Skaggs School of Pharmacy and Pharmaceutical Sciences
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Annual Research Retreat

August 1-2, 2024
Keystone, CO

Poster Presenters

Name (First)	Name (Last)	Station	Poster Title
Lauryn	**Adair**	1	Translocator Protein Ligands Inhibit Hyperexcitability and Metabolic Deficits in a Zebrafish Model of Dravet Syndrome
Sarah	**Asby**	2	Development of Novel Detection Methods for Immune Checkpoint Inhibitor-Mediated Nephrotoxicity in Cancer Patients
Stephanie	**Bersie**	3	Differential Handling of Necrotic versus Apoptotic Particulate-Laden Cellular Corpses within Phagocytes
Daniel	**Breiner**	4	Hemin Alters Pseudomonas aeruginosa Alkyl Quinolone Production
Robert	**Canfield**	5	Nanoparticle delivery of Nucleic Acids to Induce a Type 1 Interferon Response in Bladder Cancer
Nai-Chia	**Chen**	6	Association of Time in Range and the Risk of Retinopathy Among Patients with Type 1 Diabetes
Sophia	**Clune**	7	The Efficient Synthesis of CHD1L Inhibitor OTI-1100 & Derivatives as Novel Cancer Therapeutics
Bella	**Coenen**	8	Metabolomics-Based Identification of Blueberry Compounds in the Serum and Urine of Infants Consuming Blueberries as a First Food
Mouna	**Dardouri**	9	Changes in Utilization of Prescription Medications in Colorado During The COVID-19 Pandemic, 2019 – 2021: An Interrupted Time Series Analysis Using an ARIMA Model
Baharak	**Davari**	10	Immunosuppressive Activities of Sirolimus Metabolites and its Degradation Product
Anna	**Figueroa**	11	Bioenergetic Alterations and Redox Control in an in vitro model of Neuronal Hyperexcitability
Hanmant	**Gaikwad**	12	In vivo painting of tumors with cyanine lipids: structure-activity relationship
Paola	**Garcia Gonzalez**	13	Oxidative Stress Leads to An Increase In GFAP and Vimentin Expression
Shilpa	**George**	14	Phage-Like Particles for Ocular Drug Delivery: Plasmon-waveguide Resonance Spectroscopy and Evaluation Using In Vitro and Ex Vivo Cornea Models
Matthew	**Gibb**	15	Lung Inflammation and Pathology are Mast Cell Dependent in a Model of Formaldehyde and Chloropicrin Toxicity

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UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

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Hari	**Gopalakrishnan**	16	Nickel agarose microbeads for testing joint implant hypersensitivity
Emily	**Gracey**	17	Development of a CYP3A7 Fetal/Neonatal Hepatocyte-like Cell Model Derived from Induced Pluripotent Stem Cells
Jason	**Groegler**	18	Beta Cell Stress triggers formation of Hybrid Insulin Peptide through granular pH modulation
Brianna	**Hunt**	19	A Sample-Type Specific Database for Improved Fecal Metabolomics Compound Identification (A "Crappy" Rotation)
Rama	**Kant**	20	Treatment with dexamethasone ameliorates arsenical vesicant lewisite-induced toxic corneal pathophysiology
Angi	**Li**	21	Structure based design of T cell receptor like antibody against disulfide modified autoantigens of type 1 diabetes
Alexandra	**Lucas**	22	Examining the impact of NTD mutations on RBD conformational dynamics and ACE2 receptor binding affinity of the SARS-CoV-2 spike protein
Mahesh	**Maiyani**	23	Impact of COVID-19 on Timeliness of Receiving Systemic Therapy for Patients Diagnosed with Lung Cancer
Chanapa	**Mann**	24	Characterization of neuron-specific Scn1a knockout mice: A novel model for Dravet syndrome
Nicole	**McDaniel**	25	Preemptive return of clinical pharmacogenomic results to over 50,000 individuals in a population-scale biobank: phenotype and actionable medication exposure characteristics
Courtney	**McGinnis**	26	Acetylation of Proximal Cysteine-Lysine Pairs by Alcohol Metabolism
Nick	**Mendola**	27	Network Meta-analysis of Treatment Options for Adults with AQP4-IgG Seropositive Neuromyelitis Optica Spectrum Disorder (NMOSD)
Neha	**Mishra**	28	Assessing dexamethasone efficacy in treating arsenical vesicant lewisite-induced clinical impairments in rabbit corneas
Casey	**Patrick**	29	Functional Differences in the Mu Opioid Receptor SNP A118G are Dependent on Receptor Splice-Variant and Agonist Specific Interactions

Skaggs School of Pharmacy and Pharmaceutical Sciences
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Annual Research Retreat

August 1-2, 2024
Keystone, CO

Poster Presenters

Alaa	**Radwan**	30	Evaluation of Pharmacogenomic Medication Prescribing Patterns in Rural Versus Urban Settings
Shashikant	**Ray**	31	Probing the Structural and Functional Impact of Mutations in the C-Terminal Domain of Dystrophin
Angela	**Reinert**	32	Sugarcane Ash-Derived Silica Nanoparticles Induce Pulmonary Inflammation
Rick	**Reisdorph**	33	SSPPS Mass Spectrometry Core Facility
Madison	**Ricco**	34	Development and Implementation of a Holistic Course Review Process Embracing Collaboration between Assessment and Curriculum Committees
Gabriela Terue	**Rizzo Kodama**	35	Investigation of the Cytotoxic and Genotoxic Activity of the Antidepressant Vortioxetine in Human Renal Carcinoma Cell Line (786-0)
Kyle	**Ross**	36	Mechanistic Basis for Neuropathological Distributions of Dynamin-Related Protein 1, DRP1, in Mitochondrial Fission
Emily	**Ruggiano**	37	Investigating the Role of Chloropicrin in non-IgE-Mediated Mast Cell Degranulation
Lauren	**Rutt**	38	Investigating Wnt3a and Wnt5a as Factors of Hepatic Stellate Cell Activation in Models of Alcohol-associated Liver Disease
Stefanie	**Schwab**	39	Factors Affecting TFV-DP Concentrations in PBMC and Relationships with DBS in PWH on TAF-Based ART
Julieta	**Torres**	40	Per- and Polyfluoroalkyl Substances (PFAS) Inhibit Human Carboxylesterase I and II
Damien	**Trujeque**	41	Terminase Enzymes and Their Role in the Assembly of dsDNA Viruses
Shannon	**Twardy**	42	A Role for the C5 Complosome in Kupffer Cell Hyperpolarization in Alcohol-Associated Liver Disease
Vaibhav	**Upadhyay**	43	Biophysical evolution of the receptor-binding domains of SARS-CoVs
Zixuan	**Wei**	44	Pharmacokinetics of Isoniazid Metabolites During Pregnancy and Postpartum
Jon	**Woodward**	45	Next-generation PFAS and SIRT3: Defining Novel Mechanisms of PFAS Hepatotoxicity
Ram	**Nagaraj, Ph.D.**	46	Protein Aging by Glycation: Role of Thiols

Skaggs School of Pharmacy and Pharmaceutical Sciences

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

Annual Research Retreat

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Keystone, CO

Poster Presentation-Abstracts

1. **Translocator Protein Ligands Inhibit Hyperexcitability and Metabolic Deficits in a Zebrafish Model of Dravet Syndrome**

Lauryn Adair, Rebecca Han, Ruth Fulton, Anna Figueroa, and Manisha Patel

Dravet syndrome (DS), is a form of epilepsy commonly associated with de novo heterozygous loss-of function variants in the sodium voltage-gated channel Nav1.1 gene, SCN1A. Using a drug screening approach, we have previously identified PK11195 as a potential therapeutic agent that rescues metabolic deficits and seizures in a scn1Lab Zebrafish (ZF) model (Banerji et. Al., 2021). PK11195 is a ligand of the translocator protein (TSPO) which resides on the outer mitochondrial membrane and has been widely accepted as a biomarker for neuroinflammation. In this study we evaluated additional TSPO ligands for their therapeutic potential to modulate behavioral and electrographic activity in the scn1Lab mutants. First, we tested Etifoxine and XBD173, two TSPO ligands, to assess their ability to decrease seizure-like behavioral activity. We found that Etifoxine and XBD173 significantly reduced seizure-like swim behavior. Next, we utilized an multielectrode array system and found that Etifoxine and XBD173 decreased neuronal spikes and bursts resembling neuronal hyperexcitability in a ZF and primary cerebrocortical cultures. Furthermore, similar to PK11195, XBD173 significantly reversed metabolic deficits in the scn1Lab ZF. The data suggest that TSPO ligands show promise in a translational ZF model of DS and may be novel therapeutic entities for its treatment.

Supported by : NINDS grant 5R01 HD102071 (MP).

