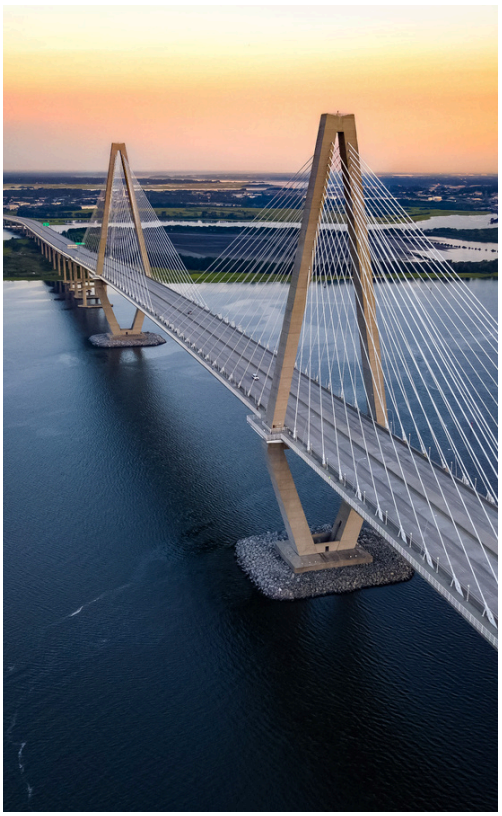

ET 19
CHARLESTON

19th International Conference on Endothelin: From New Discoveries to New Treatments

Francis Marion Hotel,
Charleston, SC, USA
September 21–24, 2025

Welcome
From the Organizers

Dear Colleagues and Friends,

On behalf of the Organizing Committee, we are excited to welcome you to the 19th International Conference on Endothelins (ET-19). As we gather with leading scientists, clinicians, and emerging researchers from around the world to share the latest advances in endothelin biology, pathophysiology, and therapeutic targeting, we are proud to continue the tradition of excellence that has characterized the ET conference series since its inception.

Beyond the scientific program, we hope you will take full advantage of the networking opportunities, foster new collaborations, and engage in stimulating discussions that will shape the future of endothelin research.

We are especially grateful to our speakers, sponsors, and partners for their invaluable support in making this conference possible. To all participants, whether you are attending for the first time or have been part of the ET community for years, we extend our warmest welcome.

We look forward to a productive and inspiring meeting and to sharing this exciting experience with you.

Sincerely,
The ET-19 Organizing Committee

Malgorzata Kasztan, PhD | **Justin Van Beusecum , PhD** | **Joshua Speed, PhD**

Malgorzata Kasztan, PhD
University of North Carolina

Justin Van Beusecum, PhD
Medical University of South Carolina

Joshua Speed, PhD
The University of Mississippi Medical Center

International Advisory Board and Administrative Committee

Endothelin International Advisory Board (ET-IAB)

Matthias Barton, MD
University of Zurich, Switzerland

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Medical University of South Carolina, U.S.A.

Donald E. Kohan, MD, PhD
University of Utah, U.S.A.

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University of Edinburgh, U.K

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Mario Negri Institute, Bergamo, Italy

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Ivana Vaněčková, PhD, DSc
Czech Academy of Sciences, Czech Republic

ET-19 Administrative Committee

Rena Becker
University of Alabama at Birmingham, U.S.A

Abigail Raczynski
University of Alabama at Birmingham, U.S.A.

Kelsi Rodriguez
University of Alabama at Birmingham, U.S.A.

General Information

Registration: Sunday Sept. 21, Francis Marion Lobby, 4:00-6:00PM
International African American Museum, 6:00-9:00PM
M-W Daily: 7 AM - 5 PM EST; Located in Prefunction Area

ATM Locations:

Nearest on Calhoun St. near the College of Charleston Bookstore

Photo/Video Policy:

Photography is not allowed during sessions unless permission provided by speaker

Wifi:

Francis Marion Meeting
PW: king1924

Coffee/Tea:

Coffee/Tea Stations located daily in Gold Ballroom Prefunction at the start of breakfast.

Transportation:

Taxi, Uber or Lyft available to and from airport and throughout Charleston
Stallion Car Service (downtown transportation) 843.330.9444

Electrical Socket:

Type A and Type B

Check out our sponsors on the bottom-right corner of select program pages, and be sure to visit their booths in the Gold Ballroom Prefunction area

Other:

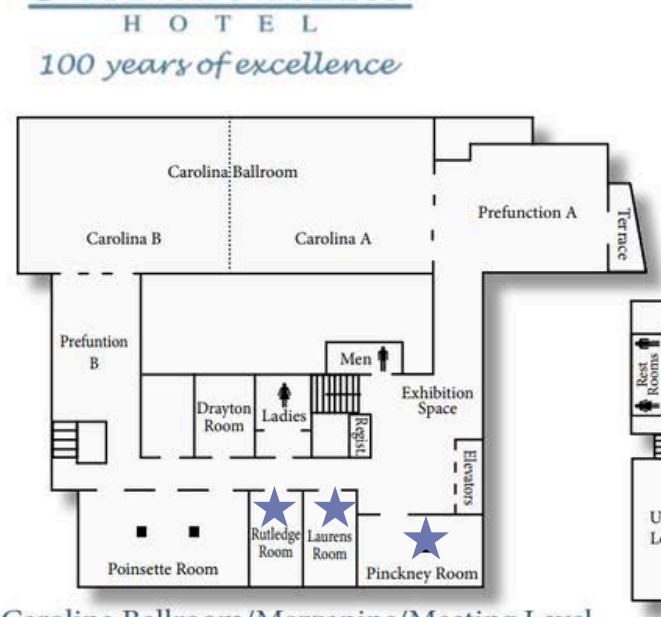
- Tipping is customary, but not required
- Please see Page 56 of Program for Safety Information
- All presentations will be located in the Gold Ballroom
- Poster Sessions will be held in the Pickney Room

Conference Map Key:

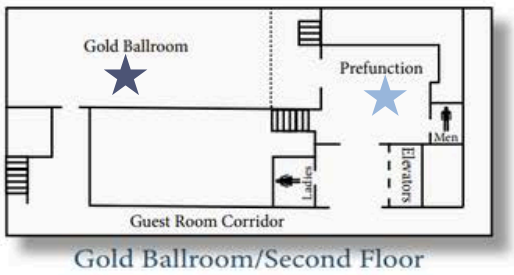
- All Sessions
- Breakfast, Lunch
- Poster Sessions

FRANCIS MARION HOTEL
100 years of excellence

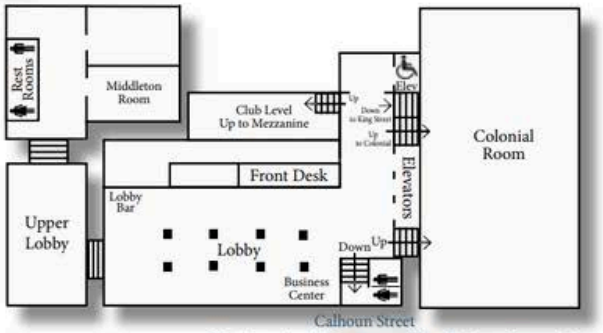
CONFERENCE ROOM INFORMATION



Carolina Ballroom/Mezzanine/Meeting Level



Gold Ballroom/Second Floor



Colonial Room/Lobby Level

Conference At a Glance

Day 1: September 21, 2025

4:00 - 6:00 PM: Registration (Francis Marion Hotel Lobby)
6:00 - 8:00 PM: Welcome Reception (International African American Museum)

Day 2: September 22, 2025

7:30 - 8:45 AM: Continental Breakfast (Gold Ballroom, Prefunction area)
8:50 - 9:00 AM: Introduction/Welcoming remarks (Gold Ballroom)
9:00 - 9:30 AM: Opening Lecture: David Webb, MD, University of Edinburgh, Sponsored by British Pharmacological Society
9:30 - 11:30 AM: Session I: Nephrology (Gold Ballroom)
11:00 - 11:30 AM: Break
11:30 - 1:00 PM: Session II: Hot Topics Vari-ET (Gold Ballroom)
1:00 - 2:00 PM: Lunch (Gold Ballroom, Prefunction area)
2:00 - 3:00 PM: Poster Session (Pickney Room)
3:00 - 4:30 PM: Session III: Vascular Biology and Hypertension (Gold Ballroom)
6:30 - 8:30 PM: Dinner for All Attendees - Francis Marion Hotel, Colonial Room, Lobby Level

Day 3: September 23, 2025

7:00 - 8:00 AM: Continental Breakfast (Gold Ballroom, Prefunction area)
8:00 - 9:30 AM: Session IV: Neurology (Gold Ballroom)
9:30 - 10:00 AM: Break
10:00 - 11:30 AM: Session V: Inflammation/Rheumatology (Gold Ballroom)
11:30 - 12:30 PM: Lunch (Gold Ballroom, Prefunction area)
12:30 - 1:30 PM: Poster Session (Pickney Room)
1:00 - 3:00 PM: Section VI: Women's Health (Gold Ballroom, Prefunction area)
3:00 - 3:30 PM: Break
3:30 - 5:00 PM: Session VII: Cardiology (Gold Ballroom, Prefunction area)

Day 4: September 24, 2025

7:00 - 8:00 AM: Continental Breakfast (Gold Ballroom, Prefunction area)
8:00 - 9:30 AM: Session VIII: Endocrinology/Metabolism (Gold Ballroom)
9:30 - 10:00 AM: Break
10:00 - 11:30 AM: Session IX: Clinical Trials/Health Disparities (Gold Ballroom)
11:30 - 12:30 PM: Lunch (Gold Ballroom, Prefunction area)
12:30 - 1:30 PM: Session X: Panel: Endothelin Receptor Antagonists: A Discussion on Challenges and Opportunities in Clinical Trials (Gold Ballroom)
1:30 - 2:00 PM: Awards Ceremony (Gold Ballroom)
2:00 - 2:45 PM: Concluding Remarks (Gold Ballroom)

Contents

Day 1: September 21, 2025

4:00-6:00 pm

Registration
Francis Marion Hotel Lobby
(International African American Museum after 6 pm)

6:00-8:00 pm

Welcome Reception
Location: International African American Museum (14 Wharfside Street, Charleston, SC 29401)

Day 2: September 22, 2025

7:30-8:45 am

Continental Breakfast - Gold Ballroom Prefunction Area

8:50-9:00 am

Introduction/Welcoming Remarks
Joshua S. Speed, PhD, Malgorzata Kasztan, PhD, Justin P. Van Beusecum, PhD
Location: Gold Ballroom, 2nd Floor, Francis Marion Hotel

9:00-9:30 am

Opening Invited Lecture: David Webb, MD
University of Edinburgh
Recent Advances in Endothelin Research
Sponsored by British Pharmacological Society

9:30-11:00am

SESSION I: Nephrology
Session Chairs: Donald Kohan, MD, PhD, University of Utah
Malgorzata Kasztan, PhD, University of North Carolina at Chapel Hill

9:30-10:00am

Keynote Lecture: Vimal Derebail, MD
University of North Carolina Chapel Hill
Endothelin antagonism in glomerular disease: A decade or more in the making

10:00-10:15 am

Rob Geletka, MD Travere Therapeutics Inc. **0-1**
Urinary Biomarker Analysis Reveals Rapid Intrarenal Anti-Inflammatory and Anti-Fibrotic Effects of Sparsentan (SPAR) in IgA Nephropathy (IgAN) in the SPARTAN Study

10:15-10:30 am

Martine Clozel, MD, Idorsia Pharmaceuticals Ltd. **0-2**
The potential of aprocitentan in kidney diseases

10:30-10:45 am

Matthew Sayer, MD, University of Edinburgh **0-3**
Dual endothelin-angiotensin antagonism improves endothelial function & fibrinolysis in ANCA vasculitis: a randomised, double blind, active control clinical trial

10:45-11:00 am

Ilse Diehn, PhD, Icahn School of Medicine at Mount Sinai **0-4**
Podocyte derived endothelin-1 in glomerular injury

11:00-11:30 am

Break

Day 2: September 22, 2025

11:30-1:00 pm	**SESSION II: Hot Topics Vari-ET** Session Chairs: Michelle Gumz, PhD, University of Florida Kelly Hyndman, PhD, University of Alabama at Birmingham	
11:30-12:00 pm	**Keynote: Maneesh Jain, PhD, University of Nebraska** *Remodeling Tumor Microenvironment via ET-Axis Antagonism to Improve Cancer Therapy*	
12:00 -12:15 pm	**Sumayah Jamal, MD, PhD, ENB Therapeutics, Inc.** *ENB-003, an ETBR antagonist, in combination with pembrolizumab for refractory advanced solid tumors: Topline data from the ENBolden-101 Phase 1B clinical study*	**0-5**
12:15-12:30 pm	**Gusty Rizky Teguh Ryanto, MD, PhD, Kobe Pharmaceutical University** *Early Inflammatory Signatures Drive Alveolar Simplification in Endothelin 2–Deficient Lung Development*	**0-6**
12:30-12:45 pm	**Novia Nurul Faizah, MD, Kobe University** *Endothelin-1 as a Downstream Effector of Activin A Signaling in Pulmonary Arterial Hypertension: Mechanistic and Therapeutic Implications*	**0-7**
12:45-1:00 pm	**Neeraj Dhaun, PhD, University of Edinburgh** *Dual endothelin-angiotensin receptor blockade improves diurnal blood pressure profile in patients with ANCA-associated vasculitis*	**0-8**
1:00-2:00 pm	**Lunch**	
2:00-3:00 pm	**Poster Session I - Pickney, Mezzanine Level, Francis Marion Hotel**	
3:00-4:30 pm	**SESSION III: Vascular Biology/Hypertension** Session Chairs: Neeraj Dhaun, MD, University of Edinburgh Ivana Vaneckova, PhD, DSc., Institute of Physiology of the Czech Academy of Sciences	
3:00-3:30 pm	**Keynote: Jennifer Pollock, PhD University of Alabama at Birmingham** *When you eat is as important as what you eat*	
3:30-3:45 pm	**Gavin Chapman, MD, University of Edinburg** *Choroidal thinning reflects systemic vascular injury in ANCA-associated vasculitis and improves with dual endothelin-angiotensin blockade*	**0-9**
3:45-4:00 pm	**Guangrong Lu, MS, Texas A&M University** *Mechanism of Porcine Coronary Arteriolar Constriction to a Clinical Level of Endothelin-1*	**0-10**

Day 2: September 22, 2025

Session III Continued..

4:00-4:15 pm — **Michael Crompton, PhD, University of Bristol** — **0-11**
Sparsentan has direct effects on the glomerular capillary wall to attenuate increased permeability in nephrotic syndrome models

4:15-4:30 pm — **Aditya Sharma, University of Cambridge** — **0-12**
Ethnic differences in associations of Endothelin-1 and novel Renin-Angiotensin-Aldosterone System biomarkers in Hypertensive individuals

6:30-8:30 pm — **Dinner for All Attendees - Francis Marion Hotel, Colonial Room, Lobby Level**

Day 3: September 23, 2025

7:00-8:00 am — **Continental Breakfast**

8:00-9:30 am — **SESSION IV: Neurology**
Session Chairs: Adviye Ergul, MD, PhD, Medical University of South Carolina
Bryan Becker, PhD, University of Alabama at Birmingham

8:00-8:30 am — **Keynote Lecture: Bryan Becker, PhD, University of Alabama at Birmingham**
Differential Expression in Sensory Neurons of Rats Deficient in Endothelin B Receptors on Normal and High Salt Diets

8:30-8:45 am — **Yasir Abdul, PhD, Medical University of South Carolina** — **0-13**
Endothelin-1 upregulates phagocytosis functions in human microglial cells

8:45-9:00 am — **Yaritza Inostroza-Nieves, PhD, San Juan Bautista School of Medicine** — **0-14**
Endothelin-1 as an Activator of Pro-inflammatory Microglia Cells"

9:00-9:15 am — **Vishnu Yelakanti, Pharmazz Inc.** — **0-15**
A Phase III Study to Assess the Safety and Efficacy of Sovateltide in Patients with Acute Cerebral Ischemic Stroke: Protocol of the RESPECT- ET_B Trial

9:15-9:30 am — **Adviye Ergul, MD, PhD, Medical University of South Carolina** — **0-16**
Intranasal treatment by ETA receptor antagonist BQ123 or ETB receptor agonist Sovateltide attenuates stroke-mediated deficits in multiple domains of behavior in diabetic rats

9:30-10:00 am — **Break**

10:00-11:30 am — **SESSION V: Inflammation/Rheumatology**
Session Chairs: Justin Van Beusecum, PhD, Medical University of South Carolina
Carmen De Miguel, PhD, University of Alabama at Birmingham

10:00-10:30 am — **Keynote Lecture: Erin Taylor, PhD, University of Mississippi Medical Center**
ET-1 in Autoimmune Disease Activity and Progression

Day 3: September 23, 2025

Session V Continued...

10:30-10:45 am
Eric George, PhD, University of Mississippi Medical Center **0-17**
Endothelin-1 Regulates Hypoxia-Induced Increases in Placental Inflammatory and Anti-Angiogenic Cytokines through the ET_A Receptor

10:45-11:00 am
Helen Butler, PhD, Medical University of South Carolina **0-18**
Endothelin Receptor Autoantibodies: Associations with Blood Pressure and Endothelial Activation in Systemic Lupus Erythematosus

11:00-11:15 am
Abigail Brooks, MS, University of Alabama at Birmingham **0-19**
The ET-1/ETA axis on T cells, not Dendritic Cells, Mediates Inflammation and Renal Dysfunction during Type 1 Diabetes

11:15-11:30 am
Megumi Mills, PhD, University of Mississippi Medical Center **0-20**
"Dual Endothelin-1 Receptor A/B Blockade Attenuates Adipose Tissue Inflammation in Mice Fed a High Fat Diet"

11:30-12:30 pm
Lunch

12:30-1:30 pm
Poster Session II - Pickney, Mezzanine Level, Francis Marion Hotel

1:30-3:00 pm
SESSION VI: Women's Health
Session Chairs: Marharyta PhD, Medical University of South Carolina
Eman Gohar, PhD, Vanderbilt University

1:30-2:00 pm
Keynote Lecture: Michelle Gumz, PhD, University of Florida
Of Mice and (Wo)Men: Recent Advances in Understanding ET-1 Physiology and Pathophysiology

2:00-2:15 pm
Eman Gohar, PhD, Vanderbilt University Medical Center **0-21**
Pregnancy regulates renal endothelin-1 and aldosterone signaling systems in aged female mice lacking G protein-coupled estrogen receptor

2:15-2:30 pm
Marice McCrorey, Medical University of South Carolina **0-22**
Pathological Upregulation of Endothelin Receptor A Signaling In Systemic Lupus Erythematosus

2:30-2:45 pm
Feng Li, MD, PhD, University of North Carolina Chapel Hill **0-23**
ET-1 impairs decidualization and angiogenesis during early pregnancy, which is mitigated by nicotinamide, in mice

2:45-3:00 pm
Tha Luong, MS, University of Alabama at Birmingham **0-24**
Endothelium-derived ET-1 stimulates Th17 cell expansion in a sex-dependent manner under high-salt diet

3:00-3:30 pm
Break

Day 3: September 23, 2025

3:30-5:00 pm
SESSION VII: Cardiology
Session Chairs: David Webb, MD, PhD, University of Edinburgh
Pedro D'Orleans Juste, PhD, University of Sherbrooke

3:30-4:00 pm
Keynote Lecture: Raj Gupta, MD, Harvard Medical School
Linking to human genetic variation to regulation

4:00-4:15 pm
Daniel Angkasa, Universitas Gadjah Mada **0-25**
Association between Endothelin-1 and Right Ventricular Remodeling in Pulmonary Hypertension Secondary to Atrial Septal Defect Patients

4:15-4:30 pm
Thomas Dempster, Medical University of South Carolina **0-26**
Mice lacking CD8+ T-cells have lower Edn1 expression post-MI

4:30-4:45 pm
Virginia Beasley, University of Alabama at Birmingham **0-27**
Lack of the ET-1/ETA axis on dendritic cells results in ameliorated hypertension in males, but not females

4:45-5:00 pm
Samarjit Das, PhD, Johns Hopkins **0-28**
Endothelin-1 mediated crosstalk between heart and skeletal muscle during obesity induced cardiac dysfunction

Day 4, September 24, 2025

7:00-8:00 am
Continental Breakfast

8:00-9:30 am
SESSION VIII: Endocrinology/ Metabolism
Session Chairs: Joshua Speed, PhD, University of Mississippi Medical Center
Sam Das, PhD, Johns Hopkins

8:00-8:30 am
Keynote Lecture: Jaquelyn Limberg, PhD, University of Missouri
Endothelin-1 as a novel target for the prevention of cardiometabolic dysfunction with intermittent hypoxia

8:30-8:45 am
Marvin Sinsakul, MD, AstraZeneca **0-29**
Effects of combined treatment with zibotentan and dapagliflozin compared to dapagliflozin alone on insulin resistance and markers of inflammation

8:45-9:00 am
Hayley Murphy, MS, University of Mississippi Medical Center **0-30**
Transcriptomics of adipose tissue from patients with obesity reveal upregulation of endothelin-1 signaling in Black Americans

9:00-9:15 am
Alexandria Juffre, University of Florida **0-31**
The circadian protein PERIOD-1 in the adrenal gland moderates endothelin-1 and aldosterone excretion in response to dietary salt in C57Bl/6J mice

9:15-9:30 am
Satrio Adi Wicaksono, MD, Kobe University **0-32**
Endothelin-2 Signaling Orchestrates Telogen-to-Anagen Transition in Hair Follicles: Implications for Alopecia Therapy

Day 4, September 24, 2025

9:30-10:00 am	**Break**	
10:00-11:30 am	**SESSION IX: Clinical Trials/Health Disparities** Session Chairs: Martine Clozel, MD, Idorsia Pharmaceuticals Ltd. Noriaki Emoto, MD, PhD, Kobe University	
10:00-10:30 am	**Keynote Lecture: Anthony Davenport, PhD, University of Cambridge** *Emerging therapeutic agents and proteomics strategies targeting the endothelin pathway*	
10:30-10:45 am	**Emmie Anderson, University of Illinois Urbana-Champaign** *Sovateltide (IRL-1620), an endothelin B receptor agonist for cerebral ischemic stroke treatment, shows an unexpected reduction in lymphedema in a lymphatic filariasis patient*	**0-33**
10:45-11:00 am	**Bruce Hendry, MD, PhD, Travere Therapeutics Inc.** *Patients (Pts) With Focal Segmental Glomerulosclerosis (FSGS) Achieved Low Proteinuria Targets Earlier and More Often With Sparsentan (SPAR) vs Irbesartan (IRB) in DUPLEX*	**0-34**
11:00-11:15 am	**Preyan Mehta, Pharmazz Inc.** *Systematic Review and Meta-Analysis of Sovateltide, Alteplase, and Tenecteplase Treatments for Acute Cerebral Ischemic Stroke*	**0-35**
11:15-11:30 pm	**Chee Kay Cheung, MD, PhD, University of Leicester** *Sparsentan (SPAR) as First-Line Treatment of Incident Patients (Pts) With IgA Nephropathy (IgAN): An Interim Analysis of the SPARTAN Trial Evaluating Efficacy and Cardiovascular (CV) Risk Variables*	**0-36**
11:30-12:30 pm	**Lunch**	
12:30-1:30 pm	**"Endothelin Receptor Antagonists: A Discussion on Challenges and Opportunities in Clinical Trials"** **Discussion Panel:** Donald Kohan, MD, PhD, University of Utah Bruce Hendry, MD, PhD, Travere Therapeutics Inc. Martine Clozel, MD, Idorsia Pharmaceuticals Ltd. Anil Gulati, MD, PhD, Pharmazz Anthony Davenport, PhD, University of Cambridge Kristin Fiorino, MD, AstraZeneca	
1:30- 2:00 pm	**Awards Ceremony**	
2:00-2:30 pm	**Highlights of the Meeting by Masashi Yanagisawa, MD, PhD, University of Tsukuba**	
2:30-2:45 pm	**Closing remarks of the Conference Chairs** **Announcement of the next ET-20 meeting location**	

Poster Sessions

Pickney Room

Monday, September 22, 2025 - 2:00 PM - 3:00 PM
Tuesday, September 23, 2025 - 12:30 PM - 1:30 PM

We are in rare for life.

At Travere Therapeutics,we come together every day to help patients, families and caregivers of all backgrounds as they navigate life with a rare disease. We know the need for treatment options is urgent - that is why our global team works with the rare disease community to identify, develop and deliver life-changing therapies.

