UK MD/PhD Program Research Day Abstracts

ORAL PRESENTATION

Abstract Title:	**Novel Applications of MRI Techniques in the Detection of Neuronal Dysfunction before Tangle Pathology in Tau Transgenic Mice.**
Author(s):	R.A. Cloyd, College of Medicine, U of Kentucky S.N. Fontaine, Department of Physiology U of Kentucky S.E. Meier, Department of Physiology U of Kentucky D.K. Powell, Department of Anatomy and Neurobiology, U of Kentucky M. Vandsburger, Department of Bioengineering, U of California, Berkley J.F. Abisambra,Department of Physiology U of Kentucky

Abstract: Background: Tauopathic patients have significant cognitive decline accompanied by severe, irreversible brain atrophy. Neuronal dysfunction is thought to occur years before diagnosis. A major obstacle in the treatment of tauopathies is that current diagnostic tools are ineffective at detecting pre-pathological changes. We previously developed a MEMRI (manganese-enhanced magnetic resonance imaging) protocol coupled with R1-mapping to measure the extent of neuronal dysfunction that occurs before appearance of cognitive deficits and tau pathology associated with the rTg4510 tau model. In this study, we performed MEMRI with mangafodipir, an FDA-approved contrast. Methods: We used MEMRI to measure neuronal dysfunction in rTg4510 mice tau transgenic mice at 2 months (no pathology/cognitive deficits), and 3 months (presymptomatic pre-tangle pathology detectable). We measured MEMRI R1 changes before (baseline) and after (time-course) injecting mangafodipir (50mg/kg) intraperitoneally. We focused on the superior cortex and hippocampal sub-regions. Results: We found mangafodipir to be an effective contrast for MEMRI of mouse brains. Optimal enhancement of the cortex and hippocampus occurs 12-24 hours post-injection. Conclusions: This study builds upon our previous work showing that MEMRI (with MnCl2) reveals important functional differences between tau transgenic and non-transgenic mice. Here we found that mangafodipir is as effective as MnCl2 in performing MEMRI. Mangafodipir exhibits less toxicity than MnCl2 due to structural similarity to EDTA (used to treat manganese toxicity), making mangafodipir a target for translation of MEMRI for tauopathy into human subjects.

Supported by:	NIH/NINDS 1R01 NS091329-01, Alzheimer's Association NIRG-14-322441, NIH/NCATS 5UL1TR000117-04, NIH NIGMS 5P30GM110787, GlaxoSmithKline, Department of Defense AZ140097, the University of Kentucky Epilepsy Center (EpiC) and NIH/NIMHD L32 MD009205-01. We acknowledge the University of Kentucky Alzheimer's Disease Center (UK-ADC) which is supported by NIH/NIA P30 AG028383. The MRISC is supported by NIH S10 shared instruments grant number 1S10 RR029541.

Primary Presenter / email:	**Cloyd, R. A.** / racl232@uky.edu University of Kentucky **Clinical Science** **Other** **PSMRF Program Participant**
Mentor / e-mail:	Abisambra, J.F. / Joe.abisambra@uky.edu

UK MD/PhD Program Research Day Abstracts

ORAL PRESENTATION

Abstract Title:	**Steroid Therapy Limits Stem Cell Activation Required to Enact Mucosal Healing in Inflammatory Bowel Disease**
Author(s):	E.B. Lynch, Dept. of Microbiology, Immunology and Molecular Genetics, College of Medicine, U of Kentucky T. Goretsky, Dept. of Internal Medicine, U of Kentucky E. Bradford, Dept. of Internal Medicine, U of Kentucky T. Barrett, Dept. of Internal Medicine, U of Kentucky

Abstract: Background/Objectives: Intestinal stem cells (ISC) primarily act in the repair of ulcerated epithelium, and their proliferative capacity relies on Wnt/β-catenin signaling. However, the role of GCs on basal epithelial cell signaling has not been fully characterized. The objective of this study was to interrogate a mechanism by which steroids may limit ISC activation. We hypothesized that GCs limit Wnt/β-catenin signaling required for ISC activation and epithelial restitution by inhibiting NFκB activation in epithelial cells. Methods: To examine the effects of GCs on intestinal epithelial cells, we NCM460 cells with dexamethasone and observed the effects on NFκB and Wnt/β-catenin signaling events. We isolated mouse epithelial cells from the distal colon for stem cell culture as 3D “organoids.” We obtained pure epithelial cell preparations from mucosal biopsies isolated from patients treated at GI clinics at the University of Kentucky and VA Medical Center. Results: In steroid-treated NCM460 cells, we saw a significant decrease in transcripts for Wnt target genes, including Axin2 and cmyc; NFκB target genes, including IFNG and IL6; and the shared NFκB and Wnt pathway co-activator CREBBP, despite unchanged transcript levels for β-catenin (CTNNB1). This data was corroborated in 3D stem cell cultures from cells isolated from mouse colon tissue, which had significant decreases in transcripts for stem cell markers Lgr5 and Ascl2, proliferative markers KI67 and PCNA, and Wnt target Axin2. NCM460s transfected with a lentivirus carrying a TCF/LEF luciferase construct showed a 2.5-fold decrease in TNF-stimulated luciferase activity with dexamethasone treatment. Interestingly, this effect can be rescued by glucocorticoid receptor (GR) blockade with RU-486. Intestinal epithelial cells from patient biopsies showed significant decreases in colitis-induced Axin2, p-LRP6 (a positive marker of Wnt Signaling) and nuclear β-catenin. Conclusion: Together, these data suggest that steroid therapy inhibits Wnt/β-catenin signaling at multiple levels, and effects stem cell proliferation in pure stem cell cultures. Decreases in TCF/LEF transcriptional activation (nuclear β-catenin’s DNA binding target) can be reversed with steroid receptor blockade with RU-486, suggesting that a receptor level interaction may be occurringWhile steroids play a significant role in regulating the amount of inflammatory damage that occurs during IBD treatment, our data suggest that they may be limiting pathways required for effective healing as well.

Supported by: 5TL1TR000115-05 University of Kentucky CTSA Pre-doctoral T32 1TL1TR001997-01 University of Kentucky CTSA Pre-doctoral T32 1I01CX001353 - VA Merit; VA Medical Center Lexington

Primary Presenter / email: **Lynch, E.B.** / evan.lynch@uky.edu University of Kentucky
Clinical Science
GI
Mentor / e-mail: Barrett, T. / t.barrett@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #24

Abstract Title:	**Low Arousal Positive Emotional Stimuli Ameliorate Working Memory Processing Dysfunction in Persons with Mild Cognitive Impairment**
Author(s):	L.S. Broster, Department of Behavioral Science, U of Kentucky S.L. Jenkins, Medical University of South Carolina S.D. Tarrant, Sanders-Brown Center on Aging, U of Kentucky G.A. Jicha, Sanders-Brown Center on Aging, U of Kentucky Y. Jiang, Department of Behavioral Science and Sanders-Brown Center on Aging, U of Kentucky

Abstract: Emotional enhancement effects have been proposed to be robust to the pathophysiology of Alzheimer's disease. Others have suggested that such effects are dysfunctional in this context, especially when other memory faculties are simultaneously engaged. Participants with and without mild cognitive impairment presumed to be due to Alzheimer's disease performed an emotionally-valenced delayed-match-to-sample repetition task while encephalography was performed to assess alterations in synaptic activity linked to discrete memory faculties in these groups. Results indicated that for persons with mild cognitive impairment, high arousal negative stimuli led to working memory processing patterns previously associated with mild cognitive impairment presumed due to Alzheimer's disease and dementia of the Alzheimer's type, but that low arousal positive stimuli evoked a processing pattern similar to MCI participants' unaffected spouses. We suggest that low arousal positive stimuli attenuate working memory processing manifestations of MCI due to Alzheimer's disease.

