP and HEKn, resulting in the inhibition of CMEP induced phosphorylation of AKT and proliferation in HEMn-LP cells. CMEP factor selectively induces activation of cMET in a non-cell type specific manner. However, cMET activation result in differential activation of the PI3K/AKT pathway, which is cell type dependent, resulting in the activation of proliferative and anti-apoptotic signaling pathways in epidermal melanocytes compared to keratinocytes. **Conclusion:** This data provides the first molecular evidence suggesting a direct role for the HGF/cMET axis in the etiology of BDUMP syndrome initiation and progression. This finding is of significant translational impact in understanding the molecular mechanism underlying BDUMP syndrome and aiding in the identification of clinically relevant molecular targets for both diagnosis and drug-based therapy. **Acknowledgement:** Funding in part WV-INBRE (P20GM103434) Cancer Biology Pilot Funding. Collaborator Jose S. Pulido, Wills Eye Institute.

## Poster Board 6

### Abstract 054

#### Alterations in Purinergic Signaling in Colorectal Cancer Impact Proliferation and Apoptosis

Ana G. Pettijohn and Kristen A. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

**Background:** Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related mortality. Emerging evidence indicates purinergic signaling plays a role in a variety of cancers. Purinergic signaling, mediated by extracellular nucleotides and P2 receptors, has been suggested to be involved in regulation proliferation, apoptosis and cell migration under normal conditions. Of the 15 purinergic P2 receptors, healthy gut epithelium has functional P2Y1 and P2Y2 receptors while several CRC derived cell lines lack functional P2Y receptors suggesting these may have an altered purinergic receptor profile. ATP activation of P2Y receptors has been shown to inhibit prostate cancer growth, however the potential role of P2Y receptor activation during CRC remains largely unknown. We hypothesize that gut epithelial purinergic receptors are altered in CRC and could be potential therapeutic targets. **Methods:** *In silico* analysis of RNA sequencing data from The Cancer Genome Atlas (TCGA) was used to assess changes in purinergic receptor expression in CRC patient tumors versus normal tissue. We performed P2 agonist treatments in CRC cell lines T84 and HCT-8 cells expressing the calcium indicator GCaMP6s. To assess the functional consequences of P2Y1 in cancer, we transduced T84 and HCT-8 cells to knock-in P2Y1. In both parental and P2Y1 knock-in T84 or HCT-8 cells, we performed live imaging microscopy of cell growth and migration. To assess proliferation, cells were treated with vehicle control or P2 receptor agonists and labeled by EdU assay. To evaluate apoptosis, cells were treated with ATP (250 $\mu$ M and 500 $\mu$ M) and imaged with propidium iodide staining after 24 and 48 hours post treatment. **Results:** Analysis of The Cancer Genome Atlas (TCGA) reveals that P2Y1 expression is significantly reduced in CRC tumors compared with healthy tissue, regardless of patient demographics, cancer stage or cancer type. Both parental T84 and HCT-8 had weak to no calcium signaling response following any P2Y select agonist activation, indicating impaired purinergic signaling. Reintroduction of P2Y1 in T84 and HCT-8 cells resulted in a significant change in calcium signaling following P2Y1 select agonist activation. P2Y1 knock-in T84 cells had clear altered morphology and exhibited delayed cell growth compared to parental cells. Furthermore, P2Y1 knock-in cells presented with increased cell death following ATP treatment compared to parental cells. **Conclusion:** Collectively this data suggests that purinergic signaling downregulation may play a role in the colorectal cancer phenotype and that pharmacologic modulation of this pathway could provide a novel therapeutic strategy for CRC treatment.

## Poster Board 7

### Abstract 055

#### Unlocking ABCC1 as a Potential Prognostic Biomarker In Laryngeal Cancer Health Outcomes

Christina Gobin<sup>1</sup>, Matthew Chang<sup>1</sup>, Jai Walker<sup>2</sup>, and Kristianna M. Fredenburg<sup>1</sup>

<sup>1</sup>Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, <sup>2</sup>Wellstar MCG Health Medical Center, Augusta, GA

**Background:** Overexpression of ABCC1 is associated with poor overall survival across many cancers and has been associated with decrease response to chemotherapy. Our *in vivo* and *in vitro* studies have shown that ABCC1 expression is higher in laryngeal squamous cell carcinoma (LSCC) tissues from individuals with African heritage. Here, we further investigate ABCC1 as a potential prognostic marker in laryngeal cancer health outcomes. **Methods:** ABCC1 gene expression was assessed on tumor tissue from 32 advanced stage LSCC patients from differing populations via RT-PCR. Immunohistochemistry (IHC) for ABCC1 protein expression was performed on tissue microarrays (TMAs) generated from 61 paraffin-embedded tissues of advanced stage LSCC from patients of differing heritages. Machine learning based digital pathology software (QuPath) was used to semi-quantitate ABCC1 IHC protein expression as H scores. Age, treatment, self-reported heritage, N stage, tumor histologic grade, and ABCC1 H-scores were evaluated as predictors of patient mortality risk via multivariable Cox proportional hazards (CPH) model. The median Cox risk score (cutoff > 0.971) was used to stratify patients into low and high mortality risk groups. The relationship among the clinical features and ABCC1 in low and high mortality risk groups was explored by Chi Square analysis. Overall survival was assessed using the Kaplan-Meier method. **Results:** LSCCs from patients with African heritage demonstrated a 2.2-fold elevation in ABCC1 gene expression and higher ABCC1 protein expression (H-score) compared with the reference population ( $p < 0.05$ ). Controlling for treatment, African heritage patients with low ABCC1 H-scores had a high mortality risk ( $p < 0.05$ ), and

patients from the reference population with metastatic disease and high ABCC1 H-scores had a low mortality risk ( $p < 0.05$ ). Overall, high-risk patients had significantly worse overall survival (HR = 2.46, 95% CI [1.27-4.74], log rank  $p < 0.01$ ) and high mortality risk was associated with low ABCC1 H-scores and regional metastatic disease ( $p < 0.05$ ). **Conclusion:** Although LSCC patients with African heritage have higher ABCC1 expression compared with the reference population, low ABCC1 H-scores carry a high mortality risk in this group. LSCC patients from the reference population with high ABCC1 H-scores have reduced mortality risk despite having regional metastatic disease. Future studies will involve exploring mechanistic implications of ABCC1 expression on treatment outcomes across differing heritages.

**Acknowledgments:** This research was supported by Congressionally Directed Medical Research Program grant (W81XWH-22-1-0935) and by the UF Health Cancer Institute (P30CA247796).

## Poster Board 8

### Abstract 056

#### From AUC to Clinical Adoption: Implementing AI in Lung Cancer Histopathology and Cytopathology

Meghdad Sabouri Rad<sup>1</sup>, Kaitlyn Macdonald<sup>2</sup>, and Patel Palak<sup>1</sup>

<sup>1</sup>Pathology Department, SUNY Upstate Medical University, Syracuse, NY, <sup>2</sup>Norton College of Medicine, SUNY Upstate Medical University, Syracuse, NY

**Background:** Artificial intelligence (AI)-assisted digital pathology tools are increasingly applied to lung cancer diagnostics, showing strong retrospective performance in malignancy detection, subtyping, grading, and biomarker prediction (AUC 0.75–0.99). However, translation into routine clinical practice remains constrained by operational, educational, and infrastructural barriers within real world laboratory environments. Precision oncology has become increasingly constrained by complex histopathologic subtyping and reflex molecular testing. There is a critical need to evaluate AI not only by accuracy metrics, but by its impact on diagnostic turnaround time (TAT), workflow distribution, case triage, and pathologist cognitive load. **Methods:** We developed a multi-dimensional implementation framework to evaluate deep learning architectures, including CNNs, Attention-based Multiple Instance Learning (ABMIL), and Pathology Foundation Models (PFMs), against a prospectively monitored "silent trial" workflow. We modeled the operational impact of three specific triage protocols: (1) negative slide exclusion, (2) urgency-based prioritization for high-grade neuroendocrine neoplasms, and (3) EGFR biomarker pre-screening. Endpoints included TAT shifts, review time per case, cross-platform accuracy, and the risk of unintended delays in non-target cases. **Results:** While PFMs effectively mitigate domain shift, their clinical utility was maximized only within a "shared-decision" triage model that preserved pathologist oversight. AI-guided triage reduced confirmatory EGFR testing volume by 43% without compromising sensitivity. A significant "non-target delay" risk was identified: cases outside the training distribution (e.g., rare sarcomatoid carcinomas) experienced a 15% increase in TAT without explicit "safety net" policies. ABMIL-based interpretability maps reduced adjudication time by 22% compared to traditional black-box architectures, directly addressing the "trust gap" in clinical adoption. **Conclusions:** AI integration in cancer pathology must pivot from algorithm benchmarking to clinical implementation using a robust and expandable framework. Silent trials and safety-net protocols can alleviate the molecular testing bottleneck and shorten time-to-treatment. This helps shift AI from a retrospective research tool into a prospective clinical system that directly enhances the delivery of precision oncology. By focusing on operational endpoints such as review burden, training time, cost efficiency, and laboratory throughput, this framework supports the sustainable adoption of AI-enabled systems that meaningfully accelerate precision oncology and reduce time-to-treatment.

## Poster Board 9

### Abstract 057

#### Harnessing Transcribed Ultraconserved Regions as Predictors of Head and Neck Cancer Treatment Outcomes

Matthew S. Chang<sup>1</sup>, Jinmai Jiang<sup>2</sup>, Christina M. Gobin<sup>1</sup>, Rui Fernandes<sup>3</sup>, Chayil C. Lattimore<sup>1</sup>, James Menefee<sup>4</sup>, Mingyi Xie<sup>5</sup>, Rolf Renne<sup>6</sup>, Thomas D. Schmittgen<sup>2</sup>, and Kristianna M. Fredenburg<sup>1</sup>

<sup>1</sup>Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, FL, <sup>2</sup>Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL, <sup>3</sup>Oral and Maxillofacial Surgery, University of Florida College of Medicine – Jacksonville, Jacksonville, FL, <sup>4</sup>Department of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, <sup>5</sup>Department of Biochemistry and Molecular Biology, <sup>6</sup>Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL

**Background:** Noncoding RNAs continue to gain momentum as predictive and prognostic biomarkers of disease. Transcribed ultraconserved regions (T-UCRs) have emerged as potential clinical biomarkers as they exhibit tissue and disease-specific expression patterns. The goal of our study was to characterize and examine T-UCR expression in tumors from patients with advanced stage head and neck squamous cell carcinoma (HNSCC). **Methods:** RNA was extracted from 32 fresh-frozen advanced-stage laryngopharyngeal tumors and 11 unpaired normal mucosal tissues. The expression of 481 T-UCRs was assessed by qPCR. 1870 miRNAs were profiled using small RNA sequencing. Differential expression of 208 UCRs and 1870 miRNAs was assessed between laryngeal tumor and normal samples via two-tailed t-tests. Benjamini-Hochberg adjustment for p-values was performed to identify significantly differentially expressed (DE) UCRs and miRNAs. Spearman Rho correlations were conducted to explore relationships between DE UCRs and miRNAs. These significantly correlated UCR-miRNA pairs were subsequently plotted in a network analysis by their binding energies (kJ/mol) according to the Fruchterman-Reingold force directed layout to visualize how they interact. Using predicted gene targets of DE miRNAs identified by the miRsystem database, a miRNA-mRNA network was plotted. Gene Ontology (GO) analysis was performed with the Database for Annotation, Visualization, and Integrated Discovery (DAVID) database on the predicted gene targets, followed by an overrepresentation analysis using the KEGG database to identify associated signaling pathways and cellular processes. **Results:** 19 UCRs and 262 miRNAs were found to be significantly DE based on adjusted p value < 0.05 and  $-1.5 < \log_{2}FC > 1.5$ . 15 UCRs were upregulated and 4 UCRs were downregulated in tumor samples with four T-UCRs exclusive to HNSCC—UC.164, UC.267, UC.424, and UC.401. 176 miRNAs were upregulated and 86 miRNAs were downregulated in tumor samples. Using significantly correlated 15 UCRs and 33 miRNAs, UCR-miRNA network analysis uncovered UC.267 as specifically associated with 8 DE miRNA. Functional enrichment of the predicted gene targets of these 8 DE miRNAs indicated involvement in oncogenic pathways, specifically linked to resistance to EGFR tyrosine kinase inhibitors. Among these targets are several genes with approved targeted therapies, including receptor tyrosine kinases KIT and RET, as well as FGFR3, PIK3CA, and VEGFA. **Conclusion:** This study provides the first characterization of T-UCRs in HNSCC, identifying a T-UCR-miRNA regulatory network unique to advanced stage HNSCC. Among them, UC.267 stands out as a promising biomarker for predicting chemotherapeutic targets for advanced-stage disease. Overall, our findings provide a basis to further examine T-UCRs as predictive biomarkers of head and neck cancer treatment outcomes. **Acknowledgments:** This research was supported by the UF Health Cancer Institute (P30CA247796).

## Poster Session – Cardiac and Vascular Pathobiology

### Poster Board 10

#### Abstract 058

#### Doxorubicin Imprints a Pro-Fibrotic Program in CD8<sup>+</sup> T-Cells that Promotes Mechanical Remodeling of Cardiac Extracellular Matrix

Ramona Emig<sup>1\*</sup>, Abraham L. Bayer<sup>2\*</sup>, Celina Kley<sup>2</sup>, Maria Antonia Zambrano<sup>1,2</sup>, Zachary Robbe<sup>1,2</sup>, Jose Max Narvaez-Paliza<sup>3</sup>, Sanam Alilou<sup>3</sup>, Sarah Powers<sup>4</sup>, Jenica N. Upshaw<sup>3</sup>, Aarti Asnani<sup>3</sup>, and Pilar Alcaide<sup>1</sup>

<sup>1</sup>Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, <sup>2</sup>Department of Immunology, Tufts University School of Medicine, Boston, MA, <sup>3</sup>CardioVascular Institute, Beth Israel Deaconess Medical Center, Boston, MA, <sup>4</sup> Molecular Cardiology Research Institute, Tufts Medical Center, Boston, MA, \*Indicates co-first authorship

**Background:** Anthracyclines, a class of highly utilized chemotherapy drugs, often lead to cardiotoxicity and chronic heart failure (HF) characterized by cardiac atrophy, fibrosis and reduced contractile function. No preventative therapies are approved for broad use in patients treated with anthracyclines. We previously showed that the anthracycline doxorubicin (DR) elicits a cardiotoxic CD8<sup>+</sup> T-cell response contributing to cardiac fibrosis through interactions with cardiac fibroblasts. However, whether T-cells directly remodel the extracellular matrix (ECM) remains unknown. Here, we tested the hypothesis that CD8<sup>+</sup> T-cells from anthracycline-treated patients and mice adopt a pro-fibrotic phenotype, enabling them to stiffen cardiac ECM. **Methods:** We performed RNA-sequencing of human circulating CD8<sup>+</sup> T-cells from patients before and 3 months after anthracycline treatment and of murine DR- or PBS-treated cardiac CD8<sup>+</sup> T-cells. *In vitro*, we used murine primary CD8<sup>+</sup> T-cells and pharmacological inhibition of HIF1 $\alpha$  (echinomycin), extracellular TGF $\beta$ 1 ( $\alpha$ TGF $\beta$ 1) or lysyl oxidation ( $\beta$ -aminopropionitrile, BAPN) to dissect the molecular mechanism involved in CD8<sup>+</sup> T-cell mediated cardiac ECM remodeling. Uniaxial tensile testing of cardiac ECM was used to characterize the mechanical properties of the ECM and the ability of CD8<sup>+</sup> T-cells to directly alter ECM mechanics. **Results:** Anthracyclines induced a

novel pro-fibrotic signature characterized by higher expression of ECM and ECM-crosslinking proteins (*Col1a1*, *Lox*, *Lox1*) in human circulating CD8<sup>+</sup> T-cells and in murine circulating, splenic and cardiac CD8<sup>+</sup> T-cells. This profibrotic gene expression was reproduced in polarized, DR-treated CD8<sup>+</sup> T-cells *in vitro* demonstrating that the direct action of DR on CD8<sup>+</sup> T-cells causes this signature. The secretome of DR-treated CD8<sup>+</sup> T-cells stiffened cardiac ECM which was fully prevented by inhibition of lysyl oxidases (LOX), HIF1 $\alpha$  or TGF $\beta$ 1. Mechanical characterization of cardiac ECM for DR-treated mice revealed that the loss of systolic function *in vivo* correlated strongly with ECM stiffness in DR-induced HF.

**Conclusion:** Our data demonstrate that DR imprints a novel intrinsic pro-fibrotic function in CD8<sup>+</sup> T-cells.

Mechanistically, DR activates HIF1 $\alpha$  signaling in CD8<sup>+</sup> T-cells, inducing the expression and secretion of TGF $\beta$ 1. In an autocrine loop, CD8<sup>+</sup> T-cell derived TGF $\beta$ 1 enhances LOX expression and secretion, which is sufficient to stiffen cardiac ECM in the absence of fibroblasts. Thereby, CD8<sup>+</sup> T-cells contribute to cardiac ECM crosslinking and mechanical remodeling leading to DR-induced HF. **Acknowledgements:** We are thankful to the patients for their participation in this study. We acknowledge Dr Albert Tai (Tufts University Core Facility Genomics) for support with sequencing experiments and analysis, and financial support from the NIH and the German Research Foundation.

## Poster Board 11

### Abstract 059

#### Immunologically Active Cardiac Fibroblasts Express MHC-II in Humans and Promotes Doxorubicin Cardiotoxicity in Mice

Maria Antonia Zambrano<sup>1,2</sup>, Abraham L. Bayer<sup>3</sup>, Ramon Bossardi Ramos<sup>4</sup>, Constanza Stortini<sup>2</sup>, Kenneth Bedi<sup>5</sup>, Kuljeet Kaur<sup>6</sup>, and Pilar Alcaide<sup>2</sup>

<sup>1</sup>Immunology Graduate Program, Tufts University Graduate School of Biomedical Sciences, Boston, MA, <sup>2</sup>Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, <sup>3</sup>Tufts Medical School, Boston, MA, <sup>4</sup>Department of Molecular and Cellular Physiology, Albany Medical College, Albany NY, <sup>5</sup>University of Pennsylvania Cardiovascular Institute, Philadelphia, PA, <sup>6</sup>Harvard University, John A. Paulson School of Engineering and Applied Sciences, Boston, MA

**Background:** Cancer patients receiving Doxorubicin (DR), one of the most widely used chemotherapy agents, often develop cardiotoxicity and heart failure and show circulating IFN $\gamma$ <sup>+</sup>CD8<sup>+</sup> cytotoxic T cells (Tc1s). In mice, Tc1 cells are necessary for pathological cardiac fibrosis and contractile dysfunction through actions on cardiac fibroblasts (CFBs). Whether CFBs adopt immune-like functions to promote cardiac inflammation in DR cardiotoxicity remains unknown. We tested the hypothesis that DR induces immunologically active CFBs that promote T cell activation and cardiotoxicity.

**Methods:** Publicly available snRNA-Seq of donor and anthracycline-treated patient hearts was used to assess CFBs populations. Human cardiac samples were tested for inflammatory and immunomodulatory genes by qPCR and immunofluorescence (IF). Primary murine and human CFBs were treated with DR, IFN $\gamma$ , or co-cultured with Tc1s in transwells and analyzed for CFB MHC-II and CXCL9 expression, and ability to trigger Tc1 migration. We used flow cytometry, immunofluorescence, and qPCR. Wild-type (WT), T cell-deficient (*Tcr $\alpha$ <sup>-/-</sup>*), *Tcf21<sup>iCre</sup>;mT/mG*, and *Tcf21<sup>iCre/+</sup>Mhcll<sup>fl/fl</sup>* mice, treated with Tamoxifen to induce specific deletion of MHC-II (CFB-*Mhcll<sup>-/-</sup>* and CFB-*Mhcll<sup>+/-</sup>*) were injected with PBS or cumulative DR (5 mg/kg/week) intraperitoneally for 4 or 8 weeks. Contractile function was assessed by echocardiography, flow cytometry used to characterize CFBs and immune cell infiltrates and proliferation by BrdU incorporation in digested hearts, qPCR to analyze whole heart gene expression, and histology to determine cardiac atrophy and fibrosis by H&E and picrosirius red staining, respectively. **Results:** Anthracycline treated patients show reduced cardiac contractile function and elevated gene expression of *HLA-DR* and *HLA-DO*. Sequencing analysis demonstrated enhanced IFN $\gamma$  responsiveness within CFB populations, and IF staining revealed colocalization of PDGFR $\alpha$ <sup>+</sup>HLA-DR<sup>+</sup> cells throughout the myocardium. DR induced CXCL9 expression in human and murine CFBs and show they are sufficient for Tc1 migration in a CXCL9-dependent manner, shown by Tc1 migration inhibition in the presence of anti-CXCL9. However, DR alone was not sufficient to induce class II expression. DR treatment of Tc1 cells enhanced IFN $\gamma$  production, and induced surface MHC-II expression on CFBs. *In vivo*, 8 weeks of DR induced the expression of MHC-II in a subset of CFBs in WT mice. This was T cell dependent, as it was not observed in *Tcr $\alpha$ <sup>-/-</sup>* mice; adoptive transfer of Tc1 cells restored CFB-MHC-II expression in the onset of DR. Lastly, CFB-*Mhcll<sup>-/-</sup>* mice developed less cardiac atrophy and fibrosis, showed improved systolic function, as well as reduced numbers of cardiac CD4<sup>+</sup> T cells in response to DR, compared to CFB-*Mhcll<sup>+/-</sup>* littermate controls. **Conclusions:** Our results demonstrate in anthracycline treated patients and using mouse models, that cardiotoxicity coincides with class II expression on CFBs. We demonstrate that DR induces a pro-inflammatory CFB state in human and murine CFBs, characterized by CXCL9 expression, which results in cytotoxic T

cell migration. DR enhancement of T cell IFN $\gamma$  is necessary for CFB-MHC-II expression *in vitro* and *in vivo*, coinciding with increased pathology. Ultimately, our data positions CFB-MHC-II as a key mediator of fibrosis, immune modulation, and contractile dysfunction in DR cardiotoxicity. **Acknowledgements:** R01HL144477 (ALCAIDE), 3R01HL144477-04S1 (ZAMBRANO) 1F31HL175911-01 (ZAMBRANO).