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MA-SP-25-0027

TRAVERE® THERAPEUTICS

Poster Session I

Monday, September 22, 2025 - 2:00 PM - 3:00 PM

A-1	**Podocyte derived endothelin-1 in glomerular injury**	*Dr. Ilse Daehn*
A-2	**Dual endothelin-angiotensin antagonism improves endothelial function & fibrinolysis in ANCA vasculitis: a randomised, double blind, active control clinical trial** (Not Presented)	*Dr. Matthew Sayer*
A-3	**ETB blockade exacerbates circadian amplitude of blood pressure in rats on a high salt diet**	*Dr. Maria Venegas*
A-4	**Sparsentan reversibly decreases mesangial IgA deposition in gddY mice: a possible role for mesangial-cell-surface autoantigen expression**	*Dr. Bruce Hendry*
A-5	**EET-A (14,15-epoxyeicosatrienoic acid analog) augments the hypotensive effects of atrasentan, and prevents edema and organ hypertrophy in spontaneously hypertensive rats**	*Dr. Ivana Vvaněčková*
A-6	**Association between Endothelin-1 and Right Ventricular Remodelling in Pulmonary Hypertension Secondary to Atrial Septal Defect Patients**	*Danniel Angkasa*
A-7	**Mice lacking CD8+ T-cells have lower Edn1 expression post-MI**	*Thomas Dempster*
A-8	**Circadian rhythms in serum ET-1 in obese individuals**	*Woojin Lee*
A-9	**ET-1 inhibits CD8+ T-cell mediated actions on the focal scar Post-Myocardial Infarction**	*Shaoni Dasgupta*
A-10	**Sovateltide (IRL-1620), an endothelin B receptor agonist for cerebral ischemic stroke treatment, shows an unexpected reduction in lymphedema in a lymphatic filariasis patient**	*Emmie Anderson*
A-11	**Systematic Review and Meta-Analysis of Sovateltide, Alteplase, and Tenecteplase Treatments for Acute Cerebral Ischemic Stroke**	*Preyan Mehta*
A-12	**Endothelin-3 induces skeletal muscle atrophy and metabolic derangements**	*Dr. Yi-Ting Chung*
A-13	**Endothelin-2 Deficiency Disrupts Adipose Tissue Structure and Thermogenic Function.**	*Dr. Saiful Hidayat*
A-14	**Endoplasmic Reticulum Stress Mediates Endothelin-1-Induced Norepinephrine Transporter Algteration in PC12 Cells Involvement of Endothelin Receptors**	*Fabian Alejandro Innamorato Costas*
A-15	**The circadian protein PERIOD-1 in the adrenal gland moderates endothelin-1 and aldosterone excretion in response to dietary salt in C57Bl/6J mice**	*Alexandria Juffre*
A-16	**ET-1-induced FGF21 promotes the formation of oxidative myofibers during skeletal muscle atrophy through activation of the ETB receptor and the endoplasmic reticulum (ER) stress signaling pathway**	*Dr. Shui-Yu Liu*
A-17	**Endothelin-1 induces FGF-21 expression in hepatocytes through activation of the endothelin type A receptor**	*Dr. Shui-Yu Liu*
A-18	**Transcriptomics of adipose tissue from patients with obesity reveal upregulation of endothelin-1 signaling in Black Americans**	*Hayley Murphy*
A-19	**Endothelin-2 Signaling Orchestrates Telogen-to-Anagen Transition in Hair Follicles:Implications for Alopecia Therapy**	*Dr. Satrio Adi Wicaksono*
A-20	**The ET-1/ETA axis on T cells, not Dendritic Cells, Mediates Inflammation and Renal Dysfunction during Type 1 Diabetes**	*Abigail Brooks*
A-21	**Choroidal thinning reflects systemic vascular injury in ANCA-associated vasculitis and improves with dual endothelin-angiotensin blockade** (Not Presented)	*Dr. Gavin Chapman*
A-22	**Dual Endothelin-1 Receptor A/B Blockade Attenuates Adipose Tissue Inflammation in Mice Fed a High Fat Diet**	*Dr. Megumi Mills*
A-23	**Endothelin Receptor Autoantibodies: Associations with Blood Pressure and Endothelial Activation in Systemic Lupus Erythematosus**	*Dr. Helen M. Butler*
A-24	**Membrane estrogen receptor activation restores nitric oxide production in human renal endothelial cells under inflammatory and lipotoxic stress**	*Mariia Stefanenko*

Poster Session II

Tuesday, September 23, 2025 - 12:30 PM - 1:30 PM

B-1	**Endothelin A and B Receptors are Differentially Regulated in a Multi-etiology Model of Alzheimer's Disease-Related Dementias (ADRD): Retinal and Cerebral Perspectives**	*Dr. Yasir Abdul*
B-2	**Endothelin-1 upregulates phagocytosis functions in human microglial cells**	*Dr. Yasir Abdul*
B-3	**Endothelin-1 as an Activator of Pro-inflammatory Microglia Cells.**	*Dr. Yaritza Inostroza-Nieves*
B-4	**High Fat Diet-induced Obesity in Alzheimer's Mice is Associated with Cerebral Hypoperfusion and ETA/ETB Receptor Imbalance**	*Pooja Pradeep*
B-5	**A Phase III Study to Assess the Safety and Efficacy of Sovateltide in Patients with Acute Cerebral Ischemic Stroke: Protocol of the RESPECT-ETB Trial**	*Vishnu Yelakanti*
B-6	**ENB-003, an ETBR antagonist, in combination with pembrolizumab for refractory advanced solid tumors: Topline data from the ENBolden-101 Phase 1B clinical study**	*Dr. Sumaya Jamal*
B-7	**Endothelin-1 as a Downstream Effector of Activin A Signaling in Pulmonary Arterial Hypertension: Mechanistic and Therapeutic Implications**	*Dr. Novia Nurul Faizah*
B-8	**Endothelin-2 is Essential for Postnatal and Adult Lung Homeostasis: Insights from Temporal Knockout Models**	*Dr. Sagita Mega Sekar Kencana*
B-9	**Early Inflammatory Signatures Drive Alveolar Simplification in Endothelin-2–Deficient Lung Development**	*Dr. Gusty Rizky Teguh Ryanto*
B-10	**Mechanism of Porcine Coronary Arteriolar Constriction to a Clinical Level of Endothelin-1**	*Guangrong Lu*
B-11	**Dual endothelin-angiotensin receptor blockade improves diurnal blood pressure profile in patients with ANCA-associated vasculitis**	*Dr. Neeraj Dhaun*
B-12	**Sparsentan has direct effects on the glomerular capillary wall to attenuate increased permeability in nephrotic syndrome models**	*Dr. Michael Crompton*
B-13	**Comparison of endothelin-1 levels in human plasma from coronary arteries measured by enzyme linked immunosorbent assay and Olink high-throughput proteomics platform**	*Dr. Anthony Davenport*
B-14	**Localization of the therapeutic targets for endothelin receptor antagonists and sodium-glucose co-transporter 2 inhibitors in the chronic liver disease, primary sclerosing cholangitis**	*Dr. Anthony Davenport*
B-15	**Analysis of the expression of genes encoding the endothelin signalling pathway using single-cell RNA sequencing (scRNA-seq) in human heart**	*Dr. Anthony Davenport*
B-16	**Endothelin type B receptor activation in the paraventricular nucleus of hypertensive DOCA salt rats. Beneficial role at the cardiovascular level**	*María Florencia Fernandez*
B-17	**Ethnic differences in associations of Endothelin-1 and novel Renin-Angiotensin-Aldosterone System biomarkers in Hypertensive individuals**	*Aditya Sharma*
B-18	**Lack of the ET-1/ETA axis on dendritic cells results in ameliorated hypertension in males, but not females**	*Virginia Beasley*
B-19	**Pregnancy regulates renal endothelin-1 and aldosterone signaling systems in aged female mice lacking G protein-coupled estrogen receptor**	*Dr. Eman Gohar*
B-20	**Endothelium-derived ET-1 stimulates Th17 cell expansion in a sex-dependent manner under high-salt diet**	*Tha Luong*
B-21	**Pathological Upregulation of Endothelin Receptor A Signaling In Systemic Lupus Erythematosus**	*Marice McCrorey*
B-22	**Sex-Specific Mitochondrial Metabolic Signatures in Human Renal Tissues**	*Dr. Marharyta Semenikhina*
B-23	**Restoration of Endothelial Function Prevents Microemboli-mediated Dysregulation of Endothelin Receptors in Diabetes: Relevance to Vascular Contributions to Cognitive Impairment (VCID)**	*Dr. Weiguo Li*

Speaker Bios

David Webb, MD

Professor of Clinical Pharmacology
University of Edinburgh

David John Webb (born 1 September 1953) is a British physician, scientist and clinical pharmacologist, who was appointed to the Christison Chair of Therapeutics and Clinical Pharmacology at the University of Edinburgh in 1995, and from 1998 to 2001 he was Head of the University's Department of Medical Sciences there. He established its Centre for Cardiovascular Science in 2000.

Webb's scientific research focuses on novel treatments for hypertension and cardiovascular risk, particularly with regards to blood vessel structure and function. His publications include first-in-human studies with renin inhibitors and endothelin antagonists, including exploration of the role of endothelin antagonists as treatment for vascular and renal disease. He has also contributed to the current understanding of arterial stiffness and its role in cardiovascular risk.

Webb was elected a Fellow of the Royal College of Physicians in 1992, a Fellow of the Academy of Medical Sciences in 1999, and a Fellow of the Royal Society of Edinburgh in 2004. He is an honorary fellow of the British Pharmacological Society and of the Faculty of Pharmaceutical Physicians. He was appointed Commander of the Order of the British Empire (CBE) in the 2020 Birthday Honours for services to clinical pharmacology, research and education.

BRITISH
PHARMACOLOGICAL
SOCIETY

Vimal Derebail, MD

Associate Professor of Medicine
University of North Carolina Chapel Hill

UNC SCHOOL OF MEDICINE

Dr. Vimal Derebail, MD received his medical degree from the Medical College of Georgia. He is currently an Associate Professor of Medicine at the University of North Carolina Chapel Hill where he is the Faculty Lead for Clinical Trials in the UNC Kidney Center. His clinical practice is focused on the care of patients with glomerular diseases and vasculitis as well as managing kidney disease in patients with sickle cell disease. As part of his research, he works closely with the Clinical Trial team that is part of the UNC Kidney Center in addition to my work with our physician and basic and translational scientists studying glomerular disease and vasculitis and those studying sickle disease and sickle cell trait. In nephrology, he also has an interest in understanding the high rates of venous thromboembolism seen in patients with nephrotic syndrome and vasculitis.

Jaqueline Limberg, PhD

Associate Professor
University of Missouri

MU

Jackie Limberg is Associate Professor of Nutrition & Exercise Physiology at the University of Missouri. She completed her PhD at the University of Wisconsin and postdoctoral fellowship at the Mayo Clinic in the lab of Dr. Mike Joyner. Jackie studies human integrative physiology with a primary focus on the role of the autonomic nervous system in cardiovascular responses to environmental stress. In particular, Dr. Limberg is interested in mechanisms that contribute to the development of cardiovascular disease and interventions that can reverse and/or prevent cardiovascular disease risk. Dr. Limberg is currently studying how blood flow and blood pressure are modulated by the nervous system, the effect of pharmacological and non-pharmacological (e.g. exercise) interventions, and how these factors may differ between men and women.

Maneesh Jain, PhD

Professor, UNMC Department of
Biochemistry and Molecular Biology

UNMC

My interest has been to develop antibody-based strategies for targeted therapy and diagnosis of diseases, particularly cancer. Our research involves development of genetically engineered antibody fragments for improved radio-immunotherapy of solid tumors. We are trying to optimize radio-immunotherapy of solid tumors by modifying the molecular design of antibody fragments and introducing sequences that will enhance the uptake and/or retention of radiolabeled antibodies in the tumor tissues without altering their distribution in non-target tissues. Recently, we demonstrated the utility of cell penetrating peptides in improving the tumor retention antibody fragments.

The other area of our research involves development of serum assays for the early diagnosis of lethal pancreatic cancer.

We are trying several approaches to develop sensitive mucin-based serum assays utilizing the antibodies that we have generated. In collaboration with several groups we are trying to develop a multimarker nanoparticle-based assay for early diagnosis of pancreatic cancer.We are also trying to use the antibodies for disrupting the signaling pathways mediated by their targets for therapeutic intervention and engineering the antibodies for human use. Additionally, we are trying to use the antibodies and antibody fragments for the delivery nanoparticle-encapsulated drugs to various cancers.

Jennifer Pollock, PhD

Professor, UAB Heersink School of Medicine,
Division of Nephrology

Jennifer Pollock, PhD, FAHA, FAPS, trained in protein biochemistry at UNC-Chapel Hill examining structure-function of prothrombin activation. Her postdoctoral studies with Dr. Ferid Murad, 1998 Nobel Laureate, were the first descriptions of NO synthase in the vasculature and provided a basis for continuing studies on the regulation of NO in cardiovascular disease. Her research has focused on endothelial function, vascular stiffness, and immune activation especially to decipher how early life stress or childhood adversity mediates vascular disease as well as control of sodium handling by the kidney. In addition, her research focuses on circadian biology and blood pressure regulation as well as timing of ood interventions in obesity and immune activation. Dr. Pollock is currently an Endowed Professor in the Department of Medicine, Co-Director of Cardio-Renal Physiology & Medicine Section and serves as the Director for the Pre-doctoral T32 Institutional Training Program of the Center for Clinical and Translational Science (CCTS) Partner Network. Dr. Pollock also founded and directs and UAB Kidney Pipeline Training Office and is the Director of the Professional Development Core for the NIDDK KUH PRIME program, NIDDK R25 Kidney Undergraduate Research Experience program, and the NIDDK R25 PROMOTE program. Dr. Pollock has held many leadership roles within her university and national societies including the 94th President of the American Physiological Society.

Erin Taylor, PhD

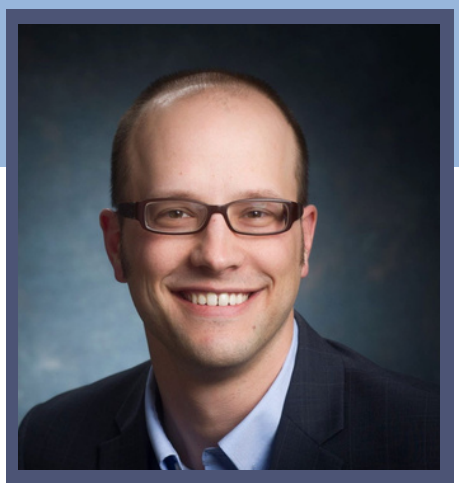
Assistant Professor of Physiology & Biophysics & Medicine
University of Mississippi Medical Center

Dr. Taylor received a PhD in Microbiology and Immunology from the University of Mississippi Medical Center (UMMC) in 2015. She is currently an NIH funded Assistant Professor at UMMC. Her primary research interest is to understand the link between the immune system dysfunction and cardiovascular disease. She is an expert in autoimmune diseases, in particularly, systemic lupus erythematosus. With over 12 years of experience with immunological techniques including flow cytometry, immune cell culture, and assays of immune cell function, her laboratory studies the interaction between immune system dysfunction and gut dysbiosis, and how these factors contribute to vascular dysfunction and hypertension in the SLE. In addition she studies the impact of central and peripheral leptin signaling on immune system function and the development of autoimmunity.

Anthony Davenport, PhD

Professor of Cardiovascular Pharmacology
University of Cambridge

Anthony Davenport is Professor of Cardiovascular Pharmacology, University of Cambridge. His research concentrates on the role of endothelin, ET_A and ET_B receptors in the human cardiovascular system and the pharmacology of selective agonists and antagonists, particularly repurposing ET compounds in new clinical conditions. Completed clinical trials include PRIZE, a precision medicine study into the efficacy of the ET_Aantagonist, zibotentan, in patients with microvascular angina, enriched with the minor G allele rs9349379 SNP. The related PRIZE ET sub-study explores patients genotyped for other genetic variants in the ET-I pathway and has identified a novel association between a coronary artery disease risk SNP, in the region of the gene encoding the ET_A receptor. Recent research has explored the action of SARS-CoV-2 on the cardiovascular system to significantly increase plasma ET in patients hospitalised during the acute phase of infection, testing the combination of ET antagonist ambrisentan with an SGLT2 inhibitor to treat COVID-19, as well as repurposing clinical compounds to reduce expression of the viral entry 'receptor' ACE2. With colleagues he has published reviews on ET including Nature Cardiovascular Research (doi: 10.1038/s44161-023-00347-2) and Pharmacological Reviews (doi: 10.1124/pr.115.011833.). He is a long standing executive member of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR), that maintains a comprehensive curated data bases of drugs and their mechanism of action including ET receptors and their ligands in Guide to Pharmacology.



Bryan Becker, PhD

Assistant Professor, UAB Heersink School of Medicine, Division of Nephrology

Bryan Becker, PhD completed his PhD in Cellular and Integrative Physiology from the University of Nebraska Medical Center in the field of autonomic nervous system control during heart failure. He joined UAB for postdoctoral training investigating contributions of endothelin signaling on autonomic control of blood pressure during hypertension under the mentorship of Dr. David Pollock. Bryan joined the faculty at UAB as Assistant Professor and continues research endeavors exploring the role of the autonomic nervous system in blood pressure control through studies investigating the role of endothelin signaling on kidney sensory nerves in the context of high salt and high fat dietary challenges. He is also involved in numerous collaborative projects including measuring markers of autonomic tone in human subjects through in clinic and ambulatory/wearable technologies and in recent work investigating circadian rhythms in the physiology of brain water homeostasis.

Michelle Gumz, PhD

Professor Department of Physiology and Aging
University of Florida

Michelle Gumz, PhD is a Professor in the Department of Physiology and Aging. Her research group focuses on the role of the circadian clock in the kidney and how it contributes to the regulation of renal function and blood pressure control. This work includes the study of sex differences in the action of the clock proteins PER1 and BMAL1 as well as the use of hypertension models. They use rodent models to better understand the integrative physiology of the circadian clock with the ultimate goal that the findings will contribute to the development of new therapies for kidney disease and hypertension in humans. Recently, the work has expanded to include investigation of circadian clock crosstalk between the kidneys and adrenal glands and how that influences renal function, blood pressure regulation, and the physiology of the aging kidney.

Rajat Gupta, MD

Assistant Professor of Medicine
Harvard Medical School

Rajat Gupta, MD is an Assistant Professor of Medicine at Harvard Medical School with a research laboratory in the Divisions of Cardiovascular Medicine and Genetics at Brigham and Women's Hospital. His research is focused on identifying new treatments for vascular disease using human genetics to discover novel biologic pathways. His post-doctoral work identified non-coding variants in the 6p24 locus as distal regulators of Endothelin-1 (Cell, 2017). Dr. Gupta's laboratory recently identified the transcriptional signature of distinct endothelial cell populations using single cell RNA-sequencing (Circulation, 2019).

Dr. Gupta graduated from the University of Pennsylvania School of Medicine (2007) where he was awarded the Adolf J. Creskoff Prize. He completed Internal Medicine residency training at Massachusetts General Hospital (2010), cardiology fellowship at Brigham and Women's Hospital (2014), and a post-doctoral fellowship in Human Genetics at the Broad Institute of Harvard and MIT (2016). Dr. Gupta has been named the Thomas W. Smith Fellow at Brigham and Women's Hospital and received the Lerner Junior Faculty Award. His research is funded by grants from the NIH (New Innovator Award), the Broad Institute, the Sperling Family Fellowship, and the Chan Zuckerberg Initiative.

ET 19
CHARLESTON

Abstracts (Oral Presentations)

01

Urinary Biomarker Analysis Reveals Rapid Intrarenal Anti-Inflammatory and Anti-Fibrotic Effects of Sparsentan (SPAR) in IgA Nephropathy (IgAN) in the SPARTAN Study

Chee Kay Cheung, William A Barratt, Sulalita Chaki, Silpa Chinnakotla, Neeraj Dhaun, Siân Griffin, Bruce Hendry, Amal A A Jama, Wenjun Ju, Ishika Khan, Radko Komers, Alex Mercer, Viji Nair, Nadia Nawaz, Matthew Sayer, Smeeta Sinha, Lisa Willcocks Matthias Kretzler, Jonathan Barratt

Presenter: **Rob Geletka, MD;** Institution: **Travere Therapeutics, Inc.**
Funding: **Travere Therapeutics, Inc.**

SPARTAN is a single-arm, exploratory trial investigating the efficacy and safety of SPAR, a dual endothelin angiotensin receptor antagonist (DEARA), as first-line therapy in IgAN. We evaluated 24-wk interim efficacy and CV risk variables. Twelve adults with biopsy-proven IgAN, proteinuria ≥0.5 g/d, eGFR ≥30 mL/min/1.73 m2, and no prior ACEi/ARB treatment were enrolled. SPAR is given for 110 wk with a 4-wk safety follow-up. One pt discontinued early due to hypotension. For CV risk factor assessments, there are occasional missing data points for 1 or 2 pts. Mean age at enrollment was 35.8 (SD, 12.2) y, with a median (IQR) proteinuria of 1.7 (0.6-3.3) g/d and mean eGFR of 70.2 (SD, 25.0) mL/min/1.73 m2 at baseline (BL). Proteinuria reductions were rapid and sustained over 24 wk (−68.9% [±SE −75.7 to −60.1] from BL to wk 24); 58% of pts achieved complete proteinuria remission (<0.3 g/d) at any time. After an initial decrease, BP and NT-proBNP remained stable over 24 wk. Minimal changes in total body water, body weight, blood lipids, triglycerides, and blood glucose were observed from BL to wk 24. Cardiac MRI results at wk 24 showed a change from BL in left ventricular mass/BSA of −3.1 (SD, 3.5) g/m2 and left ventricular ejection fraction of 0.3 (SD, 6.6) %. In newly diagnosed pts with IgAN, SPAR reduced proteinuria ≈70% over 24 wk, with CV risk factors remaining stable or improving.

02

The potential of aprocitentan in kidney diseases

Martine Clozel

Presenter: **Martine Clozel, MD;** Institution: **Idorsia Pharmaceuticals Ltd**
Funding: **Idorsia Pharmaceuticals Ltd**

Patients with chronic kidney disease (CKD) and hypertension have limited treatment options and a high morbidity and mortality risk. The dual endothelin receptor antagonist (ERA) aprocitentan markedly lowered blood pressure (BP) and was well tolerated in the PRECISION Phase 3 study in patients with resistant hypertension. As inclusion criteria allowed estimated glomerular filtration rate (eGFR) as low as 15 mL/min/1.73m2/year, 22% of the 730 patients randomized had CKD stage 3 or 4 (eGFR<60), out of which 74% also had type 2 diabetes i.e. diabetic kidney disease (DKD). The results in the entire CKD subgroup show that aprocitentan, on top of three or four anti-hypertensives including valsartan, given at maximum tolerated doses and after confirming compliance, had major effects, not only of reducing BP, but also reducing albuminuria (up to -67% reduction in urinary albumin-to-creatinine ratio (UACR) in patients with macroalbuminuria, up to 48 weeks of treatment). The data also show that the reduction of UACR was not correlated with the decrease in BP, but over-proportional; that patients with diabetic kidney disease had similar reductions; that eGFR was not decreased over the 48 weeks of the study; and that there was no risk of hyperkalemia. These data allow speculation that aprocitentan may have major potential, not only in patients with CKD or DKD who are remaining hypertensive, but also in patients with other kidney pathologies and proteinuria or nephrotic syndrome, including IgA nephropathy, focal and segmental glomerulosclerosis, other glomerular diseases, and pre-eclampsia. More studies are needed in those directions.

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03

Dual endothelin-angiotensin antagonism improves endothelial function & fibrinolysis in ANCA vasculitis: a randomised, double blind, active control clinical trial

Matthew Sayer [1] Vanessa Melville [1] Gavin Chapman [1] Bruce Hendry [2] Alex Mercer [2] Tariq Farrah [1] Neeraj Dhaun [1]
[1] British Heart Foundation/University Centre for cardiovascular Science, University of Edinburgh & Department of Renal Medicine Royal Infirmary of Edinburgh; [2] Travere Therapeutics.