Supported by:	NIH award: 5 T32 AG 242-18; UL1RR033173; UL1TR000117, and pilot funding from the Department of Behavioral Science. The project described was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH

Primary Presenter / email:	**Broster, L.S.** / lukebroster@gmail.com	University of Kentucky
	Clinical Science	
	Behavioral Science	
Mentor / e-mail:	Jiang, Y. / yjiang@uky.edu	

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #37

Abstract Title:	**The Bayesian Method for Confounding as Applied to Personality and Substance Use Data to Estimate Average Causal Effect**
Author(s):	L. Su, College of Medicine, U of Kentucky C. Wang, Department of Biostatistics, U of Kentucky C. Lee, Department of Psychology, U of Kentucky R. Milich, Department of Psychology, U of Kentucky D. Lynam, Department of Psychology, Purdue University

Abstract: Purpose: To investigate possible correlations between substance use and personality trait measurements in students attending the University of Kentucky using the Bayesian Adjustment for Confounding. Methods: The analysis was done in the statistical analysis software R using the Bayesian Adjustment for Confounding as developed by Dr. Chi Wang et al. The resulting model related the personality trait measures with substance use while accounting for a multitude of confounders. Data/Results: There were 449 individuals in the data. The dataset contained 10 different personality measurements from two different models. These variables were the exposure variables. The four outcome variables used were frequency of alcohol use, frequency of marijuana use, frequency of tobacco use, and audit total score, a measure of how harmful the subject's alcohol use is. 37 confounders were also included in the model, including sex, race, age, and quite a few variables involving the subject's friends' usage and opinions of alcohol, marijuana, and stimulants. This resulted in evaluating 40 associations/relationships, each relating one exposure variable to one outcome variable. The results showed which confounders were selected often in each model. The average causal effect (ACE) was also calculated from the models, providing a measurement of the actual level of causation between the two variables. Conclusions: Overall, the Bayesian Adjustment for Confounding is a method useful for eliminating confounders in observational studies and establishing causation with more certainty. The relationship that showed the highest positive effect was between positive urgency and audit total score. The relationship showing the most negative effect was between conscientiousness and audit total score. An example of a relationship with no effect was between marijuana use frequency and extraversion. Through the BAC method, the direct effects of personality traits on substance use can be accurately estimated.

Supported by: Supported by National Center for Advancing Translational Sciences: UL1TR000117/UL1TR001998 and UKHealthCare

Primary Presenter / email: **Su, Leon** / leon.su@uky.edu University of Kentucky
Community Science
Behavioral Science
PSMRF Program Participant

Mentor / e-mail: Wang, C. / chi.wang@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #50

Abstract Title:	**Divergence of cAMP Signaling Pathways Mediating Augmented Nucleotide Excision Repair and Pigment Induction in Melanocytes**
Author(s):	E. M. Wolf Horrell, Department of Physiology and Markey Cancer Center, U of Kentucky S. G. Jarrett, Markey Cancer Center, U of Kentucky K. M. Carter, Markey Cancer Center, U of Kentucky J. A. D'Orazio, Departments of Pediatrics, Toxicology and Cancer Biology, Physiology, Pharmacology and Nutritional Sciences, and Markey Cancer Center, U of Kentucky

Abstract: Loss-of-function melanocortin 1 receptor (MC1R) polymorphisms are common in UV-sensitive fair-skinned individuals and are associated with blunted cAMP second messenger signaling and higher lifetime risk of melanoma because of diminished ability of melanocytes to cope with UV damage. cAMP signaling positions melanocytes to resist UV injury by up-regulating synthesis of UV-blocking eumelanin pigment and by enhancing the repair of UV-induced DNA damage. cAMP enhances melanocyte nucleotide excision repair (NER), the genome maintenance pathway responsible for the removal of mutagenic UV photolesions, through cAMP-activated protein kinase (protein kinase A)-mediated phosphorylation of the ataxia telangiectasia mutated and Rad3 related (ATR) protein on the S435 residue. We investigated the interdependence of cAMP-mediated melanin upregulation and cAMP-enhanced DNA repair in primary human melanocytes and a melanoma cell line. We observed that the ATR-dependent molecular pathway linking cAMP signaling to the NER pathway is independent of MITF activation. Similarly, cAMP-mediated up-regulation of pigment synthesis is independent of ATR, suggesting that the key molecular events driving MC1R-mediated enhancement of genome maintenance (e.g. PKA-mediated phosphorylation of ATR) and MC1R-induced pigment induction (e.g. MITF activation) are distinct.

Supported by: NIH award: R01CA131071075, Melanoma Research Alliance, NIH award: P30CA177558, and NIH award: T32CA165990

Primary Presenter / email: **Wolf Horrell, E. M.** / erin.wolf@uky.edu University of Kentucky
Basic Science
Cancer
Mentor / e-mail: D'Orazio, J. A. / jdora2@email.uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #57

Abstract Title: **Lineage Tracking of Fibroblasts in the Aorta During Angiotensin II Infusion**

Author(s): Z. Chen, College of Medicine, U of Kentucky
D.L. Rateri, Saha Cardiovascular Research Center, U of Kentucky
A. Daugherty, Saha Cardiovascular Research Center, U of Kentucky

Abstract: Objective: The purpose of this study was to determine whether cells tracked with a S100A4 driven Cre retain markers for fibroblasts or expressed characteristics of smooth muscle cells. The S100A4 promoter is used to drive Cre recombination in fibroblast specific gene expression. However, the S100A4 promoter is potentially active in cell types in addition to fibroblasts. Our previous studies have demonstrated angiotensin II (AngII) infusion increases aortic medial cells expressing S100A4 promoter driven Cre in mice ubiquitously expressing a conditional LacZ gene. Approach and Results: Mice expressing Cre under the control of the S100A4 promoter were bred into transgenic mice with a repressed lacZ gene at the Rosa26 locus. At 8-10 weeks of age, mice were infused subcutaneously with either saline or AngII (1,000 ng/kg/min) for 28 days. Following infusion, aortas were dissected free and sections were obtained from the ascending, descending, and abdominal aortic regions. As noted previously, AngII infusion increased β-galactosidase tissue staining in the ascending and abdominal aortic regions, but not the descending region. β-galactosidase immunostaining was more closely colocalized with α-smooth muscle cell actin immunostaining than with ERTR7 immunostaining in all aortic regions. Conclusions: AngII infusion drives an increased expression of S100A4 in medial cells tracked with a S100A4 promoter driven Cre. Despite S100A4 being defined as a fibroblast specific gene, lineage tracked cells primarily had expression of a smooth muscle cell marker.

Supported by: NIH1 RO1HL107319-01 to Alan Daugherty

Primary Presenter / email: **Chen, Z.** / zch236@uky.edu University of Kentucky
Basic Science
Cardiovascular
Mentor / e-mail: Daugherty, A. / adaugh@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #67

Abstract Title:	**Nuanced Antibody Responses to Apolipoprotein A-I in Patients with Cardiovascular Disease**
Author(s):	D. Henson, College of Pharmacy, U of Kentucky V. J. Venditto, College of Pharmacy, U of Kentucky

Abstract: Antibodies targeting apolipoprotein A-I (ApoA-I) have been identified in patients with cardiovascular disease (CVD). Anti-ApoA-I antibodies are thought to be markers of disease, but their exact role is unclear. We hypothesize that antibodies targeting ApoA-I are both protective and pathologic and unraveling the nuanced response to ApoA-I will provide insight into improved risk stratification of patients suffering from CVD. To test our hypothesis we screened serum samples by ELISA collected from patients with CVD to identify anti-ApoA-I antibody responses toward the full length protein along with immunogenic epitopes including the lecithin cholesterol acyl transferase (LCAT) domain and the C-terminal peptide of ApoA-I. These epitopes are of particular interest due to their propensity to undergo oxidative post-translational modification. Antibodies were affinity-purified toward ApoA-I, and their role in reverse cholesterol transport elucidated. Our data indicate that serum collected from patients with CVD enrolled in multiple clinical trials possess a highly nuanced immune response. We find that these antibody responses change over time in some patients who present with an AMI and antibodies correlate with outcomes. The mechanisms of these observed effects are currently under investigation. A full report on correlations between patient characteristic and antibody level will be presented. This work highlights the complexities of anti-ApoA-I antibodies in patients, which will guide development of a CVD risk stratification tool.