## Poster Board 12

### Abstract 060

#### **Pentadecanoic Acid (C15:0) Reprograms Mice Cardiac Inflammation–Resolution Pathways altering Hepato-Cardiac Lipid Remodeling under Metabolic Stress**

Vasundhara Kain<sup>1</sup>, Nikhil Chainani<sup>2</sup>, Punith M. Sundaraswamy<sup>3</sup>, Gunjan Upadhyay<sup>1</sup>, Li Shujin<sup>3</sup>, Nitin Krishna<sup>2</sup>, Teerth Pansuria<sup>2</sup>, Shu-Ping Hui<sup>4</sup>, Divyavani Gowda<sup>4</sup>, Siddabasave Gowda B. Gowda<sup>4</sup>, and Ganesh V Halade<sup>1,5</sup>

<sup>1</sup>Heart Institute, Division of Cardiovascular Sciences, Department of Internal Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL, <sup>2</sup>College of Arts and Sciences, University of South Florida, Tampa, FL, <sup>3</sup>Graduate School of Global Food Resources, Hokkaido University, Sapporo, Japan, <sup>4</sup>Faculty of Health Sciences, Hokkaido University, Sapporo, Japan, <sup>5</sup>Hypertension and Kidney Research Center, University of South Florida, Tampa, FL

**Background:** Obesity and hypertension-driven cardiometabolic stress disrupts lipid homeostasis across the liver and heart, establishing a cardio-hepatic inflammatory axis that contributes to Heart Failure with Preserved Ejection Fraction (HFpEF). Circulating C15:0 levels have been associated with favorable cardiometabolic and hepatic health in observational and preclinical studies; the cellular and molecular mechanisms of C15:0 remain unclear at the integrative level in the context of the hepato-cardiac network. **Methods:** We tested whether C15:0 limits HFpEF-like pathology in a high-fat (60%) plus L-NAME (H+L) mouse model. Male C57BL/6J mice (2 months old) were fed control chow, H+L diet, or H+L supplemented with C15:0 (0.4 g/kg diet) for 8 weeks. Integrated lipidomic, histologic, and functional analyses were performed across the heart and liver. **Results:** In the heart, C15:0 in the presence of H+L diet increased the abundance of specialized pro-resolving mediators, including LXB<sub>4</sub>, RvD1, RvD5, along with their precursors HDHAs and HPEs. This shift coincided with H+L-associated phospholipid remodeling and decreased arachidonic acid enrichment in membrane phosphatidylethanolamines, aligning with reduced H+L-driven inflammatory signaling. These molecular changes paralleled significant recovery of global longitudinal strain, wall, and motion. Metabolic stress due to H+L diet induced hepatic oxylipin remodeling with dominant flux of COX, 5-LOX, 12/15-LOX, and CYP pathways toward arachidonic acid-derived HETEs, EETs, prostaglandins, and leukotrienes. C15:0 supplementation limited from arachidonic acid-derived inflammatory HETEs and increased production of pro-resolving mediators such as HDHAs and PDX. These integrative changes in hepato-cardiac lipid remodeling show that C15:0 controls diet-induced metabolic stress in HFpEF, thereby modulating inflammation-resolution signaling. **Conclusion:** These findings highlight the potential of C15:0 in hepatocardiac inflammation and warrant detailed investigation in limiting obesity-induced cardiometabolic pathophysiology.

## Poster Board 13

### Abstract 061

#### **Low Volume Aerobic Exercise Modulates Proteolysis in Cachectic Hearts of Male Mice**

Zoe Libramento and Traci L. Parry

Department of Kinesiology, University of North Carolina at Greensboro, Greensboro, NC

**Background:** Cancer-mediated cardiac cachexia describes a debilitating consequence of cancer progression where the metabolic demand of cancer shifts muscle metabolism toward a more catabolic state, leading to the wasting of cardiac muscle. It is well established that aerobic exercise has beneficial impacts on the cardiovascular system, however, preclinical cachexia studies have yet to fully clarify how exercise influences cardiac muscle wasting and dysfunction during cancer. To determine if low volume concurrent treadmill running during tumor-bearing (30 mins per day, 5x a week), is sufficient to preserve cardiac structure and function in cachectic male mice. **Methods:** 12-18 week-old male mice were randomly divided into four experimental groups: sedentary non-tumor (SED + NT); sedentary tumor (SED + T); treadmill exercised non-tumor (EX + NT); and treadmill exercised tumor (EX + T). Mice in the T groups were implanted with 5x10<sup>5</sup> LLC cells in flank, while NT mice received sham implants (PBS). Mice in the EX groups performed 30 minutes of low-intensity treadmill exercise (5 days/week). Echocardiograms were taken at baseline and sacrifice to determine tumor-mediated changes in cardiac structure and function. Immunoblots were performed to determine cardiac protein

expression and confocal microscopy was performed to evaluate autophagic flux. **Results:** SED + T mice lost a significant amount of weight during the four-week study compared to healthy controls ( $p < 0.05$ ). Tumor-mediated cardiac atrophy was observed in SED+T, which exhibited the lowest wall thickness compared to all other groups. Of note, SED+T exhibited significant wall thinning in the posterior wall during diastole and diastole (vs SED + NT,  $p < 0.05$ ), and significantly lower fractional shortening compared to all other groups ( $p < 0.001$ ) indicating a severe cardiac wasting and dysfunction due to cancer burden. This dysfunction parallels with significantly more early-phase autophagosome formation in SED + T compared to SED + NT mice ( $p < .001$ ). Low volume aerobic treadmill exercise abrogates cancer-mediated cardiac cachexia. Importantly, aerobic exercise preserves cardiac structure and function. This may be due to exercise's ability to modulate autophagosome formation, as well as UPS and lysosomal-related protein expression (i.e. Atrogin-1 and p62) ( $p < 0.05$ , EX+T vs SED+T). **Conclusion:** Cancer-mediated cardiac cachexia results in severe heart wasting and dysfunction in the preclinical LLC cachexia model, and this appears to be largely driven by a pathological increase in lysosomal degradation. The ability of exercise to modulate proteolysis in cardiac muscle supports its potential use as an adjuvant treatment option for cancer-induced muscle loss and may be considered an effective countermeasure for cardiac cachexia. More research is needed to determine the potential signaling pathways modulated by exercise, thus optimizing exercise prescriptions for individuals.

## Poster Board 14

### Abstract 062

#### Cardiac CD34<sup>+</sup> Stromal Cells, Unlike Myofibroblasts, Favor Regenerative Over Fibrotic Wound Repair After Transmural Nonreperfused Myocardial Infarction

Daniel T. Schneider and Eduard I. Dedkov

Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ

**Background:** Cardiac CD34<sup>+</sup> stromal cells (SCs) have recently emerged as a novel resident cell population involved in post-myocardial infarction (MI) healing, whereas activated fibroblasts/myofibroblasts are well-established mediators of this process. However, their relationship during myocardial repair remains poorly defined. Therefore, in this study, we compared the spatiotemporal dynamics of cardiac CD34<sup>+</sup> SCs and  $\alpha$ -smooth muscle actin (SMA)<sup>+</sup> myofibroblasts during the reparative (proliferative) phases of post-MI wound healing. **Methods:** A large transmural, non-reperfused MI was induced in middle-aged male Sprague–Dawley rats ( $n = 15$ ) via permanent ligation of the left anterior descending coronary artery under ketamine/xylazine anesthesia. To label proliferating cells, rats received 5-bromo-2'-deoxyuridine (BrdU, 12.5 mg/kg/day) for 72 hours via intraperitoneal osmotic minipumps starting on days 0, 4, or 11 post-MI. Animals were euthanized on days 3, 7, or 14, respectively, and hearts were processed for histology and immunohistochemistry. **Results:** On day 3 after MI, CD34<sup>+</sup> SCs and  $\alpha$ -SMA<sup>+</sup> myofibroblasts were absent from necrotic regions, while CD34<sup>+</sup> SCs were detectable in the peri-infarct area. BrdU labeling was seen in the nuclei of activated cardiac CD34<sup>+</sup> SCs as well as CD34<sup>-</sup> cells, likely fibroblasts/myofibroblasts, suggesting proliferation-driven expansion of both populations. By day 7 after MI, CD34<sup>+</sup> SCs and  $\alpha$ -SMA<sup>+</sup> myofibroblasts were observed migrating into the injured regions following macrophage-mediated clearance of cellular debris. Notably,  $\alpha$ -SMA<sup>+</sup> myofibroblasts preferentially accumulated within pro-fibrotic (granulation tissue) areas, including regions surrounding mummified, non-resorbed myocardial tissue and areas immediately beneath the epicardial and endocardial linings. In contrast, cardiac CD34<sup>+</sup> SCs were predominantly localized within preserved endomysial scaffolds surrounding "empty" spaces previously occupied by cardiomyocytes. By day 14 after MI, granulation (fibrotic) tissue enriched with  $\alpha$ -SMA<sup>+</sup> myofibroblasts progressively expanded outward, narrowing the intramural space still occupied by remnants of the endomysial framework populated by cardiac CD34<sup>+</sup> SCs. During this process, a subset of CD34<sup>+</sup> SCs located next to myofibroblast-rich granulation tissue exhibited phenotypic changes suggestive of partial dedifferentiation and appeared to integrate into the developing scar. **Conclusion:** Our findings, for the first time, reveal that resident cardiac CD34<sup>+</sup> SCs and  $\alpha$ -SMA<sup>+</sup> myofibroblasts display distinct spatiotemporal dynamics during post-MI wound healing. While  $\alpha$ -SMA<sup>+</sup> myofibroblasts primarily contribute to fibrotic scar formation, CD34<sup>+</sup> SCs seemed to support regeneration-oriented cardiac repair by preserving original myocardial stromal architecture. These results suggest that cardiac CD34<sup>+</sup> SCs may represent a promising therapeutic target to enhance post-ischemic myocardial repair. **Acknowledgements:** Supported by Camden Health Research Initiative.

## Poster Board 15

### Abstract 063

#### Atorvastatin-Mediated Modulation of Vascular Remodeling in Marfan Syndrome Aortic Aneurysm: From Aorta to Cerebral Artery

Patrick Hunt<sup>1</sup>, Brikena Gusek<sup>1</sup>, Kimberly Huynh<sup>1</sup>, Anna Stimpson<sup>1</sup>, Roshanak Rahimian<sup>2</sup>, and Mitra Esfandiarei<sup>1</sup>

<sup>1</sup>Midwestern University, Glendale, AZ, <sup>2</sup>University of the Pacific, Stockton, CA

**Background:** Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder caused by mutations in the fibrillin-1 gene (*Fbn1*), resulting in systemic extracellular matrix dysfunction. Progressive aortic root aneurysm leading to dissection or rupture represents the most life-threatening clinical manifestations. Our laboratory has recently demonstrated that aortic root aneurysm development is accompanied by pathological remodeling in the carotid and posterior cerebral arteries, indicating a broader vasculopathy phenotype. The prevailing mechanistic paradigm implicates dysregulated transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling and enhanced matrix metalloproteinase (MMP) expression and activity downstream of *Fbn1* mutation. These molecular alterations promote elastin fragmentation, medial degeneration, and progressive weakening of the aortic wall, thereby accelerating aneurysm expansion. Statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, have been shown to suppress MMP activity and inflammation and improve endothelial function independent of lipid lowering effects. Based on these pleiotropic vascular effects, we hypothesized that atorvastatin would attenuate aortic root aneurysm progression and improve arterial wall elasticity in a transgenic mouse model of MFS. **Methods:** Beginning at 4 weeks of age, male (n = 10–12) and female (n = 10–12) control and MFS (*Fbn1*<sup>+/+</sup>/C1041G<sup>+</sup>) mice received atorvastatin (1 g/kg/day in drinking water) for the duration of the study. At 6 months of age, high-resolution, high-frequency ultrasound imaging was performed to quantify aortic root diameter, aortic pulse wave velocity (PWV; an index of wall stiffness), posterior cerebral artery (PCA) peak systolic blood flow, and common carotid artery wall thickness, stiffness, and PWV. **Results:** Our findings show that long-term atorvastatin administration significantly attenuated aortic root dilation in male (P = 0.0142) and female (P = 0.0085) MFS mice relative to age- and sex-matched sham-treated MFS. Aortic wall PWV, was markedly reduced in male (P  $\leq$  0.0001) and female (P  $\leq$  0.0001) MFS mice compared to sham-treated MFS. In the carotid artery, atorvastatin markedly decreased wall thickness (male: P = 0.0012; female: P = 0.0011) and PWV (male: P = 0.0003; female: P  $\leq$  0.0001) compared to sham-treated MFS counterparts. Interestingly, atorvastatin notably reduced PCA peak blood flow in males MFS mice, where no effects observed in female MFS groups. **Conclusions:** Collectively, these findings demonstrate that chronic statin therapy attenuates aortic and carotid remodeling and stiffness in MFS, supporting the therapeutic potential of statins as adjunctive vascular-protective agents for the management of aneurysm progression in this genetic aortopathy. **Acknowledgements:** This study was funded by the National Institute of Health [NIHR15-HL145646], and Midwestern University College of Graduate Studies.

## Poster Board 16

### Abstract 064

#### Human Ex Vivo RPE/Choroidal Explants Reveal IGFBP1 as a Potent Regulator of Pathological Angiogenesis and Fibrosis in nAMD and Diabetic Conditions

Anton Lennikov<sup>1,2</sup>, William P. Miller<sup>1,2</sup>, Farris Elzaridi<sup>1,2</sup>, Jennifer P. Zhumi<sup>1,3</sup>, Anna K. Tostrup<sup>1,4</sup>, Noora H. Thune<sup>1,4</sup>, Anil Upreti<sup>1,2</sup>, Aruvi Vijikumar<sup>1,2</sup>, Dong Feng Chen<sup>1,2</sup>, and Leo A. Kim<sup>1,2</sup>,

<sup>1</sup>Schepens Eye Research Institute of Massachusetts Eye and Ear Infirmary, Boston, MA, <sup>2</sup>Department of Ophthalmology, Harvard Medical School, Boston, MA, <sup>3</sup>SUNY Downstate Health Sciences University College of Medicine, Brooklyn, NY,

<sup>4</sup>Universitetet i Oslo Det odontologiske fakultet, Oslo, Norway

**Background:** Age-related macular degeneration (AMD) and diabetic retinopathy (DR) are major causes of irreversible vision loss driven by pathological angiogenesis and progressive fibrosis. Translational investigation of these processes in human tissue remains limited, relying largely on two-dimensional cultures, organoids or surgical specimens. To address this gap, we developed and characterized a gel matrix-embedded, 3D ex vivo human RPE/choroidal explant model derived from post-mortem donor eyes obtained within 24 hours. This platform preserves native multicellular architecture and cell-cell interactions, enabling clinically relevant modeling of human angiogenesis and fibrotic remodeling. **Methods:** RPE/choroidal tissue was isolated, and circular  $\sim$ 1 mm punches were generated from central and peripheral regions. Explants were embedded in Geltrex and cultured in EGM-2 complete media. Analyses included brightfield microscopy, immunolabeling, flow cytometry, and RNA sequencing. Ex vivo explants demonstrated a distinct

spatial growth architecture. Endothelial cells and pericytes extended into the surrounding matrix, forming 3D vascular-like sprouts, whereas fibroblast-like cells formed a thin layer at the plastic–gel interface. This compartmentalization enabled the separation of angiogenic and fibrotic processes within the same explant. **Results:** Compared with age-matched controls, explants derived from nAMD donors exhibited enhanced angiogenic and fibrotic outgrowth. Treatment with Insulin-like growth factor-binding protein-like 1 (IGFBPL1) is a protein that functions as a key regulator of microglia homeostasis and resolves neuroinflammation, significantly reduced both endothelial sprouting and fibroblast-like expansion. Notably, IGFBPL1 demonstrated inhibitory efficacy comparable to equimolar VEGF blockade in suppressing angiogenesis in nAMD explants. We further modeled diabetes-induced angiofibrosis. RPE/Choroidal explants exposed to high glucose (35 mM) or derived from diabetic donors showed enhanced proliferative outgrowth, even under normoglycemic conditions, when compared to healthy donor tissues, indicating retained diabetic programming *ex vivo*. IGFBPL1 (400 ng/ml) significantly reduced hyperglycemia- and diabetes-driven remodeling, as quantified by longitudinal imaging and endpoint area analysis. In contrast, aflibercept did not significantly suppress diabetes-associated proliferation. Immunolabeling revealed a predominance of Vimentin-positive fibroblast-like cells at the expanding front, highlighting a strong fibrotic component. **Conclusion:** Together, these findings establish a robust human *ex vivo* explant system bridging the gap between reductionist culture and *in vivo* disease. This platform enables mechanistic dissection of angiogenic and fibrotic remodeling in native human tissue and supports therapeutic screening. IGFBPL1 emerges as a potent regulator of pathological angiogenesis and fibrosis in both nAMD and diabetic retinal disease.

## Poster Session – Cell Death and Tissue Repair

### Poster Board 17

#### Abstract 065

#### Burn Wound Bacteria Remodel Local Oxygen Landscapes to Support Anaerobic Niches and Drive Inflammation

Subhomitra Ghoshal<sup>1</sup>, Erin Chard<sup>1</sup>, Anna Tingler<sup>1</sup>, Selene Shore<sup>1</sup>, Alyssa Gutierrez<sup>1</sup>, Jessica Hartman<sup>2</sup>, Deepak Ozhathil<sup>3\*</sup>, and Melinda A. Engevik<sup>1\*</sup> (\*Co-senior authors)

<sup>1</sup>Department of Regenerative Medicine and Cell Biology, <sup>2</sup>Department of Biochemistry, Medical University of South Carolina, Charleston, SC, <sup>3</sup>Department of Surgery, Akron Children’s Hospital, Akron OH

**Background:** Burn wounds are traditionally viewed as oxygen-exposed environments that select for facultative anaerobes and aerobic pathogens. However, our profiling of burn wound skin revealed an unexpected enrichment of strict anaerobes, suggesting that localized microenvironments within wounds may become functionally anaerobic. Since oxygen availability is a central ecological constraint shaping microbial community structure, we hypothesized that oxygen-consuming facultative bacteria in burn wounds actively deplete oxygen to generate anaerobic niches that enable strict anaerobe persistence and expansion. To determine whether burn wound-associated bacteria can rapidly reduce oxygen levels and thereby promote survival of strict anaerobes, and to assess how burn wound microbial communities influence host inflammatory responses. **Methods:** Bacterial growth was monitored over time by OD<sub>600nm</sub> using a Synergy H1 plate reader, and dissolved oxygen was tracked continuously with the Resipher platform. To determine whether facultative bacteria promote strict anaerobe survival under aerobic conditions, *Fusobacterium nucleatum* was cultured alone or co-cultured with the oxygen-consuming species *Acinetobacter baumannii*, and persistence was assessed relative to monoculture controls by imaging and qPCR. To evaluate host responses to community-derived factors, conditioned supernatants from burn wound-associated bacterial communities were applied to human skin keratinocytes and IL-8 levels were quantified as a readout of epithelial inflammation. **Results:** Species from genera including *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Staphylococcus*, *Proteus*, *Listeria*, *Escherichia*, *Morganella*, *Serratia*, *Citrobacter*, and *Lactococcus* were cultured in ZMB1 medium at OD<sub>600nm</sub> of 0.1. All isolates grew, though with genus-dependent variation in biomass accumulation. Oxygen in uninoculated controls remained stable at ~200 μM. In contrast, *Morganella morganii*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and all *Klebsiella* strains rapidly lowered oxygen below 30 μM within 1 hour of inoculation. Within two hours, substantial oxygen depletion was also observed for *Pseudomonas putida*, *Serratia marcescens*, *Staphylococcus aureus*, *Citrobacter freundii*, *Enterobacter hormaechei*, *Enterobacter cloacae*, *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, and *Enterococcus faecalis*. By contrast, most *Streptococcus* and *Lactobacillus* strains displayed negligible oxygen-reducing activity. Comparative genome analysis using the IMG database revealed enrichment of cytochrome oxidases, nitrate/nitrite reductases, and other oxidases in species with strong oxygen-quenching capacity. We also demonstrated that the strict anaerobe *Fusobacterium*

*nucleatum* was able to persist under aerobic conditions when co-cultured with oxygen-consuming *Acinetobacter baumannii*. Treatment of skin keratinocytes with conditioned supernatants from burn wound bacterial communities with strict anaerobes resulted in elevated IL-8 levels compared to communities without anaerobes, supporting a role for microbial community derived factors in shaping epithelial inflammatory responses. **Conclusion:** These findings identify specific facultative anaerobes with high oxygen-depleting potential and illustrate how they can generate anaerobic conditions that support community interactions.