2. **Development of Novel Detection Methods for Immune Checkpoint Inhibitor-Mediated Nephrotoxicity in Cancer Patients**

Sarah Asby, BS, Lauren E. Thompson, PhD, Scott Tilden, PhD, Jordi Lanis, PhD, Zander Kostka-Newman, BS, Cindy O'Bryant, PharmD, Sarah Lindsey Davis, MD, Roberta Pelanda, PhD, Julie Lang, PhD, Lauren M. Aleksunes, PharmD, PhD, Melanie S. Joy, PharmD, PhD

Kidney immune-related adverse events (irAEs) following cancer treatment with immune checkpoint inhibitors (ICIs) have been observed in 5-25% of patients, with approximately 20% developing new onset chronic kidney disease within 5 years. Current measures of AKI using creatinine-based assessments have been found to under-estimate renal damage. As a result, the subclinical incidence of ICI-mediated nephrotoxicity is unknown, with no qualified assays or biomarkers in AKI staging. To address this significant problem, this study aimed to develop and assess methods to detect ICI-mediated nephrotoxicity in cancer patients receiving ICI treatment. As part of an ongoing study COMIRB 23-0710, cancer patients undergoing their first or second cycle of nivolumab, pembrolizumab, or ipilimumab (alone or in combination) had urine collected at 4 intervals: baseline, subclinical risk, clinical risk, and follow up periods. Urine was processed and assessed using dipstick urinalysis, sediment microscopy, targeted biomarker assays, and isolation and quantification of total cfDNA. Urine samples were additionally processed and stored for future biomarker discovery assessments. Although changes were noted over time in the several assessments of nephrotoxicity of patient urine samples, further analysis and validation is needed to qualify these methods of detecting ICI-mediated nephrotoxicity.

3. **Differential Handling of Necrotic versus Apoptotic Particulate-Laden Cellular Corpses within Phagocytes**

Stephanie Bersie, Hope Chatwin, Shannon Hott, Alexandra McCubbrey

The human lungs are exposed to inhaled xenobiotic materials with each breath. A significant portion of inhaled particulate matter (PM) makes it deep into the lower airways, reaching the alveoli, where it is engulfed by alveolar macrophages (MΦ). When MΦ die during cell turnover, their corpse is engulfed by nearby, living phagocytes. This study asks what happens to non-degradable PM contained a MΦ when it dies and how the mode of death impacts material transfer. Although apoptotic cell clearance is a well-studied cell death mechanism, necrotic clearance remains poorly understood, and the functional consequences of MΦ engulfment of PM-laden corpses is unknown. This study shows that MΦ respond preferentially to necrotic corpses, with higher engulfment and upregulation in proinflammatory, redox, and metal response genes. This transcriptional response is amplified in the presence of PM, reduced by antioxidant and metal chelators. We additionally observed unique modes of PM transfer from corpse to MΦ that were specific to corpse type. Dendritic cells (DCs), specifically a rare subset called plasmacytoid DCs, also showed a preferential response to necrotic corpses, with higher rates of engulfment and upregulation of oxidative stress and metal response genes. Future studies will address the DC response to PM-laden corpses.

4. **Hemin Alters Pseudomonas aeruginosa Alkyl Quinolone Production**

Daniel Breiner, Rachel Neve, Brent Carrillo, and Vanessa Phelan

Pseudomonas aeruginosa is an opportunistic pathogen that frequently infects people with compromised immune systems, including individuals with cystic fibrosis (CF). CF is a genetic disease marked by thick mucus, or sputum, buildup in the lungs. Sputum poses a two-fold issue: it impedes mucociliary clearance of pathogens and acts as a substrate for their growth-- providing an ideal environment for pathogens to flourish. *P. aeruginosa* is the most prevalent pathogen in CF lungs[1]. Iron is a critical nutrient for *P. aeruginosa* and higher concentrations of iron in CF sputum are correlated to increased *P. aeruginosa* population and decreased lung function.[2,3,4] Importantly, iron is a regulator *of P. aeruginosa* small molecule virulence factor production and these metabolites are critical in mediating microbe-microbe and microbe-host interactions in the CF lung.[5] Most *in vitro* studies investigating the influence of iron on *P. aeruginosa* virulence are performed using media containing free iron. However, *in vivo* iron is bound to host proteins to provide an extra layer of defense against invading pathogens via nutritional immunity. Therefore, we explored the effect of CF and healthy host iron-binding protein profiles on the production of *P. aeruginosa* small molecule virulence factors.

5. **Nanoparticle delivery of Nucleic Acids to Induce a Type 1 Interferon Response in Bladder Cancer**

Robert Canfield, Carlos Catalano, and Jared Brown

Bladder cancer is the sixth most common cause of tumor formation in the United States and the thirteenth leading cause of cancer-related deaths worldwide. Current therapies often rely on utilizing the bladders' immune responses or introducing live attenuated vaccines, such as Bacillus Calmette–Guérin (BCG). We created conjugates of the lambda phage decoration protein (gpD) with Cetuximab, a 45bp duplexed DNA strand, and fluorescein. Lambda phage particles were then decorated with all of these conjugates at a ratio of 5% Cetuximab 20% DNA and 25% fluorescein We also utilize Nano Lipid Carriers (NLC) with the same 45bp DNA or a small activating RNA (saRNA) activating the STING and RIG-I pathways respectively for a type 1 interferon response. Bladder cancer cells treated with nucleic acid conjugated particles demonstrate an increased production of interferon beta by qPCR and ELISA. Bladder cancer cells treated with the nano lipid particles also exhibit a decrease in viability and proliferation at higher concentrations. This data suggests that phage-like and lipid nanoparticles can be decorated with nucleic acids to induce a type 1 interferon response in bladder cancer cells.

6. **Association of Time in Range and the Risk of Retinopathy Among Patients with Type 1 Diabetes**

Nai-Chia Chen, MS; Eric Gutierrez, MPH; R. Brett McQueen, PhD

Objective: Rigorous evidence has established the benefits of continuous glucose monitoring (CGM) on improving glycated hemoglobin (HbA1c), but CGM metrics have yet to be used in regulatory approval studies. Our objective was to evaluate the association between continuous glucose monitoring (CGM) metrics (e.g., time in range [TIR]) and onset of diabetic retinopathy.

Methods: This was a single-center retrospective case-control study among patients with type 1 diabetes (T1D) at the University of Colorado Barbara Davis Center for Diabetes. Cases were defined as incident diagnoses of diabetic retinopathy. The 7-year CGM data was re-analyzed using a fixed-effects parametric survival-time model. Outcomes reported are hazard ratios with 95% confidence intervals

Results: A total of 162 subjects with T1D (average duration 13.7 years) were analyzed retrospectively. Mean baseline HbA1c was 8.3% for cases and 7.3% for controls. Both cases and controls showed a decreasing trend in TIR (70 – 180 mg/dL) over time. Controlling for T1D duration and baseline HbA1c suggests when the average TIR is above 70%, every 5% increase in TIR can reduce the risk of retinopathy (HR=0.82, 95% CI: 0.79, 0.85).

Conclusion: Maintaining an average TIR above 70% is significantly associated with a reduced risk of developing diabetic retinopathy in patients with T1D.

7. **The Efficient Synthesis of CHD1L Inhibitor OTI-1100 & Derivatives as Novel Cancer Therapeutics**

Sophia Clune, Paul Awolade, Qiong Zhou, Timothy Kellett, and Daniel LaBarbera

CHD1L inhibitors (CHD1Li) have proven to be a compelling new anti-tumor treatment strategy, particularly for colorectal cancer. Although these compounds can take a variety of different chemical structures, we recently reported a pyrazolopyrimidine among these novel leads. This compound, OTI-1100, reverses epithelial mesenchymal transition, inhibits cancer cell stemness, and induces cytotoxicity in tumor organoids. Several pyrazolo[1,5-a]pyrimidines have previously been identified as promising potential anti-tumor agents; however, this was the first time one has been identified as an inhibitor of CHD1L. Herein, we describe the optimization of the synthesis procedure for CHD1Li OTI-1100. We demonstrate that this compound can be produced using a simple and efficient 3 step synthesis scheme that can be adapted to accommodate the production of analogs that share the same core structure. Likewise, this study explores the potential for derivatization of the OTI-1100 pharmacophore to improve therapeutic efficacy and elucidate its structure activity relationship (SAR) with CHD1L.

8. **Metabolomics-Based Identification of Blueberry Compounds in the Serum and Urine of Infants Consuming Blueberries as a First Food**

Bella Coenen[1], Minghua Tang[2], Gabby Glime[2], Katrina Doenges[1], Cole Michel[1], Nichole Reisdorph[1], Richard Reisdorph[1]

[1] *The Skaggs School of Pharmacy and Pharmaceutical Sciences, Mass Spectrometry Core*
[2] *The University of Colorado Anschutz School of Medicine, Pediatric Nutrition*

Blueberry has been known for its anti-inflammatory effects in adults and animal models. A double-blinded study was conducted with 2 groups: infants consuming a freeze-dried blueberry powder or a placebo powder. Metabolomics using high resolution mass spectrometry was performed on blueberry powder, serum, and urine from infants and on breast milk samples from participants. Serum, urine, and breast milk data were mined to determine the presence of blueberry powder compounds. Analysis of blueberry powder compounds in urine samples found 15 statistically significant ($p \leq 0.05$) compounds of interest that were elevated in the group consuming blueberry powder. Two of these compounds are related to inflammation and immune function. Analysis of the full urine and serum datasets was used as an unbiased approach to reveal compounds of interest that were elevated in the group of infants who consumed blueberry powder. The preliminary identities of significant compounds, their relationship to metabolism and immune function, and methods used for data analysis will be presented.