Supported by:	Predoctoral TL-1 Grant supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. AHA Scientist Development Grant 17SDG32670001 (VJV). Pilot Funding from an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103527 (VJV).

Primary Presenter / email:	**Henson, D.** / david.henson0@uky.edu **Clinical Science** **Cardiovascular**	University of Kentucky
Mentor / e-mail:	Venditto, V. / vincent.venditto@uky.edu	

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #75

Abstract Title:	**Prediction of All-cause Mortality from Clinical MRI-derived Left Ventricular Ejection Fraction: 15 Years of Data from a Large Regional Health System**
Author(s):	G.J. Wehner, Department of Biomedical Engineering, U of Kentucky C.M. Haggerty, Department of Imaging Science and Innovation, Geisinger Health System J.D. Suever, Department of Imaging Science and Innovation, Geisinger Health System L. Jing, Department of Imaging Science and Innovation, Geisinger Health System J.B. Leader, Biomedical and Translational Informatics Institute, Geisinger Health System H.L. Kirchner, Biomedical and Translational Informatics Institute, Geisinger Health System J.N.A. Manus, Biomedical and Translational Informatics Institute, Geisinger Health System C.W. Good, Department of Cardiology, Geisinger Health System B.K. Fornwalt, Departments of Imaging Science and Innovation and Radiology, Geisinger Health System

Abstract: Background: Despite the widespread use of magnetic resonance imaging (MRI) to assess cardiac function, few studies have evaluated the ability of left ventricular ejection fraction (LVEF) derived from MRI to predict all-cause mortality. We used 15 years of MRI data from a large regional health system to assess the relationship between clinical MRI-derived LVEF and subsequent mortality. Methods: Records from the Geisinger Health System were reviewed to identify all instances where LVEF was measured clinically using MRI. Either date of death or last living encounter were obtained as well as patient characteristics and active diagnoses. The relationship between LVEF and mortality was assessed with Cox Proportional Hazards Regression. Results: We identified 3405 MRI studies from 3052 unique patients with clinically reported LVEF. Median follow-up time was 4.0 years. Death occurred in 707 patients representing 765 MRI studies. Including adjustments for confounders, LVEF was a significant predictor of all-cause mortality. The highest hazard ratio was observed in the lowest (<25%) LVEF interval (hazard ratio = 2.74, 95% confidence interval: 2.04 – 3.70). The hazard ratio steadily declined with increasing LVEF up to the reference 55–65% interval. There was no significant difference in the hazard ratio between the 55-65 and ≥65% intervals. Conclusions: Based on outcomes from over 3000 patients in a large regional health system, clinical MRI-derived LVEF is a significant predictor of all-cause mortality. MRI-derived LVEF can stratify patients according to their risk of all-cause mortality, with improved survival for higher LVEFs, up to an LVEF between 55-65%.

Supported by: This project was supported by a National Institutes of Health Early Independence Award (DP5 OD012132)

Primary Presenter / email: **Wehner, G.J.** / gregory.wehner@uky.edu University of Kentucky
Clinical Science
Cardiovascular

Mentor / e-mail: Fornwalt, B.K. / bkf@gatech.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #78

Abstract Title: **Elucidating Subtypes and Risk Factors of Brain Arteriolosclerosis**

Author(s):
E.T. Ighodaro, Department of Anatomy and Neurobiology, Sanders-Brown Center on Aging, U of Kentucky
E.L. Abner, Department of Epidemiology, Sanders-Brown Center on Aging, U of Kentucky
S.E. Monsell, Center for Biomedical Statistics, U of Washington, Seattle, WA
W.A. Kukull, National Alzheimer's Coordinating Center (NACC), Department of Epidemiology, U of Washington, Seattle, WA
J.H. Neltner, Department of Pathology, Division of Neuropathology, U of Kentucky
V. Smith, Department of Pathology, Division of Neuropathology, U of Kentucky
D. Fardo, Sanders-Brown Center on Aging, Department of Biostatistics, U of Kentucky
P.T. Nelson, Department of Pathology, Division of Neuropathology, Department of Anatomy and Neurobiology, Sanders-Brown Center on Aging, U of Kentucky

Abstract: Cerebrovascular pathologies are often seen in aged brains. Here, we focus on brain arteriolosclerosis (B-ASC), i.e., degenerative thickening of cerebral arterioles. We recently reported that severe B-ASC pathology is associated with global cognitive status (PMID 26738751). To study risk factors of B-ASC, we analyzed 2,390 cases with clinical and neuropathological autopsy data from the National Alzheimer's Coordinating Center. Cases were analyzed according to age at death (< 80 years and ≥ 80 years) using logistic regression modeling. Gender was associated with B-ASC pathology in both age at death groups after controlling for covariates including age at death, and conventional vascular risk factors: hypertension, diabetes, smoking, and hypercholesterolemia. In a subset of cases with genetic information (n = 925), the ABCC9 gene variant (rs704180), previously associated with hippocampal sclerosis, was also associated with B-ASC pathology in the ≥ 80 year-old group. To address in finer detail the heterogeneous arteriolar morphologies that could be classified as B-ASC, we analyzed 74 cases from the University of Kentucky Alzheimer's Disease Center (UKADC) and UK Pathology Department. Within this convenience sample, the median age at death was 56.5 years with a range of 20 – 96 years. One of the subtypes of B-ASC pathology in this cohort consisted of arteriolar profiles with multiple internal lumens, which we refer to as multi-lumen vessels (MLVs, which generally have ≥ 3 lumens in a single vascular profile). In this sample, 62.1% (n = 46) of cases had ≥ 5 MLVs per brain section, as operationalized using CD34 immunohistochemistry in the frontal neocortex (Brodmann area 9). Interestingly, MLV densities increased with advanced age of death ($r = 0.51$; $p < 0.0001$). We conclude that B-ASC is a complex pathologic phenotype in advanced age with both genetic and clinical risk factors, as well as morphologic subtypes, that require further study.

Supported by: R01 diversity supplement, F30 NIH grant

Primary Presenter / email: **Ighodaro, E.T.** / etigho2@uky.edu University of Kentucky
Clinical Science
Cardiovascular
Mentor / e-mail: Nelson, P.T. / pnels2@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #81

Abstract Title: **Modified HIV drugs to treat blindness: Novel anti-inflammatory therapeutics**

Author(s):
B.J. Fowler, Departments of Ophthalmology and Visual Sciences and Physiology, U of Kentucky
K. Ambati, Department of Ophthalmology and Visual Sciences, U of Kentucky
Y. Kim, Department of Ophthalmology and Visual Sciences, U of Kentucky
J. Fukuhara, Department of Ophthalmology and Visual Sciences, U of Kentucky
T. Yasuma, Department of Ophthalmology and Visual Sciences, U of Kentucky
R. Yasuma, Department of Ophthalmology and Visual Sciences, U of Kentucky
L. Krueger, Department of Ophthalmology and Visual Sciences, U of Kentucky
B.D. Gelfand, Department of Ophthalmology and Visual Sciences, Microbiology and Immunology, and Biomedical Engineering, U of Kentucky
J. Ambati, Department of Ophthalmology and Visual Sciences, U of Kentucky