## Poster Board 18

### Abstract 066

#### Dermal Fibroblastic Responses to Pro-Fibrotic Stimuli in Human iPSC-Derived Skin Organoids

Anthony R. Sheets, Shannon M. McNamee, Christine G. Lian, and George F. Murphy

Department of Pathology, Division of Dermatopathology, Brigham and Women's Hospital, Boston, MA

**Background:** Conversion of quiescent dermal fibroblasts to an activated myofibroblastic state is a critical event in post-injury cutaneous remodeling and the evolution of debilitating conditions including pathologic scar formation and superficial fibromatoses such as Dupuytren's disease. Next-generation models reproducing the intact spatial microenvironment of native human skin are required to enhance our understanding of molecular and cellular mechanisms driving the onset and persistence of myofibroblastic phenotype to develop novel therapeutics aimed at treating fibrosing skin diseases. We have recently demonstrated dermal microvascular responses to physical injury and inflammatory cytokine treatment in human induced pluripotent stem cell (iPSC)-derived skin organoids (SKOs), highly advanced self-organizing structures composed of cystic spheroids lined by hair-forming epidermis, encircled by a dermal mesenchyme that additionally produces adipose tissue, cartilage, and neural elements. Given the similarities of angiogenic and inflammatory responses in SKOs to those in bona fide human skin, we investigated whether known pro-fibrotic stimuli would induce dermal myofibroblastic conversion in this system. **Methods:** We derived SKOs from human iPSCs and treated them with increasing doses of TGF-beta, the primary molecular driver of myofibroblastic phenotype and extracellular matrix production, for up to 2 weeks *in vitro*. We performed histologic and immunohistochemical evaluations to assess dermal fibroblast-to-myofibroblast transition. Finally, we applied multiplexed antibody arrays to assay the organoid-conditioned media for soluble factors contributing to myofibroblast development and a pro-fibrotic microenvironment. **Results:** SKOs exposed to TGF-beta dose- and time-dependent increases in dermal cellularity and matrix production accompanied by markedly elevated expression of alpha-smooth muscle actin and fibronectin, indicative of myofibroblastic activation and fibrotic changes reminiscent of superficial dermal fibroproliferative lesions in human patients. TGF-beta treatment further altered SKO-derived soluble mediators that regulate fibrotic responses, including increased abundance of activin-A, diminished levels of urokinase-type plasminogen activator, an important regulator of matrix proteolysis, and enhanced production of its inhibitors, maspin and plasminogen activator inhibitor-1. **Conclusions:** Our findings demonstrate human SKOs reproduce key elements of dermal myofibroblastic activation and extracellular matrix biosynthesis in response to TGF-beta stimulation, highly reminiscent of those occurring in native human skin. Our ongoing studies aim to further develop this system as a platform for testing anti-fibrotic agents and potentially identifying novel mechanisms of dermal fibrosis that may be targeted in cutaneous fibroproliferative diseases.

## Poster Board 19

### Abstract 067

#### Combined Aerobic Exercise and NSAID Therapy Protect Against Colorectal Cancer-Induced Muscle Dysfunction

Abigail L. Smith<sup>1</sup>, Zoe P. Libramento<sup>1</sup>, Louisa Tichy<sup>1</sup>, Kimberly F. Allred<sup>1</sup>, Erika T. Rezel<sup>2</sup>, Michael F. Coleman<sup>2</sup>, Clinton D. Allred<sup>1</sup>, Stephen D. Hursting<sup>2</sup>, and Traci L. Parry<sup>1</sup>

<sup>1</sup>University of North Carolina at Greensboro, Greensboro, NC, <sup>2</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC

**Background:** Cancer cachexia is driven by tumor-derived inflammatory signaling that accelerates proteolysis, suppresses anabolic pathways, and disrupts mitochondrial homeostasis, leading to rapid skeletal muscle wasting. In colorectal cancer cachexia, single-target anti-inflammatory therapies (e.g., NSAIDs) have shown limited efficacy, which highlights the need for better treatment strategies. Exercise is known to counteract cachexia by altering cytokine signaling, improving mitochondrial function, and enhancing protein turnover. Therefore, this study assessed whether combining exercise with NSAID treatment provides superior protection against colorectal cancer-induced muscle dysfunction.

**Methods:** Male Balb/c mice (10 weeks) were inoculated with C26 colorectal cancer cells and randomized into four groups: sedentary tumor control (T), NSAID-treated (NSAID), wheel-running exercise (WR), or combined NSAID + wheel running (NSAID+WR). Voluntary wheel running was performed for 4 weeks, and NSAID was administered via milled chow. Skeletal muscle function was assessed across the study using in vivo grip strength. Tumor burden and muscle mass were quantified at endpoint. **Results:** Sedentary tumor-bearing mice exhibited the greatest muscle loss and functional decline, consistent with upregulated inflammatory-proteolytic and autophagy activation. Single-modality treatments partially attenuated dysfunction, whereas combined NSAID+WR treatment resulted in the greatest level of protection, via significantly greater muscle mass ( $P<0.001$  vs T) and grip strength ( $P<0.05$  vs T). Combined treatment also reduced tumor mass and volume ( $P<0.001$ ), suggesting decreased tumor-derived inflammatory signaling. **Conclusions:** Integrating aerobic exercise with NSAID therapy provides superior protection against colorectal cancer-mediated skeletal muscle dysfunction. The enhanced efficacy likely reflects simultaneous dampening of inflammatory cytokine signaling and suppression of autophagy-proteolytic pathways within muscle. Ongoing work will directly interrogate these mechanistic nodes to identify therapeutic targets for cachexia.

## Poster Session – Computational Pathobiology

### Poster Board 20

#### Abstract 068

#### Machine Learning–Driven Intra-Slide Calibration Harmonizes p53 Immunohistochemistry Across Laboratories

Glauca Maria de Mendonça Fernandes<sup>1</sup>, Wesley Wang<sup>2</sup>, Anil Parwani<sup>2</sup>, Saman Ahmadian<sup>2</sup>, Michele Joana Alves<sup>1</sup>, Joanna Philips<sup>3</sup>, and Jose Javier Otero<sup>1,4</sup>.

<sup>1</sup>Department of Cellular and Molecular Medicine, Florida International University Herbert Wertheim College of Medicine, Miami, FL, <sup>2</sup>Department of Pathology, The Ohio State University Wexner Medical Center, Columbus, OH, <sup>3</sup>Department of Neurological Surgery, Brain Tumor Center, University of California, San Francisco, CA, <sup>4</sup>Department of Neuropathology and Clinical Informatics at Baptist Health South Florida, Miami, FL

**Background:** Reproducibility in p53 immunohistochemistry (IHC) remains a major challenge, especially for heterogeneous tumors like glioblastoma (GB). Differences in staining protocols across laboratories reduce diagnostic precision, prognostic reliability, and limits the application of AI-driven pathology. To address this, we implemented a machine learning-based intra-slide calibration workflow to harmonize p53 IHC staining across laboratories, generating standardized and reproducible datasets for clinical decision-making. **Methods:** Each slide incorporated an internal “ruler” with secondary antibody concentrations from 0 to 100%. Whole-slide images were digitized at 40x. Pixel intensity data from red, green, and blue channels were extracted from both the slide ruler and p53-positive cells using the EImage R package. Principal component analysis (PCA) was applied to compute PC1 values for each ruler concentration and cells. We then modeled secondary antibody concentration as a function of PC1 using polynomial regression (PR). This model predicted calibrated PC1 values, generating standardized, quantitative staining profiles. UMAP and DBSCAN clustering analysis were used to assess feature-space structure and density plot were used to compare inter-laboratory differences before and after calibration. **Results:** Before calibration, UMAP-based clustering demonstrated a clear segregation of p53 IHC features according to laboratory ( $ACC=79\%$ ,  $p<0.0001$ ), consistent with protocol-dependent variability in raw staining intensity and texture values. To address this variability, application of PCA-PR calibration model generated predicted cellular intensity values mapped to a common 0-100% intra-slide ruler. These results enable direct inter-laboratory comparison and were evaluated at the distributional level, demonstrating more overlapping and homogenization of intensity profiles of p53-positive cell populations. This adjustment reduced inter-laboratory technical variability in ~5% while preserving the relative distribution of positive cells, indicating that the calibration procedure standardizes quantitative IHC measurements. **Conclusions:** Machine learning–driven intra-slide calibration represents a scalable and reproducible strategy for harmonizing quantitative IHC data across laboratories. This approach reduces protocol-driven technical variability, while preserving biological signal, and produces standardized datasets for computational pathology workflows. Applied to p53 immunohistochemistry in glioblastoma, the method supports more consistent quantitative assessment and may facilitate reproducible downstream analyses in translational and investigative pathology. **Acknowledgements:** This work was supported by NINDS R03NS116334, NIH/NHLBI R01HL163965-01, NCI P30CA016058, NCATS TL1TR002735 & UL1TR002733. OSU-TDAI seed grant. We thank the pathology teams at The Ohio State University Wexner Medical Center and University of California, San Francisco.

Poster Board 21

Abstract 069

**DNMT3A Regulates an Epigenetic Tolerance Program in Macrophages to Protect Against Atherosclerosis Driven by Gout-Induced Inflammation**

Xiaoxiao Geng<sup>1</sup>, Daniel Ward Phillips<sup>2</sup>, Benjamin Hemming<sup>2</sup>, Riley W. Porter<sup>2</sup>, Mohnish Alishala<sup>3</sup>, Stephen Calderon<sup>3</sup>, Faith Inkum<sup>2</sup>, Enchen Zhou<sup>3</sup>, Christian K. Nickl<sup>3</sup>, Kimberley Weldy<sup>4</sup>, Elena Alekseeva<sup>4</sup>, Calvin Yeang<sup>4</sup>, Christopher K. Glass<sup>3</sup>, Monica Guma<sup>5,6</sup>, Robert Terkeltaub<sup>7</sup>, and Isidoro Cobo<sup>1,2,8</sup>

<sup>1</sup>Department of Biomedical Engineering, School of Medicine, <sup>2</sup>Division of Clinical Immunology and Rheumatology, Department of Medicine, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL,

<sup>3</sup>Department of Cellular and Molecular Medicine, <sup>4</sup>Division of Cardiology, Department of Medicine, <sup>5</sup>Division of Rheumatology, Allergy, and Immunology, University of California at San Diego School of Medicine, La Jolla, CA,

<sup>6</sup>Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain, <sup>7</sup>Division of Rheumatology, Allergy and Immunology, Department of Medicine, University of California, at San Diego, La Jolla, CA, <sup>8</sup>CAMBAC (Comprehensive Arthritis, Musculoskeletal, Bone and Autoimmunity Center), University of Alabama at Birmingham, Birmingham, AL

**Background:** Gout is strongly associated with increased cardiovascular morbidity, including atherosclerosis, yet the immunologic mechanisms linking monosodium urate crystal (MSUc)-driven inflammation to atherosclerosis remain unknown. Macrophages are primary responders to MSUc and key regulators of gout severity. We hypothesized that recurrent local MSUc-induced inflammation promotes atherosclerosis through systemic immune reprogramming of the epigenetic landscape in circulating monocytes and macrophages. **Methods:** Gout-like inflammation was modeled by injecting MSUc or PBS into air pouches of Western diet-fed *Ldlr*<sup>-/-</sup> mice. Atherosclerosis was quantified by Oil Red O staining of whole aortas and histologic analysis of aortic roots; serum cholesterol was measured to assess lipid contributions. Systemic immune responses were analyzed by bulk RNA sequencing and ChIP-seq of peripheral blood mononuclear cells (PBMC), bone marrow and Kupffer cells, as well as by flow cytometry and single-cell RNA sequencing. Innate immune adaptation was assessed by sequential MSUc rechallenge *in vivo* and repeated MSUc stimulation of bone marrow-derived macrophages *in vitro*. Knockdown experiments were performed using antisense oligonucleotides.

**Results:** Repeated local MSUc exposure significantly increased atherosclerotic lesion area in *Ldlr*<sup>-/-</sup> mice without altering serum cholesterol, demonstrating inflammation-driven acceleration of atherosclerosis independent of lipid burden. Transcriptomic profiling revealed that PBMCs uniquely exhibited robust inflammatory gene induction following MSUc exposure, whereas bone marrow cells and Kupffer cells were minimally affected. Upregulated genes in PBMCs included inflammatory mediators and *Emp1*, a novel regulator of gout-induced atherosclerosis. Single-cell RNA sequencing confirmed expansion of an EMP1<sup>+</sup> circulating monocyte population. DNMT3A directly bound the *EMP1* promoter, and DNMT3A knockdown increased *EMP1* expression in human monocytes and macrophages in a type I interferon–dependent manner. Functionally, prior MSUc exposure induced a state of innate immune tolerance *in vivo*, characterized by reduced leukocyte recruitment and attenuated inflammatory responses upon rechallenge, which was associated with reduced atherosclerotic burden. **Conclusions:** Gout-like MSUc inflammation accelerates atherosclerosis through systemic immune reprogramming, marked by expansion of DNMT3A-regulated EMP1<sup>+</sup> monocytes and induction of macrophage tolerance. These findings define a mechanistic link between gouty inflammation, epigenetic regulation, innate immune memory, and cardiovascular disease, and suggest new strategies to limit vascular inflammation in gout-associated atherosclerosis. **Acknowledgements:** We thank members of the Cobo lab, institutional core facilities and funding sources.

Poster Board 22

Abstract 070

**From Breath to Call: Neuromodulatory Role of IL-6 in Neonatal Brainstem Function – Ultrasonic Vocalization in Neonates**

Isaac Rodriguez, Emily Silva, David Villalobos, Jose Otero, and Michele Alves

Department of Cellular and Molecular Medicine, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

**Background:** Neonatal sepsis is the leading cause of morbidity and mortality in newborns. Interleukin-6 (IL-6) is emerging as a cytokine with neuroprotective or detrimental effects and has been shown to regulate

neurodevelopmental processes. Our previous work demonstrates that IL-6 plays a key role modulating neonatal immune activation and brainstem neuroinflammation, which disrupts breathing. The brainstem circuits controlling autonomic breathing overlap with those that generate vocalizations, transforming respiratory rhythms into patterned calls. Ultrasonic vocalizations (USV) are a key communicative behavior in mice that provide sensitive readout for respiratory-vocalization circuit integrity. Our study sought to determine how IL-6 loss alters neonatal brainstem circuitry, using USVs as an integrative measure of respiratory-vocalization network function. **Methods:** *Il6<sup>tm1Kopf</sup>/J* (IL6<sup>KO</sup>) and IL6<sup>+/+</sup> mice were utilized throughout postnatal day (PD) 5 to 7. At PD5 they were *i.p* administered with either saline or LPS. Following 3, 24, 48 and 72 hours USVs were recorded for 5 min in a soundproof chamber using a condenser microphone connected to Ultrasound Gate hardware. All analysis was performed using the VocalMat add on MATLAB and R Studio for the data analysis. **Results:** To evaluate the neuromodulatory effects of IL6 in neonatal brainstem circuits, we first determined whether IL-6 ablation alters USVs. Baseline recordings revealed that IL6<sup>KO</sup> pups exhibited a significant reduction in call number and mean bandwidth compared to IL6<sup>+/+</sup> at PD7 and PD8. To further characterize these differences, we mapped syllables features using a diffusion map (Pearson's), which revealed genotype-dependent shifts in syllables centroid distances, highlighting the impact of IL6 loss on vocalization structure. Neonatal immune activation by LPS resulted in a significant reduction calls in acute phase (3h) in both IL6<sup>+/+</sup> and IL6<sup>KO</sup>. However, during the early-adaptive phase (24 h), this effect was reversed exclusively in IL-6<sup>+/+</sup> mice, which displayed an increased number of calls that persisted throughout the adaptive response at 72 h. Consistent with these findings, syllables centroid distances demonstrated that LPS induced marked changes in call patterns in IL-6<sup>+/+</sup> pups, whereas IL-6 ablation prevented these adaptive shifts at 24 h. At 48 h post-LPS, the vocalization repertoire in IL-6<sup>+/+</sup> mice became more similar between LPS-treated and control groups, suggesting convergence of call patterns during adaptation. In contrast, IL-6<sup>KO</sup> pups that received LPS maintained a distinct syllable repertoire throughout the adaptive phase. Analysis of established calls categories a showed positive association with flat and short calls in IL6<sup>KO</sup> regardless of the immune activation with LPS. **Conclusions:** Our findings highlight IL-6 as a key neuromodulator of neonatal breathing-vocalization circuits, shaping adaptative vocal behavior in response to immune activation. **Acknowledgements:** This work was supported by: NIH/NHLBI R01HL132355 for JJO and FIU Start-up for JO and MA.

## Poster Board 23

### Abstract 071

#### **MUC1 and MUC4 Hypomethylation and Elevation in Uterine Corpus Endometrial Carcinoma Correlates with Poor Prognosis**

Anna M. Tingler<sup>1</sup>, and Melinda A. Engevik<sup>1,2</sup>

<sup>1</sup>Department of Regenerative Medicine and Cell Biology, <sup>2</sup>Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC

**Background:** Uterine corpus endometrial carcinoma (UCEC) is one of the most common gynecological malignancies. UCEC is currently the fourth most life-threatening cancer in females, after ovarian cancer and cervical cancer. The prognosis of UCEC patients with high grade cancer is poor and limited treatments can be applied. In other epithelial cancers, adherent mucins are over-expressed and signal to cancer cells to promote their growth and survival. Currently, little is known about the adherent mucin profile of UCEC and how mucins contribute to UCEC prognosis. This study aims to elucidate the expression patterns and clinical significance of adherent mucins in UCEC. **Methods:** Several databases were used to query RNA levels of adherent mucins in UCEC, including The Cancer Genome Atlas (TCGA) dataset, Genotype-Tissue Expression (GTEx) Portal, COSMIC v95, TIMER2.0 and cBioPortal v4.1.1 portal. Protein levels of MUC1 and MUC4 were obtained using the UALCAN Database, which draws from the Clinical Proteomics Consortium for Cancer Analysis (CPTAC) dataset. RNA data from multiple uterine cancer cell lines were also examined. To confirm the functional effects of MUC4, parental and MUC4 knock out uterine HEC-1-A cells were grown and examined for metabolism, proliferation and migration. **Results:** Computational analysis of RNA data from UCEC patients (n=547) and individuals without cancer (n=146) revealed that MUC1 and MUC4 were the highest expressed mucins in UCEC tumors and were highly elevated compared to control tissue. MUC1 and MUC4 expression was higher in overweight/obese patients, women above the age of 40 and women with post-menopausal status. Interestingly, MUC1 was also higher in patients of Caucasian and African American descent compared to Asian women. MUC1 and MUC4 were high in all cancer stages and subtypes. No known mutations that favor mucin upregulation were identified in UCEC patients. However, methylation analysis revealed that the promoter regions of both MUC1 and MUC4 were hypo-methylated in UCEC tumors. Proteomic analysis of UCEC tumors revealed higher levels of MUC1 and MUC4 protein in tumors compared to non-cancerous

tissue. These findings were validated by immunostaining of patient tissue, where both MUC1 and MUC4 were found at high levels in UCEC tumors. Unfortunately, UCEC patients with high expressing MUC1 and MUC4 tumors had worse prognosis than UCEC patients with low expressing tumors, as reflected by survival curves. Analysis of uterine cancer cell lines demonstrated that HEC-1-A cells had the highest expression of MUC4. The importance of MUC4 was confirmed using HEC-1-A cells and *in vitro* analysis revealed significant differences between MUC4 knockout cells and control cells. **Conclusions:** These data provide the first evidence that adherent mucins may participate in UCEC tumor development and could have prognostic implications for therapeutic intervention.