Presenter: **Matthew Sayer, MD;** Institution: **University of Edinburgh, Edinburgh, Scotland, UK;**
Funding: **Travere Therapeutics, Inc.**

Background: Cardiovascular disease is a long-term complication of systemic inflammatory diseases and anti-neutrophil cytoplasm antibody-associated vasculitis (AAV) is an exemplar. Mechanisms are poorly understood, and robust risk reduction strategies are lacking. Arterial stiffness and endothelial dysfunction are independent risk factors for incident cardiovascular disease. Previously, we showed that AAV patients in long-term disease remission have arterial stiffening and endothelial dysfunction. We demonstrated that endothelin-1 contributes. Here, we examined the effects of medium-term dosing with the dual endothelin-angiotensin receptor antagonist, sparsentan, on cardiovascular risk in patients with AAV. *Method:* Thirty-two patients with AAV in disease remission entered a randomised, double-blind, active-control, parallel group study. Following withdrawal of previous renin-angiotensin system (RAS) inhibition, patients were randomised 1:1 to six weeks of once daily oral treatment with either sparsentan or the RAS inhibitor, irbesartan. The dose of sparsentan was 200mg, increased as tolerated to 400mg at one week, and 150mg and 300mg, respectively, for irbesartan. Arterial stiffness was measured by carotid-femoral pulse wave velocity (PWV). Endothelial vasomotor function was assessed by gold standard venous occlusion plethysmography following randomised intra-arterial infusions of acetylcholine (ACh, endothelium-dependent vasodilator, 7.5, 15, 30µg/min) and sodium nitroprusside (SNP, endothelium-independent vasodilator, 1, 2, 4µg/min). Endothelial fibrinolytic capacity was assessed by tissue plasminogen activator (tPA) release in response to intra-arterial bradykinin (100, 300, 1,000pmol/min). The primary endpoint was the change from baseline to week six in ACh-mediated vasodilatation. Secondary endpoints included changes in tPA release and PWV. *Results:* All 32 patients completed the study. Patients had a mean age of 64±12 years. Mean blood pressure (BP) was 134/82mmHg and mean eGFR was 58±22mL/min/1.73m2. Baseline BP, endothelial vasomotor and fibrinolytic function, and PWV did not differ between the sparsentan and irbesartan groups. Six weeks treatment with sparsentan led to a 46±48% improvement in endothelium-dependent vasodilatation at a dose of ACh 30µg/min (P=0.02). Irbesartan did not alter vasomotor function (-0.08±40%, P=0.39). Neither sparsentan nor irbesartan affected endothelium-independent vasodilatation. Sparsentan treatment was also associated with an increase in endothelial fibrinolytic capacity whereas no change was observed with irbesartan [tPA release: +277 (140–352) vs +11(-18–44)%, P<0.001]. Whereas both sparsentan and irbesartan reduced arterial stiffness [PWV, sparsentan: -1.4±0.7m/s, irbesartan: -0.8±0.5, both P<0.0001 versus baseline], the reduction was greater with sparsentan [P=0.007 for sparsentan versus irbesartan]. *Conclusions:* Dual endothelin-angiotensin receptor antagonism improves endothelial function and arterial stiffness in patients with AAV in long-term disease remission. These effects would be expected to translate to improved longer-term cardiovascular outcomes in these patients.

NOVARTIS

04

Podocyte derived endothelin-1 in glomerular injury

*Liping Yu1, Ubong S. Ekperikpe1#, Hunter W. Korsmo1#, Zhengzi Yi1, Sean Lefferts1, Kristin Meliambro1, Kirk Campbell1, Donald E Kohan2, Phillip J. McCown3, Abhijit S. Naik3, Edgar A. Otto4, Börje Haraldsson5, Weijia Zhang1, Ilse S. Daehn*1*

1Barbara T. Murphy Division of Nephrology, Department of Medicine, Icahn School of Medicine at Mount Sinai, NY, USA, 2Division of Nephrology, University of Utah Health, Salt Lake City, Utah, USA, 3Division of Nephrology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA, 4Michigan Medicine, Ann Arbor, MI, USA, 5Institute of Neuroscience & Physiology, University of Gothenburg, Gothenburg, Sweden.

Presenter: **Ilse Daehn, PhD;** Institution: **Icahn School of Medicine at Mount Sinai**

Crosstalk between activated podocytes and glomerular endothelial cells (GECs) has been demonstrated in mouse models of glomerular diseases, however the mechanistic link between podocyte activation and GEC injury that results in podocyte depletion and albuminuria remains unclear. We hypothesize that increased endothelin-1 (ET1) and endothelin receptor A (ETA) signaling could be implicated in the pathogenesis of focal segmental glomerulosclerosis (FSGS) and diabetic kidney disease (DKD). Here we developed mouse lines with endothelial cell targeted and conditional deletion of ETA using the Cre-loxP system (Scl:Cre-ETAfl/fl), as well as targeted deletion of ET1 in podocytes (Nphs2:Cre-ET1fl/fl). The absence of endothelial ETA in Scl:Cre-ETAfl/fl mice was protective in adriamycin-induced glomerular injury, as evidenced by decreased albuminuria and the prevention of podocyte depletion. RNAseq from ET1-treated mGECs showed activation of cellular crosstalk communication and alteration of matrix component deposition programs via ETA. Pre-pro ET1 expression was detected in podocytes in patient biopsies and mice with FSGS. Canonical TGFβ signaling was shown to mediate ET1 release by podocytes. Compared to adriamycin-treated control mice (Nphs2:Cre-ET1+/+), podocyte-specific knockout of ET1 (Nphs2:Cre-ET1fl/fl) mice treated with adriamycin had reduced glomerular injury, albuminuria and podocyte depletion. Finally, podocyte-specific Edn1 knockout, and inducible, podocyte-specific TGFβR1 signaling (Nphs2:Cre-ET1-Nephs1:Tgfbrl) mice treated with Dox for 7 and 14 days had no glomerular injury or albuminuria. Moreover, podocyte depletion and GEC injury was completely prevented in these mice, and there was no increase in GEC associated ETA expression. These findings provide new insights into the underlying mechanisms of FSGS pathogenesis, highlighting podocytes as an important source of ET-1 and, for the first time, in vivo demonstrating that glomerular endothelial cell ETA can mediate glomerular injury in FSGS.

Abstracts (Oral Presentations)

05

ENB-003, an ETBR antagonist, in combination with pembrolizumab for refractory advanced solid tumors: Topline data from the ENBolden-101 Phase 1B clinical study

Sumayah Jamal, MD-PhD, Selvaggi, G., MD

Presenter: **Sumayah Jamal, MD, PhD**; Institution: **ENB Therapeutics, Inc.;** Funding: **Trial sponsored by ENB Therapeutics, Inc.**

The endothelin B receptor (ETBR) prevents T-cell trafficking which may be required for anti-PD1 efficacy. The ETBR is expressed in more than 40% of cancers overall and expression correlates with cold tumors and poor survival. ETBR inhibitors (ETBRIs) enhance anti-PD1 efficacy across multiple tumor types in preclinical models. This study aimed to investigate the safety and efficacy of the combination of pembrolizumab and ENB-003, an ETBRI, in patients refractory to anti-PD1 therapy or with MSS tumors that historically do not respond to single agent anti-PD1. No dose limiting were observed across 6 dosing cohorts. The most common treatment emergent adverse events irrespective of grade or causality included fatigue (28.2%), constipation (26.1%), abdominal pain (26.1%), nausea (23.9%), anemia 17.4%, diarrhea (17.4%). Serious adverse events, grade 3 and above considered possibly related to pembrolizumab and/or ENB-003 include fatigue (n=4), diarrhea (n=3), dyspnea (n=3) constipation (n=2), rash (n=2). 15 patients with evaluable disease were enrolled in cohorts 1-5 (ENB-003 dose range 150ug-1000ug) and 15 patients with evaluable disease were enrolled in the 6th cohort (ENB-003 dose 2000ug). All patients enrolled in cohorts 1-5 were ETBR-Hi whereas in cohort 6 patients were enrolled irrespective of ETBR levels. The dosing frequency for cohort 6 was doubled to 6 doses every 3 weeks from 6 doses every 6 weeks in cohort 1-5. The disease control rate (DCR) across all cohorts irrespective of ETBR status was 33% (1 PR, 9 SD, 20 PD). The DCR in ETBR-Hi patients was 33% in cohorts 1-5 (4 SD, 1 PR, 10 PD) and 83% in cohort 6 (5 SD, 1 PD). The DCR for ETBR-Lo patients in cohort 6 was 0% (9 PD). For MSS platinum R/R ovarian cancer there was an 80% DCR across all cohorts (1 PR, 3 SD, 1 PD) with a trend for durable responses at higher doses of ENB-003. A 95% PR of 12-month duration was observed in a platinum refractory MSS OC patient. Clinical benefit was observed in an MSS PDAC patient refractory to standard of care chemotherapy and in anti-PD1 refractory melanoma and HNSCC patients with durable responses observed in all three patients. ETBRi is a novel approach to overcoming immunotherapy resistance. ENB-003 demonstrates robust preclinical proof of concept for enhancing anti-PD1 efficacy across multiple cancer indications. In the clinic, the combination is well tolerated and is demonstrating promising early signals of anti-tumor efficacy. The best efficacy signals thus far have been observed in patients expressing high levels of ETBR. Preliminary efficacy signals in platinum refractory/ resistant ovarian cancer are particularly encouraging and the therapeutic approach may also show promise in pancreatic cancer patients with TME stroma positive for ETBR. The data suggest that ETBR blockade with ENB-003 may expand the therapeutic benefit to patients who are refractory or resistant to anti-PD1 therapy.

06

Early Inflammatory Signatures Drive Alveolar Simplification in Endothelin-2–Deficient Lung Development

Gusty Rizky Teguh Ryanto, Ahmad Musthafa, Sagita Mega Sekar Kencana, Ratoe Suraya, Tetsuya Nagano, Masayuki Taniguchi, Tomoya Furuyashiki, Mitsuru Morimoto, Yoko Suzuki, Tetsuya Hara, Masashi Yanagisawa, Noriaki Emoto

Presenter: **Gusty Rizky Teguh Ryanto, MD, PhD**; Institution: **Kobe Pharmaceutical University**

Proper lung development is essential for neonatal survival and long-term respiratory function. Endothelin-2 (ET-2) deficiency has been associated with defective alveolarization, yet the molecular underpinnings of this phenotype remain poorly understood. To elucidate the mechanisms by which ET-2 regulates perinatal lung development, we investigated the temporal inflammatory landscape in ET-2 knockout (ET2-KO) mice. Single-cell RNA sequencing of lungs at postnatal day 0.5 (P0.5) revealed a marked expansion of activated neutrophils in ET2-KO lungs compared to wild-type (WT) controls, accompanied by widespread parenchymal inflammation. This was paralleled by heightened NF-κB pathway activation, indicating robust pro-inflammatory signaling at birth. By P5, the inflammatory milieu transitioned to a macrophage-dominant profile with elevated cytokine secretion. These early immune perturbations coincided with a significant reduction in proliferating alveolar type II (AT2) epithelial cells by P10, suggesting impaired regenerative capacity and alveolar maturation. Together, these findings implicate aberrant immune activation as a central driver of alveolar simplification in the context of ET-2 deficiency. Our study highlights the critical role of ET-2 in maintaining immunological quiescence during early lung development and supports its potential as a therapeutic target to mitigate developmental lung disorders.

Abstracts (Oral Presentations)

07

Endothelin-1 as a Downstream Effector of Activin A Signaling in Pulmonary Arterial Hypertension: Mechanistic and Therapeutic Implications

Novia Nurul Faizah, Gusty Rizky Teguh Ryanto, Yoko Suzuki, Tetsuya Hara, Ken-ichi Hirata, Hiromasa Otake, Noriaki Emoto

Presenter: **Novia Nurul Faizah, MD**; Institution: **Kobe University**

Pulmonary arterial hypertension (PAH) is a progressive and life-threatening disease characterized by extensive vascular remodeling. Elevated levels of endothelin-1 (ET-1), a potent vasoconstrictor and mitogen, are well documented in PAH and therapeutically targeted with endothelin receptor antagonists (ERAs). However, the upstream regulators of ET-1 expression in PAH remain poorly defined. We previously demonstrated that excessive Activin A signaling, driven by INHBA overexpression, contributes to vascular remodeling in PAH, and that Sotatercept—an ActRIIA-Fc fusion protein that traps Activin A—provides clinical benefit when added to vasodilator therapies. In this study, we explored the mechanistic link between Activin A and ET-1 during PAH pathogenesis. In vitro, human pulmonary artery endothelial cells (hPAECs) treated with recombinant Activin A or transduced with INHBA showed marked upregulation of ET-1 expression and activity. These effects were attenuated by Follistatin (FST), an endogenous Activin A antagonist, either alone or in combination with the ERA Bosentan (BOS). In vivo, endothelial cell–specific INHBA-overexpressing mice (Vecad-INHBA-Tg) exposed to chronic hypoxia exhibited increased ET-1 expression and developed PAH-like hemodynamic and histological abnormalities. These were significantly ameliorated by FST or FST+BOS treatment, whereas BOS alone had only partial effects. Our findings establish ET-1 as a downstream effector of Activin A signaling in PAH vascular remodeling and suggest that targeting Activin A upstream may offer a more effective strategy to suppress ET-1–mediated pathogenic pathways.

08

Dual endothelin-angiotensin receptor blockade improves diurnal blood pressure profile in patients with ANCA-associated vasculitis

Gavin Chapman [1] Matthew Sayer [1] Hannah Preston [1] Dan Pugh [1] Fiona Chapman [1] Vanessa Melville [1] Bruce Hendry [2] Alex Mercer [2] Tariq Farrah [1] Neeraj Dhaun [1]

Presenter: **Neeraj Dhaun, MD**; Institution: **University of Edinburgh;** Funding: **Travere Therapeutics, Inc**

ANCA-associated vasculitis (AAV) is a prototypic autoimmune disease associated with increased cardiovascular disease (CVD) risk. This is partly driven by endothelin-1 (ET-1), which promotes endothelial dysfunction and impairs blood pressure (BP) regulation, including the loss of normal nocturnal dipping - a recognised marker of vascular health. We assessed 24-hour ambulatory BP profiles in 32 newly diagnosed AAV patients (mean age 64±16 years) at initial presentation and following induction of disease remission. At diagnosis, mean BP was 127/75 mmHg, and 87% exhibited abnormal nocturnal dipping. Following immunosuppressive therapy, BP improved to 120/72 mmHg, and the proportion with abnormal dipping decreased to 72%. Separately, 32 AAV patients in sustained remission (mean age 64±12 years) were randomised to 6 weeks of sparsentan, a dual endothelin-angiotensin receptor antagonist, or irbesartan. Although baseline BP was well controlled (121/74 vs 126/75 mmHg), abnormal dipping remained common (79% vs 64%). After 6 weeks, BP improved in both groups, but only sparsentan improved dipping in a proportion of patients (36% vs 0%; P<0.05). Abnormal nocturnal BP profiles are frequent in AAV and may contribute to residual CVD risk. Dual pathway blockade with sparsentan improves this feature and may offer additional vascular protection.

09

Choroidal thinning reflects systemic vascular injury in ANCA-associated vasculitis and improves with dual endothelin-angiotensin blockade

Gavin Chapman [1] Matthew Sayer [1] Emily Godden [1] Hannah Preston [1] Dan Pugh [1] Fiona Chapman [1] Vanessa Melville [1] Bruce Hendry [2] Alex Mercer [2] Tariq Farrah [1] Neeraj Dhaun [1]

[1] British Heart Foundation/University Centre for Cardiovascular Science, University of Edinburgh & Department of Renal Medicine Royal Infirmary of Edinburgh; [2] Travere Therapeutics.

Presenter: **Gavin Chapman, MD**; Institution: **University of Edinburgh;** Funding: **Travere Therapeutics, Inc**

Novel biomarkers to identify patients at risk of cardiovascular disease (CVD) and track treatment response are urgently needed. Optical coherence tomography (OCT) non-invasively images the eye's microvasculature. We evaluated its utility in monitoring vascular injury and treatment response in ANCA-associated vasculitis (AAV), a prototypic autoimmune disease with elevated CVD risk. Ninety AAV patients (59±16 years) underwent OCT at diagnosis and post-treatment remission. Compared to matched controls (n=70), patients had thinner choroids (187±82 vs 220±84 μm; P<0.01), which thickened by ~10% after treatment (P<0.001). Next, 35 AAV patients in remission (54±13 years) underwent OCT alongside measures of arterial stiffness and endothelial function. Choroidal thinning correlated with increased arterial stiffness (r=-0.57; P<0.001), plasma ET-1 (r=0.31; P<0.05), and reduced endothelial function (r=0.45; P<0.01). Finally, 32 AAV patients in remission (64±12± years) were randomised to 6 weeks of sparsentan, a dual endothelin-angiotensin receptor antagonist, or irbesartan. Sparsentan partially reversed choroidal thinning whereas irbesartan did not (+14.3±7.6 vs -4.0±9.8 □m; P<0.001). OCT-derived choroidal metrics are promising dynamic biomarkers of vascular health in AAV. Further studies to validate OCT as a trial endpoint are warranted.

010

Mechanism of Porcine Coronary Arteriolar Constriction to a Clinical Level of Endothelin-1

Guangrong Lu, Xin Xu, Lih Kuo, Travis W. Hein

Presenter: **Guangrong Lu, MBBS, iMBA, MS**; Institution: **Texas A&M Health Science Center;** Funding: **NIH EY018420 (TWH) and Kruse Chair Endowment Fund (LK)**

Elevated endothelin-1 (ET-1) level leading to vasoconstriction via protein kinase C (PKC) activation is implicated in ischemic coronary microvascular disease (CMD) based on large coronary artery studies at ET-1 concentrations beyond clinical levels. To validate this thought in the coronary microvessels, we examined isolated, pressurized porcine coronary arterioles (<100 μm) with a clinical ET-1 level (0.1 nM). Coronary arterioles developed basal tone to 50% of maximal diameter, and ET-1 constricted them an additional 40-50%. Rho kinase (ROCK) and classical PKC inhibitors, H-1152 and Ro 32-0432, blocked constriction to ET-1 and PKC activator PDBu (0.1 μM), respectively. Increased phosphorylated myosin phosphatase target subunit 1 (pMYPT1, T850) was detected in ET-1- but not PDBu-constricted arterioles. Without extracellular Ca2+, arterioles did not constrict to ET-1 and PDBu. With extracellular Ca2+ present, L-type voltage-operated Ca2+ channel (L-VOCC) blocker nifedipine abolished constriction to PDBu but did not alter constriction to ET-1. ETA receptor (ETAR) antagonist BQ123 prevented constriction to ET-1. Thus, a clinical level of ET-1 binds coronary arteriolar ETAR, triggers extracellular Ca2+ entry independent of L-VOCC, and activates ROCK but not PKC, leading to elevated pMYPT1 and constriction. This distinct ET-1 pathway via ROCK may provide a therapeutic target for CMD.

011

Sparsentan has direct effects on the glomerular capillary wall to attenuate increased permeability in nephrotic syndrome models

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Presenter: **Michael Crompton, PhD**; Institution: **University of Bristol**

The glomerular filtration barrier (GFB) is essential for selective permeability in the kidney and consists of three critical layers: the glomerular endothelial glycocalyx (eGlx), the basement membrane, and podocytes. Dysfunction in these structures leads to proteinuric kidney diseases, including conditions that cause nephrotic syndrome (NS). Current treatments are broad and non-specific. Sparsentan, a dual endothelin type-A and angiotensin II type-1 receptor antagonist (DEARA), has received regulatory approval for long-term kidney function preservation in IgA nephropathy and is in clinical developmentfor focal segmental glomerulosclerosis (FSGS). We used in vivo and ex vivo models of NS, to evaluate whether sparsentan preserves GFB integrity. Female Lewis rats received sparsentan (60 or 120 mg/kg) or vehicle from day 0 to day 8. On day 1, an anti-nephrin antibody (mAb 5-1-6, 33 mg/kg) was used to induce proteinuria. Urinary albumin:creatinine ratio (uACR), glomerular albumin permeability (Ps'alb) and eGlx thickness were measured. Additionally, human NS plasma samples from relapse (RL) and remission (RM) phases were incubated ex vivo with healthy rat glomeruli to assess the effect of sparsentan (0.1–10 μM) on Ps'alb and eGlx thickness. Control rats developed severe proteinuria, reflected by increased uACR and Ps'alb, and reduced eGlx ($P<0.001$) and disrupted nephrin localisation ($P=0.049$). Sparsentan attenuated uACR ($P<0.001$) and Ps'alb at both doses ($P<0.001$), preserving eGlx ($P<0.001$) and ameliorated disrupted nephrin localisation indicating preserved GFB integrity. These effects were independent of blood pressure changes. In ex vivo studies, RL plasma increased Ps'alb ($P<0.001$) and reduced eGlx thickness ($P=0.004$). Sparsentan prevented these changes ($P<0.001$ for Ps'alb; $P=0.015$ for eGlx), restoring glomerular function to RM plasma-treated levels. The effect was dose-dependent, with Ps'alb changes inversely correlating with eGlx thickness ($r^2=0.83$, $P<0.001$). Sparsentan had no effect on Ps'alb in RM glomeruli, indicating no impact on healthy capillaries. Sparsentan reduces albuminuria and glomerular permeability, preserves eGlx integrity and maintains GFB function in nephrotic syndrome models through direct actions on glomerular cells.. These findings suggest that the action of sparsentan on the GFB could help maintain barrier integrity in podocytopathic proteinuric kidney diseases.

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Abstracts (Oral Presentations)

012

Ethnic differences in associations of Endothelin-1 and novel Renin-Angiotensin-Aldosterone System biomarkers in Hypertensive individuals

Aditya Sharma, Ian Wilkinson, Anthony Davenport, Luca Faconti, Phil Chowienczyk, Spoorthy Kulkarni

Presenter: **Aditya Sharma** Institution: **University of Cambridge**

Introduction: Black ethnic individuals experience higher rates of hypertension and associated cardiovascular complications than white individuals. Ethnic differences in the renin-angiotensin-aldosterone system (RAAS) may contribute to this disparity. Endothelin-1 (ET-1) may interact with RAAS pathways differently by ethnicity. This study investigates ethnic differences in the relationship between RAAS peptides and ET-1 in hypertensive (HT) and normotensive (NT) black and white individuals. *Methods and Results:* 62 HT (32 white; 45 male) and 27 NT (12 white; 20 male) were included. ET-1 levels were similar in black and white HTs (median 1.41 vs. 1.36 pg/mL; p=0.71) but were significantly higher in black NTs compared to white NTs (1.37 vs. 0.79 pg/mL; p=0.008). In black HTs, ET-1 positively correlated with renin, 24-hour urinary sodium-to-potassium ratio, angiotensin (Ang) I, Ang III, and Ang-(1–5). Inverse associations were seen with aldosterone, ARR, and aldosterone–Ang II ratio. No significant associations between ET-1 and RAAS peptides were observed in white HTs or in both NT groups. *Conclusions:* Ethnic differences in the interaction between ET-1 and RAAS peptides may exist in HT individuals. The ET-1 associations in black HTs may suggest altered ET-1 sensitivity or downstream signalling in this group, potentially contributing to hypertension pathophysiology.

013

Endothelin-1 upregulates phagocytosis functions in human microglial cells

Yasir Abdul, Sarah Jamil and Adviye Ergul

Presenter: **Yasir Abdul, PhD**; Institution: **Medical University of South Carolina** Funding: **VA Merit Review (BX000347), VA Senior Research Career Scientist Award (IK6 BX004471), NIH RF1NS083559 and RF1 NS104573 to AE, and AARFD-23-1144963 to KA and UL1TR001450/SCTR2201 to YA**

Introduction-Microglia are key regulators of immune responses and maintain homeostasis of the brain. Dysregulation of microglial phagocytic function plays a pivotal role in aging and progression of Alzheimer's disease (AD) and related dementias (ADRD). Endothelin-1 (ET-1) levels in the postmortem brain specimens of ADRD patients closely correlate with the severity of the disease. Microglia expresses both endothelin A (ETAR) and B (ETBR) receptors. How and to what extent ET-1 contributes to the microglial function are not established. We hypothesize that ET-1 upregulates microglial phagocytosis via the ETAR activation. *Methods*- Serum starved (2% fetal bovine serum) human microglia cells (HMC3) were incubated with ET-1 (100nM) in presence/absence of ETAR antagonist BQ123 (20μM; cells were treated 30 minutes prior to ET-1 treatment) under normoxia or hypoxia conditions (1% oxygen in hypoxia chamber) for either 24 or 72 hours. Cell lysate was collected for RT-PCR analysis of ET (preproET-1, ETAR and ETBR) and phagocytosis (APOE, BIN1, TREM2, PTK2B and SORT1) genes. Phagocytosis assay was performed utilizing pHRodo phagocytosis assay kit and cell migration was measured after 24 hours of treatments. Results- In normoxia, 24 hours of ET-1 treatment significantly increased the expression of both preproET-1 and ETAR genes (4.5±0.17 and 4.1±1.3-fold change, respectively) while the presence of ETAR antagonist attenuated this increase (2.43±0.59 and 2.22±0.47-fold change, respectively). However, after 72 hours, preproET-1 and ETAR genes were not different from control (1.40±0.81 and 1.68±0.51 fold, respectively). Interestingly, in this cell line ETBR gene remained undetectable in all conditions. Both 24 and 72hour treatment of ET-1 indicated an upregulating trend in phagocytosis genes (BIN1, TREM2, PTK2B, and SORT1). At the functional level, phagocytosis was increased, whereas cell migration ability was decreased with ET-1 treatment, and the presence of ETAR antagonist reversed it. Hypoxia alone significantly increased the expression of preproET-1 gene expression but there was no effect of ET-1 stimulation or ETAR antagonist at both 24 and 72 hour time points (5.04±0.08, 4.8±0.63, 4.9±0.14 fold after 24 hours and 3.25±0.65, 1.54±0.84 and 3.0±1.27 fold change after 72 hours for H, H-ET-1, H-ET-1-BQ123 respectively). Hypoxia significantly increased phagocytosis genes at both 24 and 72 hour, however ET-1 and ETA antagonist did not have any additive or inhibitory effects. Functional assays in hypoxia conditions are under investigation. *Conclusions*- In this reductionist microglia cell culture model, ET-1 upregulates the expression of preproET-1, ETAR, and phagocytosis-associated genes. ET-1 also modulates microglial phagocytosis and migration ability. While there was no detectable ETBR expression in this cell line, partial inhibition of the increases in gene expression by BQ123 suggests a role for ETBR as well, which needs be confirmed at the protein and functional levels. These preliminary findings warrant further investigation into the potential role of the microglial ET system in the onset and progression of ADRD.