Abstract: Purpose: Nucleoside reverse transcriptase inhibitors (NRTIs) are mainstay therapeutics for HIV that block retrovirus replication. Surprisingly, we found that NRTIs as a class inhibit P2X7/NLRP3-mediated inflammation independent of reverse transcriptase inhibition (Fowler et al. Science 2014). Multiple FDA-approved NRTIs, including stavudine (d4T) and zidovudine (AZT), were efficacious in a mouse model of geographic atrophy (GA), a type of age-related macular degeneration (AMD) and leading cause of blindness in the elderly. However, d4T and AZT use in humans is associated with toxicity attributed to off-target host cell polymerase inhibition, which limits their therapeutic potential. Therefore, we rationally redesigned NRTIs in order to harness their anti-inflammatory properties and abrogate “off target” polymerase inhibition. We synthesized novel methoxy-substituted NRTI variants and tested whether this modification eliminates their ability to block polymerases, and tested the efficacy of modified-NRTIs in cell culture and mouse models of AMD. Methods: Methoxy-NRTI synthesis: Methoxy-substituted 3TC, d4T, AZT were synthesized from parental NRTI and methoxy-structures confirmed by H1 NMR/LC-MS. iGluc assay and western blotting were performed to assess inflammasome activation. Enhanced green fluorescent protein cell culture L1 retrotransposition assay and lentivirus GFP assay were analyzed by flow cytometry and on Biotek plate reader. mtDNA depletion measured by real-time qPCR of total DNA from cells in culture. Mouse model of dry AMD: RPE degeneration induced by subretinal injection of a plasmid expressing Alu RNA and assessed by fundus photography/ZO-1 staining of RPE flat mounts. Results: Modified NRTIs were protective in the Alu-induced mouse model of geographic atrophy. Novel NRTI variants retained inflammasome inhibition, however, unlike their parental NRTI counterparts, did not inhibit polymerases. Conclusion: NRTIs possess two distinct functions as reverse transcriptase inhibitors and anti-inflammatory compounds. The specificity of methoxy-NRTI derivatives as anti-inflammatories bolsters their therapeutic potential as a safer alternative to NRTIs. Also, modified NRTIs are useful tools for dissecting the effect of nucleosides on inflammasome vs. polymerase inhibition and could be advantageous in targeting other P2X7/NLRP3-driven diseases.

Supported by:
This work is supported by NIH grants DP1GM114862, R01EY018350, R01EY018836, R01EY020672, R01EY022238, and R01EY024068, Doris Duke Distinguished Clinical Scientist Award, Burroughs Wellcome Fund Clinical Scientist Award in Translational Research, Ellison Medical Foundation Senior Scholar in Aging Award, Foundation Fighting Blindness Individual Investigator Research Award, Harrington Discovery Institute Scholar-Innovator Award, Dr. E. Vernon Smith and Eloise C. Smith Macular Degeneration Endowed Chair, The John Templeton Foundation, Research to Prevent Blindness departmental unrestricted grant and NIH T32HL091812. The project described was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1RR033173. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Primary Presenter / email: **Fowler, B.J.** / benjamin.fowler@uky.edu University of Kentucky
Basic Science
Drug Development
Mentor / e-mail: Ambati, J. / jamba2@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #82

Abstract Title:	**Inhibition of Human Metapneumovirus Binding to Heparan Sulfate Blocks Infection in Human Lung Cells and Airway Tissues**
Author(s):	E.M. Klimyte, Department of Molecular and Cellular Biochemistry, U of Kentucky S.E. Smith, Department of Molecular and Cellular Biochemistry, U of Kentucky P. Oreste, Glycores 2000 S.r.l. 20155 Milan, Italy D. Lembo, Department of Clinical and Biological Sciences, U of Turin, Italy R. E. Dutch, Department of Molecular and Cellular Biochemistry, U of Kentucky

Abstract: Human metapneumovirus (HMPV), a recently discovered paramyxovirus, infects nearly 100% of the world population and causes severe respiratory disease in infants, the elderly, and immunocompromised patients. We previously showed that HMPV binds heparan sulfate proteoglycans (HSPGs) and that HMPV binding requires only the viral fusion (F) protein. To characterize the features of this interaction critical for HMPV binding and the role of this interaction in infection in relevant models, we utilized sulfated polysaccharides, HS mimetics and occluding compounds. Iota-carrageenan had potent anti-HMPV activity by inhibiting binding to lung cells mediated by the F protein. Furthermore, analysis of a minilibrary of variably sulfated derivatives of Escherichia coli K5 polysaccharide mimicking HS structure revealed that the highly O-sulfated K5 polysaccharides inhibited HMPV infection, identifying a potential feature of HS critical for HMPV binding. The peptide dendrimer SB105-A10, which binds HS, reduced binding and infection in an F-dependent manner, suggesting occlusion of HS at the target cell surface is sufficient to prevent infection. HMPV infection was also inhibited by these compounds during apical infection of polarized airway tissues, suggesting these interactions take place during HMPV infection in a physiologically relevant model. These results reveal key features of the interaction between HMPV and HS, supporting the hypothesis that apical HS in the airway serves as a binding factor during infection, and HS modulating compounds may serve as a platform for potential antiviral development.

Supported by:	Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number F30AI114194 to EK and NIH grants R01AI051517 and 2P20 RR020171 from the National Center for Research Resources to RED. Funding was also provided by CCTS TL1 training program (TL1TR000115) to EK and UK University Research Professor funds to RED.

Primary Presenter / email:	**Klimyte, E. M.** / edita.klimyte@uky.edu	University of Kentucky
	Basic Science **Drug Development**	
Mentor / e-mail:	Dutch, R. E. / rdutc2@uky.edu	

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #83

Abstract Title:	**Mapping Unique Interaction Domains in the Sterol Biosynthetic Pathway for Antifungal Development**
Author(s):	K. B. Linscott, Department of Molecular and Cellular Biochemistry, U of Kentucky T. Niehaus, Horticultural Sciences Department, U of Florida X. Zhuang, Department of Pharmaceutical Sciences, U of Kentucky S. Bell, College of Pharmacy, U of Utah J. Chappell, Departments of Molecular and Cellular Biochemistry and Pharmaceutical Sciences, U of Kentucky

Abstract: Invasive mycoses are becoming a significant cause of patient morbidity and mortality, indicating a need for the development of novel antifungal therapeutics. Squalene synthase catalyzes the first committed step in sterol biosynthesis. While the overall architecture of this enzyme is similar throughout eukaryotes, it has been shown that the plant and human enzymes can only complement a knockout mutation in yeast if the non-catalytic carboxy-terminal domain is swapped for one of fungal origin. This implies that there is a region within this domain that is unique to the fungal Kingdom. In order to characterize this potential therapeutic target, we used the model organism Saccharomyces cerevisiae with a squalene synthase knockout mutation and expressed chimeric squalene synthase genes originating from fungi, plants, animals, and algae under the control of a galactose inducible promoter. We have shown that all enzymes tested can complement the knockout mutation when expression levels are low. When the promoter is induced, it appears that overexpression of non-native squalene synthases in yeast may lead to the toxic accumulation of a sterol intermediate or by-product. We have also shown that this phenotype is specific to a 26 amino acid hinge region adjacent to the catalytic domain, and that the region can be mimicked to inhibit the growth of wild-type yeast. Our results suggest that the hinge region is a promising lead for the production of a broad spectrum antifungal therapeutic that would not disrupt cholesterol synthesis is humans.

Supported by: The project described was supported by the Harold R. Burton and George A. Digenis endowed professorships

Primary Presenter / email: **Linscott, K. B.** / kristin.linscott@uky.edu University of Kentucky

Basic Science
Drug Development

Mentor / e-mail: Chappell, J. / chappell@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #87

Abstract Title:	**Evaluation of the combinational therapy cyclosporine A and phenelzine on protection of mitochondrial respiration following severe controlled cortical impact injury in rats**
Author(s):	J. R. Kulbe, SCoBIRC and Department of Neuroscience, U of Kentucky J. A. Wang, SCoBIRC and Department of Neuroscience, U of Kentucky I. N. Singh,SCoBIRC and Department of Neuroscience, U of Kentucky E. D. Hall, SCoBIRC and Department of Neuroscience, U of Kentucky