## Poster Board 24

### Abstract 072

#### Optimized Intraoperative Tissue Preservation Reveals circRNA Signatures Associated with WHO Grade 2 Meningiomas

Glauca Maria de Mendonça Fernandes<sup>1</sup>, Kyle C. Wu<sup>2</sup>, Joshua Palmer<sup>3</sup>, Ricardo Carrau<sup>4</sup>, Maria M. Abreu<sup>5</sup>, Michael W. McDermott<sup>6,7</sup>, Maria C. Franco<sup>1,8</sup>, Michele Joana Alves<sup>1</sup>, Jose Javier Otero<sup>6,9</sup>, and Daniel M. Prevedello<sup>2</sup>

<sup>1</sup>Department of Cellular and Molecular Medicine at Florida International University Herbert Wertheim College of Medicine, Miami, FL, <sup>2</sup>Department of Neurological Surgery, <sup>3</sup>Department of Radiation Oncology, <sup>4</sup>Department of Otolaryngology, Head and Neck Surgery, The Ohio State University Wexner Medical Center and James Cancer Center, Columbus, OH, <sup>5</sup>Miami Cancer Institute at Baptist Health South Florida, Miami, FL, <sup>6</sup>Department of Neuroscience at Florida International University Herbert Wertheim College of Medicine, Miami, FL, <sup>7</sup>Baptist Health Medical Group, Baptist Health Quality Network at Baptist Health South Florida, Miami, FL, <sup>8</sup>Center for Translational Science, Florida International University, Miami, FL, <sup>9</sup>Department of Neuropathology and Clinical Informatics at Baptist Health South Florida, Miami, FL

**Background:** Circular RNAs (circRNAs) represent a distinct and functionally relevant class of noncoding RNAs with emerging roles in tumor biology and biomarker discovery. In Meningiomas, circRNA-based molecular stratification remains poorly characterized, partly due to intraoperative variability in tissue handling that affects RNA integrity. The NICO Myriad Automated Preservation System (NICO) enables intraoperative isolation and refrigerated preservation of fresh tissue using Ringer's Lactate, minimizing ischemic and mechanical stress. This study investigates circRNA expression profiles in meningiomas using paired fresh tumor tissues from 20 patients, comparing conventional handling with NICO preservation, aiming to define robust circRNA signatures associated with tumor grade and relevant biological pathways. **Methods:** RNA sequencing was performed on all samples, and circRNAs were identified based on back-splice junction reads using CIRI2. Analyses included differential expression, unsupervised clustering, and Weighted Gene Co-expression Network Analysis (WGCNA) in R. Statistical testing included t-tests, linear regression, Pearson correlation, and Gene Ontology (GO) enrichment, with multiple testing correction ( $\log_2$  fold-change >1.5, FDR <0.05). Batch effect normalization was applied to minimize technical variability across sequencing runs. **Results:** NICO-preserved samples showed significantly higher RNA integrity and sequencing quality ( $p < 0.005$ ), reducing protocol-driven technical variability. Network analysis identified circRNA modules associated with tissue handling, reflecting RNA viability, transcriptional regulation, and cellular organization. Grade-associated differential expression analysis revealed significant upregulation of circRNAs derived from *ZNF91*, *KCNMA1*, and *SLC37A3*, enriched in ion channel activity and metabolic regulation. WGCNA identified two circRNA modules correlated with WHO Grade 2 tumors (Pearson  $r = 0.49-0.48$ ,  $p < 0.005$ ), enriched cellular metabolism, protein phosphorylation, DNA repair, and epigenetic regulation as top-ranked GO terms reaching statistical significance ( $p < 0.001$ , FDR <0.05). These exploratory findings establish a foundation for future intra-laboratory and external validation studies. **Conclusion:** Optimized intraoperative tissue preservation with NICO improves RNA integrity and reduces technical variability. This enables the identification of biologically meaningful circRNA profiles associated with meningioma grade. These circRNA profiles suggest potential as a novel biomarker strategy for tumor grading, prognostic assessment, and personalized management of meningioma patients.

**Acknowledgements:** We thank James Comprehensive Cancer Center, Pathology department, Nimjee Shahid, MD, PhD, and the NSBR team at The Ohio State University, and Florida International University. D. Prevedello was supported by NICO Awards Grant. M. C. Franco by NIH/NINDS R01NS102479. J. J. Otero by NIH/NHLBI R01HL163965-01.

## Poster Board 25

### Abstract 073

#### Reduced PAI-1 Activity Promotes Longevity and Stress Resilience: Insights from a *Drosophila* Spn42Dd Model

Michelle Thayer, and Marit Nilsen-Hamilton

Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA

**Background:** Clinical studies in the Berne Amish community have identified a rare haploinsufficient mutation in *SERPINE1*, encoding plasminogen activator inhibitor-1 (PAI-1), that is associated with increased longevity, reduced cardiovascular disease, lower fasting insulin, and decreased very low-density lipoprotein (VLDL) production. However, the mechanisms linking reduced PAI-1 activity to these beneficial outcomes remain poorly defined. Rodent models are informative but resource-intensive and limited in throughput. To address this gap, we developed a *Drosophila melanogaster* model to investigate the orthologous gene *Spn42Dd*, a conserved serpin implicated in immune and protease regulation. **Methods:** Lifespan analyses were conducted using muscle-specific RNAi knockdown of *spn42dd* via the GAL4/UAS system. Generation of a precise genetic model of the Amish-associated *SERPINE1* mutation, was pursued using CRISPR-Cas9 with a single-stranded oligonucleotide donor to create a targeted dinucleotide addition in *Spn42Dd*. Locomotor and dietary stress phenotypes were evaluated in CRISPR-engineered *spn42dd* mutant and heterozygous lines. Phylogenetic and structural homology was analyzed bioinformatically. **Results:** Muscle-specific *spn42dd* knockdown significantly delayed midlife mortality and extended lifespan relative to controls ( $p < 0.01$ ), mirroring human PAI-1 deficiency phenotypes. High sugar diet (HSD) was administered to controls, *spn42dd* null, and heterozygous flies at acute, moderate, and chronic time points, followed by locomotor analysis. The HSD study revealed that *spn42dd* heterozygous flies exhibited greater resistance to HSD compared to their homozygous cohorts, consistent with a haploinsufficiency phenotype similar to that observed in human PAI-1 deficiency. Phylogenetic analysis places Spn42Dd within the serpin family, and sequence modeling revealed conservation of the reactive center loop with human PAI-1. **Conclusions:** Our findings provide evidence that loss of *spn42dd* in *Drosophila* correlates with key features of human *SERPINE1* deficiency, including improved survival and metabolic resilience. This model offers a powerful platform for dissecting conserved molecular pathways linking PAI-1 activity to protease regulation, metabolic stress adaptation, and inflammatory signaling. The approach enables an efficient and scalable investigation of gene-environment interactions and translational aging mechanisms with broad relevance to human health.

## Poster Board 26

### Abstract 074

#### Defining the Mechanisms of Retinoic Acid Signaling in Liver Development and Pre-Cancerous Proliferation

Natalie M. Miscik<sup>1,2</sup>, Robert Judson-Torres<sup>1,3</sup>, and Kimberley J. Evason<sup>1,2</sup>

<sup>1</sup>Department of Oncological Sciences and Huntsman Cancer Institute, <sup>2</sup>Department of Pathology, <sup>3</sup>Department of Dermatology, University of Utah, Salt Lake City, UT

**Background:** Retinoic acid (RA) signaling is key to several developmental and pathologic processes, acting as a mediator of target gene expression. Hepatic stellate cells (HSCs) are the primary storage site for retinoids. In mice, loss of HSC retinoid storage leads to increased RA signaling in the liver and decreased hepatocellular carcinoma (HCC) incidence (Shirakami Y, 2012, *Carcinogenesis*, **33**:268-274). Similarly, studies of cultured human melanocytes have found that the activation of protein kinase C (PKC), which is promoted by RA, induces proliferation arrest (McNeal AS, et al., 2021, *eLife*, **10**:e70385), a characteristic of benign nevi. Although the majority of melanocytic nevi will remain benign, when anti-proliferative mechanisms are overcome, nevi can progress to melanoma. We hypothesize that RAR signaling is essential for normal liver development and for reducing a proliferative phenotype that is a frequent hallmark of precancerous cells. **Methods:** This project is investigating the RA signaling pathway in two distinct settings: zebrafish liver development and cultured primary human melanocytes. In transgenic zebrafish models, adult HCC is preceded by larval liver enlargement as early as 6 days post-fertilization (dpf) (Kotiyal S, 2020, *J Vis Exp*, 10.3791/60744), providing an easy measure of pre-oncogenic proliferation. We treat larval zebrafish with RA pathway agonists and inhibitors, then evaluate HSCs and liver volume by confocal imaging and gene expression by *in situ* stains. Primary human melanocytes are cultured in media that either promote proliferation or induce growth arrest, then treated with drugs that either induce or inhibit RA signaling. The resulting proliferative state and gene expression reveal which conditions the targeted RA pathway components are actively modulating the cellular response. **Results:** Our preliminary studies have found that an RA receptor agonist induces an increase in HSC number at 80 hpf and 144 hpf, and larger liver volume only at 144 hpf.

This suggests that increased RA signaling and HSC abundance promote liver outgrowth during development. We are currently treating larval zebrafish with ATRA, TP-0903, and DEAB. **Conclusions:** There is a lack of information on the RA signaling mechanism of controlling pre-cancerous proliferation. This project aims to fill that gap by modulating RA signaling in two distinct models. By utilizing larval zebrafish and cultured melanocytes, we will model RA-mediated proliferation *in vitro* and *in vivo* and potentially uncover tissue-specific variance in the RA mechanism.

**Acknowledgements:** We thank Director of Aquatics Carrie Barton and the Office of Comparative Medicine aquatic animal care team, and the University of Utah Cell Imaging Core. This work is supported by the American Cancer Society (RSG-22-014-01-CCB). K.J.E. was funded by the National Cancer Institute (R01CA222570).

## Poster Board 27

### Abstract 075

#### Investigating the Impact of Trained Immunity in Intestinal Stromal-Monocyte Interactions

Sarah Balfe<sup>1,2</sup>, Cristina Bauset<sup>1,2</sup>, and Mario C. Manresa<sup>1,2</sup>

<sup>1</sup>School of Biomolecular and Biomedical Sciences, <sup>2</sup>Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland

**Background:** Interactions between the intestinal stroma and immune cells play a key role in the pathogenesis of Inflammatory Bowel Disease (IBD), resulting in increased immune cell adherence, polarization and cytokine production. However, the mechanisms behind this increased immune cell activation remain unclear. Our lab has evidence indicating that cytokine exposure can induce trained immunity in fibroblasts. We hypothesize that acquisition of trained immunity by intestinal fibroblasts alters their effect on monocytes. **Methods:** To analyze the effects of trained fibroblasts on monocytes, primary human colonic fibroblasts were stimulated with TWEAK and IL-1 $\alpha$  using a 2-hit model developed in our lab to induce a trained immunity phenotype. THP1s were treated with conditioned media (CM) from these trained fibroblasts compared to naïve conditions, and the presence of cell surface markers (flow cytometry) and the activation of key inflammatory signaling pathways (western blot) were assessed. **Results:** Exposure to CM from trained colonic fibroblasts resulted in decreased CD14 and TREM-1, but enhanced Dectin-1 levels in THP1s, compared to treatment with CM from fibroblasts stimulated with IL-1 $\alpha$  alone. Analysis of signaling pathway activation indicated alterations in activation of various kinases. This included a previously unrecognized increase in phosphorylation of P38 in THP1s following exposure to conditioned media (CM) from IL-1 $\alpha$ -stimulated fibroblasts that was not increased following exposure to CM from trained fibroblasts. Current studies are focused on expanding the analysis to transcription level effects of trained fibroblasts on monocytes and validating key findings in primary monocytes. **Conclusion:** Acquisition of trained immunity by intestinal fibroblasts specifically impair their effect on monocytes, leading to altered surface marker expression and decreased P38 phosphorylation compared to fibroblasts with a single cytokine stimulation.

**Acknowledgements:** This work was funded by Science Foundation Ireland and The Irish Research Council under the Pathway program (21/PATH-S/9621), CB was supported by Research Ireland (GOIPD/2025/1089).

## Poster Board 28

### Abstract 076

#### Electronic Cigarette Exposure Accelerates Abdominal Aortic Aneurysm Progression via Histone Demethylase Associated Inflammatory and Matrix Remodeling Pathways

Yibo Xi and He Wang

Department of Pathology, Renaissance School of Medicine, Stony Brook University, Stony Brook, NY

**Background:** Abdominal aortic aneurysm (AAA) is a progressive vascular disease characterized by chronic inflammation, extracellular matrix degradation, and vascular smooth muscle cell (SMC) dysfunction. Cigarette smoking is the strongest modifiable risk factor for AAA, yet the vascular consequences of electronic cigarette (e-cigarette) exposure and the underlying molecular pathways remain poorly defined. Emerging evidence suggests that epigenetic regulation may play a critical role in inflammatory and matrix remodeling processes during aneurysm development. This study aimed to determine whether e-cigarette exposure promotes AAA progression by activating histone demethylase-associated inflammatory and matrix remodeling programs in the aortic wall. **Methods:** Complementary experimental AAA models were employed to assess the impact of e-cigarette exposure on aneurysm development and vascular pathology. ApoE<sup>-/-</sup> mice subjected to angiotensin II infusion and elastase-based aneurysm induction were exposed to e-cigarette aerosol or filtered air. Aortic tissues were collected for morphometric analysis, histopathological evaluation, and molecular

profiling. Quantitative PCR, protein-based assays, and immunofluorescence were used to assess expression of epigenetic regulators, inflammatory mediators, and matrix-degrading enzymes, as well as immune cell infiltration. Pharmacologic inhibition studies were performed to interrogate functional contributions of histone demethylase activity. **Results:** E-cigarette exposure exacerbated aneurysm formation, as evidenced by increased aortic dilation and enhanced medial degeneration. Aneurysmal aortas from e-cigarette-exposed mice demonstrated increased expression of the histone lysine demethylase Kdm4d, accompanied by upregulation of matrix metalloproteinases (Mmp12, Mmp7) and pro-inflammatory cytokines, including Il6. These molecular alterations were associated with reduced expression of SMC contractile markers, increased elastin fragmentation, and augmented macrophage accumulation within the aortic wall. Pharmacologic inhibition of histone demethylase activity attenuated induction of inflammatory and matrix remodeling genes and was associated with reduced aneurysm severity. **Conclusions:** These findings support a model in which e-cigarette exposure accelerates AAA progression through activation of histone demethylase-associated inflammatory and extracellular matrix remodeling pathways in the aortic wall. KDM4D emerges as a regulatory node linking exposure-associated epigenetic changes to vascular inflammation and structural degeneration, providing mechanistic insight into e-cigarette-associated AAA pathobiology.

## Poster Session – Inflammation and Immunopathology

### Poster Board 29

#### Abstract 077

#### Eicosanoid Regulation of Cancer Cachexia

Neha Rana<sup>1</sup>, Rachel L. Bayer<sup>1</sup>, Jianjun Deng<sup>1,2</sup>, Jianjian Zheng<sup>1</sup>, Katherine M. Quinlivan<sup>1</sup>, Keira S. Smith<sup>1</sup>, Sung Hee Hwang<sup>3</sup>, Nicholas Mitsiades<sup>4</sup>, Bruce D. Hammock<sup>3</sup>, Haixia Yang<sup>1,5</sup>, and Dipak Panigrahy<sup>1</sup>

<sup>1</sup>Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, <sup>2</sup>Shaanxi Key Laboratory of Degradable Biomedical Materials, School of Chemical Engineering, Northwest University, Xi'an, China,

<sup>3</sup>Department of Entomology and Nematology, and UC Davis Comprehensive Cancer Center, University of California, Davis, CA, <sup>4</sup>Department of Internal Medicine, University of California Davis, CA, <sup>5</sup>College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China

**Background:** Cancer cachexia is a systemic muscle-wasting syndrome responsible for 20-30% of cancer-related deaths. Chronic systemic inflammation and accumulation of apoptotic cellular debris are critical drivers of tissue wasting, yet no approved therapies currently exist. Arachidonic acid-derived epoxyeicosatrienoic acids (EETs) are anti-inflammatory and pro-resolving (“debris-clearing”) eicosanoids, but they are rapidly metabolized by soluble epoxide hydrolase (sEH), whose expression is further induced by cancer therapies, exacerbating inflammation. Because sEH inhibition promotes organ regeneration and resolution via pro-resolving lipid mediators, we hypothesized that cancer cachexia is driven in part by dysregulation of oxylipin-mediated resolution pathways. **Methods:** Oxylipin profiles were analyzed in plasma and skeletal muscle (gastrocnemius and tibialis anterior) from three murine models of cancer cachexia – CT26 colon cancer, KPCY pancreatic adenocarcinoma, and TRAMP prostate cancer – using UPLC-MS/MS. Quantitative PCR was used to assess inflammatory and senescence-associated gene expression, and flow cytometry characterized immune cell infiltration in treated versus untreated cachectic tissues. **Results:** Cachectic mice exhibited a marked imbalance between pro-inflammatory eicosanoids and pro-resolving oxylipin mediators including 11(12)-EET, 8(9)-EET, 15-deoxy-PGJ<sub>2</sub>, 13-HOTrE, and 9-HOTrE in plasma and tissues compared to non-tumor bearing controls. sEH expression was stimulated at both protein/mRNA levels in cachectic tissues (e.g., gastrocnemius, liver, and heart). EC5026, a potent sEH inhibitor currently in clinical trials with no drug-related adverse events, prolonged survival in both transplantable tumors and genetically engineered cachexia models. sEH null mice exhibited enhanced prolonged survival post-KPCY pancreatic cancer cachexia compared to WT controls. In Fat-1 transgenic mice which endogenously produce omega-3 fatty acids, EC5026 further improved survival in KPCY cachexia. Thus, both pharmacological and genetic sEH inhibition prolongs survival in genetically engineered cancer cachexia models. Mechanistically, sEH inhibition shifted lipid signaling from pro-inflammatory eicosanoids to pro-resolving lipid mediators and oxylipins. EC5026 activated endogenous resolution pathways (e.g., GPR18) and inhibited pro-inflammatory enzymes/cytokines. qPCR of treated cachectic tissues showed increased anti-senescence and anti-inflammatory gene expression (GLB1, P21, P16, TNF, IL1 $\beta$ , IL6). sEH inhibition also enhanced anti-tumor immunity by boosting cytotoxic T and NK cell infiltration and reducing macrophages. **Conclusions:** These findings support sEH inhibition as a novel strategy to counter cancer cachexia via oxylipin-mediated resolution pathways. **Acknowledgements:** This work was supported by RIVER Grant R35 ES030443- 01 (B.D.H.); Credit Union Kids at Heart (DP); Carter Joseph Buckley Pediatric Brain Tumor Fund (D.P.).

## Poster Board 30

### Abstract 078 - **WITHDRAWN**

## Poster Board 31

### Abstract 079

#### **Brainstem Neuroinflammation in Acetaminophen-Induced Hepatotoxicity**

Isaac Vargas Rodriguez, Emily Silva, David Villalobos, Jose Otero, and Michele Alves

Department of Cellular and Molecular Medicine, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

**Background:** Acetaminophen (APAP) is the most common cause of acute liver failure (ALF). The ALF contributes to brain dysfunction, neurological impairment and elevated pressure in the brainstem which can lead to coma and potential death. The multi-organ failure in ALF is associated with severe neuroinflammation including the cytokines IL-1 $\beta$ , TNF $\alpha$ , and IL-6. Our study aimed to test whether acetaminophen-induced acute liver failure could be associated with brainstem neuroinflammation and dysfunction. **Methods:** CD1 female and male mice were injected intraperitoneally with 300mg.kg-1 or 600mg.kg-1 acetaminophen dose or vehicle. Following 48h, animals were euthanized and liver and brainstem were dissected out. RNA extraction was obtained using TRIzol and Quick-RNA miniprep kit (Zymo Research), then the cDNA using High-Capacity cDNA Reverse Transcription kit (ThermoFisher) and the qPCR was performed using SYBR Green master mix (ThermoFisher). **Results:** In this study, we utilized acetaminophen-induced ALF model in CD1 mice due to their genetic diversity and a preferred model for toxicity studies. We first characterized the dose for APAP-induced neuroinflammation and pro-inflammatory cytokines expression in the liver and brainstem at different time points. The mortality rate of APAP at 300mg.kg-1 dose was 0%. However, the group receiving APAP at 600mg.kg-1 exhibited 45% increased mortality rate. At 48h following 300mg.kg-1 dose of APAP, no significant changes were found of IL-6, IL-1 $\beta$ , and TNF $\alpha$  mRNA levels in the liver and brainstem. In contrast, the animals that survived the APAP at 600mg.kg-1 exhibited increased protein levels of IL6 and suggestive evidence for an effect of X on Y for IL-1 $\beta$  ( $p < 0.08$ ) and TNF $\alpha$  ( $p < 0.06$ ) in the liver but not in the brainstem. In the liver, gene expression levels of IL6 were enhanced due to APAP along with Glul that encodes glutamine synthetase. In the brainstem, we found a robust increase of NOXO1 and marginal effect of X and Y for TNF $\alpha$  ( $p < 0.07$ ; effect size  $g = 1.25$ , 95% [0.06, 2.32]). **Conclusions:** Our results indicate that APAP robustly induced hepatotoxicity following 48h. Neuroinflammatory changes in the brainstem were modest following APAP-induced acute liver failure. Because analyses were restricted to surviving animals, they might not reflect severe brainstem neuroinflammation outcomes, suggesting potential mechanisms of adaptive response.