9. Changes in Utilization of Prescription Medications in Colorado During The COVID-19 Pandemic, 2019 – 2021: An Interrupted Time Series Analysis Using an ARIMA Model

Mouna Dardouri, PharmD, MPH[1], Garth Wright, MPH[1], Joseph Sasseen, PharmD[1,2], Kavita V. Nair, PhD, FAAN[1,3], Kelly E. Anderson, PhD, MPP[1]

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[2] *Department of Family Medicine, CU Medicine, Aurora, CO*
[3] *Department of Neurology, CU Medicine, Aurora, CO*

Background: The COVID-19 pandemic caused significant disruptions across the U.S. health care system, with many individuals forgoing health care including prescription medications.

Objective: To quantify the extent of forgone care for prescription medications among Colorado residents during the COVID-19 pandemic.

Methods: We analyzed Colorado all-payers claims data from January 2019 to December 2021, focusing on adults (age 18+) with health insurance coverage for at least one month during the study period. Variables included patient information, insurance and prescription details. We conducted an Interrupted Time Series (ITS) analysis using an Auto Regressive Moving Average Model to assess the changes in the number of monthly prescriptions before and after the COVID-19 pandemic onset on March 1, 2020.

Results: The study cohort included 84,405,430 pre-pandemic and 151,543,495 post-pandemic total member months. There was a significant decrease in the proportion of all drugs prescribed per monthly eligible cohort by 4.5% (95% CI: [-6.3% to -2.7%], $p < 0.001$), retail drugs by 4.35% (95% CI: [-6.47% to -2.22%], $p < 0.001$), antibiotics by 0.24% (95% CI: [-0.37% to -0.12%], $p < 0.001$), and opioids by 0.10% (95% CI: [-0.16% to -0.05%], $p < 0.001$). No significant change was observed for physician-prescribed drugs, cardiometabolic system drugs, or mental health medications.

Conclusion: The COVID-19 pandemic resulted in a significant reduction in prescription medication fills in Colorado, particularly in retail drugs, antibiotics, and opioids. Further investigation into the long-term effects of this forgone care is needed.

10. Immunosuppressive Activities of Sirolimus Metabolites and its Degradation Product

Baharak Davari, MS, Jelena Klawitter, PhD, Uwe Christians, MD, PhD

Sirolimus (rapamycin, SRL), is utilized in clinical settings as an immunosuppressant and anti-proliferative agent in cardiovascular devices. The primary mechanism of action of SRL inhibits the mTOR pathway. Despite its longstanding clinical use of 30 years, comprehensive knowledge of its human drug metabolism remains incomplete. SRL primarily undergoes metabolism by cytochrome P450 3A enzymes in both the liver and intestine. The pharmacodynamic and toxicodynamic effects of the resulting metabolites and major degradation product on the mTOR pathway are entirely unknown.

In this research, Sirolimus metabolites and its degradation product were generated using human liver microsomes and subsequently isolated using high-performance liquid chromatography with ultraviolet detection. The purity and identification of these metabolites were rigorously confirmed using quadrupole time-of-flight mass spectrometry. Prostate cancer cells were utilized to investigate the effects of these key compounds on the mTOR pathway. The findings indicate that these metabolites exhibit activity and can potentially inhibit the mTOR pathway, underscoring their pharmacological significance and potential therapeutic implications.

11. Bioenergetic Alterations and Redox Control in an in vitro model of Neuronal Hyperexcitability

Anna G. Figueroa, Christopher. Q. Huynh, Manisha Patel

Epilepsy is a common, chronic, and disabling neurological disorder that is widely associated with excessive neuronal activity. Metabolic regulation of neuronal excitability is partly understood through the effectiveness of dietary therapies that control seizures, like ketogenic diets (KD). Interestingly, glycolytic rates sharply increase during epileptic seizures, and inhibition of glycolysis with 2-deoxyglucose (2-DG) has anti-seizure effects. In this study, we explore the relationship between neuronal excitability and bioenergetic changes.

Mixed rat primary cortical cultures were exposed to 4-aminopyridine (4-AP), a non-selective potassium channel blocker. Neuronal excitability was measured using a multiple electrode array system. The rates of glycolysis and mitochondrial respiration were measured using an Agilent Seahorse XFe Analyzer.

4-AP induced neuronal-hyperexcitability and glycolysis, evidenced by a 250% increase in spontaneous neuronal-spiking and a 150% rise in glycolysis. Pre-treatment with 2-DG reduced these effects. Notably, hyperexcitability resulted in global ATP reduction, with mitochondrial ATP most affected. Furthermore, hyperexcitability caused mitochondrial dysfunction evidenced by a 60% increase in proton-leak respiration and reduction in maximal respiratory capacity. Interestingly, pre-treatment with 2-DG restored most parameters of mitochondrial function to baseline after 4-AP treatment.

While preliminary, these data suggest neuronal hyperexcitability induce bioenergetic alterations, which were reversed by pre-treatment with an anti-seizure drug 2-DG.

12. In vivo painting of tumors with cyanine lipids: structure-activity relationship

Hanmant Gaikwad[1,2,3*] Ashlynn Barnes[1,2*], David Siegel[1,2], Benedikt Haupt[4,5], Irina Balyasnikova[4,5] and Dmitri Simberg[1,2,3]

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Lipid nanoformulations, including liposomes, micelles, lipid nanoparticles, emulsions, and lipid prodrugs, have demonstrated promising potential as tumor drug delivery systems. The effect of lipid structure on accumulation in tumors remains poorly understood. Here, we designed a small library of fluorescent cyanine Cy3 lipids with various modifications of lipid tail and linkers. Utilizing the same fluorophore headgroup allowed us to compare the circulation half-life, stability in plasma and tissues, biodistribution, and tumor accumulation. The lipids were formulated with DSPE-PEG2000 into colloidally stable PEGylated nanoparticles and administered intravenously in orthotopic 4T1 breast cancer-bearing mice. Stable indocarbocyanine lipids (ICLs) exhibited the longest half-life and the best tumor accumulation. The parent molecule Cy3 and Cy3-PEG5000 conjugate exhibited minimal accumulation in tumors. Furthermore, Cy3 lipids with ester linkages displayed degradation in serum and minimal tumor accumulation. Cy3 lipids with amide linkers, while more stable in serum, exhibited instability in tissues and tumors, resulting in decreased tumor accumulation. Shorter-chain lipids and unsaturated lipids demonstrated lower accumulation compared to longer-chain saturated lipids. Ex vivo confocal imaging confirmed the widespread painting of 4T1 breast and GL261 intracranial glioma models. Our results underscore the crucial role of lipid stability in serum and tissues in determining the lipid half-life and efficiency of tumor accumulation. The lead formulations can be utilized for drug delivery and imaging applications.

*equal contribution

13. Oxidative Stress Leads to An Increase In GFAP and Vimentin Expression

Paola Garcia Gonzalez, Ruth Fulton, Ariana Crary, Manisha Patel

When there is injury to the central nervous system, astrocytes respond by undergoing a process known as astrogliosis, which is characterized by increased expression of Glial Fibrillary Acidic Protein (GFAP). However, the mechanism that leads to astrogliosis remains a mystery. Previously, our laboratory found increased astrogliosis in a mouse model with neuronal specific knockout of antioxidant enzyme, superoxide dismutase (SOD2). This led us to hypothesize that reactive oxygen species (ROS) derived from the mitochondria may be enough to induce astrogliosis. To explore this, we treated primary cortical co-cultures with mitoparaquat (MPQ), a mitochondrial-specific redox cycling agent. Additionally, cultures were exposed to hypoxic (5% O2) conditions to assess if limited oxygen availability was enough to prevent astrogliosis. Our results show that cultures treated with MPQ in normoxia (18% O2) had a significant upregulation of both GFAP and Vimentin mRNA, as well as protein expression. However, these changes were not present in cultures treated with MPQ in hypoxia. Furthermore, immunocytochemistry data showed a clear change in astrocyte morphology after treatment with MPQ as well as an increase in vimentin and GFAP colocalization. Together these findings suggest a role for mitochondrial ROS in GFAP and vimentin upregulation and astrogliosis.

14. Phage-Like Particles for Ocular Drug Delivery: Plasmon-waveguide Resonance Spectroscopy and Evaluation Using In Vitro and Ex Vivo Cornea Models

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Ocular drug delivery faces challenges due to physiological barriers, especially rapid clearance of foreign particles by the precorneal mucous layer. This study investigates bacteriophage lambda derived-Phage-like particles (PLPs) as a novel approach for ocular therapeutics. PLPs are designed with mucoadhesive and mucopenetrative properties, allowing them to adhere to and diffuse through the mucus layer toward epithelial tissues. We explored PLP-mucin interactions using Plasmon-waveguide resonance (PWR) spectroscopy, revealing distinct angle shifts for mucin-PLP binding compared to mucin-prism interactions, which demonstrates PLPs' potential to cross the mucosal barrier. In vitro studies with primary bovine corneal epithelial cells demonstrate increased PLP uptake at higher concentrations, independent of mucin layer presence. Ex vivo bovine eye experiments show higher PLP concentrations in the corneal epithelium compared to corneal stroma and aqueous humor following topical administration. We also describe the impact of surface modifications on PLP performance. Contrary to our hypothesis, hyaluronic acid (HA) decoration does not significantly enhance uptake. However, ocular fluorophotometry suggests that polyethylene glycol (PEG) conjugation might improve delivery outcomes. Further optimization of PLP design and surface modifications could lead to more effective strategies for overcoming physiological barriers in ocular drug administration.