Abstract: Traumatic brain injury (TBI) represents a significant health crisis in the United States and there are currently over five million people living with a TBI related disability. However, acute treatment of TBI remains supportive and there are no FDA-approved pharmacotherapies available to prevent the devastating neurologic consequences of TBI. Following TBI, mitochondria buffer increases in intracellular calcium in an attempt to maintain homeostasis, however, increases in intra-mitochondrial calcium lead to generation of reactive oxygen and nitrogen species (ROS/RNS), induction of lipid peroxidation (LP), and formation of the LP-derived aldehydes, 4-HNE and acrolein. 4-HNE and acrolein covalently bind mitochondrial proteins, exacerbating production of ROS/RNS, mitochondrial dysfunction, and energy impairment. Eventually, mitochondrial dysfunction leads to opening of the mitochondrial transition pore (mPTP), extrusion of calcium back into the cytosol, activation of calpain, cytoskeletal degeneration, neuronal death, and neurologic impairment. Therefore, mitochondria are promising therapeutic targets for prevention of cellular death and dysfunction following TBI. Individual administration of cyclosporine A (CsA), an immunosuppressant with the ability to inhibit mPTP, or phenelzine (PZ), an antidepressant with aldehyde scavenging properties, has been shown to partially attenuate mitochondrial respiratory function following experimental TBI. Here, the ability of the combination of CsA (15min 20mg/kg i.p. loading dose + 10mg/kg/day s.c. osmotic pump) and PZ (15min 10mg/kg s.c. loading dose + 10mg/kg/day s.c. osmotic pump) to improve mitochondrial respiration 72h following severe controlled cortical impact injury in three month old male Sprague-Dawley rats is assessed in comparison to either agent alone.

Supported by: This work was supported by 5R01 NS083405, 5R01 NS084857, and F30 NS096876

Primary Presenter / email: **Kulbe, J.R.** / Jacqueline.Kulbe@uky.edu University of Kentucky
Basic Science
Drug Development

Mentor / e-mail: Hall, E.D. / ed.hall@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #94

Abstract Title: **Using Calibrated Proton Density Imaging to Measure Blood-Brain Partition Coefficient in Aging and Alzheimer's Disease Mice**

Author(s):
S.W. Thalman, Dept. of Biomedical Engineering, U of Kentucky
D.K. Powell, Dept. of Biomedical Engineering and Magnetic Resonance Imaging and Spectroscopy Center, U of Kentucky
A. Shen, Sanders-Brown Center on Aging, U of Kentucky
A.M.S. Hartz, Sanders-Brown Center on Aging and Dept. of Molecular and Biomedical Pharmacology, U of Kentucky
A. Lin, Depts. of Pharmacology and Nutritional Sciences and Biomedical Engineering, U of Kentucky

Abstract: Purpose: In the present study, we determine the blood-brain partition coefficient (BBPC) in aging C57Bl6/N mice and the transgenic 129S6/Tg2576 mouse model of Alzheimer's disease using a calibrated proton density imaging approach. This parameter is an important coefficient in the quantification of cerebral blood flow (CBF) derived from arterial spin labeling (ASL) acquisitions. Previous studies have shown both regional and age-related differences in BBPC in humans, yet the current consensus in the field does not correct for these differences but instead assumes a single constant value for all regions and all patients. Arterial spin labeling has become particularly relevant in the study of brain aging where it has been used to image the vascular dysfunction that occurs with advanced age. In Alzheimer's disease it has also shown sensitivity to the vascular dysfunction which precedes amyloid and tau pathologies. This has been recapitulated in small animal models such as the 129S6/Tg2576 mice which have the human Swedish amyloid precursor protein (hAPP) mutation. However, the limitations of small animal scanners and the inherent low signal of ASL techniques require quantification models to be as precise as possible. Furthermore, any uncorrected variation in BBPC could potentially bias CBF measurements. For this reason, we test the hypothesis that BBPC will be reduced in aged C57Bl6/N mice and transgenic 129S6/Tg2576 mice. Methods: Imaging Protocol- Male C57Bl6/N wild type mice aged 3 months (n=8) and 12 months (n=8) as well as male 12-month-old 129S6/Tg2576 (n=6) with their 129S6 wild type controls (n=3) were imaged using a 7T Bruker ClinScan (Bruker Biospin, Ettlingen, Germany) with a 39mm diameter birdcage transmit/receive coil. Inside the coil was placed a series of phantoms with 0, 10, 20, 30, and 40% deuterium oxide in water that were also doped with gadobutrol (Gadavist, Bayer Healthcare Pharmaceuticals, Whippany NJ, USA, 0.07 mM) such that the T1 was approximately 2.0s. Blood was drawn from the facial vein of each subject and placed in a capillary tube alongside the deuterated phantoms. A series of image stacks were acquired with a phase-spoiled, FLASH-GRE sequence (FOV = 2.8cm x 2.8cm, matrix = 256 x 256, slice thickness = 1mm, number of slices = 10, flip angle = 90°) with a very short TE (3.2ms) and 6 different TR values (125, 187, 250, 500, 1000, 2000ms). Image Analysis- For each transverse slice, the TR-series was fit to the mono-exponential recovery curve S = M0*[1-e^(TR/T1)] in a voxel-wise manner yielding maps of both apparent T1 and relative proton density, M0. The relative M0 maps were calibrated to a regression line of the average M0 values in regions of interest (ROIs) drawn in the deuterated phantoms. Therefore, the calibrated M0 values represent the percent water content of each voxel. The BBPC value is then calculated by dividing by the average M0 value in the blood sample and the average density of brain tissue, i.e. BBPC = M0,brain/(M0,blood * 1.04g/mL). An ROI was then drawn manually for each transverse slice excluding any susceptibility artifacts. BBPC values were averaged over all ROIs for each mouse. Results: The calibrated proton density imaging protocol was able to produce high resolution, low noise maps of BBPC despite reducing scan time from 2 hours, as in Leithner et al., to 25 minutes. The 12 month old mice demonstrated a 5.5% reduction in BBPC (μ= 0.94±0.04 mL/g) compared to the 3 month old mice (μ= 0.99±0.04 mL/g, p = 0.02). Preliminarily, the Tg2576+ mice demonstrate an elevated BBPC (μ= 01.03±0.04 mL/g) compared to WT (μ= 1.00±0.05 mL/g), though more subjects are needed. Discussion/Conclusion: The variability of BBPC values from these data demonstrates the potential error in assuming a constant value for all patients when calculating CBF. When measuring CBF in aging mice, failing to correct for the reduced BBPC will overestimate CBF resulting in reduced sensitivity. However, it appears that in the 129S6/Tg2576 model the elevated BBPC may be a confounding factor. Scan time can be reduced further by reducing the resolution to match ASL acquisitions making this technique a potentially viable method of correcting CBF measures for differences in BBPC.

Supported by: NIH/NIA Grant # K01AG040164 to AL F. Joseph Halcomb III MD Fellowship for Engineering in Medicine to ST

Primary Presenter / email: **Thalman, S.W.** / scott.thalman@uky.edu University of Kentucky

Basic Science
Imaging

Mentor / e-mail: Lin, A. / ailing.lin@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #96

Abstract Title:	**White Matter Microstructure in the Default Mode Network Mediates Executive Function Declines Associated with Aging, Alzheimer's, and Cerebrovacular Pathology**
Author(s):	C.A. Brown, Department of Neuroscience, U of Kentucky F.A. Schmitt, Departments of Neurology, Psychiatry, and Sanders-Brown Center on Aging, U of Kentucky C.D. Smith, Department of Neurology, Sanders-Brown Center on Aging, and Magnetic Resonance Imaging and Spectroscopy Center, U of Kentucky B.T. Gold, Department of Neuroscience, U of Kentucky

Abstract: Objective: This study investigated whether white matter (WM) microstructure in the default mode network (DMN) may serve as a common marker of executive function decline due to age, Alzheimer's disease (AD), and cerebrovascular disease (CVD). Methods: 32 older adults underwent diffusion tensor imaging (DTI), FLAIR imaging, cerebrospinal fluid (CSF) sampling, and neuropsychological assessment. Fractional anisotropy (FA) was measured in WM pathways connecting DMN regions. WM lesion (WML) volume in DMN-WM was quantified using FLAIR images, and CSF was analyzed for levels of Aβ42. Cross-sectional relationships between variables were explored with additional longitudinal follow-up underway. Results: Partial correlations controlling for sex and education revealed relationships between measures of age, WML volume, and CSF Aβ42 with both executive function (r = -.39, -.32, .37) and FA in DMN-WM (r = -.38, -.39, .44), which was also associated with executive function (r = .65). Separate mediation analyses found that FA in DMN-WM mediated the effect of age (58% mediation, indirect effect (ab) = -0.16, 95% CI: [-0.36, -0.04]), CSF Aβ42 (72% mediation, ab = 0.17 [0.04, 0.34]), and WML volume (76% mediation, ab = -0.16 [-0.43, -0.03]) on executive function. Conclusion: These results point to alterations in WM microstructure as an underlying mechanism of executive declines associated with aging, AD, and CVD. Further, interventions preserving WM microstructure may protect against the negative impact of multiple pathologies.