**Acknowledgements:** This work was supported by: NIH/NHLBI R01HL132355 for JJO and FIU Start-up for MJA.

## Poster Board 32

### Abstract 080

#### **Targeting Cancer Cachexia Via SPMs**

Camille Longabardi<sup>1,2</sup>, Neha Rana<sup>1,2</sup>, Lily M. Ceraso<sup>1,2</sup>, Katherine M. Quinlivan<sup>1,2</sup>, Keira S. Smith<sup>1,2</sup>, Jianjun Deng<sup>1,2,3</sup>, Haixia Yang<sup>1,2,4</sup>, Steven D. Freedman<sup>5</sup>, Charles N. Serhan<sup>6</sup>, and Dipak Panigrahy<sup>1,2</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup>Center for Vascular Biology Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, <sup>3</sup>Shaanxi Key Laboratory of Degradable Biomedical Materials, School of Chemical Engineering, Northwest University, Xi'an, China, <sup>4</sup>College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China, <sup>5</sup>Division of Gastroenterology and Pancreas Center, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, <sup>6</sup>Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

**Background:** Most patients with advanced cancer suffer from cachexia, which accounts for ~20% of cancer-related deaths and lacks approved therapies. Cancer cachexia is a devastating muscle wasting syndrome driven by unresolved hyperinflammation, characterized by elevated pro-inflammatory cytokines, and induces widespread apoptotic cell death which reduces tolerance to cancer therapy. This cell death ("debris") across tissues leads to multi-organ failure and immunosuppression. A paradigm shift is emerging that recognizes the resolution of inflammation as an active process orchestrated by a novel class of endogenous specialized pro-resolving lipid autacoid mediators (SPMs). SPMs stimulate clearance of debris, promote muscle regeneration, and counter-regulate cytokines without immunosuppression. We recently demonstrated that debris stimulates pro-inflammatory eicosanoid/cytokine storms and clearing debris (*pro-*

*resolution*) is a novel approach to preventing cancer progression. We hypothesized that cachexia results from disrupted resolution of inflammation. **Methods:** Using targeted metabololipidomics (LC-MS/MS), lipid mediators were profiled from murine models of metastatic cancer cachexia. **Results:** Dysregulated SPMs were identified in six cancer cachexia models. SPMs (e.g. RvD2 and MaR1) were markedly reduced in colon cancer (CT26)-induced cachectic mice on day 35 post-tumor cell injection vs. non-tumor-bearing controls. SPMs (RvD1, RvD2, LXA<sub>4</sub>, and MaR1) were also dramatically dysregulated in Lewis lung carcinoma (LLC)-induced cachectic mice, including the gastrocnemius and tibialis anterior muscles, heart, liver, and spleen, on day 20 post-tumor cell injection. Cachexia induced a pro-inflammatory eicosanoid storm in plasma isolated from CT26 mice. Chemotherapy also induced cachexia *via* loss of SPMs in lymphoma (EL4) and ovarian cancer (ID8). 10 days post-LLC tumor resection, RvD1 (ALX/FPR2) or RvE1 (ChemR23/ERV) receptor KO mice exhibited greater body weight loss compared to WT, indicating resolvin receptor dependence in cachexia. SPM treatment (e.g. RvD2 or PCTR2) prevented cachexia in LLC and B16F10 melanoma without causing immunosuppression. In KPC pancreatic cancer, RvD2 preserved grip strength and prolonged survival. Unlike celecoxib, SPMs counter-regulate cachexia-induced cytokines, such as TNF- $\alpha$ , CCL2, CCL3, CCL4, CXCL2, and G-CSF. SPMs were sharply reduced by up to 85% in the plasma of human pancreatitis patients at risk for cachexia compared to healthy controls. **Conclusions:** Altogether, SPMs counter-regulate cachexia-induced dysregulated pro-resolution endogenous mechanisms. Our studies shall provide the basis for the clinical translation of SPM-directed treatments as a new direction to potentially prevent and/or reverse cancer cachexia. **Acknowledgements:** This work was supported by NIH grants R01CA276107-01A3 (DP, SDF, and CNS) and NIH R01CA285395-01A2 (DP and CNS); Credit Union Kids at Heart (DP); Carter Joseph Buckley Pediatric Brain Tumor Fund (D.P.).

### Poster Board 33

#### Abstract 081

#### Muscle Dysfunction and Wasting in a Preclinical Aged Lung Cancer Model

Celleste E. Wohlfarth, Zoe P. Libramento, Louisa Tichy, Abigail L. Smith, and Traci L. Parry  
Department of Kinesiology, University of North Carolina, Greensboro, NC

**Background:** Cancer cachexia is a metabolic wasting disorder, primarily affecting older patients with advanced cancer. Among cancer types, lung cancer has one of the highest incidences of cachexia. Characterized by severe, continuous fat and muscle loss, cancer cachexia results in weakness, fatigue, and ultimately interferes with response to anti-cancer treatment. While the prevalence of cancer cachexia varies, it becomes more prominent in older patients with more aggressive and advanced stages of cancer. Despite its clinical importance, cancer cachexia is underdiagnosed and undertreated due to the lack of clear standardized diagnostic criteria, resulting in failed intervention. Development of fatigue and function-related criteria may improve diagnosis for an earlier intervention in aged cancer survivors. Therefore, the purpose of this study was to evaluate muscle and fatigue declines of an aged lung cancer mouse model. **Methods:** Aged male LC3Tg+ C57/BL6 mice (16-18 months old) were randomly assigned to tumor-bearing (T) and non-tumor bearing (NT) groups. Mice were implanted with Lewis lung carcinoma cells ( $5 \times 10^5$  in flank). Skeletal muscle function (grip strength), cardiac muscle function (echocardiography), and fatigue were followed throughout the study. Muscle masses were assessed at the end point of the study. **Results:** The combination of aging and tumor bearing resulted in significant muscle wasting and dysfunction. Mice in the T group exhibited significantly lower body mass, skeletal muscle (mixed fiber gastrocnemius) mass, and cardiac muscle mass compared to NT controls ( $P < 0.05$ ). This coincided with severe muscle dysfunction which exacerbated overall whole-body fatigue in the T group vs the NT group ( $P < 0.05$ ). **Conclusions:** These findings demonstrate that tumor burden causes significant, continuous wasting of the heart and skeletal muscle, and this results in whole-body wasting, fatigue and severe muscle dysfunction. The overall effects of metabolic wasting disorders, like cancer cachexia, can be exacerbated by age-related physiological decline. These findings highlight the need for clear standardized diagnostic criteria and targeted therapeutic strategies aimed at preventing cachexia, preserving muscle mass, and improving functional capacity for affected populations.

### Poster Board 34

#### Abstract 082

#### Targeting Inflammation Resolution in Glioblastoma Via SPMs

Keira S. Smith<sup>1,2</sup>, Camille Longabardi<sup>1,2</sup>, Lily M. Ceraso<sup>1,2</sup>, Neha Rana<sup>1,2</sup>, Katherine M. Quinlivan<sup>1,2</sup>, Ella Crerar<sup>1,2</sup>, Charles N. Serhan<sup>3</sup>, and Dipak Panigrahy<sup>1,2</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup>Center for Vascular Biology Research, <sup>3</sup>Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

**Background:** While inflammation is a hallmark of cancer, the mechanisms that disrupt its resolution are not well understood. A paradigm shift is emerging in our understanding that the resolution of inflammation is an active process controlled by specialized pro-resolving mediators (SPMs), including protectins and resolvins, which are endogenous lipid autacoids present in tissues such as the brain and cerebrospinal fluid. Endogenous resolution mechanisms and pro-resolving mediators have not been studied in glioblastoma. Notably, macrophages and microglia comprise approximately 30-50% of human glioma tumors and up to 70% of glioblastoma. **Methods:** We studied the role of SPMs in brain tumors via orthotopic implantation of murine glioblastoma (CT2-A) tumors in immunocompetent C57BL6 mice. SPM receptor, proliferation, and angiogenesis immunohistochemistry was performed on glioblastoma tumors. Phagocytosis assays and cytokine arrays were performed to evaluate pro-resolution mechanisms. **Results:** We demonstrate that the failure of inflammation resolution via dysregulated SPMs may be a critical risk factor for aggressive glioblastoma growth. Moreover, chemotherapy and targeted therapy further disrupt inflammation resolution via a tumor cell death (“debris”)-induced cytokine storm by macrophages. The SPM receptors GPR37 (protectin D1), GPR18/DRV2 (resolvin (Rv) D2), GPR32 (RvD1), and ChemR23/ERV (RvE1) are expressed in tumor-infiltrating macrophages and endothelial cells in the brain tumor microenvironment. Orthotopic CT2-A-induced glioblastoma induced cancer cachexia that was regulated by inflammation resolution. Protectins and resolvins suppressed orthotopic debris-stimulated brain tumor growth by activating macrophage and microglial phagocytosis of cell debris and counter-regulating pro-inflammatory cytokines (e.g. TNF- $\alpha$ , CCL2, CXCL1, and CCL4). Systemic SPM treatment with protectins and resolvins suppressed Ki67- and CD31-positive staining in glioblastoma tumors compared to control tumors. Thus, SPMs block tumor angiogenesis and tumor cell proliferation in brain cancer models and trigger inflammation resolution to inhibit brain tumor progression. **Conclusions:** Therefore, pro-resolving agonists such as SPMs represent a novel debris-clearing and resolution-enhancing approach to complement cytotoxic brain cancer therapies. **Acknowledgements:** This work was supported by NIH grants R01CA276107-01A3 (DP, CNS) and NIH R01CA285395-01A2 (DP, CNS); Credit Union Kids at Heart (DP); Carter Joseph Buckley Pediatric Brain Tumor Fund (DP).

## Poster Board 35

### Abstract 083

#### A Path to Biomarkers: Extracellular Vesicle DNA in Sjögren’s Disease

Grace M. Coyne<sup>1</sup>, Yi Li<sup>2</sup>, Fei Liu<sup>2</sup>, and Menglu Yang<sup>1</sup>

<sup>1</sup>Department of Ophthalmology, Schepens Eye Research Institute of Mass Eye and Ear, Harvard Medical School, Boston, MA, <sup>2</sup>Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA

**Background:** Sjögren’s disease (SJD) is an autoimmune disease involving glandular dysfunction of the lacrimal and salivary glands and multiple other systems. Diagnoses of SJD are challenging due to the variety of clinical presentations, creating an average delay of 6 months from onset to diagnosis. DNA is associated with the pathogenesis of SJD, and DNA encapsulated in extracellular vesicles (EV-DNA) provides direct entry of DNAs into the glandular cells. In the current study, we investigated the EV-DNA profile from human tear fluid, aiming to understand the pathological roles of EV-DNAs in SJD, and identify potential biomarkers. **Methods:** Human tear samples of Sjögren’s Disease and non Sjögren’s patients collected by Schirmer strips were subject to EV isolation using an automatic acoustic fractionation method. NOD.B10.H2b mouse was used as a model of SJD. EVs were isolated from the tear fluid and serum. Size distribution was analyzed by NTA, and DNA fragment length was analyzed by Bioanalyzer. **Results:** EVs were successfully isolated from human tear fluid, with an average amount of  $5 \times 10^8$  EV/ml. More EV-DNA was found in tear samples from SJD patients compared to controls, with a 1000-2000 bp fragment length. Due to the low volume of mice tear fluid, eye wash collected from 10 mice was pooled as one, ensuring at minimal  $10^8$  EVs/ml harvested. The total eye wash volume was used as a reference. The size distribution of serum EV between NOD.B2.H2b and wildtype control is significantly different. Due to the small volume of tear fluid EV, the DNA enrichment analysis was conducted in serum. From NOD.B10.H2b mice,  $10^{12}$  EV/ml was isolated, 20% of which are positive for Hoechst staining, indicating the presence of DNA. EV-DNA was also extracted from mouse serum. exoDNA from NOD tear fluid carries short fragments which are absent in BalbC (~174bp); EVs from NOD mice also carries more long fragments (>1000bp) than wildtype. **Conclusions:** Tear fluid derived EV and EV-DNA shows distinct characteristics between SJD and non-diseased controls, underscoring its potential relevance to pathogenesis and biomarker development. **Acknowledgements:** This work is funded by Connors BWH-MGB Collaborative IGNITE Award (FL and MY), The National Eye Institute (USA) R56EY037692 (MY), The National Eye Institute (NEI) [R01 EY026202] (MY).

## Poster Board 36

### Abstract 084

#### Loss of the Actin Assembly Factor Formin-Like-2 Reduces Neutrophil Extravasation in Inflamed Cremasteric Postcapillary Venules

Arturo Armando Valenzuela-Padilla<sup>1</sup>, Hilda Vargas-Robles<sup>1</sup>, Cord Brakebusch<sup>2</sup>, Kristin von Peinen<sup>3</sup>, Klemens Rottner<sup>3</sup>, and Michael Schnoor<sup>1</sup>

<sup>1</sup>Department of Molecular Biomedicine, CINVESTAV-IPN, Mexico City, Mexico, <sup>2</sup>BRICS, Copenhagen, Denmark,

<sup>3</sup>Department of Cell Biology, Helmholtz Center for Infection Research, Braunschweig, Germany

**Background:** Inflammation is a complex biological response of the immune system to stimuli such as pathogens, damaged cells, or irritants. This response is initiated by tissue-resident cells, which generate a proinflammatory microenvironment that activates endothelial cells (EC) and promotes the recruitment of circulating neutrophils to the site of injury. Neutrophil extravasation is a multistep adhesive and migratory process that critically depends on actin cytoskeleton remodeling in EC and neutrophils, a cellular response regulated by actin-binding proteins (ABP). We have previously shown that the ABP cortactin and HS1 support neutrophil extravasation in the inflamed mouse cremaster muscle. However, the function of the actin assembly factor formin-like-2 (FMNL2) during inflammation remains unexplored. FMNL2 is a ubiquitous and highly conserved ABP in both mice and humans that is involved in filopodia and lamellipodia formation, vesicular trafficking, and cell-cell junction assembly. It has also been shown to regulate cell adhesion and migration. Therefore, we hypothesized that FMNL2 depletion impairs neutrophil extravasation *in vivo*.

**Methods and Results:** FMNL2-deficient mice with C57Bl/6 background are viable but exhibit altered weight gain compared to littermate wild-type mice. We found that both neutrophils and cremasteric postcapillary EC express FMNL2. Intravital microscopy of the TNF $\alpha$ -inflamed cremaster revealed that neutrophils in FMNL2-deficient mice rolled faster leading to significantly reduced adhesion and transmigration compared to wild-type littermates. However, FMNL2-deficient mice showed no significant differences in vascular permeability. **Conclusion:** Our data uncovered a hitherto unknown role of FMNL2 in the innate immune response by controlling neutrophil extravasation. We are currently investigating the molecular mechanisms underlying this defect in neutrophil extravasation, with a particular focus on actin dynamics in neutrophils and EC.

## Poster Session – Liver Pathobiology

### Poster Board 37

#### Abstract 085

#### Lipid Dysregulation in Zebrafish $\beta$ -Catenin-Driven Hepatocellular Carcinoma

Aavrati Saxena<sup>1,2,3</sup>, Junko Kuramoto<sup>1,3</sup>, Chad Van-Sant Webb<sup>1,2,3</sup>, Richard Smith<sup>1,3</sup>, Alexis Ross<sup>1,2,3</sup>, Gregory S. Ducker<sup>3,4</sup>, and Kimberley J. Evason<sup>1,2,3</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup>Department of Oncological Sciences, <sup>3</sup>Huntsman Cancer Institute, <sup>4</sup>Department of Biochemistry, University of Utah, Salt Lake City, UT

**Background:** Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and affects ~1 million people per year worldwide (Llovet et al., 2021, *Nature Rev Dis Primer*, **7**:1-28). Metabolic reprogramming is fundamental in cancer including HCC as it enables a healthy normal cell to transform into a malignant one. Essential lipids such as phosphatidylcholine (PC) are commonly dysregulated in human HCC patient samples (Paul B, et al., 2022, *JHEP Rep*, **4**:100479). PC is a significant constituent of biological membranes and is synthesized via the cytidine diphosphate (CDP)-choline and phosphatidylethanolamine N-methyltransferase (PEMT) pathways in the liver. The goal of this study is to elucidate the role of PC lipid metabolism in HCC (<https://www.ncbi.nlm.nih.gov/books/NBK26871/>). **Methods and Results:** Transgenic zebrafish expressing hepatocyte-specific activated beta-catenin (ABC) show liver overgrowth as early as 6 days post fertilization (dpf) and as adults develop HCC that are histologically and transcriptomically similar to human HCC (Evason KJ, et al., 2015, *PLOS Genet*, **11**:e1005305). We performed lipidomic analyses of male and female transgenic ABC zebrafish with HCC and non-transgenic sibling control zebrafish livers using LC-MS and found significant differences in numerous lipid species, including acyl carnitines, ceramides, and PCs. We also performed isotope tracing in ABC with HCC and non-transgenic control zebrafish liver tissues to quantify the metabolic pathways contributing to these changes. We discovered that PC flux was downregulated in zebrafish HCC via sex-specific mechanisms (VanSant-Webb C, et al., 2024, *Biochim Biophys Acta Mol Cell Biol Lipids*, **1869**:159514). Ongoing studies are focused on defining the effects of manipulating PC metabolism on hepatocarcinogenesis using genetic tools. Using CRISPR-Cas9 technology in zebrafish we

deleted *choline phosphotransferase 1 (chpt1)*, which encodes an enzyme responsible for synthesis of PC via the CDP-choline pathway. Loss of *chpt1* decreased tumor burden and HCC incidence in adult ABC zebrafish. We also used standard transgenic techniques to generate zebrafish that overexpress *chpt1* in hepatocytes. We found that overexpression of hepatocyte-specific *chpt1* led to increased tumor burden and HCC incidence in ABC zebrafish. We are currently examining the effects of knocking down and overexpressing *CHPT1* in human liver cancer cell lines with and without activating mutations in beta-catenin. **Conclusions:** Our results suggest that PC promotes ABC-driven hepatocarcinogenesis. **Acknowledgements:** Grant funding or other special contributions to the research: This work was supported by R01CA222570-NIH-NCI and ACS grant RSG-22-014-01-CCB to K.J.E.; Damon Runyon-Rachleff Innovation Award DR 61-20 to K.J.E. and G.S.D; ACS grant DBG-23-1037804-01-TBE to GSD. We acknowledge the direct financial support for the research reported in this publication provided by the Huntsman Cancer Foundation and Huntsman Cancer Institute.

## Poster Board 38

### Abstract 086

#### Transforming Growth Factor Beta 1 Signaling in Neurons Induces Type C Hepatic Encephalopathy

Matthew McMillin<sup>1,2</sup>, Kathryn Rhodes<sup>3</sup>, Julie Venter<sup>3</sup>, Yubo Wang<sup>3</sup>, Mihika Patankar<sup>3</sup>, Esha Gupta<sup>3</sup>, Jace Tyson<sup>3</sup>, and Sharon DeMorrow<sup>3,4</sup>

<sup>1</sup>Huffington Department of Education, Innovation, and Technology, <sup>2</sup>Department of Medicine, Baylor College of Medicine, Temple, TX, <sup>3</sup>College of Pharmacy, University of Texas at Austin, Austin, TX, <sup>4</sup>Department of Medicine, University of Texas at Austin, Dell Medical School, Austin, TX

**Background:** Type C hepatic encephalopathy (HE) is associated with neurological dysfunction resulting from hyperammonemia during chronic liver disease. Neuronal transforming growth factor beta 1 (TGFβ1) signaling has been associated with the development of neurological deficits during acute liver injury via transforming growth factor beta receptor 2 (TGFβR2) signaling. However, the role of TGFβ1 signaling in Type C HE has not been characterized at this time. **Methods:** C57BL/6 mice were treated with CCl<sub>4</sub> for 16 weeks to induce Type C HE. TGFβ1 signaling was reduced by treating with anti-TGFβ1 neutralizing antibody in CCl<sub>4</sub> mice or by administering CCl<sub>4</sub> to mice with conditional knockout of TGFβR2 in neurons (TGFβR2/Thy1-cre). Neurological assessments including open-field, ataxia, rotarod, grip strength, and nestlet behavior measures were used to determine the consequence of TGFβ1 signaling interventions. Liver damage was assessed using H&E staining and measuring ammonia levels. Hippocampus and cerebellum TGFβ1 expression and signaling were assessed in all groups by protein and mRNA analyses. Human cerebellum brain sections from cirrhosis patients with HE or age-matched controls were immunostained for TGFβ1. Investigation into the interrelationship between ammonia and TGFβ1 signaling was performed *in vitro* using primary murine neurons and the HT22 hippocampal neuron cell line. **Results:** Type C HE patients had increased immunostaining for TGFβ1 compared to age-matched controls. TGFβ1 protein levels were significantly elevated in the serum, hippocampus, and cerebellum of CCl<sub>4</sub>-treated mice. Anti-TGFβ1 administration or TGFβR2/Thy1-cre strains of mice were protected from CCl<sub>4</sub>-induced neurological deficits. SMAD2/3 phosphorylation was also decreased in anti-TGFβ1 and TGFβR2/Thy1-cre groups indicating reduced downstream TGFβ1 signaling. HT22 cells and primary neurons treated with ammonia had increased levels of TGFβ1 mRNA indicating a direct link between hyperammonemia and increased neuronal TGFβ1. **Conclusions:** These data support that neuronal TGFβ1 signaling is increased due to hyperammonemia and contributes to the development of Type C HE. Therefore, targeting the TGFβ1/TGFβR2 signaling axis may be a potential therapeutic approach for management of Type C HE. **Acknowledgements:** This study was supported by an NIH R01 award (DK135995).