014

Endothelin-1 as an Activator of Pro-inflammatory Microglia Cells

Yaritza Inostroza-Nieves1, Shakira Bou1, José Alvarado1, Diego Capo-Ruiz1,2, Jessica Garcia1, Jean P. Moliere1, Claudia P. Arenas1*

Presenter: **Yaritza Inostroza-Nieves, PhD**; Institution: **San Juan Bautista School of Medicine;** Funding: **San Juan Bautista School of Medicine Pilot Projects Program to YIN.**

Microglial cells are highly specialized central nervous system (CNS) cells that can promote the inflammation and neurodegeneration seen in Multiple Sclerosis (MS). MS is a neurodegenerative autoimmune disease characterized by inflammation, demyelination, and axonal degeneration. In MS patients, demyelination is associated with activated microglia. Endothelin-1 (ET-1) is a potent vasoconstrictor that induces cerebral vasoconstriction and inflammation. However, the mechanism of how ET-1 activates a proinflammatory response in the CNS is unknown. To investigate ET-1's role in microglia activation, HMC3 cells were treated with ET-1 in the presence or absence of endothelin receptor B antagonist, BQ788. TNFα and IL-6 levels were measured using ELISA. Nitric Oxide (NO) production was measured using Griess Reagent. Reactive oxygen species (ROS) production was measured using the MUSE Oxidative Stress kit. ET-1 increases TNFα levels by 56% ($p=0.0003$) and IL-6 levels by 86% ($p=0.0111$) in HMC3 cells, and it was decreased to basal levels in the presence of BQ788. NO and ROS production is induced by ET-1 ($p<0.05$), and treatment with BQ788 was able to decrease them. ET-1 increases STAT-1 activation by 3.5 folds compared to control ($p<0.0001$) in microglia cells. Moreover, to study ET-1 levels in MS, we used C57BL/6 mice brains with or without induced experimental autoimmune encephalomyelitis (EAE). We found that the ET-1 gene and protein were upregulated by 1.5 folds ($p=0.0199$) in EAE mice compared to the control. These data suggest that in vitro administration of ET-1-activated microglia significantly increased inflammatory cytokine levels, NO, and ROS formation.

015

A Phase III Study to Assess the Safety and Efficacy of Sovateltide in Patients with Acute Cerebral Ischemic Stroke: Protocol of the RESPECT-ETB Trial

Vishnu Yelakanti, Amaresh Ranjan and Anil Gulati

Presenter: **Vishnu Yelakanti, BS**; Institution: **Pharmazz Inc.**

Background: Cerebral tissue damage is the primary cause of long-term disability and/or death in acute cerebral ischemic stroke (ACIS). Available drugs in the market dissolve blood clots but do not repair brain injury. Sovateltide (IRL-1620), with neuroregenerative, neuroprotective, and angiogenic potential, is being developed as a "first-in-class" ACIS therapy. Clinical trials in India have demonstrated the drug's strong safety and efficacy, and it is now approved and marketed in the country. A global phase III RESPECT-ETB trial in the USA, Germany, Spain, Australia, and the UK was planned and approved by the US-FDA. The protocol aims to assess the safety and efficacy of sovateltide treatment in patients with ACIS. *Method:* A total of 514 patients (age 18–80 years) with stroke onset within 24 hours will be enrolled in the study. Patients will be randomized 1:1 to receive 3 doses of sovateltide (0.3 µg/kg body wt. per dose) or placebo per day at an interval of 3±1 hour on days 1, 3, and 6 along with standard care. Stratification is based on time from stroke onset within <12 vs. ≥12 hours, and thrombolytic use. The primary endpoint is the proportion of ACIS patients achieving a good functional outcome, as measured by a modified Rankin Scale (mRS) score of 0-2, on day 90 post-randomization. Secondary endpoints include the proportions of ACIS patients having functional outcomes with National Institutes of Health Stroke Scale (NIHSS) score of <6, Barthel Index (BI) score of ≥90, and mRS score of 0-1 on day 90 post-randomization. Other secondary endpoints are change in quality-of-life scores (EQ-5D-5L, SS-QOL) from baseline to day 30, 60 and 90, proportion of patients with recurrent stroke within 90 days, mortality, proportion of patients with symptomatic or radiographic intracranial hemorrhage within 24 ± 6 hours of randomization, change in Montreal Cognitive Assessment (MoCA) at day 30 and 90, and proportion of patients with adverse events (AEs) and serious adverse events (SAEs). *Anticipated Results and Conclusions*: This protocol achieved the Special Protocol Assessment (SPA) agreement with the USFDA, suggesting that the USFDA concurs with the adequacy and acceptability of the protocol design, which would help endorse the future marketing application of sovateltide. Positive results from this trial may support regulatory approval in several countries, including the USA.

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Intranasal treatment by ETA receptor antagonist BQ123 or ETB receptor agonist Sovateltide attenuates stroke-mediated deficits in multiple domains of behavior in diabetic rats

Yasir Abdul, Mia Edgerton-Fulton, Sarah Jamil, Weiguo Li, Eda Karakaya, Justin Van Beusecum and Adviye Ergul

Presenter: **Adviye Ergul, MD, PhD;** Institution: **Medical University of South Carolina**

Funding: **VA Merit Review (BX000347), VA Senior Research Career Scientist Award (IK6 BX004471), NIH RF1 NS083559 and RF1 NS104573 to AE**

Diabetes increases the risk of Alzheimer's Disease Related Dementias (ADRD), including Vascular Contributions to Cognitive Impairment & Dementia (VCID). Diabetes also dysregulates the endothelin (ET) system. Elevated brain ET-1 levels correlate with tissue perfusion status and disease severity in patients with ADRD. There is emerging evidence that ETB receptor agonism improves outcomes in patients with cerebral ischemic stroke, but long-term effects, especially in diabetes, are unknown. We hypothesized that intranasal treatment with ETA antagonist or ETB agonist would prevent cognitive decline in diabetic animals in a post-stroke cognitive impairment model of VCID. Male rats underwent 60-min middle cerebral artery occlusion surgery and animals that met the preset inclusion criteria were randomized to ETA antagonist BQ-123 (BQ, 3 µg/100 µl PBS), ETB agonist Sovateltide (SVL, 5 µg/kg), or vehicle intranasal treatment for 3 days per week until 8 weeks post-stroke (n=6-8). Nondiabetic and diabetic naïve rats served as controls for stroke surgery. Sensorimotor, emotional, and cognitive outcomes were monitored over an 8-week period. Blood biomarkers were measured using Meso Scale Discovery (MSD) Neurology, Angiogenesis and Amyloid beta panels. Data was analyzed by one-way ANOVA. Diabetic rats developed anxiety-like behavior after stroke (mean ± SEM, z-score, 1.2 ± 0.3), which was prevented by ETAR antagonism (-0.3 ± 0.3, p=0.027). Risk-taking behavior z-score was also higher in the stroke vehicle group (1.4 ± 0.8) as compared to all other groups (p=0.001). Exploratory behavior was impaired as evidenced by lower exploration time in diabetic rats after stroke (36.3 ± 2.1 s), which was restored to levels in naïve groups by both treatments (BQ, 51.3 ± 4.0 and SVL, 45.2 ± 4.7 s). Both treatments also prevented global memory deficits (z-score: 0.4 ± 0.2, -0.5 ± 0.07,-0.08 ± 0.1 and 0.3 ± 0.2 in naive, vehicle, BQ and SVL groups, respectively). The sucrose preference test indicated depression-like symptoms in the stroke vehicle group and only BQ-123 prevented this effect (%sucrose consumed, %12.9 ± 6.4 vs 67.1 ± 13, p=0.03). The plasma total tau in the stroke vehicle group (16.3 ± 4.5 pg/ml) was significantly lowered by SLV treatment (3.5 ± 0.5, p=0.01). Z-score for combined neurology markers (Neurofilament Light Chain, Glial Fibrillary Acidic Protein and total tau) was elevated in the stroke vehicle group (1.3 ± 0.4) as compared to other groups (p=0.005), indicating both treatments prevented this increase. Similarly, amyloid beta levels were higher in the stroke vehicle group than in other groups (z-score: 0.2 ± 0.2, 1.5 ± 0.4,0.09 ± 0.4 and -1.6 ± 0.2 in naive, vehicle, BQ and SVL groups, respectively). These results suggest that stimulation or inhibition of brain ETB and ETA receptors, respectively, prevent cognitive impairment after stroke, and intranasal administration is effective in targeting the brain ET system. While further studies are required to better understand how the brain ET system impacts stroke recovery in diabetes, our findings provide novel insights into potential neurovascular protective and restorative therapies for ADRD.

Abstracts (Oral Presentations)

017 Endothelin-1 Regulates Hypoxia-Induced Increases in Placental Inflammatory and Anti Angiogenic Cytokines through the ET_A Receptor

Heather Chapman, Emmy Pruett, and Eric M George

Presenter: **Eric George, PhD;** Institution: **University of Mississippi Medical Center, Jackson, MS**

Preeclampsia (PE) is one of the most common obstetrical disorders, affecting ~5-7% of all pregnancies in the United States. Hallmarked by new-onset hypertension, vascular dysfunction, end-organ injury, and fetal growth restriction, it is a leading cause of maternal and fetal morbidity and mortality. While the initiating causes of the disorder are unclear, failure to remodel the maternal arteries to allow for sufficient blood flow to the placenta, leading to chronic placental ischemia, is thought to be a central feature. In response to this ischemia, the placenta secretes a series of anti-angiogenic and inflammatory cytokines, which cause the maternal symptoms. Previous research has shown that maternal vascular dysfunction is associated with aberrant Et-1 expression, and that placental ischemia-induced hypertension can be attenuated by ET_A antagonists, presumably through maternal vascular mechanisms. What is less clear is the role of Et-1 in the placenta itself. We hypothesized that increased Et-1 signaling in placental cells/tissues directly leads to increased inflammatory/antiangiogenic cytokine production, and that it is a key mediator of hypoxia-induced increases in these factors.BeWo placental trophoblasts were treated in oxygen concentrations mimicking healthy (8%) or ischemic (1%) placentas. Hypoxia induced a significant increase in the mRNA of PPET (~twofold) and EDNRA (2.5-fold) with $p<0.05$. When cells were treated with recombinant Et-1, there was a significant 50% increase in TNF-α production, with a threefold increase in secretion of the anti-angiogenic protein sFlt-1; both factors known to be dysregulated in PE. This effect could be completely attenuated by administration of an ET_A antagonist (ABT-627), but an ET_B antagonist (BQ-788) had no inhibitory effect. Here, we show for the first time that Et-1 signaling through ET_A in the placenta has a direct role in regulating trophoblast-derived inflammatory and anti-angiogenic cytokines.

018 Endothelin Receptor Autoantibodies: Associations with Blood Pressure and Endothelial Activation in Systemic Lupus Erythematosus

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Presenter: **Helen Butler, PhD;** Institution: **Medical University of South Carolina**
Funding: **VA BLRD IK2BX005605, P30AR072582, T32GM132055-5, U54DA016511-22, T32-AR050958-19, 25PRE1372738, LRA Innovation Award**

The pathophysiology of systemic lupus erythematosus (SLE) involves increased autoantibody levels and systemic IgG deposition in multiple organs and the vasculature. Crucially, SLE patients have high incidence of CVD complications, including hypertension (HTN), much earlier in life relative to healthy counterparts. While the risk of CVD mortality in SLE patients is known, the cause of vascular complications is not well understood. The vascular pathology of SLE can involve dysregulation of the endothelin system, which is comprised of endothelin-1 (ET-1) and its two G-coupled protein receptors (ETAR and ETBR), resulting in endothelial activation and dysfunction. Importantly, autoantibodies targeting ETAR (ETAR-AAs) are associated with pulmonary arterial hypertension in systemic sclerosis and SLE. However, their relevance to SLE-associated hypertension and vascular activation remains unknown. To investigate this, we used patient samples from recruited non-SLE and SLE subjects in both a small pilot as well as a larger validation cohort to allow for associations with blood pressure. We measured ETAR-AA and ETBR-AA levels as well as markers of endothelial activation, soluble vascular adhesion molecule-1 (sVCAM-1) and intracellular adhesion molecule-1 (sICAM-1) by ELISA. We found ETAR-AAs and ETBR-AAs were increased in SLE subjects relative to non-SLE in both the pilot and validation cohorts (ETAR pilot: $p=0.002$, validation: $p<0.0001$; ETBR pilot: $p=0.011$, validation: $p<0.0001$), as was sVCAM-1 (pilot: $p<0.0001$, validation: $p<0.0001$). We further ran a principal component analysis (PCA) and a Spearman's correlation matrix to investigate the relationship between ETAR/ETBR-AA levels and blood pressure. The PCA revealed distinct clustering of subject samples driven primarily by blood pressure (PC1), VCAM-1 (PC2) and ETAR-AAs (PC3). The correlation matrix further revealed significant positive correlations between ETAR/ETBR-AA levels and systolic and diastolic blood pressure, suggesting ETAR/ETBR-AAs have a role in SLE-associated HTN. To evaluate functionality of subject IgG on the vasculature, we isolated primary human cortical endothelial cells (HRECs) from transplant kidneys (n=4) and evaluated calcium signaling following acute exposure to pooled (n=6) non-SLE or SLE isolated IgG. Both non-SLE and SLE IgG resulted in a calcium flux. However, dual blockade of ETAR and ETBR by BQ123 and BQ788, respectively, reduced the signal in 50% of SLE IgG exposed cell lines ($p=0.011$, $p=0.027$), reflective of donor heterogeneity and the clinical population of SLE, suggesting ETAR and ETBR activation by SLE IgG is a key driver of calcium signaling in these cells. In addition, primary HRECs (n=10) were incubated with non-SLE and SLE isolated IgG for 24 hours prior to measuring media ET-1 levels. Incubation with SLE IgG resulted in an increase in ET-1 secretion relative to non-SLE IgG ($p=0.022$), which was reduced by ETAR ($p=0.006$) and ETBR (0.006) antagonism. Overall, we show that receptor activation by autoantibodies may contribute to the pathogenesis of SLE and HTN through the initiation or exacerbation of vascular inflammation and dysfunction.

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The ET-1/ETA axis on T cells, not Dendritic Cells, Mediates Inflammation and Renal Dysfunction during Type 1 Diabetes

Abigail J. Brooks, Melissa Rodriguez, Sara N. Biswal and Carmen De Miguel

Presenter: **Abigail Brooks, MS**; Institution: **University of Alabama at Birmingham;** Funding: **Deep South KUH PRIME U2C DK133422 & TL1 DK139566 to AJB and Diabetes Research Connection Funds and UAB Diabetes Research Center Pilot Funds to CDM**

Renal inflammation is a hallmark of diabetic kidney disease. Endothelin-1 (ET-1) is critically implicated in diabetes and is elevated in patients with diabetic kidney injury and animal models of the disease. ET-1 is pro-inflammatory in the kidney via activation of its ETA receptor. Immune cells like T cells and dendritic cells (DCs) express ETA and ETB receptors; yet, the role of the immune cell ET-1/ETA axis in driving kidney inflammation and damage during type 1 diabetes (T1D) is unclear. We hypothesized that stimulation of the ET-1/ETA axis on immune cells is crucial in the development of T1D and the associated renal injury. Male mice lacking ETA receptor on T cells (T cell ETA KO) or DCs (DC ETA KO) and floxed ETA controls were diabetic for 10wks (streptozotocin, 50 mg/kg, i.p., 5 consecutive days). Urinary protein excretion and glomerular filtration rate (GFR) via FITC-sinistrin clearance were evaluated at baseline and after 10wks of T1D. Flow cytometry studies on spleen, blood, and kidney identified changes in the immune profiles both systemically and organ-specific. In response to T1D, T cell ETA KO mice showed markedly lower protein excretion compared to controls (Floxed ETA vs. T cell ETA KO: 9.66±2.4 vs. 5.57±0.48 mg/day, n=4-8/group, p=0.0083). Diabetic floxed ETA controls showed a prominent decline in GFR, while diabetic T cell ETA KO mice sustained renal function (baseline vs. 10 wks T1D: Floxed ETA: 324.1±18 vs. 236.87±17 uL/min/b.w., n=6-7/group, p=0.0034; T cell ETA KO: 318.0±20 vs. 295.9±21 uL/min/b.w., n=6/group, p=0.43). Although % of CD4+ and CD8+ T cells were similar between genotypes, we found distinct alterations in activation/differentiation markers in CD4+ and CD8+ T cell subtypes within kidney, spleen, and blood (by mean fluorescence intensity (MFI), n=3-6/group). Compared to diabetic floxed ETA controls, diabetic T cell ETA KO mice exhibited lower activation of kidney CD4+ T cells, as shown by lower CD44 (MFI: 25612.3±2960 vs. 16172.5±2040, p=0.031) and CD69 (MFI: 12018.0±597 vs. 7770.0±920, p=0.010). Similar trends were seen systemically, with lower splenic CD4+CD44+ (p=0.009), CD8+CD69+ (p=0.014), CD4+RORγt+ (p=0.046) and CD8+RORγt+ (p= 0.030) cells. The circulatory immune phenotype also showed decreased CD4+ T cell MFI of CD44 (p=0.0045) and CD69 (p=0.025), and lower CD8+ T cells MFI of CD44 (p=0.0091), CD69 (p=0.049), and RORγt (p=0.032). On the contrary, no differences in lymphoid or myeloid populations nor their activation status were found in diabetic DC ETA KO mice compared to controls or T Cell ETA KO mice. Our findings demonstrate that the ET-1/ETA axis on T cells is key for regulating T cell maturation, activation, and differentiation in the diabetic setting, and that activation of the T cell ETA receptor drives renal dysfunction during T1D. Based on this evidence, targeting the ET-1/ETA axis on T cells may represent a novel therapeutic approach to mitigating the progression of diabetic kidney disease.

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Abstracts (Oral Presentations)

O 20 Dual Endothelin-1 Receptor A/B Blockade Attenuates Adipose Tissue Inflammation in Mice Fed a High Fat Diet

Megumi Mills, Caroline Miller, Bridget Konadu, Natalie Wilson, Madilyn Lewis, Joshua Speed

Presenter: **Megumi Mills, PhD**; Institution: **University of Mississippi Medical Center;** Funding: **National Institute of General Medical Sciences (U54 GM115428), the National Institute of General Medical Sciences (P30 GM149404), the National Heart, Lung, and Blood Institute (T32 HL105324), and the National Institute on Diabetes, Digestive, and Kidney Diseases (R01 DK124327)**

Endothelin-1 (ET-1), a potent vasoconstrictor, is increased in adipose tissue of high fat diet (HFD) fed mice and contributes to cardiovascular and metabolic disease risk in obesity. Obesity causes adipose tissue hypoxia leading to low grade peripheral inflammation that most likely starts in adipose tissue, which becomes infiltrated with pro-inflammatory T cells and macrophages as adiposity increases. This environment is further exacerbated by high levels of plasma triglycerides, cholesterol and non-esterified free fatty acids that characterize dyslipidemia in obesity. Our lab recently reported that treatment with bosentan, a dual ETA/ETB antagonist attenuates the increased proinflammatory immune cell infiltration in visceral adipose and several parameters of dyslipidemia in obese mice. Interestingly, adipocyte knockout of the ETB receptor led to a more robust attenuation of adipose tissue inflammation in response to HFD feeding in mice, suggesting bosentan may not reach adipocyte ETB receptors. We hypothesized that macitentan, also a dual ETA/ETB antagonist, better at penetrating into the interstitial space and with significantly fewer side effects, will reduce adipose tissue inflammation and reduce cardio-metabolic disease risk in obese mice. To test this hypothesis, C57BL/6J mice were fed either normal diet (NMD) or high fat diet (HFD) for 8 weeks followed by 2 weeks of treatment with either vehicle or macitentan (30mg/kg/day). HFD mice had significantly higher fat mass than NMD mice, with no significant effect of treatment with macitentan. In addition, HFD fed mice had a significant increased tumor necrosis alpha mRNA (241.5+/-50.1 vs. 847.2+/-117.4 copies per 50 ng RNA; p<.0001 NMD vs. HFD) that was attenuated in mice treated with macitentan (847.2+/-117.4 vs. 430.5+/-44.3 copies per 50 ng RNA; p=.0009 vehicle vs. macitentan treated HFD). Hypoxia inducible factor alpha (Hif-1a) message, which increases as oxygen tension decreases, was elevated in visceral adipose of HFD fed mice compared to NMD; (27968+/-2504 vs. 53045+/-5448 copies per 50 ng RNA; p <.0009 NMD vs. HFD), which was attenuated with macitentan treatment (27611+/-1707 vs. 32353+/-5061 copies per 50 ng RNA; p=0.0054 vehicle vs. macitentan treated HFD). In addition, HFD feeding increased plasma total cholesterol, HDL and LDL-C, which was attenuated with macitentan treatment. Taken together, these data indicate that ET-1 receptor blockade with macitentan improves the proinflammatory environment in visceral adipose tissue of obese mice, in part, by increasing oxygen delivery to the adipose tissue and by reducing dyslipidemia.

O 21 Pregnancy regulates renal endothelin-1 and aldosterone signaling systems in aged female mice lacking G protein-coupled estrogen receptor

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Kidney disease is one of the top ten leading causes of death for women. In female rodents, estrogen elicited protective responses against various kidney injuries through G protein-coupled estrogen receptor 1 (GPER1). GPER1-induced vasodilation in rats has been shown to be increased with pregnancies. We recently found that GPER1 and pregnancy protect aged female mice against renal injury. However, the mechanism underlying these protective effects remains unclear. Of note, links have been established between GPER1 and the renal endothelin-1 (ET-1) and aldosterone signaling systems. Given the established contribution of ET-1 and the renin angiotensin aldosterone system (RAAS) to both the progression and potential treatment of kidney diseases, we hypothesized that GPER1 and previous pregnancies protect against renal injury in aged female mice via regulating the renal ET-1 and aldosterone signaling pathways. To test our hypothesis, we collected 24-hour urine samples and kidneys from 16-20 month-old GPER1 wild-type (WT) and global knock-out (KO) female mice with or without a history of former pregnancies (n=7-8/group). We measured urinary levels of ET-1 and aldosterone. We also assessed the renal cortical expression levels of ET receptors subtypes and key components of RAAS by RT-PCR. We found that the deletion of GPER1 increased urinary excretion of ET-1 [KO: 0.36 ± 0.07 vs WT: 0.173 ± 0.01 ng/day; p=0.0063] in the aged nulliparous mice. Interestingly, aged parous KO mice elicited lower levels of excretion of ET-1 [0.16 ± 0.02 ng/day; p=0.0048] and aldosterone, compared to nulliparous KO mice. Renal cortical mRNA expression of ET receptor subtype A (ETA) was comparable in the aged mice regardless of their genotype or pregnancy history. However, GPER1 deletion increased the mRNA expression of ETB receptor in kidneys obtained from aged parous, but not nulliparous, mice [1.05 ± 0.10 vs 0.68 ± 0.12; p=0.0294]. No genotypic nor pregnancy-related differences were observed in the renal cortical mRNA expression levels of Agtr1a, which encodes type-1 angiotensin II receptor-associated protein, Agtrap, which encodes angiotensin II type 1a receptor, Ace, which encodes angiotensin-converting enzyme, and Nr3c2, which encodes the mineralocorticoid receptor. Our data indicates that GPER1 and former pregnancies regulate ET-1 and aldosterone signaling in the kidneys of aged female mice, which may contribute to the renal protective effects of GPER1 and pregnancy. This data could provide insight into the development of renoprotective therapeutic agents for postmenopausal women.