Supported by: The project described was supported by National Center for Advancing Translational Sciences and National Institute on Aging of the National Institutes of Health, through Grants TL1TR001997, P30AG028383, P01AG0301228, and R01AG033036. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Primary Presenter / email: **Brown, C.A.** / cabr237@uky.edu University of Kentucky
Clinical Science
Imaging
Mentor / e-mail: Gold, B.T. / brian.gold@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #97

Abstract Title:	**Novel Applications of MRI Techniques in the Detection of Neuronal Dysfunction before Tangle Pathology in Tau Transgenic Mice.**
Author(s):	R.A. Cloyd, College of Medicine, U of Kentucky S.N. Fontaine, Department of Physiology U of Kentucky S.E. Meier, Department of Physiology U of Kentucky D.K. Powell, Department of Anatomy and Neurobiology, U of Kentucky M. Vandsburger, Department of Bioengineering, U of California, Berkley J.F. Abisambra,Department of Physiology U of Kentucky

Abstract: Background: Tauopathic patients have significant cognitive decline accompanied by severe, irreversible brain atrophy. Neuronal dysfunction is thought to occur years before diagnosis. A major obstacle in the treatment of tauopathies is that current diagnostic tools are ineffective at detecting pre-pathological changes. We previously developed a MEMRI (manganese-enhanced magnetic resonance imaging) protocol coupled with R1-mapping to measure the extent of neuronal dysfunction that occurs before appearance of cognitive deficits and tau pathology associated with the rTg4510 tau model. In this study, we performed MEMRI with mangafodipir, an FDA-approved contrast. Methods: We used MEMRI to measure neuronal dysfunction in rTg4510 mice tau transgenic mice at 2 months (no pathology/cognitive deficits), and 3 months (presymptomatic pre-tangle pathology detectable). We measured MEMRI R1 changes before (baseline) and after (time-course) injecting mangafodipir (50mg/kg) intraperitoneally. We focused on the superior cortex and hippocampal sub-regions. Results: We found mangafodipir to be an effective contrast for MEMRI of mouse brains. Optimal enhancement of the cortex and hippocampus occurs 12-24 hours post-injection. Conclusions: This study builds upon our previous work showing that MEMRI (with MnCl2) reveals important functional differences between tau transgenic and non-transgenic mice. Here we found that mangafodipir is as effective as MnCl2 in performing MEMRI. Mangafodipir exhibits less toxicity than MnCl2 due to structural similarity to EDTA (used to treat manganese toxicity), making mangafodipir a target for translation of MEMRI for tauopathy into human subjects.

Supported by:	NIH/NINDS 1R01 NS091329-01, Alzheimer's Association NIRG-14-322441, NIH/NCATS 5UL1TR000117-04, NIH NIGMS 5P30GM110787, GlaxoSmithKline, Department of Defense AZ140097, the University of Kentucky Epilepsy Center (EpiC) and NIH/NIMHD L32 MD009205-01. We acknowledge the University of Kentucky Alzheimer's Disease Center (UK-ADC) which is supported by NIH/NIA P30 AG028383. The MRISC is supported by NIH S10 shared instruments grant number 1S10 RR029541.

Primary Presenter / email:	**Cloyd, R. A.** / racl232@uky.edu University of Kentucky
	Clinical Science
	Imaging
	PSMRF Program Participant
	Von Allmen Competition Participant
Mentor / e-mail:	Abisambra, J.F. / Joe.abisambra@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #100

Abstract Title:	**Pol Versus Env Genetics in SHIV-Infected Macaques Highlights Importance of Phylogenetic Signal**
Author(s):	C. Coomer, National Cancer Institute, NIH, Frederick, MD E.M. Anderson, National Cancer Institute, NIH, Frederick, MD L. Smith, National Cancer Institute, NIH, Frederick, MD W. Shao, National Cancer Institute, NIH, Frederick, MD J. Spindler, National Cancer Institute, NIH, Frederick, MD J.W. Mellors, Div. of Infectious Disease, Dept. of Medicine, U of Pittsburgh, Pittsburgh, PA J.M. Coffin, Dept. of Molecular Biology and Microbiology, Tuffs U, Boston, MA A. Ambrose , Div. of Infectious Diseases, Dept. of Medicine, U of Pittsburgh, Pittsburgh, PA M.F. Kearney, National Cancer Institute, NIH, Frederick, MD

Abstract: Previously we investigated HIV pol populations in SHIV-infected macaques by single-genome sequencing to determine if low-level replication was a source of residual viremia during ART and to investigate viral compartmentalization across tissues. Using this approach, we found no evidence for evolution during suppressive ART and little evidence of viral compartmentalization. To investigate the possibility that the low diversity in pol masked the emergence of new viral variants and/or compartmentalization, we applied the same methods to the more diverse env gene in the infected macaques. Two macaques (M03250 and K02396) received 20 weeks of ART (TNF, FTC, EFV) and one macaque (6760) was untreated. Longitudinal plasma samples (N=11) from treated macaques were analyzed by single-genome sequencing of a 1 kb pol fragment and a 2.5 kb env fragment. Tissues were collected at necropsy after infection for 30 weeks of infection and single-genome sequences were obtained from a plethora of tissues. The entire 2.5kb env fragment and the 101 nucleotide V3 region alone were evaluated separately for population diversity, divergence, and compartmentalization using phylogenetic and panmixia analyses, and compared to results from pol. Phylogenetic and panmixia analyses of 2.5kb env sequences in plasma did not reveal the emergence of new variants during ART, showing that the lack of evolution in pol was not due to low phylogenetic signal in this region. Env populations analyzed in tissues from 6760 were highly diverse but showed similar population structures to pol and a lack of tissue compartmentalization. By contrast, phylogenetic analyses of only the V3 env region showed very weak phylogenetic signals and little diversity, indicating that the V3 region is not appropriate to evaluate intra-individual populations for diversity, evolution, and phylogenetic structure. These findings highlight the importance of performing single-genome and deep sequencing on regions of the viral genome with strong phylogenetic signal.

Supported by: This work was supported in part with federal funds from the National Cancer Institute, National Institutes of Health, the NIH Bench to Bedside Program, Contracts No. 12XS547 and 13XS110 through Leidos Biomedical Research, Inc.