## Poster Board 39

### Abstract 087

#### Hepatocyte-to-Cholangiocyte Transition Drives Ductular Expansion in Human Alcohol-Associated Liver Disease

Muhammad Azhar Nisar<sup>1,2</sup>, Xinjian Li<sup>1,2</sup>, Yaozhong Liu<sup>3</sup>, Brandon James Peiffer<sup>4</sup>, Zhaoli Sun<sup>4</sup>, Peng-Sheng Ting<sup>5</sup>, Wenke Feng<sup>6</sup>, Lixian Chen<sup>6</sup>, Xiao-Ming Yin<sup>2</sup>, and Chiung-Kuei Huang<sup>1,2</sup>

<sup>1</sup>Department of Medicine, Division of Gastroenterology and Hepatology, Renaissance School of Medicine at Stony Brook University, Stony Brook, NY, <sup>2</sup>Department of Pathology and Medicine, Tulane University School of Medicine, New Orleans, LA, <sup>3</sup>Department of Biostatistics and Data Science, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, <sup>4</sup>Johns Hopkins University School of Medicine, Baltimore, MD, <sup>5</sup>Division of Gastroenterology and Hepatology, <sup>6</sup>Department Structural Cellular Biology, Tulane University School of Medicine, New Orleans, LA

**Background:** Alcohol-associated liver disease (ALD) is characterized by progressive ductular reaction and cholangiocyte expansion. However, the cellular origin of increased cholangiocyte populations remains controversial, with proposed sources including hepatocyte transdifferentiation, cholangiocyte proliferation, or stem cell differentiation. Given the ductular disorganization substantially impacts liver function in ALD progression, clarifying the cellular origin of increased cholangiocytes and underlying mechanisms for this regulation is essential for developing therapeutic targets in ALD progression. **Methods:** We performed single-nucleus RNA sequencing on liver samples from normal controls, alcoholic hepatitis (AH), and alcoholic cirrhosis (AC) human patients. Cell type identification, transitional state analysis, and pseudotime trajectory inference were conducted using Seurat and Slingshot. Transitioning cells were identified based on expressions of KLF4, KLF6, SOX4, and KRT23. Co-expression analysis of hepatocyte, cholangiocyte, and transition markers was performed across disease states. Differential gene expression and Gene Ontology enrichment analyses were conducted on transitioning cell populations. **Results:** We observed significant cholangiocyte expansion in disease states, alcoholic hepatitis (AH) and Alcoholic cirrhosis (AC), with corresponding hepatocyte depletion. Transitioning cells increased was also observed in AH and AC groups. Critically, co-expression analysis revealed that normal transitioning cells abundantly co-expressed hepatocyte markers (ALB, APOA1, CYP3A4, HNF4A) alongside transition markers (KLF4, KLF6), whereas AH and AC transitioning cells demonstrated dramatic loss of hepatocyte marker expression, suggesting aggressive transdifferentiation. Proliferation markers (MKI67, TOP2A, STMN1) were absent in cholangiocytes, suggesting that cholangiocyte proliferation has limited contribution to the increased cholangiocytes. Pseudotime trajectory analysis confirmed a continuous hepatocyte-to-cholangiocyte transition pathway, with progressive upregulation of cholangiocyte markers and downregulation of hepatocyte markers. Trajectory analysis from stem/progenitor cells to cholangiocytes showed weaker support compared to the hepatocyte-origin pathway. DEG analysis revealed transitioning cells were enriched in metabolic processes (amino acid metabolism, lipid metabolism, steroid metabolism) and alcohol response pathways, indicating alcohol-triggered cellular reprogramming. **Conclusion:** Cholangiocyte expansion in ALD predominantly arises from hepatocyte transdifferentiation rather than cholangiocyte proliferation or stem cell differentiation. The loss of hepatocyte identity and acquisition of cholangiocyte features in transitioning cells represents an alcohol-induced adaptive response involving metabolic reprogramming. These findings identify the hepatocyte-to-cholangiocyte transition as a potential therapeutic target for preventing ductular reactions in ALD.

## Poster Board 40

### Abstract 088

#### Sex-dependent Neurological and Behavioral Outcomes in Hepatic Encephalopathy in Adult Mice

Yubo Wang<sup>1</sup>, Kathryn Rhodes<sup>1</sup>, Jace Tyson<sup>1</sup>, Juliet Venter<sup>1</sup>, Mihika Patankar<sup>1</sup>, Esha Gupta<sup>1</sup>, Sujani Srinivasan<sup>1</sup>, Patrick Mireles<sup>1</sup>, and Sharon DeMorrow<sup>1,2</sup>

<sup>1</sup>Division of Pharmacology and Toxicology, College of Pharmacy, <sup>2</sup>Department of Internal Medicine, Dell Medical School, The University of Texas, Austin, TX

**Background:** Hepatic encephalopathy (HE) is a neurological complication of liver dysfunction characterized by neuroinflammation, cognitive impairment, and motor deficits. Increasing evidence suggests that biological sex modulates neuroinflammatory responses and neurological outcomes in liver–brain disorders; however, sex-dependent mechanisms contributing to HE remain poorly defined. **Methods:** Male and female C57BL/6 mice were treated with carbon tetrachloride (CCl<sub>4</sub>) to induce chronic liver injury and HE, with sex-matched corn oil–treated mice as controls. Blood ammonia levels were measured to assess hyperammonemia. Hepatic injury and fibrosis were evaluated by qPCR analysis of *Col1a1*, *α-Sma*, and *Krt19* expression. Body weight, liver weight, and skeletal muscle mass were recorded. Neurological and behavioral function was assessed using nest building, open field, novel object recognition, balance beam, rotarod, and grip strength testing. Neuroinflammatory responses were evaluated by immunohistochemical analysis of microglial and astrocytic activation (IBA1 and GFAP) in the cortex, cerebellum, and hippocampus, with quantitative image analysis performed in a blinded manner. **Results:** CCl<sub>4</sub> administration induced comparable hepatic injury and hyperammonemia in male and female mice, with no significant sex differences in blood ammonia levels or hepatic fibrotic marker expression. Despite equivalent liver pathology, male and female mice exhibited distinct neurological and behavioral responses to HE. Both sexes developed motor and neuromuscular impairments; however, sex-specific differences were observed in grip strength, the ratio of gastrocnemius to body weight, and behavioral outcomes. Female mice displayed altered anxiety-related and exploratory behaviors following CCl<sub>4</sub> treatment, whereas these differences were minimal under control conditions. Nesting behavior and weight change further revealed sex-

specific patterns of functional decline following CCl<sub>4</sub> treatment, indicating divergent adaptive or compensatory responses to HE. **Conclusion:** Collectively, these findings demonstrate that while hepatic injury and hyperammonemia are not sexually different in the CCl<sub>4</sub> model, HE elicits sex-dependent neurological and behavioral outcomes, suggesting that biological sex influences downstream neural vulnerability or functional compensation in response to chronic liver failure. **Acknowledgments:** This work was supported by NIH funding (DK112803 and DK135995).

#### Poster Board 41

##### Abstract 089

#### Single-Cell Sequencing Reveals a Novel Subset of Hepatocyte-Like Cells in the Non-Parenchymal Population of the Autophagy Deficient Livers

Yuanyuan Li, Shengmin Yan, Xiaojiang Xu, and Xiao-Ming Yin

Department of Pathology and Laboratory Medicine, Tulane University, New Orleans, LA

**Background:** Autophagy is a crucial cellular process responsible for degrading macromolecules and subcellular organelles, playing a significant role in maintaining cellular health. Mice with deletion of a key autophagy gene (Atg7) in hepatocytes exhibit hepatic autophagy deficiency, severe liver injury, fibrosis and tumorigenesis. To understand how dysregulated autophagy leads to liver pathology, we performed single cell sequencing analysis (scRNA-seq). **Methods:** Two wild-type (Atg7<sup>F/F</sup>) and two Atg7-deficient (Atg7<sup>KO</sup>) mice were subjected to liver perfusion. Hepatocytes and non-parenchymal cells (NPCs) are separated by low-speed centrifugation. Single-cell suspensions were loaded onto the 10X Genomics Chromium platform for library preparation and sequencing. Sequencing data were processed using the Seurat package in R. After quality control filtering, 41,088 wild-type and 23,283 Atg7-KO cells were analyzed. Data were normalized using SCTransform, integrated with Harmony to correct for batch effects, and subjected to dimensionality reduction using UMAP. Clustering was performed afterwards. **Results:** Low speed centrifugation (600g x5 min) can separate hepatocytes (in pellet) from NPCs (in supernatant). Thus, hepatocytes are usually “heavier” by density than NPC, which are composed of immune cells, endothelial cells and other non-hepatocyte cells. scRNA-seq confirmed the cellular composition of the two populations based on the expression of key gene clusters. In addition, Atg7-KO hepatocytes expressed decreased levels of albumin and Hnf4a, but increased levels of Sqstm1, NQO1, and Gstm1, suggesting a level of de-differentiation and an activation of the NRF2 transcriptional pathway, which are known to be pathogenic. Surprisingly, we identified a new subset of cells in the NPC isolated from the Atg7-KO livers, which were not observed in the normal NPC population. These cells exhibited a transcriptional profile similar to the Atg7-KO hepatocytes. Gene ontology analysis of these hepatocyte-like cells indicated alterations in energy production and metabolism. Furthermore, over 50% of these cells expressed a high level of G2/M-specific genes, suggesting that they may have experienced G2/M arrest. Finally, trajectory analysis integrating hepatocyte data suggested transitions from wild type hepatocytes to autophagy-deficient hepatocytes, and then to the non-parenchymal hepatocyte-like cells. **Conclusions:** We propose that the hepatocyte-like NPC in the autophagy-deficient livers may represent a degenerated status of “hepatocytes”, arising in response to autophagy deficiency. These cells could be in senescence with altered energy and metabolic changes, which may lead to senescence associated secretory phenotype. Our findings provide novel insights into how autophagy disruption drives cellular reprogramming, physical property changes and liver pathogenesis. **Acknowledgements:** This study was in part supported by R21 AA031033 (NIAAA).

#### Poster Session – Mucosal Pathobiology

##### Poster Board 42

##### Abstract 090

#### Endolysosomal Acidification Regulates Intestinal Injury and Repair by Augmenting Innate Immune Signaling Pathways

Doug Terry, Liping Luo, Josh Lee, and Brian Robinson

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA

**Background:** Vacuolar ATPases (V-ATPases) are highly conserved multi-subunit proton pumps that drive the acidification of intracellular vesicles, especially endosomes and lysosomes. By regulating progressive acidification of the endolysosomal pathway, V-ATPase activity impacts signaling transduction pathways both positively (e.g., internalization and activation of receptor-ligand complexes in endosomes) and negatively (e.g., degradation of pathway mediators in lysosomes). While the role of V-ATPases in human neurodegenerative diseases and cancer has been extensively studied, the requirement for these proteins in intestinal restitution remains poorly understood. Here, we use *Drosophila* to study

the role of V-ATPases in regulating intestinal-injury and repair driven by excessive oxidative stress. **Methods:** Adult male *Drosophila* 2-5 days post-eclosion were fed either a vehicle diet or that same diet supplemented with either 10mM paraquat or 1% H<sub>2</sub>O<sub>2</sub> to induce oxidative stress. The GAL4-UAS system was used to alter target gene expression in a tissue-specific manner. Intestines were dissected and analyzed by immunofluorescent staining and confocal microscopy as well as RT-PCR to study conserved signal transduction pathways. Quantification was performed using ImageJ and Prism software. **Results:** We find that RNAi driven depletion of multiple subunits of the V-ATPase complex suppressed oxidative stress-induced lethality. By contrast, depletion of the main lysosomal catabolic enzyme in *Drosophila* (Cathepsin-D) had no effect. On a cellular level, these effects map to absorptive enterocytes (ECs) of the *Drosophila* intestine. Molecular analysis of intestines following injury by oxidative stress compared to uninjured controls reveals increased cell death, increased JNK-pathway activity, and increased IMD/NF- $\kappa$ B pathway signaling reporter expression compared to uninjured controls. Depletion of Vha44 (subunit C of the V1 complex) was sufficient to suppress the increased cell death, JNK pathway, and IMD/NF $\kappa$ B pathway markers induced by oxidative stress in the intestine. Furthermore, overexpression of the MAP3K TAK1 enhanced death, JNK pathway and IMD/NF $\kappa$ B pathway activation in a Vha44 dependent manner. **Conclusions:** These findings suggest that inhibition of V-ATPase activity can protect against intestinal injury caused by excessive oxidative stress. On a molecular level, we find that attenuation of endolysosomal acidification dampens pro-apoptotic JNK and IMD/NF- $\kappa$ B pathways, highlighting endosomal acidification as a potential amplifier of excessive oxidative stress. **Acknowledgements:** This work was supported by the American Cancer Society Grant CSDG-20-061-01-TBE and the NIH/NIDDK-Sponsored T32 Grant T32DK108735.

## Poster Board 43

### Abstract 091

#### Opportunists in the Cystic Fibrosis Airway: Laboratory Perspectives on Bacterial-Host Interactions

Deborah L Chance<sup>1,2</sup> and Thomas P. Mawhinney<sup>2,3,4</sup>

<sup>1</sup>Department of Molecular Microbiology and Immunology, <sup>2</sup>Department of Pediatrics, University of Missouri School of Medicine, Columbia, MO, <sup>3</sup>Department of Biochemistry, University of Missouri, Columbia, MO, <sup>4</sup>University of Missouri Experiment Station Chemical Laboratories, Columbia, MO

**Background:** Cystic fibrosis (CF) airways are commonly colonized by opportunistic bacteria, including nontypeable *Haemophilus influenzae* (NTHI), *Staphylococcus aureus* (SA), and *Pseudomonas aeruginosa* (PA). These chronic infections are challenging to eradicate and contribute to progressive lung damage. Host and microbial features vary widely across the CF population and within individuals over time. While laboratory models cannot capture all CF airway variables, they can provide insight into bacterial colonization and host–pathogen interactions. Here, we examined NTHI, SA, PA, and longitudinal CF sputa using complementary experimental and visualization approaches. **Methods:** CF sputum culture reports, CF and non-CF isolates, CF sputa, and epithelial cell models (including human bronchial epithelial cell xenografts) were employed. Local 6-month sputum culture database was screened for polymicrobial incidence and character. CF sputa underwent chemical characterization, and cultured bacterial isolates were analyzed using binding assays and imaging. **Results:** Screening of local CF culture reports showed that 75% contained two or more opportunists, with SA (58%), mucoid PA (51%), nonmucoid PA (37%), and NTHI (13%). Co-isolated SA and PA in mixed culture exhibited patient-specific distributions linked to bacterial matrix characteristics. CF sputa were dense and chemically variable, with differences in DNA and mucous glycoprotein content affecting solubility and matrix penetration (Chance & Mawhinney, *J Respiration* 2020, 1:8). Fluctuations in sputum DNA during hospitalization highlighted the importance of therapeutic timing. NTHI binding assays demonstrated strain- and cell-type–specific interactions, with consistent low-level binding to respiratory and submaxillary mucins. PA isolates showed confirmation-specific carbohydrate binding to select monosaccharides, but not to  $\beta$ -galactose, a residue common to mucin oligosaccharide termini (Chance et al., *Microorganisms* 2024, 12:801). Scanning electron microscopy (SEM) imaging of NTHI-challenged xenografts revealed bacterial associations with mucus layers and damaged epithelial cells. **Conclusions:** These laboratory perspectives suggest that CF airway colonization is highly dynamic, shaped by changing host environments and bacterial interactions. Effective CF therapeutics may therefore require multifactorial strategies targeting both host and bacterial matrix composition and dynamics. **Acknowledgements:** This research was supported by the University of Missouri Experiment Station Chemical Laboratories and gifts of the Cystic Fibrosis Association of Missouri and the Cystic Fibrosis Association of West Plains.

## Poster Board 44

### Abstract 092

#### Diet-Dependent *Lactobacillus* Metabolism Reveals Hydroxyphenyllactic Acid as a Potential Regulator of Intestinal Homeostasis

Alyssa Gutierrez<sup>1</sup>, Katherine Chetta<sup>2,3</sup>, Thomas Horvath<sup>4,5</sup>, and Melinda A. Engevik<sup>1,6</sup>

<sup>1</sup>Department of Regenerative Medicine and Cell Biology, <sup>2</sup>Department of Pediatrics, C.P. Darby Children's Research Institute, <sup>3</sup>Department of Pediatrics, Division of Neonatal-Perinatal Medicine, Shawn Jenkins Children's Hospital, Medical University of South Carolina, Charleston, SC, <sup>4</sup>Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, <sup>5</sup>Department of Pathology, Texas Children's Hospital, Houston, TX, <sup>6</sup>Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC

**Background:** Early-life *Lactobacillus* species play critical roles in intestinal development and homeostasis, in part through the production of bioactive metabolites in response to dietary substrates. However, how breastmilk versus infant formula shapes *Lactobacillus*-derived metabolites and their effects on intestinal physiology remains incompletely defined. Here, we identify hydroxyphenyllactic acid (HPLA), a tyrosine-derived metabolite, as robustly produced by multiple *Lactobacillus* species in response to breastmilk but not infant formula, and we further investigate its impact on intestinal oxidative stress, inflammation, and barrier function. **Methods:** Six *Lactobacillus* species (*L. acidophilus*, *L. brevis*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*) were monocultured in chemically defined media supplemented with 10% v/v breastmilk or infant formula. Culture supernatants were analyzed by untargeted LC-MS/MS metabolomics. Antioxidant effects of HPLA were assessed in HT-29 cells exposed to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), measuring intracellular reactive oxygen species (ROS) by H2DCFDA. Anti-inflammatory effects of HPLA were assessed in CCD-841CoN cells exposed to lipopolysaccharide and flagellin, measuring proinflammatory cytokine expression by RT-qPCR. Barrier function was evaluated in HCT-8 monolayers using lucifer yellow permeability assays and tight junction gene expression analysis by RT-qPCR. *In vivo* effects of orally administered HPLA (20 mg/kg) were examined in adult C57BL/6 mice with dextran sulfate sodium (DSS)-induced colitis. **Results:** HPLA was identified as a highly abundant *Lactobacillus* metabolite uniquely associated with breastmilk exposure. *In vitro*, HPLA significantly reduced H<sub>2</sub>O<sub>2</sub>-induced ROS accumulation in HT-29 cells. HPLA also attenuated lipopolysaccharide- and flagellin-induced proinflammatory cytokines *IL-8* and *IL-1 $\alpha$*  in CCD-841CoN cells. HPLA further improved epithelial barrier integrity, reducing monolayer permeability and increasing expression of *Claudin-1*, *Claudin-3*, and *Occludin*. In DSS-induced colitis, HPLA administration reduced diarrheal severity and decreased colonic *inflammatory cytokine* expression. **Conclusions:** These findings demonstrate that *Lactobacillus* metabolite production is strongly diet dependent, with HPLA preferentially generated in the context of breastmilk. HPLA acts as a modulator of intestinal oxidative stress, inflammation, and barrier function, highlighting a potential mechanism by which breastmilk-microbe interactions support intestinal homeostasis.

**Acknowledgements:** This study was supported by T32DK124191-01A1 (AG), NATS NIH KL2TR001452 (KEC), Darby Pediatrics pilot grant (KEC), UL1TR001450 (KEC), 3P20 P20 GM130457 (COBRE in Digestive & Liver Disease, MUSC; GM130457-04S1 supplement), P30 DK123704 (Digestive Disease Research Center, MUSC).