15. Lung Inflammation and Pathology are Mast Cell Dependent in a Model of Formaldehyde and Chloropicrin Toxicity.

Matthew Gibb, Alison Bauer, Jared M. Brown
Department of Pharmacy and Pharmaceutical Sciences, CU Anschutz Medical Campus, Aurora, CO 80045

The mucosal surface of the respiratory tract is directly and continually exposed to the external environment, including potential toxicants. Mast cells are located throughout the lung and are readily capable of rapid activation, degranulation, and cytokine release after exposure to xenobiotics, leading to inflammation and disease. Formaldehyde, a nearly ubiquitous chemical pollutant, and chloropicrin, a fumigant and chemical agent for use in riots and warfare with known pulmonary toxicity, are known to cause airway hyperresponsiveness with subsequent neutrophil recruitment and activation. However, little is known about the contribution of mast cells on cell recruitment, inflammation, and lung pathology following acute dose exposure to formaldehyde or chloropicrin. To assess mast cell contributions to these chemical exposures, we utilized wild type (C57BL/6) and B6.Cg-KitW-sh/HNihr-JaeBsm ($Kit^{w\text{-}sh}$), mast cell deficient) male mice. Data show increased pulmonary toxicity related to cellular influx, immune cell activation, and increased lung pathology in BL/6 mice that was significantly reduced in $Kit^{w\text{-}sh}$ mice. $Kit^{w\text{-}sh}$ mice showed decreased percentages of airway cellular infiltration and immune cell activation from lavage fluid and flow cytometric analyses. In $Kit^{w\text{-}sh}$ mice, BAL fluid revealed leukotrienes and downstream lipid mediators in the arachidonic acid pathway were significantly increased while lipids associated with ROS and immune activation were increased in BL/6 mice. H&E staining showed decreased pathology in $Kit^{w\text{-}sh}$ compared to BL/6 mice with dose-dependent increases in mast cell and neutrophilic influx in BL/6 mice. Results indicate that mast cells may play a significant role in cell recruitment, inflammation, and pathology in the lung after acute exposures to chemical toxicants.

16. Nickel agarose microbeads for testing joint implant hypersensitivity

Hari Gopalakrishnan, Munendra Tomar, Shaodong Dai

Nickel hypersensitivity is thought to be the foremost contact hypersensitivity by incidence globally, with rates as high as 19% among the non-patient population.[1] Nickel is relevant medically as a component of joint implants (including at the knee and hip), which are increasing in prevalence.[2] Due to inconsistencies in testing and the fact that nickel hypersensitivity is primarily a $CD4^+$ T-cell-mediated Type IV hypersensitivity, there is both an unmet need and an opportunity for innovation in testing patients.[2, 3] The project described here aims to develop a method by which nickel-reactive $CD4^+$ T-cells can be screened for using nickel-chelating agarose beads on the micrometer scale. The use of agarose beads is motivated by earlier findings in the lab of Dr. Shaodong Dai suggesting that some $CD4^+$ T-cells can be activated efficiently by nickel presentation from beads alone. The current work includes synthesis of agarose beads for future customization, genetic study of nickel-reactive transgenic mice T-cells, and the collection of mononuclear cells from arthroplasty patients, While T-cell activation by agarose beads is not a new idea, investigation of nickel-chelating agarose beads shows promise towards improved understanding of nickel reactivity and standards of care in joint implant patients.

17. Development of a CYP3A7 Fetal/Neonatal Hepatocyte-like Cell Model Derived from Induced Pluripotent Stem Cells

Emily G Gracey, Sylvie E Kandel, and Jed N Lampe

Fetal and neonatal populations receive many drugs off-label, yet there is limited safety information due to the general exclusion of pregnant and pediatric populations from clinical trials as well as a lack of appropriate model systems. Adult primary human hepatocytes (PHHs) represent a gold standard for *in vitro* drug metabolism and toxicity testing. However, fetal/neonatal PHHs are scarce, come with ethical concerns, and display altered enzyme profiles compared to adult PHHs. Fetal/neonatal-specific CYP3A7 metabolizes endogenous and exogenous substrates and has the potential to be involved in drug-hormone interactions. Our goal was to develop a sustainably-sourced hepatocyte-like cell (HLC) model derived from stem cells to study CYP3A7 *in vitro*. Our HLC differentiation protocol mimics hepatocyte development *in utero*, starting with induced pluripotent stem cells obtained from adults. HLCs were assessed for their gene expression via qPCR, and CYP3A activity through DHEA hydroxylation incubations measured via LC-MS/MS. Our results suggest 26 days as the optimal differentiation timing and 100 nM dexamethasone as the best glucocorticoid to maximize CYP3A7 gene expression and activity. After a rigorous characterization and comparison to adult and neonatal PHHs, this work will yield a sustainable model to gauge fetal/neonatal xenobiotic metabolism and interactions in these understudied populations.

18. Beta Cell Stress triggers formation of Hybrid Insulin Peptide through granular pH modulation

Jason Groegler, Mylinh Dang, Thomas Delong

The Eisenbarth model of type 1 diabetes (T1D) fails to explain variable disease progression in at-risk individuals. Hybrid insulin peptides (HIPs), formed when proinsulin peptides link to other beta cell protein fragments, are potential disease triggers. These peptides, not expressed in the thymus, allow HIP-reactive T cells to escape negative selection.

We investigated HIP formation by cathepsin D (CatD) in beta cell insulin granules, hypothesizing that different pH optima for CatD activity explain the consistent detection in murine islets but sporadic detection in human islets. We treated human islets with cytokines, glucose, or C381 (a vATPase activator) to activate HIP formation.

LC-MS/MS analysis revealed a novel HIP in islets treated with IL-1β, high glucose, or C381, suggesting specific stressors can upregulate HIP formation by decreasing granular pH. This research may modify the

Eisenbarth model and offer new insights into T1D progression. Future studies will explore how stressors affect immune targeting of HIP-containing beta cells and potential gradual beta cell depletion.

19. A Sample-Type Specific Database for Improved Fecal Metabolomics Compound Identification (A "Crappy" Rotation)

Brianna Hunt, Katrina Doenges, Cole Michel, Richard Reisdorph, Kevin Deane, Marie Feser, Catherine Lozupone, Nichole Reisdorph

Compound identification (ID) remains a major challenge in LC/MS-based metabolomics. The Reisdorph lab addresses compound ID issues through sample type-specific databases (STSDBs), which are built using empirically derived data from different biosamples. The goal of this research was to develop a method to detect compounds in human stool samples and then build a STSDB of compounds detected in healthy control and rheumatoid arthritis (RA) samples. During method development, three different sample preparation methods were tested: MeOH extraction, liquid-liquid extraction with MTBE, and solid phase extraction with Captiva cartridges. Each sample preparation method was assessed for total number of compounds, metabolome coverage, number of unique compounds detected, as well as recovery of internal standards. The optimal method was used to prepare the human fecal samples for analysis by LC/MS, and detected compounds were annotated and added to the STSDB. Preliminary data on mouse fecal samples indicate that the three sample preparation methods are relatively equal in number of features detected. Collection of human data is currently underway, and these results will be presented. The overall result of this project will be an established fecal metabolomics method for human samples, and a compound library which will be made available for free distribution.

20. Treatment with dexamethasone ameliorates arsenical vesicant lewisite-induced toxic corneal pathophysiology

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Lewisite (LEW), an arsenical-vesicant developed as a chemical warfare agent, is a cellular poison especially for moist surfaces, including eyes, skin, and respiratory tract. In eyes, cornea is most susceptible to LEW-induced injuries due to its aqueous nature/anterior location. LEW exposure causes immediate pain/foreign body sensation, induces inflammatory and immune responses, corneal opacity, ulceration, and neovascularization, that can cause partial/total blindness. Currently, no therapeutic treatment is approved for LEW-induced injuries. Thus, we assessed dexamethasone (DEX) efficacy against LEW-induced corneal injuries using histopathological and molecular parameters. Young New Zealand male rabbits (groups: control, LEW exposed, and LEW+DEX; n=4-6/group), were exposed to LEW. DEX was given 2 h post LEW exposure and every 8 h thereafter either for up to 14 or 28 days. Histopathological and molecular expression analysis of samples confirmed DEX efficacy against LEW-induced injuries. The 28-day DEX treatment more effectively reversed LEW-induced corneal thickness, stromal-epithelial separation, and epithelial degradation as compared to the 14-day treatment; LEW induced COX-2 and MMP-9 was reversed comparably by both DEX treatments at day 28. Continuous DEX administration for 28 days more effectively countered LEW-induced corneal injuries and hance can be used as an effective therapeutic countermeasure.