Primary Presenter / email: **Coomer, C.A** / charles.coomer@uky.edu National Cancer Institute
Basic Science
Infectious Disease
Mentor / e-mail: Kearney, M. / kearneym@mil.nih.gov

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #104

Abstract Title:	**Detection and Handling of Spectral Artefacts in Fourier Transform Mass Spectra of Metabolomics Experiments**
Author(s):	J.M. Mitchell, Dept. of Molecular and Cellular Biochemistry, Markey Cancer Center, Center for Environment and Systems Biochemistry and the Resource Center for Stable Isotope Resolved Metabolomics, U of Kentucky R.M. Flight, Markey Cancer Center, Center for Environment and Systems Biochemistry and the Resource Center for Stable Isotope Resolved Metabolomics, U of Kentucky Q. Wang, Markey Cancer Center, Department of Ophthalmology and Visual Sciences, U of Kentucky A.N. Lane, Markey Cancer Center, Center for Environment and Systems Biochemistry and the Resource Center for Stable Isotope Resolved Metabolomics, Department of Toxicology and Cancer Biology, U of Kentucky H.N.B. Moseley, Department of Molecular and Cellular Biochemistry, Markey Cancer Center, Center for Environment and Systems Biochemistry and the Resource Center for Stable Isotope Resolved Metabolomics, Institute for Biomedical Informatics, U of Kentucky

Abstract: Fourier-transform mass spectrometry (FTMS) allows for the high-throughput detection of thousands of metabolites. Conservatively, over 90% of the observed spectral features do not correspond to known metabolites and cannot be placed into existing metabolic networks. Without accurate assignment of these features, discerning their roles within living systems is effectively impossible. Assignment remains difficult due to the low concentrations of some detected metabolites, the volume of data produced by FTMS and the small m/z differences between isotopologues. Additional phenomena producing large numbers of spectral artefacts further complicate FTMS assignment. Assignments made to these artefact peaks can create large interpretative errors. We have observed three types of artefacts unique to FTMS that often result in regions of abnormally high peak density which we collectively refer to as high peak density artefacts. 1 - Fuzzy sites: small regions of m/z space with a 'fuzzy' appearance due to the extremely high number of peaks. 2 – Ringing: where a very intense peak produces side bands of decreasing intensity that are symmetrically distributed around the main peak. 3 - Partial ringing: where only a subset of the side bands are observed for an intense peak. Fuzzy sites and partial ringing appear to be novel artefacts previously unreported in the literature and we hypothesize that all three artefact types derive from Fourier transformation defects. We have developed a set of tools to detect these artefacts and are developing new methods to mitigate or eliminate their effects on spectra and downstream analyses.

Supported by: This research was supported by NIH 1R01ES022191-01, 1U24DK097215-01A1, and NSF 1252893 grants.

Primary Presenter / email: **Mitchell, J. M.** / joshuamerrittmitchell@gmail.com University of Kentucky

Basic Science
Informatics

Mentor / e-mail: Moseley, H. N. B. / hunter.moseley@gmail.com

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #105

Abstract Title: **Automated High-Content Analysis of Skeletal Muscle Immunohistology**

Author(s):
Y. Wen, Department of Physiology, U of Kentucky
K. Murach, College of Health Sciences, U of Kentucky
I. Vechetti, Jr., Department of Physiology, U of Kentucky
C. Vickery, Paul Laurance Dunbar HS
C. Peterson, College of Health Sciences, U of Kentucky
J. McCarthy, Department of Physiology, U of Kentucky
K. Campbell, Department of Physiology, U of Kentucky

Abstract: High volume analysis of skeletal muscle histological cross sections is often necessary for studying muscle physiology. As automation improves for immunohistochemistry and fluorescence microscopy, preparation and imaging of muscle sections is performed with ever increasing speed and efficiency. As such, high content image data analysis represents the most significant bottleneck in workflow, especially for large-scale studies. To date, no fully automated, accurate, and reliable software is yet available to muscle researchers. Therefore, we introduce FiberVision, a software that 1) improves upon previously reported algorithms, 2) achieves > 94% accuracy for myofiber detection, size measurement, type classification, and myonuclear counting without human input, and 3) is available with a readily usable interface. FiberVision is the most robust, intuitive and free software available for muscle histological analysis, and will greatly improve analysis efficiency for the spectrum of muscle researchers.

Supported by: NIH award: R01AR061939

Primary Presenter / email: **Wen, Y.** / ywen2@g.uky.edu University of Kentucky
Basic Science
Informatics
Mentor / e-mail: Campbell, K. S. / k.s.campbell@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #113

Abstract Title: **Effect of Neurotensin on Hepatic Fatty Acid Synthesis**

Author(s): J. A. Johnson, Department of Toxicology & Cancer Biology, U of Kentucky
J. Ling, Markey Cancer Center, U of Kentucky
B.M. Evers, Markey Cancer Center, U of Kentucky

Abstract: Neurotensin (NT) is a gut peptide that is released from enteroendrocrine cells of the small intestine after fat ingestion. Our hypothesis is that NT deficiency protects hepatocytes from high fat diet (HFD)-induced hepatic steatosis by: (1) decreasing fatty acid (FA), triglyceride (TG), and cholesterol synthesis via activation of AMP-activated protein kinase (AMPK) and (2) increasing FA oxidation by enhancing mitochondrial activity. Primary mouse hepatocytes were isolated from murine livers and were treated with 0, 10, 100, 1000, 2000, and 4000 nM of NT. Western blots will be run to determine the effect on levels of phosphorylated AMPK (p-AMPK) and phosphorylated extracellular signal-regulated kinases 1 and 2 (p-ERK 1/2). Increases in p-ERK 1/2 and decreases in p-AMPK will indicate that these hepatocytes contain NT receptors. Future work will determine which NT receptor (NTR)—NTR1 or NTR3—plays a more significant role. Additionally, wild type (WT) and NT knockout (KO) mice were fed a low fat diet (LFD) or HFD for 24 weeks. After sacrifice, the average liver mass of the NT-KO mice on a HFD was significantly less than the average liver mass of the WT mice. Western blots and real time polymerase chain reactions (RT-PCR) of the liver tissue will be conducted for the following enzymes: p-AMPK, steroid response element binding protein 1-c (SREBP-1c), fatty acid synthase (FASN), and HMG-CoA reductase. If liver tissues from NT KO mice fed a HFD have increases in p-AMPK and SREBP-1c and decreases in FASN and HMG-CoA reductase, then this will suggest that NT deficiency protects the liver from HFD-induced obesity through inhibition of FA synthesis.

Supported by: R01 T32

Primary Presenter / email: **Johnson, J. A.** / jeremy-johnson@uky.edu University of Kentucky
Basic Science
Nutrition
Mentor / e-mail: Evers, B. M. / mark.evers@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #124

Abstract Title: **Gender Specific Inflammasome Activation in the Trigeminal Ganglion**

Author(s): B.C. Farmer, College of Medicine, U of Kentucky
J. Cho, College of Medicine, Ophthalmology, U of Kentucky
R. Albuquerque, College of Medicine, Ophthalmology, U of Kentucky

Abstract: The effect of gender on pain and analgesia has been the subject of important studies for decades. It is widely known that common forms of painful conditions, such as neuropathic pain, are more prevalent in females. Determining the cellular and molecular mechanisms underlying such differences will enhance our basic understanding of pain biology, and will also help target therapy that addresses these gender discrepancies. Recently our lab detected unprecedented gender dependent differences in inflammasome activation in satellite glial cells (SGCs) cultured from the trigeminal ganglion (TG). The TG houses the cell bodies of corneal sensory fibers and SGCs and represents an important relay station for corneal sensory input. We hypothesize that activation of the NLRP3 inflammasome in trigeminal SGCs is gender specific. To test this, trigeminal ganglia were harvested from male and female mice and grown in cell culture. Alu-like RNAs (B1 and B2 RNAs), known to activate the inflammasome, were administered to the cultured SGCs. Twenty-two hours after B1/B2 RNA stimulation, quantitative PCR was performed to evaluate changes in inflammasome activation markers in male versus female cells. Notably in B1-treated TG SGCs, males showed a significant increase in inflammasome effector protein known as ASC compared to females while females showed a significant increase in IL-18 compared to males ($p<0.05$). In B2 treated TG SGCs, female mice showed significantly higher levels of IL-18 and Dicer-1 ($p<0.05$). These findings suggest that there may be intrinsic cellular mechanisms that modulate inflammasome activation in male versus female SGCs. Future studies should build upon these findings and further investigate these cellular pathways as a potential mechanism of gender specific differences in pain behavior.

Supported by: The project described was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998.