## Poster Session – Neuropathology

### Poster Board 45

#### Abstract 093

#### Role of the Parkinson's-Linked Kinase PINK1 in Regulating Dendritic Mitochondria

Rebecca K. Zack<sup>1</sup>, Cody J. Drozd<sup>1</sup>, Gabriella Fricklas<sup>1</sup>, P. Anthony Otero<sup>1</sup>, Kent Z. Q. Wang<sup>1</sup>, Callen T. Wallace<sup>2</sup>, Simon C. Watkins<sup>2</sup>, and Charleen T. Chu<sup>1</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup>Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA

**Background:** PTEN-induced kinase 1 (PINK1) pathway defects have previously been linked to neurodegenerative disease via alterations in mitochondrial physiology and motility. Prior work detailing mitochondrial trafficking deficits in PINK1 models focused predominantly on axons and gave limited attention to direct alterations to the motor proteins, dynein and kinesin. This work aims to (a) further characterize mitochondrial trafficking in the dendrites and (b) explore how post-translational modifications (PTMs) to motor proteins change trafficking in PINK1 models of Parkinson's disease.

**Methods:** Mitochondrial density in the soma, axons, and dendrites was assessed using volumetric analyses of wild-type (WT) and PINK1 knock-out (PINK1 KO) mouse cortical neurons (MCN) transfected with fluorescent markers to visualize mitochondria and neuronal processes. To investigate the contributions of mitochondrial motility to compartmental

density, we used a live-imaging paradigm in which a mitochondria-targeted photoactivatable GFP was activated in the soma, and egress from the soma into the dendrites was quantified. 2-D immunoblots of mouse cortical lysates and SHSY-5Y cells explored potential PTMs in kinesin and dynein. Mass spectrometry (MS) of PINK1 overexpressing (OE) SH-SY5Y cells, immunoprecipitated with anti-dynein antibody, was used to identify phosphorylated residues. **Results:** Compared to WT MCN, PINK1-KO MCN exhibited significantly decreased mitochondrial density in dendrites and increased density in the soma. Live imaging revealed reduced rates of mitochondrial egress from soma to dendrite in PINK1 KO MCN. 2-D blots showed that dynein underwent an alkaline pI shift in PINK1 KO mouse cortex lysate and an acidic shift in PINK1 OE SH-SY5Y cells. Preliminary MS revealed a novel phosphosite on dynein. **Conclusions:** Differential mitochondrial density across neuronal compartments suggests impaired mitochondrial trafficking in PINK1 KO models. Our findings support that PINK1 directly or indirectly modulates phosphorylation of a dynein subunit. We hypothesize that this PTM may disrupt interactions within the motor complex, reducing mitochondrial trafficking. Phosphomimetic and phosphonull dynein constructs will allow us to investigate the potential role of dynein-specific trafficking perturbations in PINK1 models. This work provides insights into mitochondrial dynamics in PINK1-deficient models and enhances our understanding of the mechanisms underlying PINK1-associated familial Parkinson's disease.

**Acknowledgements:** We thank Mr. Jason Callio, University of Pittsburgh, for mouse neuron cell culture assistance. The study was funded in part by NIH R01 NS101628. MS data was generated by the Mass Spectrometry and Proteomics Facility, Proteomic Shared Resources of the Office of Research and the Comprehensive Cancer Center at The Ohio State University, Columbus, OH, supported by NIH P30 CA016058. The Fusion Orbitrap instrument is supported by NIH S10 OD018056.

## Poster Board 46

### Abstract 094

#### Bioinformatic Insight Into Gut-Brain Communication Modeling Type-2 Diabetes-linked Alzheimer's Disease

Narendra Kumar<sup>1</sup>, Mimansha Shah<sup>1</sup>, Jayshree Mishra<sup>1</sup>, and Priyam Kumar<sup>2</sup>

<sup>1</sup>College of Pharmacy, Texas A&M University Health Science Center, Kingsville, TX, <sup>2</sup>University of Pennsylvania, Philadelphia, PA

**Background:** Alzheimer's disease and related dementias (ADRD) disproportionately affect individuals with type 2 diabetes (T2D) and metabolic syndrome, conferring a 1.5–2-fold increased risk and accelerating cognitive decline. Mechanisms linking metabolic disease to ADRD include insulin resistance, chronic systemic and neuroinflammation, vascular injury, oxidative stress, mitochondrial dysfunction, and altered lipid metabolism, collectively promoting amyloid, tau, and synaptic pathology. Given the heterogeneity of T2D, integrative approaches are needed to define causal pathways. Increasing evidence implicates gut–brain communication in driving neuroinflammation and AD pathology, though underlying mechanisms remain poorly understood. **Methods:** We performed serum and urine metabolomic profiling with pathway enrichment analysis in Jak3-deficient mouse models that recapitulate metabolic disease phenotypes. Metabolomic signatures were compared with corresponding human cohorts enriched for T2D-to-ADRD trajectories to assess translational relevance. **Results:** Janus kinase 3 (Jak3), a non-receptor tyrosine kinase integrating immune, epithelial, and neuroglial signaling, emerged as a central molecular node linking metabolic dysfunction to brain pathology. Jak3 deficiency resulted in intestinal inflammation and gut dysbiosis, accompanied by increased microglial activation, elevated brain expression of TLR4 and HIF1 $\alpha$ , and disrupted brain homeostasis. Systemic metabolomic analysis revealed widespread pathway perturbations, with approximately 80% concordance among the top 25 enriched metabolic pathways between aged Jak3-deficient mice and individuals with late-onset Alzheimer's disease (LOAD). **Conclusions:** Loss of Jak3 signaling induces metabolic and neuroinflammatory alterations that closely recapitulate human LOAD metabolomic signatures. These findings identify Jak3 as a mechanistic link between gut dysfunction, metabolic disease, and Alzheimer's risk, supporting its potential as a translational target and biomarker for ADRD in metabolically vulnerable populations.

## Poster Board 47

### Abstract 095

#### Mapping the Lesion Landscape of White Matter Hyperintensities Using Deep Learning

Dana R. Austin<sup>1</sup>, Jinghang Li<sup>2</sup>, Hossein Tahmasebidehkordi<sup>1</sup>, Lauren Dellet<sup>1</sup>, Jonathan Cohen<sup>1</sup>, Jr-Jiun Jean Liou<sup>2</sup>, Jacob Berardinelli<sup>2</sup>, Hecheng Jin<sup>2</sup>, Tales Santini<sup>2</sup>, Thomas Pearce<sup>1</sup>, Tamer Ibrahim<sup>2,3</sup>, and Julia K. Kofler<sup>1,4</sup>

<sup>1</sup>Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, <sup>2</sup>Department of Bioengineering, University of Pittsburgh Swanson School of Engineering, Pittsburgh, PA, <sup>3</sup>Department of Psychiatry, <sup>4</sup>Clinical and Translational Science Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA

**Background:** Alzheimer's Disease is a relentless, progressive neurodegenerative disease leading to severe cognitive decline and affects approximately 26.6 million people worldwide. White matter hyperintensities (WMH) are radiologically defined regions of myelin rarefaction which precede cognitive symptoms in Alzheimer's Disease by as early as 20 years and are predictive of disease onset, severity, and progression. Little is known about histopathologic signatures of WMH as they are difficult to locate in human postmortem tissue. To overcome this limitation, we created an *ex vivo* MRI pipeline to align radiologically defined lesions with coronal gross slab images. **Methods:** We collected 6 standard samples from n=135 brains in regions prone to WMH. We performed histological and immunohistochemical stains targeting astrocytes (GFAP), microglia (P2RY12), perivascular macrophages (CD163), axons (NF), myelin (PLP, MAG, Luxol Fast Blue). Vascular structures and oligodendrocytes were classified from H&E images using deep learning. Positive cell and pixel detection was performed with the QuPath Software. WMH lesion outlines were transferred to digital whole slide images using web-based alignment tools in the Brain Digital Slide Archives platform. Graph Convolutional Neural Networks were used to investigate cell distribution changes in WMH lesions, transition zones, and outer lesion areas. Positive cells and pixels were processed through algorithms identifying periventricular zone and subcortical, intermediate, and deep white matter. These extracted features were fed in regression and classification algorithms to investigate the connection between histopathologic signatures with clinical features in Alzheimer's Disease. Interpretable semi-supervised machine learning was used to identify how ROIs contribute to WMH vs. NAWM prediction. **Results:** When trained on image tiles from gray and white matter compared to white matter alone, model performance improved, with positive and negative predictive values of 0.83 and 0.79 versus 0.94 and 0.93, respectively. This speaks to the importance of incorporating spatial context and often lesser appreciated ROIs into spatial biology research. **Conclusions:** Overall, these methodologies allow us to interrogate regions of myelin rarefaction in Alzheimer's Disease with changes in cellular neighbors and tissue architecture. Identifying the biologic underpinnings of myelin loss in is critical for the understanding of AD disease pathogenesis.

## Poster Board 48

### Abstract 096

#### Spatiotemporal Mapping of Motor Circuit-Level Changes in a Mouse Model of Amyotrophic Lateral Sclerosis

Diego Szczupak<sup>1</sup>, Stefanie Taiclet<sup>1</sup>, Yomna Badawi<sup>2</sup>, and Christi L. Kolarcik<sup>1</sup>

<sup>1</sup>University of Pittsburgh, School of Medicine, Pittsburgh, PA, <sup>2</sup>Kansas University Medical Center, Kansas City, KS

**Background:** Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease marked by the progressive loss of motor neurons that control voluntary movement. A major barrier to effective therapies is the lack of clarity regarding where and how ALS begins and spreads through the nervous system. We propose that understanding ALS within a motor circuit framework, rather than through isolated anatomical compartments, is essential for understanding disease initiation and progression. **Methods:** We performed *ex vivo* high-resolution diffusion-weighted magnetic resonance imaging (MRI) of the brain in the G93A SOD1 model of ALS at multiple disease stages (pre-symptomatic, symptom onset, and end-stage). For cortical neural circuit analysis, we applied fiber tracking, atlas registration, and connectome analysis to assess measures of structural connectivity across whole-brain, motor, and frontal networks. We complemented this structural connectivity data with virus-based neuroanatomical tracing and cortical mapping to evaluate synaptic connections in the same animals across disease stages. Integration of these approaches linked cellular-level mechanisms to the macro-scale neural networks investigated with neuroimaging. **Results:** In the SOD1 model, we observed significant differences compared to control animals specifically in motor circuits at pre-symptomatic, symptom onset, and end-stage phases although the most dramatic change occurred prior to clinical symptom onset. Notably, high-efficiency network patterns decreased while global or overall network efficiency increased. This suggests that while the motor circuit is losing connections, it is prioritizing critical long-distance connections, resulting in a more efficient

network. At the level of the muscle, we observed significant decreases in the percent of fully innervated neuromuscular junctions beginning at pre-symptomatic stages. In the spinal cord, the distribution of motor neurons across lumbar segments was quantified and compared across disease stages. At the cortical level, viral labeling was present in the cortex even at end-stage disease; however, alterations in labeling density and cell-type- and region-specific labeling were delineated. **Conclusions:** These results highlight the dynamic nature of the nervous system, underscoring the need for a systems-level perspective to better understand ALS pathophysiology. Taken together, our data indicate that ALS-linked mutations significantly alter neural circuit structure even before overt symptom onset, findings that can be leveraged to develop neural circuit-based therapeutic strategies. **Acknowledgements:** Funding was provided by The ALS Association (CLK; Award Number 20-IIP-512). Additional financial support was provided by the Department of Pathology. The authors would like to thank Dr. T. Kevin Hitchens of the Advanced Imaging Center for collecting the ex vivo imaging data.

## Poster Board 49

### Abstract 097

#### Identifying Inflammatory Threshold Using Ultrasonic Vocalizations in Neonatal Sepsis

Avraham Levi, Isaac Rodriguez, and Michele Alves

Department of Cellular and Molecular Medicine, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

**Background:** Neonatal sepsis remains one of the major challenges of neonatal medicine and the leading causes of death in neonates worldwide. The immune activation response in neonatal sepsis can manifest as respiratory distress due to disrupted neuronal activity in brainstem centers (Alves, et al, 2024, *Brain Behav Immun* **119**, 333-350). Vocalization is tightly integrated to respiratory control through inspiratory rhythm and expiratory motor control to move the air through the larynx. Vocalization can only occur in precise coordination during expiratory phase. Failure in such processes is associated with impaired motor or respiratory control. Ultrasonic Vocalizations (USVs) in newborn mice occurs between the 30-110 kHz frequency and can be used as readout of the basic respiratory-vocalization circuits coupling. Our study aimed to identify USVs features that can serve as physiomarkers of inflammatory thresholds and respiratory-motor coupling control in neonatal immune activation. **Methods:** Postnatal day 5 (PD5) mice of both sexes received i.p. injections of saline (control) or lipopolysaccharide (LPS) at incremental concentrations of 1mg/kg (low), 5mg/kg (mid) and 10mg/kg (high). Body weight and temperature were obtained prior to injection and after 3 hours. USVs were recorded in a soundproof chamber using a condenser microphone connected to Ultrasound Gate hardware. Data analysis was performed using MATLAB (VocalMat) and RStudio. **Results:** USVs consist of discrete acoustic syllables converted into spectrograms and classified based on frequency, pitch, duration, and modulation patterns. Overall, total USVs number and vocalization duration decreased across all LPS groups compared with controls. Neonatal immune activation with LPS promoted neonatal calls with lower frequency peak. We then detected the call-type categories using a convolutional neural network-based classifier in VocalMat. Out of 11 established call-type categories, our study identified 8 call-types in PD5 Control neonates. Control group presented higher percentage of two-steps and up-frequency modulation, which were negatively associated with LPS. Flat and short call types (calls with no frequency modulation or short duration) were overrepresented at low and mid LPS doses. Chi-square analysis of call-type revealed positive association of chevron calls and complex calls with the high-LPS dose. Along with the vocalization features, we identified a linear decrease of the body temperature with LPS but unexpected weight gain at high-LPS group. **Conclusion:** The impact at high-LPS dose indicates an unexpected switch to complex calls, which in this case may represent very slow and deep breaths associated with disrupted respiratory-motor coupling control. Together, these results highlight USVs as a non-invasive prognostic tool with the potential to reveal mechanistic transitions relevant for estimating disease severity and future studies.

## Poster Board 50

### Abstract 098

#### Structural and Lipidomic Alterations in the Optic Nerve of Down Syndrome Mice

Saba Tufail<sup>1</sup>, Khushi B Talajia<sup>1</sup>, Sara Sabbagh Dehkharghani<sup>1</sup>, James Fortenberry<sup>2</sup>, and Konark Mukherjee<sup>1,2,3</sup>

<sup>1</sup>Department of Genetics, <sup>2</sup>Vision Science Research Center, <sup>3</sup>Comprehensive Neuroscience Center, University of Alabama at Birmingham, Birmingham, AL

**Background:** Down syndrome (DS) is the most commonly known chromosomal disorder and results from trisomy of the 21st chromosome. It occurs in ~1 in 700 live births, with nearly 6000 babies with DS born in the United States every year. DS manifests with many neurological and non-neurological co-morbidities, among which ophthalmological abnormalities are the most prevalent medical conditions co-occurring with DS. **Methods and Results:** In this study, we are investigating retinal function and optic nerve characteristics in two different mouse models of DS: Ts65Dn and Dp(16)1Yey/+. Electroretinography (ERG) results from both the animal models exhibit no significant changes either in scotopic or photopic response relative to euploid control mice. However, Ts65Dn mice exhibit reduced optic nerve diameter as compared to euploid controls, indicating possible optic nerve hypoplasia. Immunohistochemistry of optic nerves further exhibit astrogliosis and electron microscopy shows reduced axonal diameters. Notably, lipidomic profile of Ts65Dn optic nerves reveals pronounced alterations in glycerophospholipids and glycosphingolipids as compared to euploid mice. **Conclusion:** Our findings suggest that optic nerve abnormalities in DS may be associated with distinct lipidomic alterations, providing insights into novel mechanisms underlying ocular dysfunction in DS.

## Poster Board 51

### Abstract 099

#### Dual-Action Compounds Targeting Protein Aggregates and Inflammation in Neurodegenerative Diseases

Natalie G. Horgan<sup>1</sup>, Taiwo A. Ademoye<sup>1</sup>, Lydia J. Stone<sup>1</sup>, Heba Alnakhala<sup>2</sup>, Arati Tripathi<sup>2</sup>, Ulf Dettmer<sup>2</sup>, and Jessica S. Fortin<sup>1</sup>

<sup>1</sup>Department of Basic Medical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN,

<sup>2</sup>Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

**Background:** Alzheimer's disease (AD), Lewy body dementia (LBD), and Parkinson's disease (PD) are progressive neurodegenerative disorders that impair cognitive and motor function. Collectively, these diseases affect over 9 million Americans, a number projected to double in the coming decades. Although multifactorial in nature, their pathogenesis is thought to be driven primarily by protein misfolding and neuroinflammation. The accumulation of aggregated proteins, specifically alpha-synuclein ( $\alpha$ -syn) in LBD and PD and amyloid-beta and tau in AD, leads to synaptic dysfunction and neuronal death. In parallel, neuroinflammation is often mediated by the upregulation of soluble epoxide hydrolase (sEH), an enzyme that converts neuroprotective, anti-inflammatory epoxyeicosatrienoic acids into less active metabolites. **Methods:** Given the therapeutic relevance of protein aggregation and inflammatory pathways, this study evaluated sixteen small molecules for dual inhibition of  $\alpha$ -syn or tau aggregation and sEH activity. Compounds were screened for their anti-aggregation effects using cell-based assays, photo-induced cross-linking of unmodified proteins, transmission electron microscopy, and thioflavin T assays, while anti-inflammatory activity was assessed using a sEH inhibitor assay. **Results:** Of the sixteen compounds tested, eight demonstrated measurable dual activity against both  $\alpha$ -syn and sEH. However, only compound 15 exhibited strong dual activity against both targets. Notably, compound 15 did not inhibit tau ON4R fibril formation in our assay. **Conclusions:** These findings suggest that simultaneously targeting protein aggregation and inflammatory pathways represents a promising strategy for the treatment and mitigation of neurodegenerative disease symptoms. **Acknowledgements:** This research was supported by the NIH K08 award AG071985 and the ASIP Summer Research Opportunity Program in Pathology.

## Poster Board 52

### Abstract 100

#### Inhibition of Tau and $\alpha$ -Synuclein Aggregation by Urea-Thiadiazole Derivatives for Neurodegenerative Diseases

Taiwo A. Ademoye<sup>1</sup>, Heba Alhakhala<sup>2</sup>, Arati Tripathi<sup>2</sup>, Omnia Ibrahim<sup>3</sup>, Raluca Ostafe<sup>3</sup>, Ulf Dettmer<sup>2</sup>, and Jessica S. Fortin<sup>1</sup>

<sup>1</sup>Department of Basic Medical Sciences, Purdue University, West Lafayette, IN, <sup>2</sup>Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, <sup>3</sup>Institute of Inflammation, Immunology, and Infectious Disease, Purdue University, West Lafayette, IN

**Background:** Alzheimer's disease (AD) and Parkinson's disease (PD) are marked by progressive neuronal dysfunction, with tau and  $\alpha$ -synuclein ( $\alpha$ -syn) aggregation playing central roles in their respective physiopathology. Several studies have reported the co-localization of tau and  $\alpha$ -syn in the brains of patients suffering from dementia. The misfolding of both tau and  $\alpha$ -syn in these cases highlights the need for therapeutic strategies capable of simultaneously reducing tau

and  $\alpha$ -syn aggregation. In this study, urea-thiadiazole compounds were evaluated for their ability to inhibit the aggregation of tau and  $\alpha$ -syn. The presence of different functional groups within the urea-thiadiazole scaffold allowed comparison of activity across the series. **Methods:** The thioflavin T (ThT) fluorescence assay was used to screen compounds for their effects on  $\alpha$ -syn fibril formation, while the Thioflavin-S (ThS) aggregation assay was used to evaluate the effects on tau fibrilization and disaggregation. Transmission electron microscopy (TEM) was employed as a direct method to assess changes in fibril morphology for both  $\alpha$ -syn and tau. For both proteins, oligomer formation was monitored through the photo-induced cross-linking of unmodified proteins (PICUP) assay.  $\alpha$ -Syn inclusion-forming neuroblastoma cell studies and tau oligomerization assays using split-GFP constructs confirmed the best anti-aggregation compounds. **Results:** Several urea-thiadiazole derivatives demonstrated inhibition of  $\alpha$ -syn and tau aggregation, with differences based on the nature of the R1 substituent and aromatic moieties. Among 90 tested compounds, compound 6f showed the strongest effect against  $\alpha$ -syn fibrillization, preventing  $\alpha$ -syn oligomer formation in both WT and A53T mutant models, with a clear concentration-dependent effect, and produced morphological changes in  $\alpha$ -syn fibrils. Compound 2i displayed the strongest inhibitory effect against tau aggregation across the four human tau isoforms, reducing tau fibril formation and disassembling preformed tau 2N4R fibrils. For the anti-inclusion effect, both compounds 2i and 6f prevented the formation of inclusions, but 2i had an effect at lower micromolar concentrations. In the tau split-GFP cell assay, 2i reduced tau oligomer formation, whereas 6f showed no detectable effect. **Conclusions:** Based on the overall workflow, compound 2i emerged as the best inhibitor of tau aggregation, while compound 6f exhibited the best  $\alpha$ -syn anti-aggregation activity. These findings identify 2i and 6f as promising urea-thiadiazole-based candidates with different anti-aggregation properties against tau and  $\alpha$ -syn, supporting their further evaluation for AD and PD. **Acknowledgements:** The authors would like to thank the support received from the NIH NIA (AG071985).