022

Pathological Upregulation of Endothelin Receptor A Signaling In Systemic Lupus Erythematosus

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Funding: **VA BLRD IK2BX005605, P30AR072582, T32GM132055-5, U54DA016511-22, T32-AR050958-19, 25PRE1372738, LRA Innovation Award**

Endothelin-1 (ET-1) was reported to be elevated in systemic lupus erythematosus (SLE) in 1991. Despite the improvements in the management of hypertension (HTN), a disease highly prevalent in SLE, the associated vascular inflammation and plasma ET-1 remain elevated and positively correlated, highlighting the need for exploration of ET-1 signaling in SLE-associated cardiovascular disease (CVD). Therefore, in this study, we utilize human subjects, primary human renal endothelial cells (HRECs), and the B6.Nba2 murine model of SLE-associated CVD. Firstly, we recruited human subjects with non-SLE, non-HTN, SLE non-HTN, and SLE HTN (n=36-49/group). All subjects were on current therapies for HTN and/or SLE. SLE subjects showed increased plasma ET-1 levels compared to non-SLE ($p=0.0151$). Interestingly, stratification by HTN showed SLE HTN subjects were elevated over Non-SLE Non-HTN controls ($p=0.0090$), indicating a potential interaction between HTN, SLE, and plasma ET-1. A positive association was found between plasma ET-1 and systolic blood pressure in this cohort ($r=0.2691$; $p=0.0035$). Utilizing female HRECs, we mimicked an HTN environment by exposing HRECs to pathological HTN-like biaxial stretch in the presence of Non-SLE or SLE plasma. Endothelin Receptor B (ETBR) expression was unchanged while Endothelin Receptor A (ETAR) expression was significantly increased in HRECs exposed to SLE plasma ($p=0.0159$). This coincided with an increase in the endothelial inflammation marker VCAM-1 in SLE plasma-exposed HRECs ($p = 0.0009$). In vivo, female B6.Nba2 mice exposed to TLR7 activation via R848 (100ug/30ul; topically) demonstrated significant cardiac dysfunction measured via reduced ejection fraction compared to acetone vehicle controls ($p<0.0001$). Furthermore, measurement of cardiac ET-1 levels was increased in R848 mice compared to vehicle controls ($p=0.0009$). Additionally, plasma sVCAM-1 was increased in R848 mice ($p=0.0028$). A trending positive association with cardiac ET-1 and plasma sVCAM-1 was found ($r=0.4693$; $p=0.0573$). Furthermore, ETAR staining in the kidney showed positive staining in glomerular endothelial cells, a finding that has been observed under pathological conditions in the kidney. Blockade of ETAR demonstrated beneficial effects by slowing the reduction in ejection fraction in female B6.Nba2 mice cotreated with sitaxsentan compared to only R848 ($p=0.0343$) and coincided with an increased glomerular filtration rate in mice given sitaxsentan ($p=0.0095$). Overall, these findings show that within SLE, there is a dysregulation of endothelin receptor expression that promotes ETAR signaling and endothelial inflammation. Furthermore, this study warrants further exploration into the beneficial effects of ETAR antagonism in SLE-associated CVD.

UNC SCHOOL OF MEDICINE Kidney Center

023 ET-1 impairs decidualization and angiogenesis during early pregnancy, which is mitigated by nicotinamide, in mice

Feng Li

Presenter: **Feng Li, MD, PhD**; Institution: **University of North Carolina at Chapel Hill;** Funding: **NIH**

Endothelin-1 (ET-1) is involved in pregnancy complications, including preeclampsia (PE). We have reported that female mice (Edn1H/+) having ~3-4x higher plasma ET-1 levels than wild type (WT) developed the full-spectrum PE-like phenotype during pregnancy in a maternal genotype-dependent manner. Impaired embryo implantation resulting from insufficient decidualization during early pregnancy plays an important role in PE. In this study, we investigate whether decidualization is impaired in Edn1H/+ dams, and whether a potent inhibitor of ET-1 (e.g. nicotinamide, amide form of vitamin B3, NAM) executes any beneficial effect. We compared the implantation site at 7.5 days post coitus (dpc) between WT and Edn1H/+ dams with or without NAM treatment. Implantation sites of Edn1H/+ dams exhibited abnormal ectoplacental cone (EPC) and irregular sinusoids with reduced vascular density in mesometrial regions. There was more VEGF expression in implantation sites of Edn1H/+ dams than those of WT dams. The marker of decidualization (BMP2, prolactin) was decreased in Edn1H/+ dams. Nam corrected the abnormality present in the implantation sites of Edn1H/+ dams. Additionally, during differentiation (decidualization) of cultured human endometrial stomal cells, ET-1 halved the upregulated expression of markers of decidualization (PRL, IGFBP-1 and WNT4), and decreased the upregulated expression of angiogenesis-related genes (ANG, PAPPA). On the other hand, Nam normalizes the expression of these genes. Our data indicates that Nam counteracts ET-1's detrimental effects on decidualization and angiogenesis, therefore, NAM supplementation has potential to improve embryo implantation.

024 Endothelium-derived ET-1 stimulates Th17 cell expansion in a sex-dependent manner under high-salt diet

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Presenter: **Tha Luong, MS** Institution: **University of Alabama at Birmingham;** Funding: **U2C/TL1 Deep South KUH PRIME U2C DK133422 & TL1 DK139566 from the NIH/NIDDK to TL, NIH F31 HL151264-0 to PAM, P01HL136267 to JSP and DMP, and K01HL145324 to CDM.**

Endothelin-1 (ET-1) regulates renal sodium handling in a sex-specific manner and is elevated in salt-sensitive hypertension—a condition also characterized by increased Th17 cell activity. However, the cellular source of ET-1 and its specific role in Th17 cell differentiation remain unclear. We hypothesized that endothelium-derived ET-1 promotes Th17 cell differentiation in mice on a high-salt diet in a sex-dependent manner. To test this, we placed male and female vascular endothelial cell ET-1 knockout (VEET KO; 8–14-weeks old) on normal or high-salt diet (HS, 4% NaCl) for 3 weeks and measured kidney Th17 cells by flow cytometry. We found that HS induced less Th17 cell accumulation in male kidneys from VEET KO compared to floxed controls (ΔTh17: 6.7 ± 57.9 vs. 171.1 ± 95.9, respectively; $p < 0.001$; n=5-10). We observed no significant genotype difference in females. To determine if ET-1 directly affects Th17 differentiation, splenic naïve CD4+ T cells from VEET KO and floxed mice were isolated and cultured under Th17 polarizing conditions (TGFβ and IL-6) with ET-1 at 0, 500, or 1000nM. We found that ET-1 directly increased IL17A+CD4+ T cell frequency only in VEET KO males. Indeed, in these mice, Th17 cell frequency rose from 9.4±1.0% (media control) to 13.9±1.2% (500nM ET-1; $p=0.041$; n=3-5) and 17.7±1.8% (1000nM ET-1; $p=0.0007$; n=3-5). Interestingly, regardless of ET-1 concentration, CD4+ T cells from VEET KO females showed greater Th17 polarization (3.1±1.5%) compared to floxed controls (1.3±0.4%; $p < 0.02$). In both sexes, CD4+ T cells from VEET KO mice produced more IL-17A than floxed controls under Th17 polarizing conditions. At baseline, CD4+ T cells from VEET KO produced more IL-17A compared to those from floxed controls (males: 427±162 pg/mL, $p = 0.033$; females: 215±29 pg/mL, $p=0.017$). Upon ET-1 treatment, IL17A levels stayed elevated in VEET KO, but did not increase further in either sex (males, 500 nM ET-1: 387±190 pg/mL, $p = 0.049$; females, 1000 nM ET-1: 235±57 pg/mL, $p=0.011$). These results demonstrate that endothelium-derived ET-1 promotes Th17 differentiation only in male mice and suggest a mechanistic ET-1/Th17 axis that contributes to the inflammatory response to high salt diets.

025

Association between Endothelin-1 and Right Ventricular Remodelling in Pulmonary Hypertension Secondary to Atrial Septal Defect Patients

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Background: Atrial septal defect (ASD) is one of the most prevalent types of congenital heart disease in children. In this condition, blood pressure increase on the right ventricle leads to right ventricular (RV) remodelling. Certain biomarkers like endothelin-1 (ET-1) promote vasoconstriction, promoting RV dilatation. Pulmonary hypertension (PH) patients secondary to ASD have higher ET-1 levels than normal. Overtime, heart failure may occur, causing lower functional capacity. The extent in which ET-1 affects the right ventricle diameter remains unclear. Objectives: Investigate the association between plasma ET-1 and right ventricular remodeling, and explore ET-1 levels and functional capacity in adults patient with PH secondary to ASD. Methods: This was a retrospective cohort study, involving 37 ASD patients with PH from the COngenital HeARt Disease in adult and Pulmonary Hypertension (COHARD-PH) registry. Plasma ET-1 levels were obtained by enzyme-linked immunosorbent assay (ELISA), and right ventricle diameter was measured by echocardiography. 6-minute walk test (6MWT) was performed and used to assess functional capacity. Spearman's test was performed to analyze the relationship between ET-1 and right ventricle diameter and ET-1 and 6MWT. According to the European Society of Cardiology (ESC) in advanced heart failure, impaired functional capacity is taken as 6MWT ≤300 m. Logistic regression was done by dichotomizing 6MWT values with low functional capacity (6MWT ≤300 m) and ET-1. For all statistical analyses two tailed tests were used, and a p-value of < 0.05 was taken as significant. Variability of absorbance in lower-range ET-1 concentrations along with high blank absorbance were noted and considered during data collection. Results: 37 samples were collected, and the population was dominated by females, at 36 females (97.3%) and 1 male (2.7%). The age (mean ± SD) of subjects was 32.5 ± 11.3 years, right ventricle diameter (mean ± SD) was 4.67 ± 8.18 cm, and ET-1 levels (mean ± SD) were 16.3 ± 3.55 pg/ml. A significant weak positive correlation was found between ET-1 levels and RV diameter with Spearman's correlation ($r = 0.342$, $p = 0.019$). This showed that ET-1 had an effect on RV dilatation. ET-1 levels had no significant association with 6MWT with Spearman's correlation ($r = -0.249$, $p = 0.069$), and logistic regression (OR 1.12, $p = 0.253$). Tricuspid annular plane systolic excursion (TAPSE) was used to analyze RV systolic function in relation with ET-1 and RV diameter. There were no significant correlations found between ET-1 and TAPSE, Pearson correlation ($r = 0.031$, $p = 0.855$) and RV diameter and TAPSE ($r = 0.248$, $p = 0.138$). Future studies may be done to investigate if ET-1 could be a biomarker used to predict functional decline in PH patients. Conclusion: In ASD patients associated with PH, ET-1 is significantly associated with RV dilatation but not with reduced functional capacity nor functional contractility. These findings suggest that ET-1 may serve as a potential biomarker of cardiac remodeling in congenital heart disease associated with pulmonary hypertension.

026

Mice lacking CD8+ T-cells have lower Edn1 expression post-MI

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Presenter: **Thomas Dempster, MS;** Institution: **Medical University of South Carolina**
Funding: **NIH T32GM123055, NIH R01HL173273, VA I01BX005848**

CD8+ T-cells have been shown to be adverse regulators of left ventricular inflammation following myocardial infarction (MI). However, the mechanisms by which CD8+ T-cells contribute to inflammation and cardiac remodeling after injury are not entirely understood. While direct cytotoxic action on the myocardium likely plays an important role, CD8+ T-cells also negatively regulate post-MI healing through the release of inflammatory mediators and have been linked to endothelin-1 upregulation. Endothelin-1 is associated with adverse outcomes, including mortality and heart failure, in MI patients. We hypothesized that CD8+ T-cells exacerbate endothelin signaling in the infarcted area of left ventricle (LV), and that mice lacking functional CD8+ T-cells (CD8atm1mak; CD8-/-) would have reduced gene expression of Edn1, Ednra, and Ednrb compared to wild-type (WT) mice. WT or CD8-/- mice underwent surgical induction of MI by permanent ligation of the left ascending coronary artery. The infarct of LV was collected at terminal timepoints (day 1, day 7, or day 14 post-MI; n=3/genotype/timepoint) and tissue was dissociated and sequenced for bulk RNA. Transcriptomic analysis was performed by first aligning FASTQ files to the reference genome (mm10) and identifying counts using Elysium, then analyzing differentially expressed genes with DESeq2. We found that Edn1 transcripts in the infarct were 1.6-fold lower in CD8-/- mice compared to WT ($p=0.003$). Interestingly, Ednrb was 3.2-fold higher (0.04) whereas Ednra was unchanged. When comparing specific timepoints post-MI, Edn1 transcripts were 1.8-fold lower in CD8-/- mice at day 1 post-MI ($p=0.02$) and 2.2-fold lower at day 7 post-MI ($p=0.01$) compared to WT. At day 14 post-MI, there were no differences in endothelin system transcripts between CD8-/- and WT mice. Our data suggests a potential connection between CD8+ T-cells and the endothelin system that is temporally regulated. This in turn may modulate post-injury angiogenesis and left ventricular healing. Further in vitro co-culture experiments inhibiting endothelin interactions between CD8+ T-cells and endothelial cells will help elucidate specific cross-talk mechanisms after MI.

027

Lack of the ET-1/ETA axis on dendritic cells results in ameliorated hypertension in males, but not females

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Presenter: **Virginia Beasley, BS**; Institution: **University of Alabama at Birmingham;** Funding: **UAB/UCSD O'Brien Center Summer Research Scholar Training U54 DK137307 to VB, Deep South KUH PRIME U2C DK133422 & TL1 DK139566 to AJB, and K01HL145324, R25 HL145817 Future Faculty of Cardiovascular Disease (FOCUS) and UAB Diabetes Research Center pilot funding**

Immune cell dysregulation and exaggerated levels of endothelin-1 (ET-1) are critical for the development of hypertension. Yet, there is an important gap of knowledge regarding the involvement of the immune ET-1 system in the progression of this condition. Dendritic cells (DCs) are deeply involved in the onset of hypertension, and express ETA and ETB receptors on their surface. However, if activation of the ET-1/ETA axis in DCs is critical for rising blood pressure and deteriorating cardio-renal function during hypertension is unclear. We hypothesized that stimulation of the ET-1/ETA axis on DCs drives the development and progression of hypertension, as well as the deterioration of cardio-renal function. We induced hypertension in male and female mice lacking ETA receptor specifically on DCs (DC ETA KO) and floxed ETA control mice with the nitric oxide synthase inhibitor L-NAME (0.5 mg/mL, drinking water) for 3 wks. Systolic blood pressure (SBP) was monitored via daily tail cuff plethysmography. Changes in renal function were assessed via glomerular filtration rate (GFR) at baseline and after L-NAME treatment, and cardiac function and pulse wave velocity were studied via echocardiography. Lack of DC ETA receptor resulted in decreased SBP in male mice at baseline (Floxed ETA vs. DC ETA KO: 120.2±2.2 vs. 113.2±1.6 mmHg, n=10/group, p=0.25) and led to ameliorated responses to L-NAME treatment in males (Floxed ETA vs. DC ETA KO: 140.6±3.6 vs. 124.9±2.5 mmHg, n=10/group, p=0.003). No difference in SBP among genotypes was found in females. Despite the important difference in blood pressure with L-NAME treatment, no changes in renal function were detected between the groups. Similarly, urinary markers of proximal tubular damage were not different after L-NAME, although the absence of DC ETA receptor tended to ameliorate urinary levels of NGAL in males after L-NAME (floxed ETA vs. DC ETA KO: 144.2±18.1 vs. 109.6±15. 7 ng/ml, n=5-7/group, p>0.05). Our findings demonstrate that activation of the DC ET-1/ETA axis is important for increasing SBP in response to a hypertensive insult, but only in males. Our data indicate that the use of ETA receptor antagonists may be more effective in men for protection against hypertension and its associated renal damage.

028

Endothelin-1 mediated crosstalk between heart and skeletal muscle during obesity induced cardiac dysfunction

Samarjit Das

Presenter: **Sam Das, PhD**; Institution: **Johns Hopkins School of Medicine**

IObesity-induced cardiac dysfunction is growing alarmingly, dramatically increasing in global prevalence. During obesity, cardiac injury starts much before the onset of decreased cardiac contractility. However, there is another type of heart failure often associated with obesity, where the ejection fraction is preserved (HFpEF), which often initially goes undiagnosed—creating the unmet need for mechanistic insight into the development and progression of obesity-induced cardiac dysfunction. While 20% of total US healthcare budget are spent on obesity, HFpEF complications are the major contributors to these costs. Lifestyle changes and early implementation of drug therapy can significantly counter the disease. However, this is only effective if high-risk individuals are identified at early stages. While it's easy to detect individuals with heart failure with reduced ejection fraction (HFrEF) through routine analysis, individuals with HFpEF frequently remain undiagnosed until much later in the progression of the disease. It has been observed that HF has more global consequences, as it has detrimental effects on skeletal muscle. This points to the importance of communication between cardiomyocytes and skeletal myocytes. Adults who have HFpEF experience significant exercise intolerance, disability, and heightened mortality. Skeletal muscle metabolism is impaired in heart failure, where insulin resistance also occurs and may contribute to exercise intolerance in heart failure. Recently, we discovered a molecular pathway that provides important mechanistic insight into insulin resistance in HFpEF. We identify that HF causes upregulation of miR-133b in skeletal muscle due to elevated level of circulating Endothelin-1 (ET-1). The data indicate that the upregulation of miR-133b, previously shown to bind to the 3'-UTR of Klf15, transcription factor for GLUT4, inhibits glucose uptake into skeletal muscle by lowering GLUT4 expression and impairing mitochondrial energy production. Importantly, our data show that skeletal myocytes develop insulin resistance with the overexpression of miR-133b. Further, the treatment with ET-1Ar antagonist (BQ-123; 1 mM) in vitro, and daily Atrasentan (ABT-627) treatment (10 mg/kg b.wt.) for 8-weeks during the development of HF mitigates ET-1 induced miR-133b upregulation and its consequences. Together, the data from this study allow us to undertake future studies to reveal the mechanisms governing communication between the heart and skeletal muscle in the pathophysiology of HFpEF.

029 Effects of combined treatment with zibotentan and dapagliflozin compared to dapagliflozin alone on insulin resistance and markers of inflammation

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Presenter: **Marvin Sinsakul, MD;** Institution: **AstraZeneca**

Background: Alteplase and Tenecteplase are commonly used therapeutics for acute cerebral ischemic stroke (ACIS), while sovateltide is a novel therapeutic under development. Sovateltide demonstrated high safety and efficacy in Phase II and III trials with an extended therapeutic window of up to 24 hours. This study aims to systematically review and meta-analyze the impacts of sovateltide and thrombolytic treatments—alteplase and tenecteplase. *Methods:* Clinical studies were retrieved from PubMed and Google Scholar up to June 2025. Retrieved studies were screened using the PICOT framework (Patient- study participants with AIS, Intervention- Alteplase, Tenecteplase, or Sovateltide, Comparison- control treatment, Outcome- neurological assessments, Timeline- 90 days). A total of 29 studies comprising 15,616 ACIS patients (alteplase-10,595, tenecteplase-4,823 and sovateltide-198) were selected. The primary outcomes were excellent (mRS 0 or 0-1) or good (mRS 0-2 or 0-3) functional outcomes at 90 days. Their associations were calculated for the overall subgroups using the ordinary odds ratios (ORs) and compared. *Results:* The synthesized results from the meta-analysis demonstrated that sovateltide (2.7 µg/kg) had superior outcomes compared to alteplase (0.9 ± 0 mg/kg) at mRS 0–2 (OR 3.05 ± 0.78 vs 1.09 ± 0.11; 95% CI 1.14 to 2.79; p=0.0002) and mRS 0–3 (OR 5.58 ± 1.08 vs 1.02 ± 0.16; 95% CI 3.42 to 5.7; p=0.0001), and non-inferior at mRS 0 (OR 1.89 ± 0.26 vs 1.32 ± 0.20; 95% CI -0.49 to 1.64; p=0.26) and mRS 0–1 (OR 1.82 ± 0.45 vs 1.17 ± 0.11; 95% CI -0.1 to 1.39; p=0.085). In patients not given thrombolytics, sovateltide had better outcomes (OR 5.85 ± 4.18 vs 1.32 ± 0.19; 95% CI 1.33 to 7.7; p = 0.0098), even at mRS 0. Sovateltide outperformed Tenecteplase (0.324 ± 0.025 mg/kg) at mRS 0-1 (p=0.02), 0-2 (p=0.002), and 0-3 (p=0.0002). Mortality and intracranial hemorrhage (ICH) incidence were similar among the studies. Mean Tau2= 0.0485 ± 0.029, I2 = 15.50% ± 8.38%, and mean H2 = 1.077 ± 0.250 and Galbraith plots for mRS 0, 0-1, 0-2 and 0-3 indicated acceptable heterogeneity and variability, respectively, while funnel plots indicated minimal bias in the included studies. *Conclusions:* Sovateltide has the potential to be developed as a new therapeutic for ACIS, which may lead to improved functional outcomes compared to currently used thrombolytics, such as Alteplase and Tenecteplase. However, the limited patient population treated with sovateltide underscores the need for larger-scale global trials in the future. Registration - https://doi.org/10.17605/OSF.IO/P7FNJ.

030 Transcriptomics of adipose tissue from patients with obesity reveal upregulation of endothelin-1 signaling in Black Americans.

Hayley Murphy, Natalie Wilson, and Joshua S. Speed

Presenter: **Hayley Murphy, MS;** Institution: **University of Mississippi Medical Center;** Funding: **NIH (U54 GM115428); (P30 GM149404), (T32 HL105324), and (R01 DK124327).**

Obesity causes adverse health outcomes including hypertension, dyslipidemia, and type II diabetes. While obesity affects over 40 percent of the United States population, genetic and socioeconomic determinants of health lead to increased obesity disease risk and treatment outcomes in Black population; Our lab has recently demonstrated that ET-1promotes adipose tissue inflammation and insulin resistance in obese animals. It is well-documented that circulating ET-1 is increased in human Black (BA) compared to White (WA) populations. Therefore, we hypothesized that ET-1 and markers of inflammation are significantly higher in BA with obesity compared to WA patients. To test this hypothesis, we performed single nuclear RNA sequencing on visceral adipose tissue from age and weight matched BA and WA patients undergoing bariatric surgery (n=3 each with >30,000 total cells. Annotation analysis indicated a higher number of pro-inflammatory cells, including macrophages and T cells in BA tissue compared to WA. Tissue hypoxia, evidenced by increased Hif1a expression, was higher in all cell types of BAs compared to WAs, and most notably ECs, VSMCs, adipocytes, and macrophages. Interestingly, ET-1 expression was significantly higher in ECs of BA adipose compared to WA where it was hardly detected by single nuclear RNA seq. KEGG pathway analysis of differentially express genes in adipocytes revealed significant down regulation of PPAR signaling pathway, Insulin resistance, and Adipocytokine signaling pathways and upregulation of MAPK signaling, HIF-1 signaling, and TNF signaling, all common pathways of ET-1 signaling. These data suggest that ET-1 may play an important role in promoting increased disease risk in BA with obesity.

031 The circadian protein PERIOD-1 in the adrenal gland moderates endothelin-1 and aldosterone excretion in response to dietary salt in C57Bl/6J mice

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Presenter: **Alexandria Juffre, MS;** Institution: **University of Florida**

Background: Our lab previously identified the circadian protein PERIOD-1 (PER1) as a transcriptional regulator of endothelin-1 (ET-1) in several cell culture and rodent models, including global and kidney-specific PER1 knockouts (KO) in mice and a global PER1 KO in salt-sensitive rats. Rodents lacking PER1 also display increased ET-1, abnormal sodium handling, increased aldosterone, and dysregulated blood pressure (BP). In this study, our lab generated an adrenal gland-specific knockout of PER1 (AsPer1 KO) on a C57Bl/6J mouse background by crossing floxed Per1 mice with aldosterone synthase Cre mice (gift of David Breault). This KO is specific to the zona glomerulosa cells of the adrenal gland. The goal of this study was to test the response of these mice to dietary salt challenges and treatment with the aldosterone analog desoxycorticosterone pivalate (DOCP) to determine the adrenal gland-specific role of PER1 in maintaining sodium balance. *Methods:* Male mice (n=6 per group) were placed in metabolic cages for 2 days of acclimation. Mice were then administered various gel diet treatments: 3 days of normal salt (NS), 5 days of low salt (LS), 3 days of high salt (HS), and finally 3 days of HS + DOCP. Body weight, food and water consumption and urine output were measured throughout, and urine was collected every 12 or 24 hrs depending on diet. Total protein was collected from flash frozen kidney tissue. ELISA was used to measure ET-1 levels in kidney tissue lysate and urine from LS Day 5 and HS + DOCP Day 4. ELISA was used to measure aldosterone levels in urine from NS Day 3, and the first and last day of LS, HS, and HS + DOCP. Flame spectrophotometry was used to measure sodium (Na) levels in all urine samples. Statistical analysis was performed using student's t-test or 2-Way ANOVA, with post-hoc analysis by Šídák's multiple comparisons test in GraphPad Prism 10. Results: There was a significant diet and interaction effect in aldosterone excretion (p_{diet} = <0.0001, $p_{interaction}$ = 0.0277), and a significant increase in aldosterone levels in AsPer1 KO mouse urine from LS Day 5 (p = 0.0057). These increases were also seen in excreted ET-1 levels from AsPer1 KO mice on LS Day 5 (p = 0.0038) and HS + DOCP Day 4 (p = 0.0458). At the end of the study, renal ET-1 levels measured from kidney tissue lysates were not significantly different between groups (p = 0.4070). When normalized to body weight, Na and water balance both changed depending on dietary treatment and increased after HS Day 1 (p_{diet}=0.0016; p_{diet}=0.0006), but there was no significant genotype effect for Na or water balance ($p_{genotype}$=0.1495; $p_{genotype}$=0.2959). *Conclusions:* ASPer1 KO mice were able to respond to dietary sodium challenges to regulate Na and water balance similarly to controls; however, this came at the expense of increased urinary excretion of aldosterone and ET-1. The BP response of these mice as well as the systemic effects of increased aldosterone and ET-1 excretion in response to dietary salt will be investigated in the future. These results provide further evidence that PER1 represses aldosterone and ET-1 to maintain homeostasis in response to changes in dietary salt.