21. Structure based design of T cell receptor like antibody against disulfide modified autoantigens of type 1 diabetes

Anqi Li, Wei Li, Yan Zhang , Yang Wang, Ronghui Li, Donald Evans, and Shaodong Dai

T cells reactive to beta cell antigens play an important role in the development of type 1 diabetes (T1D). Islet amyloid polypeptide (IAPP) is an autoantigen in human T1D and the non-obese diabetic (NOD) mouse. The first 20 amino acids of IAPP, known as KS20, is the target antigen (Ag) for a highly diabetogenic CD4 T cell clone BDC5.2.9. Our crystal structure shows that KS20 is disulfide modified on the face of the major histocompatibility complex (MHC) class II molecule IAg7, forming the major epitope of T cells. We hypothesize that antibodies against IAg7- KS20 complex would inhibit T1D without interfering with recognition of other IAg7-presented self-antigens. To test this hypothesis, we generated mAb96.24, which specifically binds to IAg7-KS20 complexes with high affinity (KD=6.64nM). MAb96.24 blocks binding of IAg7-KS20 tetramers to cognate T cells and specifically inhibits T cell responses to KS20 peptides. We then treated female NOD mice with mAb96.24 starting at the age of 4 week. The results showed that intervention with mAb96.24 at early stages can prevent diabetes onset up to 24 weeks. These results indicate that mAb96.24 is a potential therapeutic or diagnostic antibody for the treatment of type 1 diabetes.

22. Examining the impact of NTD mutations on RBD conformational dynamics and ACE2 receptor binding affinity of the SARS-CoV-2 spike protein

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The structural composition of SARS-CoV-2 spike protein is highly complex and a critical area of study. Antiviral therapies that target the spike protein are highly successful at treating infection. However, spike proteins are prone to frequent mutations, resulting in the consistent emergence of viral variants that escape life-saving therapies. The most frequent and dangerous mutations occur in the receptor binding domain (RBD) and the N-terminal domain (NTD). During infection, viral spike proteins bind to human cells when RBD transitions from an inaccessible “down” conformation to a receptor-accessible “up” conformation, a process that is conserved among other coronaviruses. Mutations in RBD have been well studied and are known to improve infectivity. Likewise, mutations outside the RBD, which increase RBD-up conformations, also increase viral infection and transmission. The impact of NTD mutations on RBD binding and conformations is poorly understood. Given its proximity to RBD, we hypothesize that NTD mutations improve host cell binding by increasing RBD transitions and native up-states. Our results show that spikes engineered to contain only NTD mutations with wild-type RBD had a greater binding affinity for ACE2 than wild-type spikes when measured by surface plasmon resonance. Spikes with both RBD and NTD mutations had the greatest receptor affinity, implying that NTD and RBD mutations work together to increase viral infectivity. Understanding how SARS-CoV-2 is evolving will help develop better therapies with broad efficacy against current and future variants.

23. Impact of COVID-19 on Timeliness of Receiving Systemic Therapy for Patients Diagnosed with Lung Cancer

Mahesh Maiyani, MBA, Kris Wain, PhD, Nikki M. Carroll, MS, Debra P. Ritzwoller, PhD

Background: There is little information on how COVID-19 affected the timeliness of receipt of first-course systemic therapy treatment after a lung cancer diagnosis. We examined changes in the timeliness of first-course systemic therapy before, during, and after the COVID-19 pandemic among patients diagnosed with lung cancer.

Methods: We identified patients diagnosed with stage I-IV lung cancer between 1/1/2018 and 9/30/2021. Time periods were grouped as pre-COVID (01/01/2018 to 03/14/2020), early-COVID (03/15/2020 to 06/30/2020), and late-COVID (07/01/2020 to 09/30/2021). We calculated the number of days between the date of diagnosis and first-course systemic therapy and used interrupted time series to estimate the average number of days from diagnosis to first-course systemic therapy for each time period.

Findings: The proportion of patients who received first-course systemic therapy changed from 56.8% in pre-COVID to 7.5% in early-COVID to 35.7% in post-COVID. There was an immediate effect during early-COVID with a 9.6 (95% CI: 4.8 to 14.5) day increase from diagnosis to treatment. We saw a non-significant 4.9-day (95% CI: -1.5, 11.4) increase in early-COVID and 1.3-day (-2.3, 4.8) increase in late-COVID compared to pre-COVID. Additional research needs to be done on the outcomes of patients diagnosed in each period.

24. Characterization of neuron-specific Scn1a knockout mice: A novel model for Dravet syndrome

Chanapa Mann, Rebecca Han, Ruth Fulton, Manisha Patel

De novo heterozygous loss-of-function mutations of the SCN1A gene account for the majority of pathogenic variants identified in patients with Dravet Syndrome (DS), a severe developmental and epileptic encephalopathy. Here we developed a novel neuron-specific Scn1a knockout mouse model to investigate the role of principal neurons in DS pathogenesis. EEG recording of a Scn1a KO mouse showed waveform patterns indicative of hyperexcitability in addition to behavioral abnormalities. Induction of febrile seizures was only observed in the Scn1a homozygous KO at 39.5 °C, while no seizures were observed in the Scn1a heterozygous KO up until 40 °C. Immunohistochemical analysis of parvalbumin-positive and somatostatin-positive interneuron populations showed no differences in the Scn1a KO compared to the WT. Our results provide preliminary evidence suggesting that the epileptic phenotype observed in our model is independent of differences in major interneuron subpopulations, which support the idea that principal neurons may be involved in the mechanism of DS pathogenesis.

25. Preemptive return of clinical pharmacogenomic results to over 50,000 individuals in a population-scale biobank: phenotype and actionable medication exposure characteristics

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Introduction: Among population-scale biobanks, some offer the opportunity to return clinical pharmacogenomic (PGx) results to the electronic health record (EHR) for use in clinical care. Here, we describe PGx phenotype and actionable medication exposure characteristics of >50,000 participants who have received results from the biobank at the Colorado Center for Personalized Medicine (CCPM biobank).

Methods: The CCPM biobank preemptively returns clinical PGx results electronically to the EHR for *CYP2C19*, *SLCO1B1*, *DPYD*, *CYP2C9*, *TPMT*, *NUDT15*, and *ABCG2*. Automated drug-gene interaction (DGI) EHR alerts provide clinicians guidance when prescribing medications to participants with at-risk phenotypes. We evaluated CCPM biobank participant demographic, phenotype, and alert characteristics from 12/01/21-02/25/24.

Results: Over the 27-month period, 51,649 distinct participants had at least one PGx result returned to the EHR, totaling 325,469 distinct PGx results (Epic Genomic Indicators). The cohort was 61.6% female, 85.2% of European descent, and 9.4% Hispanic; mean ± SD age at the time of result return was 53.7 ± 16.7 years, and 99.2% of participants were alive as of 2024. As expected, the frequency of non-normal phenotypes was highest for CYP2C19 (58.8%), followed by CYP2C9 (34.6%), SLCO1B1 (27.1%), and ABCG2 (21.8%). There were 42,947 (83.2%) distinct participants with results returned for all seven genes. The percentage of participants with ≥1, ≥2, or ≥3 non-normal phenotypes for at least one of the seven genes was 90%, 50.6%, and 15.4%, respectively. Of the 51,649 participants with at least one PGx result returned, 5,794 (11.2%) had at least one distinct DGI alert, indicating a potentially actionable medication exposure. Of all distinct DGI alerts (n=7,247), the most common were omeprazole/*CYP2C19* (26.5%), pantoprazole/*CYP2C19* (21.1%), atorvastatin/*SLCO1B1* (14.6%), rosuvastatin/*SLCO1B1*/*ABCG2* (9.4%), and escitalopram/*CYP2C19* (6.8%).

Conclusion: Our findings demonstrate that population biobanks can be successfully leveraged to deliver precision medicine at scale, with immediate clinical impact across a variety of drug classes.

26. Acetylation of Proximal Cysteine-Lysine Pairs by Alcohol Metabolism

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Alcohol consumption induces hepatocyte damage through complex processes involving oxidative stress and disrupted metabolism. These factors are associated with increased protein acetylation and a reduced cysteine proteome providing a critical opportunity to identify therapeutic targets of alcohol-associated liver disease (ALD) pathogenesis. Interestingly, recent evidence suggests lysine acetylation occurs when a proximal cysteine residue is within ~15Å of a lysine residue, referred to as a cysteine-lysine (Cys-Lys) pair. Here, acetylation can occur through the transfer of an acetyl moiety via an S→N acetyl transfer reaction. Alcohol-mediated hepatic redox stress is known to occur coincident with lysine acetylation, but cysteine and lysine crosstalk within ALD remains unexplored. The hepatic redox state and lysine acetylation were quantified in a chronic murine model of ALD. Interrogating both cysteine redox and lysine acetylation datasets, 1,281 proteins were mapped by AlphaFold2 to quantify distances between cysteine and lysine residues. Our analysis reveals that alcohol metabolism induces redox changes and acetylation selectively on proximal Cys-Lys pairs with an odds ratio of 1.89 ($p < 0.0001$) on proteins critical to ALD pathology. Furthermore, these unique Cys-Lys redox signatures are translationally relevant as demonstrated by orthologous comparison with severe alcohol-associated hepatitis (SAH) explants, revealing numerous pathogenic thiol redox signals.

27. Network Meta-analysis of Treatment Options for Adults with AQP4-IgG Seropositive Neuromyelitis Optica Spectrum Disorder (NMOSD)

Nicholas D. Mendola, MPH, Jeffrey L. Bennett, MD, PhD, Kavita V. Nair, PhD, R. Brett McQueen, PhD, Tianjing Li, MD, PhD

Objective: Neuromyelitis optica spectrum disorder (NMOSD) is a rare neurological disease, with three recently approved new therapies with NMOSD specific indications for the first time ever. This work looked to perform a network meta-analysis evaluating indirect comparisons of treatments for AQP4-IgG seropositive NMOSD.