Primary Presenter / email: **Farmer, B. C.** / bcfa223@uky.edu University of Kentucky

Basic Science
Pain
PSMRF Program Participant

Mentor / e-mail: Abuquerque, R. / rjalbu2@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #129

Abstract Title:	**Neonatal dendritic cells alter the immunodominance hierarchy of the CD8 T cell response during influenza infection**
Author(s):	L.H. Heil, Department of Microbiology, Immunology, and Molecular Genetics, U of Kentucky S.N. Oliphant, Department of Microbiology, Immunology, and Molecular Genetics, U of Kentucky J.L. Lines, Department of Microbiology, Immunology, and Molecular Genetics, U of Kentucky M.L. Hollifield, Department of Microbiology, Immunology, and Molecular Genetics, U of Kentucky B.A. Garvy, Department of Microbiology, Immunology, and Molecular Genetics and Division of Infectious Diseases, U of Kentucky

Abstract: Neonates are more susceptible to influenza virus infection than adults, resulting in increased morbidity and mortality as well as delayed clearance of the virus. Multiple differences between the adult and neonatal immune response to influenza help explain this vulnerability. Dendritic cells are of particular interest in this process as their decreased function in neonates results in the poor T cell activation observed during neonatal influenza infections. We sought to understand how differences in neonatal dendritic cells shape CD8 T cell specificity and immunodominance during influenza infection as well as how this may affect memory formation and viral clearance. We found that neonatal C57/B6 mice display an altered CD8 T cell immunodominance hierarchy, preferentially responding to the influenza protein PA rather than the dominant adult epitope in the NP protein. Additionally, upon secondary infection, mice first infected as pups suffered increased morbidity compared to mice infected previously as adults. Finally, transfer of influenza infected adult dendritic cells to pups resulted in increased T cell activation and enhanced viral clearance. Taken together, these data suggest that neonatal dendritic cells alter CD8 immunodominance, and this may compromise viral clearance and memory formation.

Supported by:

Primary Presenter / email:	**Heil, L. H.** / luke.heil@uky.edu University of Kentucky **Basic Science** **Pediatrics**
Mentor / e-mail:	Garvy, B. A. / beth.garvy@uky.edu

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #138

Abstract Title:	**Nucleoside Reverse Transcriptase Inhibitors Suppress Laser-Induced Choroidal Neovascularization in Mice**
Author(s):	T. Mizutani, Department of Ophthalmology and Visual Sciences, U of Kentucky B.J. Fowler, Department of Ophthalmology and Visual Sciences, U of Kentucky Y. Kim, Department of Ophthalmology and Visual Sciences, U of Kentucky R. Yasuma, Department of Ophthalmology and Visual Sciences, U of Kentucky L.A. Krueger, Department of Ophthalmology and Visual Sciences, U of Kentucky B.D. Gelfand, Department of Ophthalmology and Visual Sciences, U of Kentucky J. Ambati, Department of Ophthalmology and Visual Sciences, U of Kentucky

Abstract: Nucleoside reverse transcriptase inhibitors (NRTIs), widely used to treat HIV infection, have been shown to be therapeutic in a mouse model of dry AMD, through their intrinsic anti-inflammatory activity that targeted the purinergic receptor P2X7 and the NLRP3 inflammasome pathway. One NRTI, stavudine (d4T) was found to suppress laser-induced choroidal neovascularization (CNV) in mice in a P2X7-dependent fashion. Here we evaluate the efficacy of three other NRTIs in the laser-induced mouse model of CNV. We evaluated the NRTIs lamivudine (3TC), zidovudine (AZT), and abacavir (ABC), and the P2X7 antagonist A438079. CNV was induced by laser injury in C57BL/6J wild-type, Nlrp3–/–, and P2rx7–/– mice, and CNV volume was measured after 7 days by confocal microscopy. Drugs were administered by intravitreous injection immediately after the laser injury. VEGF-A in RPE-choroid lysates was measured three days after laser injury by ELISA. HEK293 cells expressing human and mouse P2X7 were exposed to the selective P2X7 receptor agonist, 2', 3'-(benzoyl-4-benzoyl)-ATP (Bz-ATP) with or without 3TC, and VEGF-A levels in media were measured by ELISA. Intravitreous injection of 3TC, AZT, and ABC significantly suppressed laser-induced CNV in C57BL/6J wild-type and Nlrp3–/– mice ($P < 0.05$), but not in P2rx7–/– mice. Intravitreous injection of A438079 also suppressed the laser-induced CNV ($P < 0.05$). 3TC, AZT and ABC blocked VEGF-A levels in the RPE/choroid after laser injury in wild-type ($P < 0.05$) but not P2rx7–/– mice. Moreover, there was no additive effect of 3TC on CNV inhibition when co-administered with a neutralizing VEGF-A antibody. Stimulation of human and mouse P2X7-expressing HEK293 cells with Bz-ATP increased VEGF secretion ($P < 0.001$), which was abrogated by 3TC ($P < 0.001$). Stimulation of primary human RPE cells with Bz-ATP increased VEGFA and IL6 mRNA levels, which was abrogated by 3TC. Concluding, multiple clinically relevant NRTIs suppressed laser-induced CNV, and down-regulated VEGF-A, via P2X7.

Supported by:	NIH Award: DP1GM114862, R01EY018350, R01EY018836, R01EY020672, R01EY022238, and R01EY024068; Doris Duke Distinguished Clinical Scientist Award; Burroughs Wellcome Fund Clinical Scientist Award in Translational Research; Ellison Medical Foundation Senior Scholar in Aging Award; Foundation Fighting Blindness Individual Investigator Research Award; Harrington Discovery Institute Scholar-Innovator Award; American Heart Association and International Retinal Research Foundation.

Primary Presenter / email:	**Krueger, L.A.** / laura.krueger@uky.edu	University of Kentucky
	Basic Science **Stress** **PSMRF Program Participant**	
Mentor / e-mail:	Ambati, J. / therese.stearns@uky.edu	

UK MD/PhD Program Research Day Abstracts

POSTER PRESENTATION #146

Abstract Title:	**Peripheral Nerve Grafts to the Brain of Patients With Parkinson's Disease: Microscopic, Biochemical, and Immunohistochemical Characterization**
Author(s):	A.S. Welleford, Department of Neuroscience, U of Kentucky C.G. van Horne, Departments of Neuroscience and Neurosurgery, U of Kentucky J. E. Quintero, Department of Neuroscience, U of Kentucky Y. Ai, Department of Neuroscience, U of Kentucky G. A. Gerhardt, Departments of Neuroscience and Neurosurgery, U of Kentucky

Abstract: Currently two clinical trials (NCT01833364 and NCT02369003) are underway which feature the implantation of a peripheral nerve autograft to the brain (targeted either to the Substantia Nigra or the Nucleus Basalis of Meynert) in combination with Deep Brain Stimulation (DBS) for the treatment of patients with Parkinson's disease. This nerve tissue is harvested from the sural nerve (a sensory nerve located in the ankle) of patients undergoing DBS surgery. Two tissue samples per patient are collected for study (one during the Stage I surgery, another during the Stage II surgery 5-14 days later) in addition to the tissue used for the graft. As of 2/27/16, 40 patients have received a graft. This study examines several aspects of the peripheral nerve tissue; including microscopic appearance, levels of neurotrophic factors, morphology of Schwann Cells, and presence of macrophages. Techniques used include H&E and MCOLL histological staining, immunohistochemistry, and ELISA. These results are supplemented by immunohistochemical analysis of the brain of non-human primates that have undergone an analogous procedure. The results of this model show growth of tyrosine hydroxylase-containing nerve fibers, which are a marker of dopamine-producing neurons, into the area of the peripheral nerve graft. In addition, results in this model show the presence of S100beta-containing cells as well as GFAP-containing cells within and surrounding the graft, which is a marker of peripheral nerve regeneration. These findings suggest that the nerve graft in human patients may also display a regenerative phenotype which has the potential to alter the course of neurodegeneration in the brain.

Supported by: Grant/Other Support: Gifts to the Brain Restoration Center Grant/Other Support: Tom Dupree for Parkinson's Disease Research Grant/Other Support: University of Kentucky start-up funds Grant/Other Support: National Center for Advancing Translational Sciences grant UL1TR000117

Primary Presenter / email: **Welleford, A. S.** / aswell4@uky.edu University of Kentucky
Clinical Science
Surgery
Mentor / e-mail: Gerhardt, G. A. / gregg@uky.edu