032 Endothelin-2 Signaling Orchestrates Telogen-to-Anagen Transition in Hair Follicles: Implications for Alopecia Therapy

Satrio Adi Wicaksono, Gusty Rizky Teguh Ryanto, Yoko Suzuki, Tetsuya Hara, Takeshi Fukumoto, Masashi Yanagisawa, Hiromasa Otake, Noriaki Emoto

Presenter: **Satrio Adi Wicaksono, MD**; Institution: **Kobe University**

Hair loss, or alopecia, extends beyond cosmetic concern and often reflects underlying dysfunctions in immune homeostasis and tissue regeneration. The dermal papilla plays a central role in hair follicle (HF) cycling, yet the upstream regulatory signals driving its remodeling remain incompletely defined. Here, we identify endothelin-2 (ET-2), a lesser-studied member of the endothelin family, as a critical modulator of HF regeneration. We observed that epidermal ET-2 expression is upregulated following fur shaving, coinciding with increased dermal expression of fibroblast growth factor-7 (FGF-7). This signaling cascade promotes the transition of HFs from telogen to anagen phase via activation of the pAKT/Wnt-β-catenin pathway. In inducible ET-2 knockout (ET2-iKO) mice, fur regrowth was markedly impaired, accompanied by diminished FGF-7 expression and dysregulated local immune responses. Mechanistically, ET-2 loss led to enhanced transforming growth factor-β (TGF-β) signaling, which in turn suppressed FGF-7 expression—a defect that was reversible with pharmacologic TGF-β inhibition. Moreover, microarray analysis of alopecia patient scalp samples from the public database revealed a consistent downregulation of ET-2 expression, aligning with our in vivo findings. Taken together, our study uncovers ET-2 as a previously unrecognized regulator of HF cycling and immune modulation, providing a potential therapeutic target for alopecia and related disorders of hair regeneration.

033 Sovateltide (IRL-1620), an endothelin B receptor agonist for cerebral ischemic stroke treatment, shows an unexpected reduction in lymphedema in a lymphatic filariasis patient

Rutul Shah, Abhishek Gohel, Emmie Anderson, Manish Lavhale, and Anil Gulati

Presenter: **Emmie Anderson, BS;** Institution: **University of Illinois Urbana-Champaign**

Funding: **Travere Therapeutics, Inc.**

Elevated levels of endothelin-1 (ET-1), a vasoconstrictor with pro-inflammatory properties, have been reported in patients with chronic lymphatic conditions, suggesting endothelin's involvement in lymphatic disease progression. Lymphatic filariasis is one such condition, caused by filarial worms that inhabit lymphatic vessels and impair fluid drainage. Current treatment with diethylcarbamazine citrate (DEC) targets immature and adult worms but offers limited relief for chronic symptoms, such as lymphedema or elephantiasis. In this case report, sovateltide, a selective endothelin B receptor (ETBR) agonist, marketed in India, was administered to an 84-year-old male patient with acute cerebral ischemic stroke with a 50-year history of lymphatic filariasis and persistent lower left limb lymphedema. The patient was treated with sovateltide over a 6-day treatment (0.3 µg/kg) intravenously within 24 hours of the stroke onset in three doses at intervals of 3 hours on days 1, 3, and 6. The patient was discharged after 6 days of hospitalization with 80% recovery from the stroke. The patient exhibited a substantial reduction in leg swelling. Prior treatments, including surgery, demonstrated no significant reduction in leg swelling. Mild leg swelling recurred approximately 4 months after sovateltide treatment. This case study suggests a novel therapeutic application for sovateltide in the treatment of filarial lymphedema, which warrants further exploration.

034 Patients (Pts) With Focal Segmental Glomerulosclerosis (FSGS) Achieved Low Proteinuria Targets Earlier and More Often With Sparsentan (SPAR) vs Irbesartan (IRB) in DUPLEX

Vladimir Tesar, Hernán Trimarchi, James Tumlin, Laura Kooienga, Radko Komers, Jula Inrig, Edward Murphy, Eva Rodríguez García

Presenter: **Bruce Hendry, MD, PhD;** Institution: **Travere Therapeutics, Inc.;** Funding: **Travere Therapeutics, Inc.**

SPAR, a dual endothelin angiotensin receptor antagonist (DEARA), lowered proteinuria in pts with FSGS in DUPLEX. We assessed low proteinuria targets with SPAR vs IRB and impact on kidney failure (KF). DUPLEX was a 108-wk, phase 3 study of SPAR (n=184) vs IRB (n=187) in FSGS. Urine protein-to-creatinine ratio (UPCR) <0.3 (complete remission [CR] of proteinuria), <0.5, <1.0, or <1.5 g/g and FSGS partial remission endpoint (UPCR ≤1.5 g/g and >40% reduction from baseline) were evaluated. Pooled treatment-agnostic analyses assessed impacts of CR or FSGS partial remission endpoint on KF (eGFR <15 mL/min/1.73 m2 or kidney replacement therapy). Pts reached low proteinuria earlier and more often with SPAR vs IRB: 18.5% vs 7.5% (relative risk [RR], 2.47 [95% CI 1.37-4.45]), 31.0% vs 14.4% (2.15 [1.44-3.20]), 53.3% vs 35.8% (1.49 [1.19-1.86]), 69.0% vs 50.8% (1.36 [1.16-1.59]), and 64.7% vs 43.9% (1.48 [1.23-1.78]) reached CR, UPCR <0.5, <1.0, or <1.5 g/g, and FSGS partial remission endpoint, respectively. Irrespective of treatment, KF was less common in pts who did vs did not reach CR (2.1% vs 9.9%; RR, 0.23 [95% CI 0.03-1.85]) or FSGS partial remission endpoint (3.0% vs 15.9%; RR, 0.33 [95% CI 0.11-0.95]). SPAR was well tolerated with no new safety concerns. Together, achievement of low proteinuria with SPAR and associated lower risk of KF support SPAR's nephroprotective benefit in FSGS.

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035 Systematic Review and Meta-Analysis of Sovateltide, Alteplase, and Tenecteplase Treatments for Acute Cerebral Ischemic Stroke

Preyan Mehta, Amaresh Ranjan and Anil Gulati

Presenter: **Preyan Mehta, BS;** Institution: **Pharmazz, Inc;** Funding: **Pharmazz, Inc.**

Background: Alteplase and Tenecteplase are commonly used therapeutics for acute cerebral ischemic stroke (ACIS), while sovateltide is a novel therapeutic under development. Sovateltide demonstrated high safety and efficacy in Phase II and III trials with an extended therapeutic window of up to 24 hours. This study aims to systematically review and meta-analyze the impacts of sovateltide and thrombolytic treatments—alteplase and tenecteplase. *Methods:* Clinical studies were retrieved from PubMed and Google Scholar up to June 2025. Retrieved studies were screened using the PICOT framework (Patient- study participants with AIS, Intervention- Alteplase, Tenecteplase, or Sovateltide, Comparison- control treatment, Outcome- neurological assessments, Timeline- 90 days). A total of 29 studies comprising 15,616 ACIS patients (alteplase-10,595, tenecteplase-4,823 and sovateltide-198) were selected. The primary outcomes were excellent (mRS 0 or 0-1) or good (mRS 0-2 or 0-3) functional outcomes at 90 days. Their associations were calculated for the overall subgroups using the ordinary odds ratios (ORs) and compared. *Results:* The synthesized results from the meta-analysis demonstrated that sovateltide (2.7 µg/kg) had superior outcomes compared to alteplase (0.9 ± 0 mg/kg) at mRS 0–2 (OR 3.05 ± 0.78 vs 1.09 ± 0.11; 95% CI 1.14 to 2.79; p=0.0002) and mRS 0–3 (OR 5.58 ± 1.08 vs 1.02 ± 0.16; 95% CI 3.42 to 5.7; p=0.0001), and non-inferior at mRS 0 (OR 1.89 ± 0.26 vs 1.32 ± 0.20; 95% CI -0.49 to 1.64; p=0.26) and mRS 0–1 (OR 1.82 ± 0.45 vs 1.17 ± 0.11; 95% CI -0.1 to 1.39; p=0.085). In patients not given thrombolytics, sovateltide had better outcomes (OR 5.85 ± 4.18 vs 1.32 ± 0.19; 95% CI 1.33 to 7.7; p = 0.0098), even at mRS 0. Sovateltide outperformed Tenecteplase (0.324 ± 0.025 mg/kg) at mRS 0-1 (p=0.02), 0-2 (p=0.002), and 0-3 (p=0.0002). Mortality and intracranial hemorrhage (ICH) incidence were similar among the studies. Mean Tau2= 0.0485 ± 0.029, I2 = 15.50% ± 8.38%, and mean H2 = 1.077 ± 0.250 and Galbraith plots for mRS 0, 0-1, 0-2 and 0-3 indicated acceptable heterogeneity and variability, respectively, while funnel plots indicated minimal bias in the included studies. *Conclusions:* Sovateltide has the potential to be developed as a new therapeutic for ACIS, which may lead to improved functional outcomes compared to currently used thrombolytics, such as Alteplase and Tenecteplase. However, the limited patient population treated with sovateltide underscores the need for larger-scale global trials in the future. Registration - https://doi.org/10.17605/OSF.IO/P7FNJ.

036 Sparsentan (SPAR) as First-Line Treatment of Incident Patients (Pts) With IgA Nephropathy (IgAN): An Interim Analysis of the SPARTAN Trial Evaluating Efficacy and Cardiovascular (CV) Risk Variables

Chee Kay Cheung, Stephanie Moody, Neeraj Dhaun, Matthew Graham-Brown, Siân Griffin, Alexandra Howson, Radko Komers, Bruce Hendry, Alex Mercer, Kelly Parke, Matthew Sayer, Smeeta Sinha, Lisa Willcocks, Jonathan Barratt

Presenter: **Chee Kay Cheung, MD**; Institution: **University of Leicester**

SPARTAN is a single-arm, exploratory trial investigating the efficacy and safety of SPAR, a dual endothelin angiotensin receptor antagonist (DEARA), as first-line therapy in IgAN. We evaluated 24-wk interim efficacy and CV risk variables. Twelve adults with biopsy-proven IgAN, proteinuria ≥0.5 g/d, eGFR ≥30 mL/min/1.73 m2, and no prior ACEi/ARB treatment were enrolled. SPAR is given for 110 wk with a 4-wk safety follow-up. One pt discontinued early due to hypotension. For CV risk factor assessments, there are occasional missing data points for 1 or 2 pts. Mean age at enrollment was 35.8 (SD, 12.2) y, with a median (IQR) proteinuria of 1.7 (0.6-3.3) g/d and mean eGFR of 70.2 (SD, 25.0) mL/min/1.73 m2 at baseline (BL). Proteinuria reductions were rapid and sustained over 24 wk (−68.9% [±SE −75.7 to −60.1] from BL to wk 24); 58% of pts achieved complete proteinuria remission (<0.3 g/d) at any time. After an initial decrease, BP and NT-proBNP remained stable over 24 wk. Minimal changes in total body water, body weight, blood lipids, triglycerides, and blood glucose were observed from BL to wk 24. Cardiac MRI results at wk 24 showed a change from BL in left ventricular mass/BSA of −3.1 (SD, 3.5) g/m2 and left ventricular ejection fraction of 0.3 (SD, 6.6) %. In newly diagnosed pts with IgAN, SPAR reduced proteinuria ≈70% over 24 wk, with CV risk factors remaining stable or improving.

A 1

Podocyte derived endothelin-1 in glomerular injury

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A 2

Dual endothelin-angiotensin antagonism improves endothelial function & fibrinolysis in ANCA vasculitis: a randomised, double blind, active control clinical trial

Matthew Sayer [1] Vanessa Melville [1] Gavin Chapman [1] Bruce Hendry [2] Alex Mercer [2] Tariq Farrah [1] Neeraj Dhaun [1]

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Presenter: **Matthew Sayer, MD;** Institution: **University of Edinburgh**

Funding: **NIH TL1DK139566, P01HL158500 and R01DK134562**

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A 3

ETB blockade exacerbates circadian amplitude of blood pressure in rats on a high salt diet.

Maria Venegas, Hiruni Aponso, David M. Pollock

Presenter: **Maria Venegas, PhD;** Institution: **University of Alabama at Birmingham**

Funding: **NIH TL1DK139566, P01HL158500 and R01DK134562**

Circadian rhythms are ~24-hour processes which control a variety of physiological processes including blood pressure and renal function. It is known the molecular clock directly controls the Edn1 gene which codes for endothelin-1 which is a potent vasoconstrictor. In addition, endothelin-1 receptors A and B (ETA and ETB) show time of day dependent expression further strengthening evidence that the endothelin axis is in part regulated by the circadian clock. Preliminary studies conducted in our lab found ETB deficient rats housed in constant conditions demonstrated a phase advance in MAP compared to control rats when challenged with a high salt diet suggesting a reciprocal interaction of the endothelin system with the circadian clock. To further determine how the endothelin axis interacts with the molecular clock we implanted blood pressure telemetry transmitters in male and female Sprague Dawley rats at 8-12 weeks of age and allowed to recover for at least 7 days. Rats were then maintained under constant dark conditions (DD) and fed ad libitum to eliminate external time cues for 3 weeks. Rats were either fed normal salt (NS, 0.49% NaCl, n=9) or high salt (HS, 4% NaCl, n=8) diets starting the day of release into DD. After a week of maintenance in DD for acclimation rats were given an ETB antagonist (A-192621) in the food (n=5 NS; n=6 HS). Circadian analysis was performed on the telemetry data for the last 10 days to ensure acclimation to the diet and treatments. Amplitude and MESOR were determined via cosinor analysis. Clocklab software was used to determine acrophase and circadian period. For body temperature, we found no differences in the amplitude, MESOR, goodness of fit to a cosinor wave (R2), circadian period (Two-Way ANOVA), or acrophase (Watson-two test) between any of the groups. A similar pattern was observed for heart rate data for all parameters. We found no differences between the circadian period (Two-way ANOVA), or acrophase (Watson two-test) between the groups for mean arterial pressure (MAP), and systolic blood pressure (SBP). However, we observed a significantly higher amplitude for MAP in HS diet fed animals regardless of treatment ($p_{diet}=0.0174$) compared to NS fed animals. MAP MESOR was highest in HS and antagonist treated animals compared to all other groups ($p_{diet}= 0.058$, $p_{drug}=0.0202$ $p_{interaction}<0.0001$, post-hoc $p<0.0001$). Additionally, HS and antagonist treated animals had higher MAP rhythmicity compared to NS fed animals regardless of antagonist treatment ($p_{diet}=0.0413$, post-hoc $p=0.0356$, and $p=0.0326$). As expected SBP analysis revealed the same findings to those found in MAP rhythms. Overall, our data suggest that ETB receptors regulate the amplitude and MESOR of blood pressure rhythms. ETB antagonism and HS diet also improved the rhythmicity of blood pressure which was unexpected as functional circadian rhythms are associated with increased risk of cardiovascular and renal disease. Future studies will delve into the mechanisms by which ETB antagonist combined with a high salt diet induces more robust blood pressure rhythms.

A 4

Sparsentan reversibly decreases mesangial IgA deposition in gddY mice: a possible role for mesangial-cell-surface autoantigen expression

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Presenter: **Bruce Hendry, MD, PhD;** Institution: **Tavere Therapeutics, Inc.**
Funding: **Travere Therapeutics, Inc.**

Background and Aims: IgA nephropathy (IgAN) is characterized by mesangial IgA deposition, but the mechanism remains elusive. We recently identified IgA autoantibodies in the sera of IgAN patients that specifically recognize mesangial cell (MC)-surface autoantigens, β2-spectrin and CBX3 (Nihei, Sci Adv. 2023; Higashiyama, Life Sci Alliance. 2024). Although these autoantigens are typically localized intracellularly, the mechanisms by which they are expressed on the MC surface remain unclear. Previously, we reported that Sparsentan (SP), a dual endothelin and angiotensin receptor antagonist (DEARA) approved in the U.S. and Europe for the treatment of IgAN, rapidly reduced proteinuria in gddY mice, a spontaneous IgAN model (Nagasawa, Nephrol Dial Transplant. 2024). This effect prompted us to investigate the mechanisms underlying SP's IgAN-specific effects. In this study, we examine whether endothelin-1 (ET-1) and angiotensin II (Ang II) regulate surface expression of these autoantigens in human MCs (HMCs), and whether SP treatment reduces mesangial IgA deposition in gddY mice. *Method:* Four-week-old gddY mice were divided into three groups and treated as follows: (1) control chow (CC) for 12 weeks; (2) chow containing Sparsentan at 900 ppm (SP900) for 12 weeks; or (3) SP900 for 8 weeks followed by CC for 4 weeks (SP900/CC) (n = 4 per group; one outlier was removed from 12-week CC group and one animal in each of the other groups died). Blood samples were collected to measure serum IgA (sIgA) levels. Kidney tissues were harvested to quantify glomerular IgA deposition using digital image analysis software (KS-400; Carl Zeiss). Primary cultured human mesangial cells (HMCs) were starved in medium containing 0.5% FBS for 24 hours, followed by stimulation with either ET-1, Ang II or both for 48 hours. Cell-surface expression of β2-spectrin and CBX3 was assessed by immunofluorescence microscopy after fixation with 4% paraformaldehyde. Three random images per group were quantified using the Hybrid Cell Count application (BZ-H4C; KEYENCE), calculating positive signals per total cell area. *Results:* In vivo, glomerular IgA deposition was significantly reduced in the SP900-treated mice compared to the CC group (6.0% vs 45.7%, P = 0.043). In contrast, the SP900/CC group exhibited 44.5% IgA deposition, which was comparable to the CC group (SP900 vs SP900/CC, P = 0.072). There was no statistically significant difference in serum IgA (sIgA) levels (P= 0.15) between the SP900 and CC groups. In vitro, stimulation with Ang II, ET-1 or both significantly upregulated β2-spectrin expression on the HMC surface. ET-1 alone or in combination with Ang II significantly upregulated CBX3 expression, while Ang II alone showed a trend toward increased CBX3 expression. *Conclusion:* The ET-1- and Ang II–induced upregulation in β2-spectrin and CBX3 expression on the surface of HMCs may underlie the significant reduction of mesangial IgA deposition observed in gddY mice after 12 weeks of SP900 treatment, despite no change in sIgA levels. Further investigation is warranted to elucidate the mechanisms underlying these novel findings.

A 5

EET-A (14,15-epoxyeicosatrienoic acid analog) augments the hypotensive effects of atrasentan, and prevents edema and organ hypertrophy in spontaneously hypertensive rats

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Presenter: **Ivana Vaněčková PhD;DSc.;** Institution: **Institute of Physiology**
Funding: **National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) - Funded by the European Union - Next Generation EU.**

ETA receptor blockade is often associated with edema, while epoxyeicosatrienoic acids (EETs) have natriuretic and vasodilatory activity, explaining their therapeutic potential in patients with hypertension and end-organ damage. We evaluated the effectiveness of atrasentan (ETA receptor blocker, ATR) alone or in combination with the 14,15-EET analog EET-A, on blood pressure and kidney function in spontaneously hypertensive rats. Blood pressure was measured by telemetry in rats that received ATR, EET-A, ATR+EET-A, or control solvent in drinking water. Urine and blood samples were collected weekly. At the end, the animals were euthanized, and the organs were harvested. Moreover, the effectiveness of a single intragastric drug application on renal electrolyte and water transport was tested. After a two-week treatment with ATR+EET-A, SBP decreased significantly more than after treatment with ATR alone. Decreases in plasma sodium and osmolality were significant only in the ATR group, which was associated with the greatest increase in body weight. In the ATR+EET-A and EET-A alone groups, organ weights were significantly lower than in the ATR alone group. Our results suggest that EET-A addition augments the hypotensive effect of ATR and prevents post-ATR body fluid expansion. Importantly, EET-A alone or in combination with ATR exhibits robust cardio- and reno-protective activity.

A 6

Association between Endothelin-1 and Right Ventricular Remodelling in Pulmonary Hypertension Secondary to Atrial Septal Defect Patients

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Presenter: **Danniel Angkasa;** Institution: **Universitas Gadjah Mada;** Funding: **Damas FK-KMK UGM**

See O25, Page 35 for abstract.

A 7

Mice lacking CD8+ T-cells have lower Edn1 expression post-MI

Thomas Dempster, Miguel Troncoso, Kristine DeLeon-Pennell

Presenter: **Thomas Dempster, MS;** Institution: **Medical University of South Carolina**

Funding: **NIH T32GM123055, NIH R01HL173273, VA I01BX005848**

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A 8

Circadian rhythms in serum ET-1 in obese individuals

Woojin Lee, Binli Tao, Jack Colson, Maria Venegas, Tamara Al-Daghastani, Alexis Lambert, Karen L. Gamble, Orlando M. Gutierrez, Jennifer S. Pollock, David M. Pollock

Presenter: **Woojin Lee, MS, BS;** Institution: **The University of Alabama at Birmingham**

Funding: **NIH T32GM123055, NIH R01HL173273, VA I01BX005848**

Introduction: Endothelin plays an important role in regulating the response to high salt intake and is regulated by the circadian clock. Prior animal studies have shown that rats lacking the ETB receptor have a delayed response to an acute salt load that is time of day dependent and become hypertensive when maintained on a high salt diet. We theorized that serum concentrations of ET-1 in human participants will follow a circadian pattern. The current study was designed to determine whether the time of day for high salt intake will modify serum ET-1 in obese individuals who are at risk for salt-dependent hypertension. *Methods:* Thirty-four obese adults (mean age 35±6, mean BMI 38±6 kg/m2, 74% female, 68% Black), were fed a fixed salt diet for 7 days (2.5g/day) and then randomly assigned to adding either an early salt load (2g Na) or a late salt load diet to the fixed salt diet for 9 days. After a 4-week washout period, participants crossed over to the other arm. The salt load was given in the form of chicken broth with either the morning or evening meal. On the morning of day 8 during the intervention period, 24-hour ambulatory blood pressure monitoring was conducted, and on day 9, participants reported to the UAB Clinical Research Unit to initiate 24-hour admission for physiological testing. During the final 24-hours, participants had blood drawn every 2 hours through an intravenous catheter, and urine collected in 12-hour segments. Serum endothelin-1 (ET-1) was measured by ELISA (R&D Systems, Human Endothelin-1 Catalog #: DET100). Urine electrolytes and creatinine were measured by ion selective electrodes and mass spectroscopy, respectively. *Results:* Circadian analysis of serum ET-1 data was performed by determining the goodness of fit value (R2) followed by a Spearman's nonparametric correlation test using Graph Prism. The goodness-of-fit to a cosine curve was statistically significant for both morning (R2= 0.23,p=0.038) and evening (R2= 0.3227, p=0.038) salt load arms of the study. The lowest serum ET-1 was observed at 2200 hrs for both groups. However, we did not observe any significant difference between the two salt intake periods. Urine excretion of water, sodium, and potassium all displayed a significantly higher level of excretion during the day versus night. However, daytime sodium excretion was significantly higher when the salt load was given in the morning compared to the evening salt load (133±9 versus 107±9 mmol/L, respectively; $p<0.016$, 2-way ANOVA). No significant differences were observed between the nighttime sodium excretion when comparing the two salt groups. *Discussion:* This study demonstrated that human serum ET-1 levels in obese participants followed a significant circadian rhythm. Additional studies are on-going to determine the impact of time-of-day salt intake on the renal ET-1 system and blood pressure.