Methods: A systematic literature review was conducted synthesizing randomized controlled trials that enrolled patients aged 18 years and older diagnosed with AQP4-IgG seropositive NMOSD. A fixed-effects Bayesian network meta-analysis was performed to compare the relative treatment effects of four treatments of interest: inebilizumab, eculizumab, satralizumab, and rituximab. The primary outcome of interest was time-to-first NMOSD relapse, measured using hazard ratios. Surface Under the Cumulative Ranking (SUCRA) scores were calculated to rank treatments based on their efficacy.

Results: The network meta-analysis included five studies, allowing indirect comparisons among the treatments. Rituximab had the highest SUCRA score of 0.663, indicating superior efficacy in preventing NMOSD relapses, followed by inebilizumab (0.578), eculizumab (0.468), and satralizumab (0.420).

Conclusions: Rituximab demonstrated the highest efficacy in preventing NMOSD relapses, potentially offering a cheaper yet clinically superior treatment option. However, the limited evidence base and small sample sizes underscore the necessity for more comprehensive and larger-scale studies to confirm these findings.

28. Assessing dexamethasone efficacy in treating arsenical vesicant lewisite-induced clinical impairments in rabbit corneas

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Lewisite (LEW), first developed as a chemical warfare agent (CWA) during WWI, is a powerful arsenical vesicant. Eyes are uniquely susceptible to LEW toxicity, particularly the cornea. LEW exposure may cause pain/discomfort, inflammation, edema, neovascularization (NV), and vision impairment, depending on the exposure dose and duration. Though the threat of LEW exposure is high, there are no approved, targeted treatments for LEW-induced ocular injuries. Dexamethasone (DEX), an FDA approved anti-inflammatory steroid, was effective in treating mustard-vesicant induced corneal injuries in our studies. Thus, the efficacy of DEX in treating LEW-induced corneal injuries using assessment of clinical parameters in rabbits (n=4-6/group/time point; 2.5-4.0 kg; ~3 months old; male NZW) was performed. Continuous DEX treatment was done either for 14 days or 28 days post LEW-exposure, initiated 2 h post-exposure and every 8 h thereafter. Clinical assessments (corneal thickening, opacity, ulceration, and NV) were done on day 1, 3, 7, 14, 28, 42, and 56 post LEW-exposure, determining both continuous and sustained effects of DEX. DEX 28-day treatment was more effective in mitigating LEW-induced corneal injuries; sustained effects were apparent until the study endpoint. Whereas the effects of DEX 14 day-treatment depreciated upon treatment termination (supported by U01 EY030405).

29. Functional Differences in the Mu Opioid Receptor SNP A118G are Dependent on Receptor Splice-Variant and Agonist Specific Interactions

Casey Patrick, Utibe Ettah, Vu Nguyen, Caitlin Hart, Evan Atchley, Krishna Mallela, Rob Scheinman, Andrew Monte

The OPRM1 gene codes for the mu opioid receptor (MOR) and polymorphisms are associated with complex patient clinical responses. The most studied single nucleotide polymorphism (SNP) in OPRM1 is adenine (A) substituted by guanine (G) at position 118 (118A>G, rs1799971) leading to a substitution of asparagine (Asn) for aspartic acid (Asp) at position 40 in the N-terminus of the resulting protein. To date, no structural explanation for the associated clinical responses resulting from the 118A>G polymorphism has been proposed. Using molecular docking and post-docking energy minimizations with morphine, we show that the extracellular substitution of Asn at position 40 alters the cytoplasmic C-terminal conformation. A real time BRET assay measuring G-protein and β-arrestin association with MOR generated data that tested this prediction. Consistent with this in-silico prediction, we show changes in morphine mediated β-arrestin association with receptor variants with little change in morphine mediated G-protein association comparing MOR-1 wild type (WT) to MOR-1 118A>G . We tested the system with different opioid agonists, the OPRM1 118A>G SNP, and different MOR splice variants (MOR-1 and MOR-1O). In conclusion the 118A>G substitution alters receptor responses to opioids through variable C terminal domain movements that are agonist and splice variant dependent.

30. Evaluation of Pharmacogenomic Medication Prescribing Patterns in Rural Versus Urban Settings

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Background: To decrease disparities in pharmacogenomic (PGx) testing, it is essential to understand medication prescribing patterns in different settings, particularly rural and underserved communities. The objective of this study was to compare the prescribing frequencies of PGx medications among patients residing in rural versus urban areas in Colorado (CO).

Methods: We conducted a retrospective analysis of adult patients prescribed at least one PGx medication in 2021 at UCHealth. We evaluated the prescribing frequencies of CPIC level A, A/B, and B medications. Geographic area was ascertained using an individual's last reported zip code and by assigning Rural-Urban Commuting Area (RUCA) codes. Urban areas included zip codes with RUCA codes of 1-3 and rural areas included zip codes with RUCA codes from 4-10. We used logistic regression to assess the relationship between rural vs urban setting and being prescribed three or more PGx medications (yes/no), while adjusting for covariates such as, age, gender, race, ethnicity and comorbidity.

Results: The study included 451,725 patients, with 7.5% residing in rural areas in CO. Compared to urban areas, patients in rural areas were older (mean ± SD=56.3±18.1 vs 51.2±19 years, $p<0.001$), more likely to be male (45.6% vs 41.6%, $p<0.001$), more likely to be white (88.4% vs 78.3%, $p<0.001$), and less likely to be Hispanic (10.6% vs 14.3%, $p<0.001$). The mean±SD number of unique CPIC PGx meds prescribed in this cohort was 2.2±1.6. After adjusting for covariates, residing in a CO rural area was associated with significantly higher odds of being prescribed three or more CPIC PGx medications compared to urban areas (OR=1.27, 95%CI:1.24-1.30, $p<0.001$). The most prescribed CPIC PGx medication in our cohort was ondansetron.

Conclusion: Individuals residing in rural areas in CO had a higher yearly PGx medication burden compared with urban areas, highlighting the potential clinical utility of PGx testing in these communities.

31. Probing the Structural and Functional Impact of Mutations in the C-Terminal Domain of Dystrophin

Shashikant Ray, Vaibhav Upadhyay, Sudipta Panja, Jeffrey Kearns, and Krishna M.G. Mallela Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045.

Duchenne muscular dystrophy (DMD) is the most common and lethal type of muscular dystrophy, caused by deletions, duplications, or point mutations in the dystrophin gene. The dystrophin protein comprises four domains: an N-terminal domain that interacts with actin, a rod domain containing 24 spectrin repeats, a cysteine-rich domain, and a C-terminal (CT) domain. The CT domain consists of two polypeptide chains that form an α-helical coiled-coil structure, interacting with the coiled-coil motifs of other proteins and providing binding sites for dystrobrevin and syntrophins. Numerous single amino acid substitutions in the CT domain are reported to cause either DMD or Becker muscular dystrophy (BMD). In this study, we examined the impact of six disease-causing mutations on the CT domain of dystrophin. Surprisingly, these mutants exhibited similar secondary structures and thermal stability compared to the wild-type CT domain. Moreover, our investigation into the interaction of these mutant proteins with α-dystrobrevin revealed no discernible alteration in the strength of interaction compared to the wild-type CT domain. However, the functional implications of these mutations and their effects on protein interactions and

muscle function remain subjects of ongoing research. Understanding the structure-function relationships of these mutant proteins is crucial for elucidating the molecular mechanisms underlying DMD and may provide insights for potential therapeutic interventions.

32. Sugarcane Ash-Derived Silica Nanoparticles Induce Pulmonary Inflammation

Angela Reinert, Matthew Gibb, and Jared M. Brown

Sugarcane, also known as *Saccharum officinarum*, is the largest produced cash crop worldwide with approximately 177 million metric tons propagated at the end of 2023. Optimal conditions for this crop include hot and humid climates, generally found in areas such as Brazil, India, Mesoamerican countries, and even parts of the United States. Prior to harvest, a prescribed burn is done to ease the process of cutting and cultivating. During this process, sugarcane ash-derived silica nanoparticles (SAD SiNPs) are released into the atmosphere and as these airborne particles are roughly 200 nm in diameter, they can travel approximately 1,500 meters in ambient conditions. Additionally, the dust from sugarcane bagasse, agricultural waste used in various products like alternative fuel, cement, and pulp-based paper products, contain SAD SiNPs that are also released into the surrounding environment. The continual exposure that occupational workers and habitants of local communities' encounter has contributed to the increase of acute and chronic respiratory complications. Recent studies have elucidated several key mechanisms through which mast cells (MCs) influence pulmonary inflammation. Understanding the intricate role of mast cells in SAD SiNP-specific pulmonary inflammation can potentially open avenues for more effective therapeutic interventions for chronic respiratory conditions.