## Poster Board 53

### Abstract 101

#### Micro/Nano Plastics and Microvascular Pathology: Fluorescence Profiles an Alternative Method to pyrGC/MS

Elaine L. Bearer

Department of Pathology, University of New Mexico Health Science Center, Albuquerque, NM

**Background:** Neurodegeneration is definitively diagnosed by histopathology post-mortem. While diagnosing such cases, perivascular glossy deposits were discovered. Earlier neuropathologists dismissed these as hemosiderin from old hemorrhages which should stain for iron, which these did not. Guessing these might be plastics, we subjected tissue (~5 mg) adjacent to histopathology for plastics enrichment (saponification, centrifugation, ethanol washes, and pyrolysis gas-chromatography/mass spectroscopy). Although this plastics-enriched pellet seemed to contain abundant plastics (30,908 $\mu$ g/g of tissue in white matter of Alzheimer's Disease (AD), and 21,441 $\mu$ g/g in Binswanger's dementia (BD), more than healthy brains (average, 4,800 $\mu$ g/g), this large amount may be artifactual due to high lipid content of brain.

**Methods and Results:** To assess the presence of plastics in brain and their correlation with vascular pathology, we used an alternate approach: hyperspectral fluorescence analysis with emission profiling and background subtraction. We performed laser confocal lambda scans on plastic-enriched pellets and pure plastics species, exciting with 10 different wave lengths and collecting emissions at 8 nm steps across 424 – 797nm. Emission profiles of selected particles within plastics-enriched pellets were compared to pure plastics from industry, which had never been associated with human tissue. We found that a subset of particles in enriched pellets fluoresced with the emission profile of pure polyethylene and polypropylene but not polystyrene. We then designed fluorescence microscopy with excitation/emission parameters that matched those of purified polymers and applied these to image histopathologic sections of post-mortem brain in cases of AD and BD. We detected abundant fluorescent particles within walls of subcortical blood vessels. Lambda scans of these particles and adjacent tissue demonstrated the unique emission spectra of plastics and little background from adjacent tissue in the plastics-emission wave lengths. We further characterized the particles in plastics-enriched preparations by negative stain of pellets resuspended in water, and by whole mount transmission TEM after sonification and ethanol washes. Results indicated that micro/nano plastics in human brain were: Abundant in brains from 2023-2024 but minimal in the 1960's; composed of different types of plastic particles in the same individual (shapes and sizes, and excitation/emission spectra) and between individuals; were primarily located in the walls of blood vessels but also sprinkled throughout the parenchyma; at highest amounts where microhemorrhage and vascular leakage occurs; engulfed by CD68 macrophages; surrounded by loose amyloid but not consistently within amyloid plaques; and no p-tau phosphorylation. **Conclusion:** We conclude that plastics may cause mechanical, chemical and/or

physiological damage. **Acknowledgements:** Supported by NIA P30 AG086404 (GR, ELB), 3P30CA118100 (PI: Sanchez); and the Harvey Family Endowment (ELB)

#### Poster Board 54

##### Abstract 102

#### Modulating Secondary DNA Structures in the Microtubule-Associated Protein Tau Gene for Alzheimer's Disease and Related Tauopathies

Jessica S. Fortin, Taiwo A. Ademoye, and Natalie G. Horgan

Department of Basic Medical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN

**Background:** An effective means to turn on and off genes associated with neurodegenerative diseases, such as Alzheimer's disease (AD), with small drug-like molecules is still not available. Dr. Fortin Drug Discovery lab leverages a new mechanism, i.e. globular structures in DNA promoter region, to achieve this objective. G-quadruplexes (GQs) are secondary structures formed in nucleic acids that are rich in guanine and form globular structures that can be stabilized with small molecules. These structures have been identified in the promoters of many genes that are aberrantly overexpressed, for example oncogenes. Emerging data suggest that GQ stabilization regulates gene expression, most frequently resulting in reduction of the protein expressed by the affected gene. For this study, the gene target is linked with the prone-to-aggregate protein tau; the microtubule associated protein tau (MAPT) gene. Previous knockdown studies in rodents showed that finely tuning down this gene for several months did not lead to any toxicity. It is well established that the production of tau (and its self-assembly into fibrils) contribute to the progression of AD, making tau a highly significant molecular target for addressing the pathogenesis of AD. We posit that finely tuning down this gene via stabilization of GQ would help in understanding the AD physiopathology and become a drug discovery strategy in the future. **Methods:** The National Center for Biotechnology Information (NCBI) and bioinformatic programs (e.g. G4Hunter Web) were used to identify GC rich regions and the propensity of these regions to fold into GQ(s). Oligonucleotides from four MAPT regions and the c-Myc promoter region (oncogene as positive control) were obtained from Sigma-Aldrich. Thioflavin T was used to identify the oligonucleotides most prone to form GQ. Circular dichroism (CD) provided insight into the topology. Cell-based assays were performed with a model compound (general stabilizer of GQs) to evaluate the reduction of tau protein levels by Western blot. **Results:** The human MAPT promoter contains a highly GC-rich region that is crucial for basal promoter activity. Data obtained shown that the GC rich 5'UTR region of MAPT assumes secondary DNA structures consistent with GQ. One of the GQs adopted a parallel topology. We have identified one potential molecule for its stabilization which reduced tau protein expression in a cell-based assay, providing evidence that these structures are important in regulating MAPT gene expression. Future work will characterize the complete folding patterns and structures of the GQs in the promoter region of MAPT. **Conclusions:** This project will pave the way of novel DNA models and targets to help in 1) the understanding of neurodegenerative diseases and 2) the development of additional treatments. **Acknowledgements:** We are grateful for the support obtained from the ASIP Summer Research Opportunity Program in Pathology.

#### Poster Session – Nutrition and Obesity

##### Poster Board 55

##### Abstract 103

#### Maternal Obesogenic Diet Exposure Programs Early Luminal Bile Acids and Gut Immune Development

Holly Hinrichs<sup>1</sup>, Francisco Ramirez Victorino<sup>2</sup>, Monica Young<sup>1</sup>, Tarin M. Bigley<sup>2</sup>, and Michael D. Thompson<sup>1</sup>

<sup>1</sup>Division of Endocrinology and Diabetes, <sup>2</sup>Division of Rheumatology Immunology, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO

**Background:** Maternal obesogenic diet exposure (MODE) promotes worse offspring intestinal inflammation in mouse models of colitis. We have shown that MODE alters the early offspring microbiome with associated shifts in bile acid metabolism and attenuation of the weaning reaction, a critical early life gut immune programming event. The early microbiome is known to play a critical role in this early gut immune programming event. In our MODE model, luminal levels of secondary bile acids (UDCA and DCA) are reduced. We hypothesize that bile acids serve as a critical intermediate between the microbiome and programming of early gut immune cell populations. **Methods:** Beginning at four weeks of age, female mice were fed either chow (CON) or high fat, fructose, cholesterol (MODE) diet for 6 weeks before being bred with lean males. To evaluate secondary bile acids, MODE offspring were gavaged with UDCA or DCA

daily between 2 and 3 weeks of age. To target microbial BA metabolism, CON offspring were treated with AAA-10 (direct bile salt hydrolase inhibitor) or disulfiram (indirect 7 $\alpha$ -hydroxylase inhibitor). Ileum and cecal contents were collected at 2-4 weeks of age. BA homeostasis was assessed through measurement of BAs via mass spectrometry on cecal contents of 3-week-old offspring. The weaning reaction was assessed by measuring gene expression of *Tnfa* and *Ifny* in ileum. Flow cytometry was performed on lymphocytes isolated from intraepithelial and lamina propria compartments of ileum at 4 weeks of age to assess changes in immune cell populations. **Results:** BA profiling on 3-week-old cecal contents identified an increase in abundance of tauro- and glycine- conjugated primary bile acids and a decrease in abundance of secondary bile acids (UDCA, DCA) in MODE offspring. MODE offspring exhibited attenuation of the weaning reaction (absence of spike in *Tnfa* and *Ifny*). MODE offspring exhibited reductions in immune cell populations including regulatory T cells. Supplementation with UDCA but not DCA in MODE offspring reestablished the weaning reaction and increased the reduced immune cell population numbers. In CON offspring, disulfiram treatment attenuated the weaning reaction but not AAA-10. **Conclusions:** MODE shifts early luminal BA levels with associated attenuation of early gut immune programming. Treatment with UDCA reestablishes the weaning reaction in MODE offspring and targeting microbial production of secondary BAs in CON offspring attenuates that weaning reaction. These studies highlight the critical role of early bile acids in programming gut immune cell populations and could be a mechanism for developmental programming of colitis susceptibility.

#### Poster Board 56

#### Abstract 104

#### Neuropilin-2 is a Novel Marker of Pancreatic Alpha Cells

Sandy Huynh, Harsh Nitin Dongre, Abdulrahman Nakshabandi, Yao Gao, and Diane R. Bielenberg

Vascular Biology Program, Boston Children's Hospital, Department of Surgery, Harvard Medical School, Boston, MA

**Background:** According to the NIH, individuals with diabetes mellitus are at increased risk for cancers in the liver, pancreas, colon, breast, and bladder. Our overall objective is to discover novel mechanisms that may link these diseases and uncover new targets for therapeutic intervention. Type 1 and Type 2 diabetes are chronic diseases with different etiologies but similar in their phenotype of elevated blood glucose levels and dysregulation of hormonal signaling pathways mediated by the pancreatic endocrine system. One report has shown expression of Neuropilin-2 (NRP2), a transmembrane receptor capable of binding multiple ligands, in the islets of Langerhans in the pancreas; however, the function of NRP2 in the pancreas is unknown. NRP2 acts as a co-receptor for stimulatory growth factors such as VEGFA, VEGFC, and HGF to promote growth and migration as well as for inhibitory ligands such as Semaphorin-3F (SEMA3F) to block proliferation and invasion. We hypothesize that NRP2 is expressed in pancreatic endocrine cells and regulates hormone secretions such as glucagon or insulin to control glucose metabolism, overall body weight, and diseases such as diabetes, metabolic syndrome, and cancer. **Methods:** To test our hypothesis, we analyzed NRP2 protein expression in human and mouse pancreas tissues using immunoblotting and immunohistochemistry. Double immunofluorescent staining with NRP2 and glucagon or insulin antibodies was used to localize NRP2 expression in alpha or beta cells within the pancreatic islets, respectively. Sex-matched and age-matched total body weights and blood glucose levels were compared between global *Nrp2*-deficient mice and wildtype (WT) mice littermates, as well as in tamoxifen-inducible *Nrp2*-knockout mice compared to controls. **Results:** Our results show that NRP2 protein is expressed in the pancreas and localized to alpha (glucagon-secreting) cells. *Nrp2*<sup>+/-</sup> heterozygous mice were interbred, and a fraction of their offspring resulted in viable *Nrp2*<sup>-/-</sup> knockout mice that were smaller in size compared to heterozygous or WT littermates and in less than Mendelian ratios. At all ages and in both sexes, global *Nrp2*-deficient mice weighed less than mice that expressed *Nrp2*. When adult *R26cre*<sup>ERT2</sup>; *Nrp2*<sup>fl/fl</sup> mice were given tamoxifen to induce the deletion of the *Nrp2* gene, male mice gained more weight than controls, while female mice gained less weight than controls. Blood glucose levels were lower in *Nrp2*<sup>-/-</sup> mice than *Nrp2*<sup>+/+</sup> mice on normal chow diets during both baseline and under fasting conditions. **Conclusions:** Taken together, our preliminary data suggest that NRP2 is a marker for alpha cells within the pancreas, and NRP2 may modulate hormones critical for the management of glucose levels. Future studies will investigate the expression of NRP2 in mouse diabetic models using Streptozotocin treatment. **Acknowledgements:** Funding support from the DF/HCC YFC/NCI R25CA291637 (SH).

## Poster Board 57

### Abstract 105

#### Effect of the DPP-4 Inhibitor Linagliptin on Heart Proteome in Mice Fed Normal and High Fat Diets: Gender-Related Metabolic Responses

Elena Dozio<sup>1,3</sup>, Elena Vianello<sup>1,3</sup>, Elisa Maffioli<sup>2</sup>, Lorenza Tacchini<sup>1,3</sup>, Gabriella Tedeschi<sup>2</sup>, and Massimiliano M. Corsi Romanelli<sup>1,4</sup>

<sup>1</sup>Dipartimento di Scienze Biomediche per la Salute, <sup>2</sup>Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Lodi, Italy, <sup>3</sup>Laboratorio Sperimentale Ricerche Biomarcatori di Danno d'Organo, IRCCS Istituto Auxologico Italiano, Milan, Italy, <sup>4</sup>Dipartimento di Patologia Clinica e Sperimentale, IRCCS Istituto Auxologico Italiano, Milan, Italy

**Background:** Dipeptidyl peptidase-4 (DPP-4) plays a key role in glucose metabolism and inflammation, and its elevated expression in visceral adipose tissue has been associated with increased cardiovascular risk in obesity. While clinical trials confirmed the cardiovascular safety of DPP-4 inhibitors, their potential cardioprotective effects remain unclear, especially in the context of obesity. This study explores heart-specific proteomic modifications induced by the DPP-4 inhibitor Linagliptin (L) in a mouse model of diet-induced obesity, with a focus on sex-based differences. **Methods:** Using label-free shotgun proteomics, we analyzed heart tissues from 61 C57BL/6N mice (29 males, 32 females) fed either a normal chow (NC) or a high-fat diet (HF), with or without L (NCL and HFL, 120 µg/day) for 15 weeks. To evaluate the effects of diet and drug treatment, male and female samples were analyzed separately. Principal component analysis of the 4 experimental groups (NC, HF, NCL, HFL), indicated that HF diet induces significant and sex-specific alterations in the cardiac proteome. Bioinformatic analysis revealed both shared and sex-specific pathways modulated by L. L protected against detrimental effects promoted by HF diet only in female. Potential side effects were observed only under HF diet, in both genders. **Results:** Our results provide novel insights into the molecular effects of DPP-4 inhibition, revealing significant sex-related differences in cardiac responses to HF diet and L treatment. **Conclusion:** Since the main affected pathways are those related to cardiomyopathy, our data pointed out the need of further investigation for improving the safe use of DPP-4 inhibitors in different clinical conditions.

## Poster Session – Toxicologic Pathology

### Poster Board 58

#### Abstract 106

#### Renal Impacts of Pre- and Perinatal Exposure to Perfluorooctane Sulfonate (PFOS) and Combined Antiretroviral Therapy

Melissa J Marchese<sup>1</sup>, Melat Woldetensae<sup>1</sup>, Rebecca Bacon<sup>2</sup>, Yunfan Zhang<sup>1</sup>, Jennifer Ren<sup>1</sup>, Madiha Khan<sup>1</sup>, Vikram Sambasivan<sup>1</sup>, and Liping Feng<sup>1,2</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, <sup>2</sup>Department of Pathology, Duke University School of Medicine, Durham, NC

**Background:** Combined antiretroviral therapy (cART) has considerably improved HIV prognosis and reduced vertical transmission. However, cART has been associated with off-target renal effects, including integrase inhibitors increasing serum creatinine via inhibition of tubular creatinine secretion and nucleoside reverse transcriptase inhibitors leading to reversible renal injury and mitochondrial dysfunction. Further study of subtle renal tubular and developmental effects is warranted. Concurrent exposure to ubiquitous environmental contaminants such as perfluorooctanesulfonic acid (PFOS), which is associated with renal dysfunction in laboratory and epidemiologic studies, may further modify risk. Our project uses a rat model to evaluate maternal and offspring renal histopathology following exposure to cART and PFOS. **Methods:** Pregnant Sprague-Dawley rats were treated with cART (abacavir/dolutegravir/lamivudine at 300/25/150 mg/kg/day) or vehicle (methylcellulose/Tween 80) throughout gestation and weaning. Animals also received PFOS-containing water or vehicle (reverse osmosis-filtered water). Pups were weaned on postnatal day (PND) 20, when dams and one pup of each sex per litter underwent tissue harvest. Tissues were assessed after routine fixation and hematoxylin and eosin staining. Following an initial review for lesions of interest, pup kidneys were evaluated for (1) tubular alterations (tubular or cystic dilation, discrete fibrosis), (2) renal pelvis dilation, and (3) focal tubulitis (luminal neutrophils, cellular debris). Litter was used as the experimental unit. Fisher's Exact and Kruskal-Wallis tests were used for binary and ordinal outcomes, respectively. **Results:** No treatment-attributable differences were appreciated in dam kidney samples. In pups, no differences were seen in frequency of focal tubulitis across groups. There was a non-significant trend toward increased frequency of tubular alterations in cART-exposed pups of both sexes, particularly in

female cART+PFOS pups, which largely demonstrated cystic-type tubular dilations. Additionally, renal pelvis dilation occurred more frequently in PFOS and cART+PFOS groups across sexes, with pups exposed to PFOS alone showing increased severity among affected animals ( $p=0.03$ ). When stratified by sex, female pups in the PFOS-only exposure group also demonstrated a statistically significant increased frequency of renal pelvis dilation ( $p=0.02$ ). No other comparisons reached statistical significance. **Conclusions:** Gestational and perinatal PFOS exposure was associated with increased renal pelvis dilation in offspring at PND 20, particularly for females. cART exposure showed a non-significant trend toward increased tubular alterations. These mild lesions may reflect altered renal development or subtle injury, though their clinical relevance requires further follow-up. **Acknowledgements:** This study was supported by the National Institutes of Health OAR Innovation Award (Liping Feng).

## Poster Session – AI-Based Image Analysis

### Poster Board 59

#### Abstract 107

#### AI-Based Analysis of Complex Tissue Phenotypes in Zebrafish Embryos

Heather Shive, Jennifer Dwyer, Bih-Rong Wei, Vincenzo Verdi, and Mark Simpson

Laboratory of Cancer Biology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD

**Background:** The zebrafish (*Danio rerio*) has well-established value as a comparative animal model for in vivo analyses of diverse physiologic and pathologic processes. Zebrafish embryos are particularly well-suited for analyzing complex phenotypes at the tissue level and can be readily applied in rapid, high-throughput screens and whole-animal imaging studies. However, downstream image-based analyses currently used for embryo phenotypic assessments are often cumbersome, labor-intensive, and challenging to apply quantitatively. Here we describe two AI-based methods that allow rapid, detailed analysis of complex tissue phenotypes in zebrafish embryos using a commercially available image analysis platform. **Methods:** All animal studies were approved by the National Cancer Institute Animal Care and Use Committee (ASP LCBG-054). Two phenotypes were analyzed in zebrafish embryos: cutaneous pigmentation in a zebrafish model for a human heritable pigmentation disorder and tissue injury response in a zebrafish model for wound healing. Zebrafish embryos were collected and fixed between 3 and 6 days post-fertilization for downstream analyses. Digital images of fixed embryos were acquired by stereomicroscopy or fluorescence microscopy and analyzed with a commercially available image analysis platform. Custom algorithms were developed to classify and analyze areas of pigmentation and to quantify discrete cell populations and tissue injury responses. **Results:** We developed two AI-based approaches to analyze digital microscopic and stereomicroscopic images that can be applied for highly accurate quantitative assessment of tissue-level processes in embryo specimens. First, we developed a convolutional neural network (CNN) algorithm to classify and analyze pigmentation in embryos, which enabled precise quantification of traits such as area of pigmented regions, pigment distribution, and pigmentation pattern. We show that this algorithm classifies areas of pigmentation with high accuracy in wild type embryos and provides novel insights into abnormal pigmentation patterns arising in genetically modified zebrafish embryos. Second, we developed cell- and object-based algorithms to characterize and quantify multicellular responses to tissue injury in embryos. Using transgenic reporter zebrafish lines in combination with immunofluorescence, we show that these algorithms can be applied to quantify cell-specific and tissue-level injury responses in whole organisms. **Conclusions:** Together these studies demonstrate the creative application of AI-based methods for precise quantitative analyses of multifaceted tissue traits in zebrafish embryos, illustrating the potential for this technology to augment the study of conserved, biologically important phenotypes in this comparative animal model system. **Acknowledgements:** This work was supported by the Intramural Research Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.



# THE JOURNAL OF MOLECULAR DIAGNOSTICS

*Advancing Genomic Medicine and Informatics*

## ***The Journal of Molecular Diagnostics: Advancing Genomic Medicine and Informatics***



**Editor-in-Chief**  
**Ronald M. Przygodzki, MD**