National Heart, Lung, and Blood Institute
NIH R13HL182313

A 9

ET-1 inhibits CD8+ T-cell mediated actions on the focal scar Post-Myocardial Infarction

Shaoni Dasgupta and Kristine Y. DeLeon-Pennell

Presenter: **Shaoni Dasgupta, BS;** Institution: **Medical University of South Carolina;**

Funding: **Travere Therapeutics, Inc.**

Background: Patients with advanced heart failure have been shown to have elevated CD8+ T-cells. Our previous studies demonstrated CD8+ T-cells exacerbate left ventricular dilation and impair ejection fraction post-myocardial infarction (MI) due in part to decreased stiffness of the focal scar. Endothelin-1 (ET-1) is a known vasoconstrictor associated with chronic myocardial remodeling after MI and more recently linked to immune cell regulation. Both ETA and ETB receptors are expressed on CD8+ T-cells, however what role ET-1 may have on cell function is not known. Our hypothesis is that ET-1 is inhibiting CD8+ T-cell actions on the focal scar by inhibiting protease release. *Methods:* To determine potential actions of ET-1, we developed an in vitro cleavage assay. Splenic CD8+ T-cells were isolated (n=3 technical replicates) and plated on a 96-well plate (10^4 cells/ well) coated with 2% gelatin. Cells were cultured in either RPMI media alone, CD3/CD28 (1µg/mL, 2 µg/mL respectively), IL-12, (20ng/mL) or in combination to stimulate cell activation. ET-1 (100nM) was added to CD8+ T-cells in media alone or CD3/CD28 to determine potential effects on gelatin cleavage. After 24 hours, cleavage of gelatin was determined by staining wells with Coomassie blue and measuring absorbance at 595 nm. Tissue clearance was calculated by normalizing absorbance of the negative controls (wells without cells) using the following formula: 1-[(sample Abs/(negative control Abs)]. *Results:* Assessment for gelatin cleavage demonstrated CD8+ T-cells were able to cleave collagen when exposed to CD3/CD28, IL-12, or left unstimulated ($p<0.05$ for all). When CD3/CD28 was given in combination with IL-12, cleavage efficiency significantly increased ($p=0.038$). Interestingly, ET-1 stimulation decreased CD8+ T-cells' ability to cleave gelatin by 1.5 fold even after stimulation with CD3/CD28 ($p<0.05$ vs all). *Conclusion:* Our data demonstrates that ET-1 decreases CD8+ T-cell gelatin cleavage capacity.

A 10

Sovateltide (IRL-1620), an endothelin B receptor agonist for cerebral ischemic stroke treatment, shows an unexpected reduction in lymphedema in a lymphatic filariasis patient

Rutul Shah, Abhishek Gohel, Emmie Anderson, Manish Lavhale, and Anil Gulati

Presenter: **Emmie Anderson, BS;** Institution: **University of Illinois Urbana-Champaign**

Funding: **Travere Therapeutics, Inc.**

See O33, Page 39 for abstract.

A 11

Systematic Review and Meta-Analysis of Sovateltide, Alteplase, and Tenecteplase Treatments for Acute Cerebral Ischemic Stroke

Preyan Mehta, Amaresh Ranjan and Anil Gulati

Presenter: **Preyan Mehta, BS;** Institution: **Pharmazz, Inc;** Funding: **Pharmazz, Inc.**

See O35, Page 40 for abstract.

A 12

Endothelin-3 induces skeletal muscle atrophy and metabolic derangements

Yi-Ting Chung, Yi-Chieh Chang, Chi-Chang Juan

Presenter: **Yi-Ting Chung, PhD;** Institution: **National Yang Ming Chiao Tung University**

Funding: **National Yang Ming Chiao Tung University**

Skeletal muscle comprises over 40% of total body weight and functions as a major organ for energy metabolism. Muscle atrophy disrupts this function by contributing to mitochondrial dysfunction and impaired energy homeostasis. Endothelins (ETs) are 21-amino-acid peptides that exist in three isoforms: ET-1, ET-2, and ET-3. Our previous studies have shown that ET-1 induces skeletal muscle atrophy and inhibits muscle growth via activation of the ETB receptor. Additionally, ET-3 exhibits a higher affinity for the ETB receptor compared to the ETA receptor. In this study, we aimed to determine whether ET-3 also induces muscle atrophy and impairs energy metabolism in vivo. Mice were administered ET-3 via subcutaneously implanted osmotic pumps for two weeks. ET-3-infused mice significantly reduced food intake, oxygen consumption, and carbon dioxide production, indicating suppressed metabolic activity. Results of real-time PCR analysis showed upregulation of muscle atrophy-associated genes, including Trim63 (MuRF1) and Fbxo32 (Atrogin-1) in the gastrocnemius (GA) muscle. Consistently, results of western blot analysis showed decreased expression of myosin heavy chain (MyHC) and increased levels of the atrophy-related factors MuRF1 and Myostatin in GA muscle. Histological examination further confirmed a reduction in muscle fiber cross-sectional area after two-week ET-3 infusion. Therefore, these findings demonstrated that ET-3 induces skeletal muscle atrophy and disrupts energy homeostasis in mice.

A 13

Endothelin-2 Deficiency Disrupts Adipose Tissue Structure and Thermogenic Function

Saiful Hidayat, Pranindya Rinastiti, Gusty Rizky Teguh Ryanto, Yoko Suzuki, Tetsuya Hara, Masashi Yanagisawa, Hiromasa Otake, Noriaki Emoto

Presenter: **Saiful Hidayat, MD;** Institution: **Kobe University**

Among the three endothelin isoforms, endothelin-2 (ET-2) remains the least characterized in terms of physiological function. While recent reports have implicated ET-2 in lipid homeostasis and thermoregulation, the mechanistic underpinnings remain elusive. In this study, we investigated the role of ET-2 in adipose tissue maintenance and function using both constitutive (ET2-cKO) and tamoxifen-inducible (ET2-iKO) ET-2 knockout mouse models. Most ET2-cKO mice exhibited early postnatal lethality; however, surviving adults displayed visibly reduced lipid droplet size in visceral white adipose tissue (vWAT). Similarly, adult ET2-iKO mice showed significant weight loss and reduced core body temperature by 6 weeks post-tamoxifen induction. Abdominal CT imaging revealed a marked reduction in visceral fat volume, paralleled by histological evidence of smaller lipid droplets in vWAT compared to controls. In brown adipose tissue (BAT), no overt morphological changes were observed at 6 weeks post-induction. However, by 14 weeks, ET2-iKO mice developed BAT hypertrophy with enlarged lipid droplets and significant downregulation of thermogenic gene expression, including Ucp1 and Pgc1α. These findings demonstrate that ET-2 is essential for maintaining adipose tissue architecture and thermogenic function in both vWAT and BAT. Loss of ET-2 disrupts lipid storage dynamics and impairs thermal regulation, positioning ET-2 as a critical modulator of metabolic homeostasis. Further mechanistic studies are warranted to explore ET-2–mediated signaling pathways in adipose biology.

A 14

Endoplasmic Reticulum Stress Mediates Endothelin-1-Induced Norepinephrine Transporter Algteration in PC12 Cells Involvement of Endothelin Receptors

Fabian A Innamorato Costas1, Nicolas Giangreco1,2, Guadalupe Alvarez1, Ana Paula Courreges1, Clarisa Marotte1, María F Fernández1,2, Liliana G Bianciotti1,3, Marcelo S Vatta1,2.

Presenter: **Fabian Alejandro Innamorato Costas, BS;** Institution: **Institute of Immunology, Genetics and Metabolism (INIGEM) Buenos Aires, Argentina;** Funding: **Conicet - Universidad De Buenos Aires (UBA) - Anpcyt**

The neuronal norepinephrine (NE) transporter (NET) is responsible for the clearance of NE from the synaptic cleft, and its impairment is associated with various cardiovascular diseases. Increasing evidence supports the involvement of endoplasmic reticulum stress (ERE) in the pathogenesis of cardiovascular disorders. Previously, we reported that blockade of endothelin receptors (ETA and ETB) with a dual antagonist restores hemodynamic parameters and ERE markers in the adrenal medulla of salt-dependent hypertensive rats. The aim of the present study was to reveal the underlying mechanisms of these effects in PC12 cells exposed to ET-1 for 48 h. The expression of NET, phosphorylated forms of tyrosine hydroxylase, Bak, Bax, Bcl2, and ERE markers were determined by Western blotting, NET mRNA levels by real-time PCR, apoptosis and necrosis by flow cytometry, and NE uptake was assessed by ET-1. The results showed that ET-1 increased the expression of non-glycosylated NET compared to glycosylated NET and decreased NET mRNA levels and NE uptake. ET-1 increased tyrosine hydroxylase activity assessed by the expression of TH phosphorylated at Ser 40 sites. The expression of ERE markers and the proapoptotic proteins Bak and Bax were potentiated by ET-1 while Bcl-2 was decreased. Furthermore, to increase the levels of oxidative stress. ET-1 reduced cell viability and increased apoptosis and necrosis assessed by flow cytometry. Treatment with specific antagonists of ETA (BQ610) and ETB (BQ788) showed that both receptors differentially mediated the effects of ET-1. The finding that ET-1 increases non-glycosylated NET suggests that misfolded and nonfunctional proteins accumulate in the endoplasmic reticulum, leading to ERE and apoptosis in PC12 cells. The observed effects of ET-1 were selectively mediated by the ETA and ETB receptors.

A 15

The circadian protein PERIOD-1 in the adrenal gland moderates endothelin-1 and aldosterone excretion in response to dietary salt in C57Bl/6J mice

Alexandria Juffre1,2, Jermaine Johnston1,2,3, Sophia Eikenberry1,2, Kit-Yan Cheng1, Michelle L. Gumz1,2

Presenter: **Alexandria Juffre, MS;** Institution: **University of Florida**

See O31, Page 38 for abstract.

A 16

ET-1-induced FGF21 promotes the formation of oxidative myofibers during skeletal muscle atrophy through activation of the ETB receptor and the endoplasmic reticulum (ER) stress signaling pathway

Shui-Yu Liu, Yi-Chieh Chang, Yi-Ting Chung, Lou-Hui Kuo, Chi-Chang Juan

Presenter: **Shui Liu, PhD;** Institution: **Institutes of Physiology, College of Medicine, National Yang Ming Chiao Tung University;** Funding: **Travere Therapeutics, Inc.**

Endothelin-1 (ET-1), a potent vasoconstrictor, has been implicated in the pathogenesis of skeletal muscle atrophy, contributing to impaired myogenesis and reduced muscle mass. Fibroblast Growth Factor 21 (FGF21) is associated with myogenic differentiation and is known to promote the formation of oxidative myofibers during muscle atrophy. Skeletal muscle mass is tightly regulated by the balance between protein degradation and muscle regeneration. This study aimed to determine whether ET-1 induces muscle atrophy and FGF21 expression, potentially contributing to myofiber type remodeling. Our results showed that ET-1 administration led to a significant reduction in the cross-sectional area of the gastrocnemius (GA) muscle in mice, indicating muscle atrophy. This was accompanied by marked downregulation of myosin heavy chain (MyHC) isoforms, particularly the fast-twitch types MYH2 and MYH4, along with upregulation of the muscle-specific E3 ubiquitin ligase atrogin-1. Additionally, ET-1 treatment increased the expression of FGF21, MyoD, and MyoG, and enhanced succinate dehydrogenase (SDH) activity in the GA muscle, suggesting a shift toward oxidative metabolism. In vitro, ET-1 treatment of C2C12 myotubes significantly suppressed the Akt-mTOR-p70S6K signaling pathway, indicating inhibition of protein synthesis. In addition, Treatment with FGF21 upregulated the expression of oxidative MyHC isoforms, MYH7 and MYH2 in differentiated C2C12 myotubes. Notably, blocking the ER stress signaling pathway downstream of the ETB receptor attenuated ET-1-induced upregulation of FGF21. Collectively, our findings uncover a novel mechanism driving oxidative myofiber formation and identify FGF21 as a pivotal regulator of muscle development and a key mediator of ET-1-induced muscle atrophy.

A 17

Endothelin-1 induces FGF-21 expression in hepatocytes through activation of the endothelin type A receptor

Shui-Yu Liu, Chi-Chang Juan

Presenter: **Shui Liu, PhD;** Institution: **Institutes of Physiology, College of Medicine, National Yang Ming Chiao Tung University**

Endothelin-1 (ET-1) is a strong vasoconstrictor involved in cardiovascular dysfunction and systemic diseases. It acts via endothelin type A (ETA) and type B (ETB) receptors, whose expression varies by tissue. ET-1 binding to receptors on smooth muscle cells increases Ca^{2+} influx and induces constriction, while ETB receptors on endothelial cells aid in vasodilation. In addition to its pivotal role in cardiovascular homeostasis, ET-1 may also regulate metabolism homeostasis. Studies have demonstrated that ET-1 can activate glycogenolysis in the liver and stimulate hepatic glucose production. Fibroblast growth factor 21 (FGF21), a member of the fibroblast growth factor family, is synthesized mainly in the liver. It regulates lipid and glucose metabolism and has shown efficacy in reducing body weight, improving insulin resistance, and lowering blood glucose and lipid levels in conditions such as type 2 diabetes and obesity. The purpose of present study is to investigate the regulatory mechanism of ET-1 on FGF21 expression in hepatocytes. Confluent AML12 hepatocytes were treated with 10^{-7} M ET-1 for varying durations (0-24 hours) or with increasing ET-1 concentrations (0-10^{-7} M) for 24 hours, followed by analysis of FGF21 expression via western blotting. To identify the receptor subtype involved in ET-1-induced FGF21 expression, cells were pretreated for 1 hour with 10^{-5} M BQ610 (ETA receptor antagonist) or 10^{-5} M BQ788 (ETB receptor antagonist), then exposed to 10^{-8} M ET-1 for 24 hours in the continued presence or absence of the antagonists. FGF21 expression was again assessed by western blotting. FGF21 expression significantly increased in ET-1-treated cells after 8 hours, with levels continuing to rise up to 24 hours. Treatment with 10-8 M ET-1 also led to a significant increase compared to controls, with maximal expression reaching 50% above baseline. This ET-1-induced FGF21 upregulation was completely blocked by BQ610, but not by BQ788, indicating that the effect is mediated via the ETA receptor. Additionally, inhibition of the mTOR-p70S6K signaling pathway and PPARα significantly reduced ET-1-induced FGF21 expression. In summary, ET-1 upregulated hepatic FGF21 expression in a time- and dose-dependent manner via the ETA receptor, potentially through modulation of the mTOR-p70S6K signaling pathway and PPARα activity.

A 18

Transcriptomics of adipose tissue from patients with obesity reveal upregulation of endothelin-1 signaling in Black Americans.

Hayley Murphy, Natalie Wilson, and Joshua S. Speed

Presenter: **Hayley Murphy, MS;** Institution: **University of Mississippi Medical Center;** Funding: **NIH (U54 GM115428); (P30 GM149404), (T32 HL105324), and (R01 DK124327).**

See O30, Page 37 for abstract.

A 19

Endothelin-2 Signaling Orchestrates Telogen-to-Anagen Transition in Hair Follicles: Implications for Alopecia Therapy

Satrio Adi Wicaksono, Gusty Rizky Teguh Ryanto, Yoko Suzuki, Tetsuya Hara, Takeshi Fukumoto, Masashi Yanagisawa, Hiromasa Otake, Noriaki Emoto

Presenter: **Satrio Adi Wicaksono, MD**; Institution: **Kobe University**

See O32, Page 38 for abstract.

A 20

The ET-1/ETA axis on T cells, not Dendritic Cells, Mediates Inflammation and Renal Dysfunction during Type 1 Diabetes

Abigail J. Brooks, Melissa Rodriguez, Sara N. Biswal and Carmen De Miguel

Presenter: **Abigail Brooks, MS**; Institution: **University of Alabama at Birmingham;** Funding: **Deep South KUH PRIME U2C DK133422 & TL1 DK139566 to AJB and Diabetes Research Connection Funds and UAB Diabetes Research Center Pilot Funds to CDM**

See O19, Page 31 for abstract.

A 21

Choroidal thinning reflects systemic vascular injury in ANCA-associated vasculitis and improves with dual endothelin-angiotensin blockade

Gavin Chapman [1] Matthew Sayer [1] Emily Godden [1] Hannah Preston [1] Dan Pugh [1] Fiona Chapman [1] Vanessa Melville [1] Bruce Hendry [2] Alex Mercer [2] Tariq Farrah [1] Neeraj Dhaun [1]
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Presenter: **Gavin Chapman, MD**; Institution: **University of Edinburgh;** Funding: **Travere Therapeutics, Inc**

See O9, Page 25 for abstract.

A 22

Dual Endothelin-1 Receptor A/B Blockade Attenuates Adipose Tissue Inflammation in Mice Fed a High Fat Diet

Megumi Mills, Caroline Miller, Bridget Konadu, Natalie Wilson, Madilyn Lewis, Joshua Speed
Presenter: **Megumi Mills, PhD**; Institution: **University of Mississippi Medical Center;** Funding: **National Institute of General Medical Sciences (U54 GM115428), the National Institute of General Medical Sciences (P30 GM149404), the National Heart, Lung, and Blood Institute (T32 HL105324), and the National Institute on Diabetes, Digestive, and Kidney Diseases (R01 DK124327)**

See O20, Page 32 for abstract.

A 23

Endothelin Receptor Autoantibodies: Associations with Blood Pressure and Endothelial Activation in Systemic Lupus Erythematosus

Helen M. Butler, Ph.D., Marice K. McCrorey, B.S., Ryan S. Lacey, B.S., Marharyta Semenikhina MSc, Ph.D., C. Alex Colvert, B.S, Kennedy P. Hawkins, B.S., Oleg Palygin MSc, Ph.D., Yasir Abdul, Ph.D., Adviye Ergul, M.D. Ph.D., Melissa A. Cunningham, M.D. Ph.D., Jim C. Oates, M.D. Ph.D., Justin P. Van Beusecum, Ph.D.

Presenter: **Helen Butler, PhD;** Institution: **Medical University of South Carolina**
Funding: **VA BLRD IK2BX005605, P30AR072582, T32GM132055-5, U54DA016511-22, T32-AR050958-19, 25PRE1372738, LRA Innovation Award**

See O18, Page 30 for Abstract.

A 24

Membrane estrogen receptor activation restores nitric oxide production in human renal endothelial cells under inflammatory and lipotoxic stress

Mariia Stefanenko[1,2], Mykhailo Fedoriuk[1], Nicolas Ancona[2], Marice McCrorey[1], Justin Van Beusecum[1,4], Oleg Palygin[1,3], Tammy Nowling[2], DeAnna Baker Frost[2]

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Presenter: **Mariia Stafenenko, MS**; Institution: **University of Mississippi Medical Center;** Funding: **National Institute of General Medical Sciences (U54 GM115428), the National Institute of General Medical Sciences (P30 GM149404), the National Heart, Lung, and Blood Institute (T32 HL105324), and the National Institute on Diabetes, Digestive, and Kidney Diseases (R01 DK124327)**

Background: Endothelial dysfunction, including injury to renal endothelial cells, contributes to kidney damage and increased cardiovascular risk in lupus nephritis (LN). Key features include reduced nitric oxide (NO) bioavailability and increased pro-inflammatory pathways, often driven by circulating saturated fatty acids and inflammatory cytokines. Estrogen receptor alpha (ERα) modulates vascular function through genomic and rapid membrane-initiated signaling pathways. However, the role of membrane-localized ERα in renal endothelial cells exposed to combined inflammatory and lipotoxic stress relevant to LN remains poorly understood. The present study aimed to evaluate whether selective activation of membrane ERα signaling using a pathway preferential estrogen agonist could restore NO production and reduce inflammatory responses in human renal endothelial cells. *Methods:* Primary human renal endothelial cells (hRECs) were obtained from rejected kidney transplants through the MUSC Kidney Translational Research Center and cultured in hormone-stripped media. IL-6 and MCP-1 secretion were measured by ELISA following 24 hours exposure to TNFα (100 ng/ml) or IL-1β (25 ng/mL). Live-cell confocal imaging was used to investigate NO production in hRECs loaded with the DAF-FM diacetate fluorescent probe. Cells were treated for 6 hours with palmitic acid (PA, 100 μM), TNFα (100 ng/mL), or both. In designated groups, 17β-estradiol (E2, 10 nM) or pathway preferential estrogen (PaPE, 5 μM), an agonist specific for membrane ERα signaling, was added. Maximum NO fluorescence amplitude following angiotensin II (Ang II) stimulation (30 μM) was quantified to assess endothelial NO bioavailability under control and treatment conditions. Statistical analysis was conducted using one-way ANOVA with post hoc correction. *Results:* Treatment with TNFα or IL-1β significantly increased IL-6 and MCP-1 secretion by hRECs after 24 hours ($p<0.001$). Exposure of hRECs to PA or TNFα for 6 hours significantly reduced Ang-II mediated NO production. Combined PA and TNFα treatment promoted an additive effect with up to 53% reduction compared to control conditions (N=3 independent experiments, $n\geq37$ cells per group, $p<0.001$, $p<0.0001$). In rescue experiments, cells exposed to PA alone or PA+TNFα and treated with PaPE to activate membrane ERα signaling resulted in significant improvement in NO release (up to 80% of control; $p<0.001$). Conversely, treatment with E2, activating both nuclear and membrane ERα pathways, failed to rescue NO signaling (N=3, $n\geq24$ cells per group). *Conclusion:* Activation of membrane ERα by PaPE restores NO bioavailability and reduces the inflammatory response in hRECs exposed to lipotoxic and inflammatory stimuli. These findings suggest that targeting membrane ERα signaling may offer a therapeutic strategy to preserve renal endothelial function in lupus nephritis.

B 1

Endothelin A and B Receptors are Differentially Regulated in a Multi-etiology Model of Alzheimer's Disease-Related Dementias (ADRD): Retinal and Cerebral Perspectives

Yasir Abdul, Kareem Abdelsaid, Weiguo Li, Sarah Jamil, Justin Van Beusecum and Adviye Ergul

Presenter: **Yasir Abdul, PhD**; Institution: **Medical University of South Carolina** Funding: **VA Merit Review (BX000347), VA Senior Research Career Scientist Award (IK6 BX004471), NIH RF1 NS083559 and RF1 NS104573 to AE, and AARFD-23-1144963 to KA and UL1TR001450/SCTR2201 to YA**

Vascular Cognitive Impairment & Dementia (VCID) is a leading ADRD and presents with multiple vascular pathologies, including hypoperfusion and microinfarcts. Brain endothelin-1 (ET-1) levels, the most potent vasoconstrictor, closely correlate with the degree of hypoperfusion in ADRD. ET-1 is increased in diabetes, which is a leading risk factor for VCID. We hypothesized that contractile and proinflammatory ETA receptors are upregulated in a novel multi-etiology model of ADRD. Given that the eye serves as a window to the brain, we investigated ET receptors in the retina and brain. Methods: Control and diabetic rats were subjected to microemboli (ME) injection and unilateral common carotid occlusion (UCCAO) to mimic microinfarcts and hypoperfusion as a novel model of multi-etiology VCID. Animals were followed for 22 weeks by several behavioral tests. Hematoxylin/Eosin and Luxol-fast blue staining were used to assess brain pathologies. ETRs and hypoxia markers were assessed by immunoblotting and immunohistochemistry. Functional tests included a battery of cognitive tests and an electroretinogram (ERG). Results: Tissue damage in the striatum was greater, and the corpus callosum myelination score was lower in the diabetic (d) UCCAO+ME group. Plasma ET-1 level of dUCCAO+ME group (2.25±0.52pg/ml) was significantly increased ($p<0.05$) compared to dSham (0.67±0.07pg/ml) animals. Brain tissue protein levels of ETAR (0.909±0.059 vs 0.69±0.006) and HIF-1α (2.75±0.10 vs 0.71±0.152) significantly increased in dUCCAO+ME vs dSham ($p<0.05$ and $p<0.01$, respectively), while ETBR levels in dUCCAO+ME (0.551±0.025) group was significantly lower as compared to cUCCAO+ME (0.859±0.071, $p<0.05$). Histological analyses revealed increased ETAR expression in various cell types across both the brain and the eye. Functionally, there was a significant decline in ERG amplitudes (a-wave and b-wave) in both control and diabetes groups after UCCAO+ME (a and b-wave amplitudes in cSham; 140±8.09 and 352±17.4 vs cUCCAO+ME; 31.31±9.9 and 268.6±19.64, $p<0.05$), and it was more pronounced in diabetic animals (a and b-wave amplitudes in dSham; 87.68±10.42 and 186.7±29.63 vs dUCCAO+ME; 14.38±5.3 and 63.4±12.37, $p<0.01$). Open Field revealed anxiety-like behavior (number of central visits,13.2±3.4 vs 5.37±1.8 in control vs diabetic UCCAO+ME, respectively, $p<0.05$) and an increase in overall inactivity (active time, 2051±156 sec vs 1681±228 sec in control vs diabetic UCCAO+ME, respectively, $p<0.05$) at week 22. Novel object recognition test showed a distinctive decline in the recognition and discrimination indices in the dUCCAO+ME group (RI: 0.44±0.49 and DI: -0.05±0.12) compared to the baseline dUCCAO+ME (RI: 0.70±0.44 and DI: 0.41±0.08) and WK22 dSHAM (RI: 0.71±0.06 and DI: 0.43±0.12) groups. Conclusions: This multi-etiology model of VCID, especially in comorbid disease models, may serve as a more clinically relevant model of VCID. Given that post-mortem brain ET-1 levels correlate with tissue hypoxia and disease severity in patients with dementia, further evaluation of the ET receptors and retinal markers may provide new insights and therapeutic targets in ADRD research.