33. SSPPS Mass Spectrometry Core Facility

Cole Michel, Michael Armstrong, Katrina Doenges, Beth Mitchell, **Rick Reisdorph**, Nichole Reisdorph

The SSPPS Mass Spectrometry Facility provides a wide range of metabolomics, proteomics and quantitative small molecule services. We conduct metabolomics differential analysis on virtually all tissue and biofluid types. Proteomics applications include global proteome characterization and comparative quantitative analysis, post-translational modifications including acylation, and protein intact mass determination. We offer quantitative assays encompassing over 200 target compounds including lipid mediators, nucleotides, acylcarnitines, fatty acids, amino acids and cannabinoids. Custom assays can be developed upon request; in fact, we have a patent pending on one novel method developed by our Associate Director. Our equipment includes a state-of-the-art Orbitrap Eclipse with capabilities that can improve coverage of proteins, proteomes, and PTMs. The sensitivity offered by the Eclipse can be used to detect low abundance proteins and small molecules. Our liquid handling robot can be used for metabolomics and small molecule projects with large sample sizes. We also have metabolomics, protein identification, imaging, network/pathway analysis, qualitative and quantitative analysis software packages as described below. We provide training in every aspect of proteomics and metabolomics. The Facility is generally open-access, including an open door policy, although varying degrees of training are required for use of individual pieces of equipment. Data analysis packages are available with no restrictions. Since 2015, MS Facility personnel have authored/co-authored over 90 peer reviewed manuscripts and review articles, contributed effort to over 30 grants, and supports ~50 projects each year.

34. Development and Implementation of a Holistic Course Review Process Embracing Collaboration between Assessment and Curriculum Committees

Madison Ricco, PharmD; Heather Anderson, PhD; Jacci Bainbridge PharmD; Meghan Jeffres, PharmD; Robert Page, PharmD; Laura Saba, PhD; Jennifer Trujillo, PharmD

Objective: To implement a holistic course review process that utilized data, self-assessment, and peer assessment to provide support to faculty and assure quality of our curriculum.

Methods: Our existing process was analyzed and opportunities for improvement were identified. Literature and peer institution processes were reviewed to identify best practices. A joint Curriculum Committee (CC) and Assessment Committee (AC) working group was charged to develop a new process. The CD self-assessment tool was refined based on feedback.

Results: A holistic, collaborative, systematic course review process was developed, piloted and ultimately implemented in our PharmD Program. The CD self-assessment consists of 32 items assessing content, structure, teaching/learning methods, assessments, and course-directorship. The working group completes a peer review based on the CD self-assessment, student performance data, and student evaluations. Action items, including accolades or areas in need of revision, are presented to the CC and AC. CDs are sent a final review with suggestions, concerns, or commendations for the course prior to submission of the next year's syllabus. Faculty feedback from the pilot indicated support for this more holistic and collaborative process.

Conclusions: The pilot served to validate a more collaborative course review process that includes a reflective CD self-assessment tool.

35. Investigation of the Cytotoxic and Genotoxic Activity of the Antidepressant Vortioxetine in Human Renal Carcinoma Cell Line (786-0)

Gabriela Terue Rizzo Kodama, G. T. R.

The current usage of antidepressants has been significantly increasing for the treatment of depression, with Vortioxetine being one of the most commonly used medications. Considering that antidepressants and drugs, in general, may have distinct applications and go beyond merely treating diseases and alleviating symptoms, in vitro assays with tumor cell lines can be useful for a rapid and systematic investigation through various toxicological parameters. Among the tumor cell lines, renal cell carcinoma (786-O lineage) stands out, enabling studies on in vitro chemotherapy tests, leading to a better understanding of renal cell cancer in humans. Accordingly, based on the aforementioned information, the objective of this study was to evaluate the cytotoxic and genotoxic potential of the antidepressant Vortioxetine using the MTT Cytotoxicity Assay and Comet Assay on the 786-O renal carcinoma cell line. In conclusion, the MTT Cytotoxicity Assay and Comet Assay results showed that this antidepressant was able to induce cytotoxicity and genotoxicity in the cells tested at the concentrations evaluated. This suggests that Vortioxetine may have other pharmacological uses besides the treatment of depression, thus demonstrating the importance of such studies for pharmacological and oncological research.

36. Mechanistic Basis for Neuropathological Distributions of Dynamin-Related Protein 1, DRP1, in Mitochondrial Fission

Kyle A Ross; Megan C Harwig, PhD; Monika Oláhová, PhD; R Blake Hill, PhD

Dynamin-related protein 1 (DRP1) is the primary GTPase mechanoenzyme that mediates mitochondrial fission, and *de novo heterozygous* mutations cause neonatal lethality. Productive fission events are contingent on DRP1 self-assembly into a "collar" around the mitochondrion–visible as discrete puncta by immunofluorescence microscopy. However, several pathologic DRP1 variants are impaired in functional self-assembly yet remain capable of forming subcellular puncta, suggesting puncta formation is distinct from DRP1 self-assembly. As expected, puncta reside on mitochondria consistent with the widely held view that puncta are pre-scission complexes of highly-ordered DRP1. By contrast, DRP1 puncta are also in the cytoplasm, and these puncta are more mobile by fluorescence recovery after photobleaching (FRAP) than predicted for highly-ordered pre-scission complexes. Moreover, DRP1 puncta reversibly respond to 1,6-hexanediol, a compound shown to disrupt weak interactions but not ordered assemblies. Interestingly, disease-causing assembly-deficient DRP1 variants also appear as puncta and display greater sensitivity to 1,6-hexanediol compared to wild-type DRP1. Recombinant DRP1 also undergoes liquid-liquid phase separation (LLPS) that appears enhanced for assembly-deficient pathologic DRP1 variants, suggesting a link between DRP1 oligomeric state and LLPS. Thus, these data support a model in which DRP1 puncta arise from LLPS that are requisite for its recruitment and activity in mitochondrial fission.

37. Investigating the Role of Chloropicrin in non-IgE-Mediated Mast Cell Degranulation

Emily Ruggiano, Elizabeth Boyer, Jared Brown

Mast cells are critical mediators of both the innate and adaptive immune response through their release of pro- and anti-inflammatory mediators to neutralize pathogens. Repeated and exacerbated responses from mast cells can lead to multiple disease states ranging from seasonal allergies to anaphylaxis. The incidence of allergic and pseudo-allergic diseases has greatly increased in the last 50 years and some patients are not responsive to drugs such as omalizumab that target immunoglobulin- E (IgE) - mediated pathways of mast cell degranulation. Environmental toxicants have been found to activate mast cells at mas-related G-protein-coupled receptor X2 (MRGPRX2) without a priming response and production of IgE. Chloropicrin, a fumigant used as a chemical warfare agent and pesticide for high value crops like strawberries, has garnered more attention with its suspected use in the Ukraine and Russia war. Although it is a known skin, eye, and respiratory tract irritant, the role mast cells play in chloropicrin injury is not characterized. P815 murine mastocytoma mast cells were utilized to determine the extent of mast cell activity upon exposure to chloropicrin. Beta-hexosaminidase, an enzyme within mast cell granules, was measured to determine the extent of mast cell degranulation in the presence and absence of MRGPRX2 inhibitor, QWF. Ethidium homodimer-1 was used to determine if mast cells are being activated or undergoing cell death.

38. Investigating Wnt3a and Wnt5a as Factors of Hepatic Stellate Cell Activation in Models of Alcohol-associated Liver Disease

Lauren Rutt, Rebecca McCullough

Alcohol-associated Liver Disease (ALD) is a leading cause of liver-related mortality involving 61% of the American population. Moreover, patients with progressive ALD have stage 3-4 fibrosis, yet there remains no effective antifibrotic therapies available for patients. Hepatic stellate cells (HSC) play a key role in initiating and progressing liver fibrosis by accumulation of extracellular matrix. However, detailed mechanisms driving HSC activation during fibrosis are not well understood. Wnt proteins are morphogens that elicit a multitude of cellular responses via canonical b-catenin-dependent and noncanonical, b-catenin-independent signals and have been implicated in HSC activation. We hypothesize alcohol increases the expression of Wnt transducers, leading to Wnt-dependent activation of b-catenin, promoting HSC transdifferentiation during the early pathogenesis of fibrosis in ALD.

Using a murine model of moderate ethanol (2%v/v) exposure superimposed with carbon tetrachloride (CCl4) and primary mouse HSCs, the expression and nuclear localization of b-catenin expression was increased by ethanol in vivo. In vitro, a-SMA expression was increased in mHSCs following exogenous challenge of TGF-b, Wnt3a, and Wnt5a, which was further increased by the combination of ethanol.

These data reveal that ethanol induces Wnt-mediated activation of mHSCs and that Wnt/b-catenin may play causative roles in regulating the fibrogenic program in ALD.

39. Factors Affecting TFV-DP Concentrations in PBMC and Relationships with DBS in PWH on TAF-Based ART

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Relationships between tenofovir-diphosphate (TFV-DP) steady-state concentrations (Css) in peripheral blood mononuclear cells (PBMCs), dried blood spots (DBS), and adherence have not been well-described in people with HIV (PWH) on tenofovir-alafenamide (TAF)-containing antiretroviral therapy (ART). Our objectives were to assess the influence of adherence and other factors on TFV-DP Css in PBMCs, and to examine relationships between TFV-DP Css in DBS and PBMC.

Data were available in 84 PWH (55% White, 24% Black, 21% Hispanic/Latino) on TAF for median (IQR) 32 (18, 45) months (79 unboosted [un/], 5 boosted [b/]). Median (IQR) age was 54 (43, 59) years; most were males (89%) on INSTIs (88%). Median TFV-DP Css in PBMC in PWH on un/ART were 32% higher for 9-10 vs. 5-8 days of dosing (Fig. A).
Median TFV-DP Css for b/ART was 72% higher than un/ART (1007 [IQR: 868, 1297] vs. 585 [IQR: 459, 780] fmol/10^6 cells, respectively). Ten-day adherence was the only significant predictor, with every 10% adherence increase associated with an average TFV-DP in PBMC increase of 32 (95% CI: 14, 49; P=0.0005) fmol/10^6 cells. Week 4 TFV-DP Css in DBS
and PBMC were moderately correlated.

40. *Per-* and Polyfluoroalkyl Substances (PFAS) Inhibit Human Carboxylesterase I and II

Julieta Torres, Michaela Hvizdak, Sylvie E. Kandel, Jed N. Lampe

Per- and polyfluoroalkyl substances (PFAS) are known for their C-F bonds allowing them to be resistant to degradation. PFAS exposure in humans is associated with kidney and testicular cancer, and increased cholesterol levels. PFAS are structurally similar to fatty acids and endogenous ligands of carboxylesterase (CES) enzymes. CES I and II play a role in drug and lipid metabolism. CES I is found in the liver, lungs, and brain. CES I has been shown to be a drug target in treating metabolic diseases. Compared to CES II, it's expressed in the intestine, liver, and kidney. Much is still unknown for CES II, but it's thought to be involved in liver and gut lipid signaling pathways. CES I and II inhibition can lead to impaired glucose tolerance. We hypothesize that PFAS inhibit hepatic CES I and II activity. We utilized fluorescent probes 4-nirtophenol acetate and fluorescein diacetate to screen 14 prominent PFAS for their ability to inhibit CES I and II activity. Our findings indicate that perfluorodecanoic acid (PFDA) is the most potent inhibitor of CES I (IC_{50}=5.93μM, R^2=0.973) and perfluoroundecanoic acid (PFUnDA) is the most potent inhibitor of CES II (IC_{50}=21.7μM, R^2=0.983) activity.

41. Terminase Enzymes and Their Role in the Assembly of dsDNA Viruses

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Terminase refers to a class of highly-conserved enzymes involved in the assembly of dnDNA viruses. The enzymes serve multiple functions are critical for the propagation of many bacteriophages including lambda, P22, T4, Gifsy-1 as well as several prominent human viruses such as Herpes Simplex Virus-1, Epstein-Barr Virus, and human Cytomegalovirus. Specifically, terminases are responsible for cleaving a single copy of the viral genome from a concatemeric DNA substrate and packaging of the genome within the viral capsid. Further there is recent evidence that terminase can display tRNase activity under certain conditions. To perform these functions, terminase must assemble multiple complexes with viral DNA, the capsid, and other assembly proteins. Here we have utilized analytical ultracentrifugation and size-exclusion chromatography to observe changes in terminase complex assembly under various conditions.

42. A Role for the C5 Complosome in Kupffer Cell Hyperpolarization in Alcohol-Associated Liver Disease

Shannon Twardy and Rebecca McCullough

Complement activation and inflammation play critical roles in the pathogenesis of alcohol- associated liver disease (ALD). Complement activation product C5a activates immune cells upon binding to its GPCR, C5a Receptor 1 (C5aR1). In addition to serum-operative complement, a newly described intracellular system ("complosome") can coordinate proinflammatory effector functions in immune cell types during other model of sterile inflammation. In ALD, the liver resident macrophages, Kupffer cells (KCs), drive chronic inflammation and tissue injury. Chronic alcohol exposure induces KC hyperpolarization, characterized by production of reactive oxygen species, cytokines, and suppression of homeostatic functions, but the mechanisms coordinating these phenotypes are not fully understood. It is our working hypothesis that the C5 complosome contributes to KC hyperpolarization and effector functions during ALD. C5 and C5aR1 expression were increased following EtOH and LPS exposure and colocalization of C5aR1 to the mitochondria was driven by ethanol after 24 hours in immortalized mouse KCs (ImKCs). Ethanol- and LPS-induced proinflammatory polarization, defined by increased CD86 and iNOS expression,

was attenuated in C5-/- and C5aR1-/- ImKCs. Consistently, C5-/- and C5aR1-/- ImKCs are protected against ethanol- and LPS-induced suppression of phagocytosis. These data suggests the complosome may be an important mediator of KC hyperinflammatory responses during ALD.

43. Biophysical evolution of the receptor-binding domains of SARS-CoVs

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With hundreds of coronaviruses (CoVs) identified in bats that can infect humans, it is essential to understand how CoVs that affected the human population have evolved. SARS-CoV and SARS-CoV-2 belong to the same family, follow the same receptor pathway, and use their receptor-binding domain (RBD) of spike protein to bind to the ACE2 receptor on the human epithelial cell surface. The sequence of the two RBDs is divergent, especially in the receptor-binding motif that directly interacts with ACE2. Our results show that, despite RBDs having a similar three-dimensional structure, they differ in their thermodynamic stability. RBD of SARS-CoV-2 is significantly less stable than that of SARS-CoV. Correspondingly, SARS-CoV-2 RBD shows a higher aggregation propensity. Regarding binding to ACE2, less stable SARS-CoV-2 RBD binds with a higher affinity than more stable SARS-CoV RBD. In addition, SARS-CoV-2 RBD is more homogenous in terms of its binding stoichiometry toward ACE2 compared to SARS-CoV RBD. These results indicate that SARS-CoV-2 RBD differs from SARS-CoV RBD in terms of its stability, aggregation, and function, possibly originating from the diverse receptor-binding motifs.

44. Pharmacokinetics of Isoniazid Metabolites During Pregnancy and Postpartum

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IMPAACT P1078 evaluated the safety of isoniazid (INH) preventative therapy (IPT) initiated antepartum (AP) or postpartum (PP) in women with HIV (WWH). Hepatotoxicity occurred at high rates (~6-7%) across both arms during the PP period and a higher risk of adverse pregnancy outcomes was also identified among NAT2 slow acetylators. This study reports on the intensive PK of INH and its major metabolites in AP and PP WWH receiving IPT. WWH received 300 mg of INH daily for at least 2 weeks before intensive PK assessment, conducted at ≥28 weeks gestation (AP) and 16 (±2) weeks PP. Data from 31 WWH were analyzed (10 AP and PP, 4 AP only, 17 PP only). Higher exposures of AcINH were observed in intermediate and fast acetylators, while higher exposures of INH, INA, and AcHz were noted in intermediate and slow acetylators. Hz was more frequently quantifiable and had higher AUCs PP. Adjusted for NAT2 status, AUCs for INH were generally lower AP compared to PP. These findings highlight the impact of NAT2 status on INH metabolite formation and the generally higher exposures PP. Ongoing analyses are examining population-level factors and associations with hepatotoxicity.

45. Next-generation PFAS and SIRT3: Defining Novel Mechanisms of PFAS Hepatotoxicity

Jon M Woodward and Rebecca L. McCullough

Per or polyfluoroalkyl substances (PFAS) are an emerging class of ubiquitous environmental contaminants that are found in daily consumer-based products and contaminated groundwater. Next-generation PFAS compounds, such as PFAS Sulfonamides (PFASS), are accumulating in groundwater at increasing rates and the relative health effects of PFASS remain unknown. PFAS can be detected in 98% of the United States population due to the compound's prevalence and resistance to biodegradation. Epidemiologic data have identified the PFAS exposure is associated with hepatoxicity and hyperlipidemia;[1] while the mechanisms are not fully defined, several transcription factors including peroxisome proliferative activated receptor alpha (PPARα) have been implicated.[2] Recent findings have identified that Sirtuins, a class of protein deacetylases, may be a target of PFAS.[3] Given the role of Sirtuin 3 (Sirt 3) as a regulator of post-translational modifications in the mitochondria, and because mitochondrial health can control lipid metabolism in the liver, the goal of this project is to define if PFASS can functionally impact Sirt 3 *in vitro*.

References:

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3. Duan et al., *Environmental Pollution*, 2021

46. Protein Aging by Glycation: Role of Thiols

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Advanced glycation end products (AGEs) are one of the major protein modifications in aging proteins. In this process, lysine and arginine residues in proteins are glycated after exposure to sugars and α-dicarbonyl compounds. N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) are two major AGEs produced from glyoxal (GO) and methylglyoxal (MGO). AGEs bind to RAGE and promote the pathogenesis of several aging and diabetes-associated complications. Human αA-crystallin (αAC) and αB-crystallin (αBC) were treated with GO or MGO. We observed greater levels of CML and CEL in αAC compared to αBC. Specifically, K70 and K166 in αAC were found to be heavily carboxyalkylated, located close to C142. CML and CEL formation was significantly lower in cysteine-lacking αBC. Supplementation with glutathione or N-acetyl cysteine enhanced AGE accumulation in αBC. The introduction of a cysteine residue near a lysine residue in αBC and the mutation of cysteine to alanine in αAC validated the critical role of cysteine residues, emphasizing their importance in AGE formation. The hemithioacetal, formed between cysteine residues with GO, plays a crucial role in forming CML. These findings deepen our understanding of AGE formation in tissue proteins and hold promise for developing AGE inhibitors to mitigate age- and diabetes-associated complications.