B 2

Endothelin-1 upregulates phagocytosis functions in human microglial cells

Yasir Abdul, Sarah Jamil and Adviye Ergul

Presenter: **Yasir Abdul, PhD**; Institution: **Medical University of South Carolina** Funding: **VA Merit Review (BX000347), VA Senior Research Career Scientist Award (IK6 BX004471), NIH RF1NS083559 and RF1 NS104573 to AE, and AARFD-23-1144963 to KA and UL1TR001450/SCTR2201 to YA**

See O13, Page 27 for abstract.

B 3

Endothelin-1 as an Activator of Pro-inflammatory Microglia Cells

Yaritza Inostroza-Nieves1, Shakira Bou1, José Alvarado1, Diego Capo-Ruiz1,2, Jessica Garcia1, Jean P. Moliere1, Claudia P. Arenas1*

Presenter: **Yaritza Inostroza-Nieves, PhD**; Institution: **San Juan Bautista School of Medicine;** Funding: **San Juan Bautista School of Medicine Pilot Projects Program to YIN.**

See O14, Page 28 for abstract.

B 4

High Fat Diet-induced Obesity in Alzheimer's Mice is Associated with Cerebral Hypoperfusion and ETA/ETB Receptor Imbalance

Pooja Pradeep, Nicolas Ancona, Kaitlyn Pinckney, Catrina Robinson, DeAnna Baker Frost

Presenter: **Pooja Pradeep, MS**; Institution: **Medical University of South Carolina;** Funding: **Alzheimer foundation grant**

Background: Cerebral blood flow is tightly regulated for optimal brain function. It is crucial in Alzheimer's disease (AD) as impaired blood flow can promote the onset and disease progression. The Endothelin Receptor Type A (ETA) interacts with endothelin-1, facilitating vasoconstriction associated with decreased cerebral blood flow and vascular impairment in models of AD and Endothelin Receptor Type B (ETB) has a protective function by facilitating vasodilation, serving as a compensatory mechanism in response to excessive ETA activity. The balance between these receptors is critical for vascular homeostasis and may be disrupted under metabolic stress such as high-fat diet (HFD)-induced obesity. *Hypothesis:* We hypothesize that midlife obesity induced by HFD in APP/PS1 mice disrupts the ETA/ETB receptor balance in brain endothelial cells, leading to cerebrovascular dysfunction and associated cognitive and behavioral deficits. *Methods:* APP/PS1 mice were administered either a HFD or a standard diet (STD) for 12 weeks to induce midlife obesity. Cerebral blood flow was measured at baseline and 6 and 12 weeks post-diet using laser speckle. We administered behavioral tests, including novel object recognition, novel location, and open-field tests at 6 and 12 weeks. At the end of 12 weeks, we euthanized the animals and harvested brain tissue. ETA and ETB receptor expression was evaluated with immunofluorescence (IF) and co-stained with CD31, a marker for endothelial cells, in brain tissue. *Results:* There was a significant decrease in global perfusion in APP/PS1 animals on HFD over time, with a significant decrease between baseline and 12 weeks, and 6- and 12-week measurements. Specifically, there was a statistically significant decrease in perfusion in the left occipital and left temporal regions when animals fed a STD at baseline as compared to a HFD for 12 weeks. Behavioral assessments indicated a trend to declining memory performance and increased anxiety-like behavior, in the HFD group relative to the STD group by 12 weeks. IF suggests an altered ETA and ETB receptor balance in the HFD condition, where receptor colocalization with CD31 is markedly reduced—particularly for ETA —indicating a potential downregulation of ETA and a slight decrease in ETB. *Conclusion:* These findings suggest that HFD-induced midlife obesity exacerbates cerebrovascular dysfunction in an AD mouse model. The shift in ETA/ETB receptor balance within endothelial cells may contribute to endothelial dysfunction under HFD conditions. This may represent a key mechanism linking metabolic stress to cerebral hypoperfusion, leading to cognitive decline, highlighting the importance of vascular targets in the context of AD and obesity.

B 5

A Phase III Study to Assess the Safety and Efficacy of Sovateltide in Patients with Acute Cerebral Ischemic Stroke: Protocol of the RESPECT-ETB Trial

Vishnu Yelakanti, Amaresh Ranjan and Anil Gulati

Presenter: **Vishnu Yelakanti, BS**; Institution: **Pharmazz Inc.**

See O15, Page 28 for Abstract.

B 6

ENB-003, an ETBR antagonist, in combination with pembrolizumab for refractory advanced solid tumors: Topline data from the ENBolden-101 Phase 1B clinical study

Sumayah Jamal, MD-PhD, Selvaggi, G., MD

Presenter: **Sumayah Jamal, MD, PhD**; Institution: **ENB Therapeutics, Inc.;** Funding: **Trial sponsored by ENB Therapeutics, Inc.**

See O5, Page 24 for abstract.

B 7

Endothelin-1 as a Downstream Effector of Activin A Signaling in Pulmonary Arterial Hypertension: Mechanistic and Therapeutic Implications

Novia Nurul Faizah, Gusty Rizky Teguh Ryanto, Yoko Suzuki, Tetsuya Hara, Ken-ichi Hirata, Hiromasa Otake, Noriaki Emoto

Presenter: **Novia Nurul Faizah, MD**; Institution: **Kobe University**

See O7, Page 25 for abstract.

B 8

Endothelin-2 is Essential for Postnatal and Adult Lung Homeostasis: Insights from Temporal Knockout Models

Sagita Mega Sekar Kencana, Gusty Rizky Teguh Ryanto, Ahmad Musthafa, Yoko Suzuki, Tetsuya Hara, Masashi Yanagisawa, Ken-ichi Hirata, Hiromasa Otake, Noriaki Emoto

Presenter: **Sagita Mega Sekar Kencana, MD**; Institution: **Kobe University**

The precise physiological role of endothelin-2 (ET-2) in the lung remains elusive. However, its global deletion has been linked to impaired lung development and altered susceptibility to pulmonary diseases. We hypothesized that ET-2 contributes critically to lung tissue homeostasis beyond development. To investigate this, we generated tamoxifen-inducible ET-2 knockout mice (ET2-iKO) and temporally ablated ET-2 expression at multiple postnatal stages. Neonatal deletion was induced via intragastric tamoxifen administration for 3 consecutive days at the saccular (P1), early alveolar (P5), and late alveolar (P14) stages. All groups exhibited variable degrees of alveolar simplification, ultimately resulting in early lethality. In adult ET2-iKO mice (6–8 weeks old), tamoxifen was administered intraperitoneally for 5 days. Although acutely tolerated, ET2-iKO mice developed progressive emphysematous changes and lung function decline by 6 weeks post-induction. Histological analyses revealed increased collagen deposition and elastin fragmentation in the absence of overt pulmonary fibrosis, accompanied by dynamic shifts in inflammatory mediators. By 20 weeks post-induction, ET2-iKO lungs showed more extensive alveolar destruction and a marked reduction in proliferating alveolar type II cells. Collectively, our findings identify ET-2 as a critical regulator of alveolar maintenance across the postnatal and adult lifespan. Ongoing studies aim to delineate the downstream pathways involved in ET-2–mediated lung homeostasis, with implications for chronic lung disease pathogenesis and therapy.

B 9

Early Inflammatory Signatures Drive Alveolar Simplification in Endothelin-2–Deficient Lung Development

Gusty Rizky Teguh Ryanto, Ahmad Musthafa, Sagita Mega Sekar Kencana, Ratoe Suraya, Tetsuya Nagano, Masayuki Taniguchi, Tomoya Furuyashiki, Mitsuru Morimoto, Yoko Suzuki, Tetsuya Hara, Masashi Yanagisawa, Noriaki Emoto

Presenter: **Gusty Rizky Teguh Ryanto, MD, PhD**; Institution: **Kobe Pharmaceutical University**

See O6, Page 24 for abstract.

B 10

Mechanism of Porcine Coronary Arteriolar Constriction to a Clinical Level of Endothelin-1

Guangrong Lu, Xin Xu, Lih Kuo, Travis W. Hein

Presenter: **Guangrong Lu, MBBS, iMBA, MS**; Institution: **Texas A&M Health Science Center;** Funding: **NIH EY018420 (TWH) and Kruse Chair Endowment Fund (LK)**

See O10, Page 26 for abstract.

B 11

Dual endothelin-angiotensin receptor blockade improves diurnal blood pressure profile in patients with ANCA-associated vasculitis

Gavin Chapman [1] Matthew Sayer [1] Hannah Preston [1] Dan Pugh [1] Fiona Chapman [1] Vanessa Melville [1] Bruce Hendry [2] Alex Mercer [2] Tariq Farrah [1] Neeraj Dhaun [1]

Presenter: **Neeraj Dhaun, MD**; Institution: **University of Edinburgh;** Funding: **Travere Therapeutics, Inc**

See O8, Page 25 for abstract.

B 12

Sparsentan has direct effects on the glomerular capillary wall to attenuate increased permeability in nephrotic syndrome models

Michael Crompton1, Holly Stowell-Connolly1, Jack Mills1, Viktoriia Vasylchenko1, Judy J. Watson1, Elizabeth Colby1, Wilmelenne Clapper3, Celia Jenkinson3, Bruce Hendry3, Radko Komers3, Hiroshi Kawachi2, Matthew J. Butler1, Moin A. Saleem1, Gavin I. Welsh1, Rebecca R. Foster1, Simon C. Satchell1
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See O11, Page 26 for Abstract.

B 13

Comparison of endothelin-1 levels in human plasma from coronary arteries measured by enzyme linked immunosorbent assay and Olink high-throughput proteomics platform

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Presenter: **Anthony Davenport, PhD, ScD**; Institution: **University of Cambridge;** Funding: **PlaqueTec Ltd, the National Institute for Health and Care Research, Cambridge Biomedical Research Centre Pump Priming Award and the Royal Papworth Innovation fund.**

The Olink Explore 3072 high-throughput proteomics platform enables simultaneous quantification of ~3000 proteins typically in six µl of human plasma. This technology is increasingly applied in clinical trials and large-scale biobanks. For example, Sun et al. (Nature 2023,622:329–338) demonstrated in ~50,000 UK Biobank participants an association between plasma 'EDN1' and over 70 diseases. Olink uses paired antibodies targeting recombinant protein encoded EDN1 (preproET-$1_{169–212}$), each conjugated to unique DNA oligonucleotides that are amplified and quantified. In addition to ET-1, EDN1 also encodes N-terminal proET-1, endothelin-like domain peptide, and C-terminal proET-1 that are expressed by endothelial cells and may contribute to the Olink signal. Our aim was to validate the Olink assay to enable interpretation of these studies. We assessed the concordance between Olink-derived EDN1 values (Olink Explore 3072 in ≤6 µl of plasma) versus the well-validated Quantikine ELISA specific for biologically active ET-1 (using 150 µl plasma) in coronary samples from 29 patients with stable coronary artery disease. There was a significant correlation between Olink and ELISA measurements ($r = 0.53$, $p = 0.003$) and Olink values significantly predicted ELISA results ($p = 0.003$). Bland-Altman analysis demonstrated good agreement between the two assays, with no evidence of systematic bias. These findings support the use of the Olink assay for detecting changes in plasma levels of ET-1 in pathophysiological conditions. Olink-derived EDN1 values may serve as a surrogate biomarker for ET-1 release in clinical and translational research settings.

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

B 14

Localization of the therapeutic targets for endothelin receptor antagonists and sodium-glucose co-transporter 2 inhibitors in the chronic liver disease, primary sclerosing cholangitis.

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Presenter: **Anthony Davenport, PhD, ScD**; Institution: **University of Cambridge;** Funding: **Astra Zeneca, Wellcome Trust Programme in Metabolic and Cardiovascular Disease (203814/Z/16/A, A.P.D, T.L.D); Cambridge Biomedical Research Centre Biomedical Resources Grant (University of Cambridge, Cardiovascular Theme)**

Primary sclerosing cholangitis (PSC), chronic liver disease of unknown cause, contributes to cirrhosis and cancer but has no cure. PSC is characterized by inflammation with ductal fibrosis, and progressive bile duct narrowing and loss, with damage to cholangiocytes, epithelial cells affecting bile production and liver repair. ET-1, produced by cholangiocytes, contributes to fibrosis, vasoconstriction, and inflammation via ETA receptors. In patients, ET-1 and ETA gene expression are elevated and ETA antagonists reduce disease progression in PSC animal models. A new treatment strategy combining ETA-selective antagonist zibotentan with SGLT2 inhibitor dapagliflozin, to reduce fluid retention, is currently being tested in clinical trials of portal hypertension in liver disease. We compared the localization of ET receptors and SGLT2 transporter to assess the feasibility of this therapeutic strategy. In ethically sourced healthy human liver, ETA immunofluorescence was primarily found in bile duct epithelial cells within the portal tract, smooth muscle of the central vein, with low levels in hepatocytes. SGLT2 immunofluorescence was mainly detected on bile duct epithelial cells and hepatocytes. ETA co-localized with smooth muscle cells in large arteries and veins, while ETB immunoreactivity was present in hepatocytes and endothelial cells. Crucially, both drug targets are therefore retained in the key hallmarks of PSC pathology, ETA and SGLT2 staining within cholangiocytes undergoing ductal transformation and cells within the fibrotic septa, supporting the proposed benefit of combination treatment strategy.
The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

B 15

Analysis of the expression of genes encoding the endothelin signalling pathway using single-cell RNA sequencing (scRNA-seq) in human heart.

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We analysed the expression of ET pathway signalling genes in single-cell RNA sequencing data from adult hearts (n = 14, Nextera scRNA-seq) obtained with ethical approval and informed consent. Of the receptors, EDNRA was expressed by all contractile cells: cardiomyocytes in all four chambers, smooth muscle cells, and pericytes. These results are consistent with the physiological role of ET-1 acting principally by ETA receptors on cardiomyocytes to increase cardiac contraction, and on smooth muscle to constrict coronary vessels. The gene encoding ETB receptor, EDNRB, was localised principally to endothelial cells of the arterial and venous endothelium including the microvasculature, mixed clusters of fibroblasts, epicardial mesothelial clusters and vascular endothelial cells. EDN1 was the most abundantly gene of the three isoforms, seen in endothelial cells of larger vessels and lymphatics as well as in endocardial endothelial cells. The results provide information on the expression of genes encoding the ET signalling pathway within individual cells of the heart.

B 16

Endothelin type B receptor activation in the paraventricular nucleus of hypertensive DOCA salt rats. Beneficial role at the cardiovascular level

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Presenter: **María Florencia Fernandez, BS**; Institution: **Institute of Immunology, Genetics and Metabolism, UBA-CONICET;** Funding: **UBA, CONICET and ANPCyT**

The paraventricular nucleus (PVN) regulates cardiovascular physiology and expresses both endothelin receptors (ETA and ETB). At the peripheral level ETB activation exerts cardiovascular protection however at the brain level its effect is unknown. Several reports support that central mechanisms trigger sympathetic overstimulation leading to hypertension and later deleterious heart effects like ventricular hypertrophy and cardiac dysfunction. Additionally, hypertension is associated with chronic inflammation in diverse tissues and organs involved in cardiovascular regulation. The aim of the present study was to evaluate in the paraventricular nucleus (PVN) of DOCA-salt hypertensive rats the role of ETB activation on cardiac hypertrophy and fibrosis, the inflammatory response and redox status. Hypertension was induced was induced by weekly subcutaneous injections of 30 mg/kg DOCA and the administration of 1% saline in the drinking water for 5 weeks. At week 4 a group of animals received BQ-3020 through an osmotic pump via cannulas placed in both PVN. Systolic blood pressure (SBP) and heart rate (HR) were assessed by plethysmography and left ventricular (LV) function by echocardiography. Following euthanasia, the left ventricle was removed for later studies. ETB activation by BQ-3020 decreased SBP and heart rate in DOCA-salt hypertensive rats. Further BQ-3020 reduced hypertrophy and fibrosis and improved cytokine balance (anti-inflammatory vs. proinflammatory). Superoxide dismutase and catalase activities were reduced following BQ-3020 administration, but carbonyl content remained unchanged. Present findings show that BQ-3020 in the PVN of hypertensive rats decreased SBP and HR, the cardiac inflammatory response and oxidative stress and improved cardiac hypertrophy and fibrosis supporting a beneficial effect of brain ETB activation in hypertension.

B 17

Ethnic differences in associations of Endothelin-1 and novel Renin-Angiotensin-Aldosterone System biomarkers in Hypertensive individuals

Aditya Sharma, Ian Wilkinson, Anthony Davenport, Luca Faconti, Phil Chowienczyk, Spoorthy Kulkarni

Presenter: **Aditya Sharma** Institution: **University of Cambridge**

See O12, Page 27 for abstract.

B 18

Lack of the ET-1/ETA axis on dendritic cells results in ameliorated hypertension in males, but not females

Virginia Beasley, Abigail J. Brooks*, Sara N. Biswal, Emma Q. Rosenkoetter, Melissa Rodriguez and Carmen De Miguel*

Presenter: **Virginia Beasley, BS**; Institution: **University of Alabama at Birmingham;** Funding: **UAB/UCSD O'Brien Center Summer Research Scholar Training U54 DK137307 to VB, Deep South KUH PRIME U2C DK133422 & TL1 DK139566 to AJB, and K01HL145324, R25 HL145817 Future Faculty of Cardiovascular Disease (FOCUS) and UAB Diabetes Research Center pilot funding**

See O27, Page 36 for abstract.

B 19

Pregnancy regulates renal endothelin-1 and aldosterone signaling systems in aged female mice lacking G protein-coupled estrogen receptor

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See O21, Page 32 for abstract.

B 20

Endothelium-derived ET-1 stimulates Th17 cell expansion in a sex-dependent manner under high-salt diet

Tha Luong, Patrick A. Molina, Sara N. Biswal, David M. Pollock, Jennifer S. Pollock, and Carmen De Miguel.

Presenter: **Tha Luong, MS** Institution: **University of Alabama at Birmingham;** Funding: **U2C/TL1 Deep South KUH PRIME U2C DK133422 & TL1 DK139566 from the NIH/NIDDK to TL, NIH F31 HL151264-0 to PAM, P01HL136267 to JSP and DMP, and K01HL145324 to CDM.**

See O24, Page 34 for abstract.

B 21

Pathological Upregulation of Endothelin Receptor A Signaling In Systemic Lupus Erythematosus

Marice K. McCrorey, B.S., Helen M. Butler, Ph.D., Yasir Abdul, Ph.D., Adviye Ergul, M.D. Ph.D., Melissa A. Cunningham, M.D. Ph.D., Jim C. Oates, M.D. Ph.D., Justin P. Van Beusecum, Ph.D

Presenter: **Marice McCrorey, PhD;** Institution: **Medical University of South Carolina**
Funding: **VA BLRD IK2BX005605, P30AR072582, T32GM132055-5, U54DA016511-22, T32-AR050958-19, 25PRE1372738, LRA Innovation Award**

See O22, Page 33 for abstract.

B 22

Sex-Specific Mitochondrial Metabolic Signatures in Human Renal Tissues

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Presenter: **Marharyta Semenikhina, MS, PhD**; Institution: **Medical University of South Carolina**

Background: The kidney is a mitochondria-rich organ with regionally distinct metabolic demands, as the cortex and medulla differ in function, transport, and energy requirements. Importantly, sex differences in mitochondrial function between men and women are increasingly recognized in the kidney. Our studies in rodents suggest that male renal mitochondria exhibit higher respiration, although mitochondrial efficiency is higher in females. ROS production and substrate preference also differ between male and female mitochondria. However, these mitochondrial differences have not been defined in human kidneys, and the metabolic basis for sex-specific mitochondrial function remains uncharacterized. To address this gap, we hypothesized that mitochondrial metabolic profiles differ by sex and region in the human kidney, independent of comorbidities. *Methods:* Targeted metabolomic analysis of mitochondria-related metabolic pathways using the kidney cortex was performed. Kidney cortex and medulla from 8 male and 5 female human subjects, average age 54.3±2.7 years, were analyzed. A semi-automatic approach was used for the analysis of pathway-specific alterations. Metabolic profiles were generated using UHPLC-HRMS; metabolites were identified in El-MAVEN using exact mass and retention time with an in-house library. MetaboAnalyst 6.0 was used for statistical analysis (cutoff fold change 0.5, $p<0.05$). *Results:* Targeted mitochondrial metabolomics revealed pronounced sex differences in metabolic pathways in the human kidney, particularly within the cortical region. In the cortex, females and males differed in amino acid and nucleotide-related pathways, including arginine biosynthesis ($p<0.001$), pyrimidine metabolism ($p<0.001$), alanine, aspartate and glutamate metabolism ($p<0.001$), and one-carbon metabolism via folate ($p<0.01$). Additional sex-dependent alterations were observed in pathways involved in cysteine and methionine ($p<0.01$), phenylalanine ($p<0.05$), and glycine metabolism ($p<0.01$), highlighting broad differences in nitrogen handling and redox balance. In the medulla, sex differences were also detected in purine and pyrimidine metabolism ($p<0.001$), one-carbon metabolism ($p<0.01$), and several overlapping amino acid pathways. As expected, both sexes exhibited strong regional metabolic variations between the cortex and medulla. *Conclusion:* Our data demonstrate that mitochondrial metabolism in the human kidney exhibits clear sex-specific signatures, particularly within the cortical region. These differences suggest distinct mitochondrial bioenergetic and biosynthetic profiles between male and female kidneys and provide a critical foundation for understanding sex-specific susceptibility to kidney injury.

B 23

Restoration of Endothelial Function Prevents Microemboli-mediated Dysregulation of Endothelin Receptors in Diabetes: Relevance to Vascular Contributions to Cognitive Impairment (VCID)

Weiguo Li, Eda Karakaya, Jazlyn Edwards, Sarah Jamil, Yasir Abdul, Justin Van Beusecum, Adviye Ergul

Presenter: **Weiguo Li, MD, PhD**; Institution: **Medical University of South Carolina;** Funding: **NIH, AHA, VA Merit Grants**

Cerebrovascular dysfunction, promoting brain hypoxia, is a common finding in all forms of dementia. Post-mortem brain levels of endothelin-1 (ET-1), the most potent vasoconstrictor, correlate with tissue hypoxia and disease severity in patients with dementia. However, the relative roles of ET receptors remain unknown. We reported that the restoration of endothelial function by the combination of isosorbide mononitrate (ISMN) and cilostazol (CZL), a treatment paradigm we adapted from the LACI clinical trials, prevents microemboli (ME)-mediated VCID in diabetes. Given that diabetes mediates early endothelial dysfunction, activates the ET system, and doubles the risk of VCID, the goals of the current study were to investigate 1) the relationship between the brain ET system and the degree of hypoxia and neuroinflammation in a clinically relevant model of VCID, and 2) the impact of improving endothelial function on the brain ET system. At 10 weeks after the onset of diabetes, control and diabetic rats received cholesterol crystal ME (40-70 μm) injections and were monitored for 16 weeks. Another cohort received ISMN (75 mg/kg/day) and CZL (60 mg/kg/day) combination treatment for 2 weeks before the ME injection. Behavioral tests included novel object recognition (NOR) and Y-maze. After termination, brain tissue levels of ET-1/ETAR/ETBR, hypoxia markers myelin-associated glycoprotein (MAG), proteolipid protein-1 (PLP-1), and hypoxia-inducible factor-1 (HIF-1), and microglia activation were analyzed by ELISA and immunoblotting. Diabetic animals had baseline deficits in specific cognitive domains that progressively worsened, and treatment with (ISMN/CZL) prevented decline in cognitive function. HIF-1 levels were higher, and the MAG/PLP-1 ratio was lower in the diabetes ME cohort, indicating tissue hypoxia. ISMN/CZL treatment significantly prevented brain hypoxia. While there were no ME-mediated changes or differences between control and diabetic groups in plasma and brain tissue ET-1 levels, brain ET-1 levels correlated with HIF-1 ($r^2=0.53$, $p=0.0073$) and MAG/PLP ($r^2=0.45$, $p=0.0167$). ETAR levels did not change with ME, but treatment reduced expression. On the other hand, ETBR expression was lower in the sham diabetic group than in sham controls, and ME injection did not have a further effect. The treatment restored ETBR expression to comparable levels in the control sham group. Strategies to improve vascular function by modulation of the ET system blockade can be a preventive and therapeutic strategy for VCID.

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