

# Summer Research Program

## Poster Abstracts



**Thursday, July 26, 2018**

**Medical Education Building  
1<sup>st</sup> Floor Lobby**

**9:00 – 10:30 am, Judges and Students Only  
10:30 – 11:30 am, Open to the Public  
11:30 – 12:00 pm, Award Ceremony**

# 2018 Summer Research Internship Program

The Summer Research Internship Program provides research opportunities for medical students, undergraduates and high school students. The program directors, Dr. Paula Gregory and Dr. Fern Tsien, match high school and undergraduate students with mentors in laboratories or clinics at LSU Health Sciences Center and the Stanley S. Scott Cancer Center. The Summer Research Internship Program allows students to cultivate their interest in pursuing careers in either basic or clinical sciences. During the program students conduct their own research project or work on part of an on-going research project. Support for this program comes from:

- **Baptist Community Ministries**
- **Entergy**
- **LSUHSC School of Medicine, Office of the Dean**
- **LSUHSC Stanley S. Scott Cancer Center**
- **National Science Foundation (REU Program)**

Drs. Gregory and Tsien would like to extend their special appreciation to mentors, laboratory and administrative personnel, and poster session judges who helped make the Summer Research Internship Program a success! Their assistance with this project affords each student a chance to be part of a bigger, ongoing research project and allows them an opportunity to pursue their career goals.



**Jonathan C. Abshier**

Undergraduate

The University of Texas Rio Grande Valley, Brownsville, Texas

Mentor: Dr. Jorgelina Calandria PhD

Louisiana State University Health Science Center, Neuroscience Center of Excellence

**“Role of Phospholipase A2 group VI in the survival of retinal pigment epithelial cells”**

**Background:** Phospholipase A2 group VI activity is critical for the survival of dopaminergic cells. Mutations in the Calmodulin binding site is proposed to induce a dysregulation of PLA2G6 activity and a progressive degeneration of dopaminergic neurons located in Substantia Nigra pars compacta (SNpc) and thus Parkinson’s- like symptoms at early age (Paisan-Ruiz et al., 2009; Karkheiran et al., 2015; Zhou et al., 2016). Retinal pigment epithelial cells are dopaminergic cells that share several neuronal features because of their common ancestral lineage.

**Objective:** Under the hypothesis that PLA2G6 activity dysregulation interferes with the survival and function of retinal pigment epithelial (RPE) cells our goal was to test the apoptosis in two conditions: 1) overexpression of PLA2G6 open reading frame and 2) impairment of Calmodulin-mediated inhibition of its activity. We sought to identify changes in amount and localization of the phospholipase in RPE cells under these conditions.

**Materials and Methods:** In order to overexpress PLA2G6 we transfected RPE cells with an expression plasmid carrying the open reading frame for the phospholipase fused to green fluorescent protein. The dysregulation of endogenous- and overexpressed-PLA2G6 was achieved using SKF96365, an inhibitor of TRPC1 which regulates the store operated calcium entry (SOCE) and depletes the internal pools of calcium. To visualize apoptotic cells Hoechst staining was used along with an unbiased protocol set up in Metamorph software for imaging and analysis. Localization and quantification of PLA2G6 endogenous and overexpressed was assessed using immunocytochemistry and western blot techniques.

**Results:** Overexpression and dysregulation of PLA2G6 increased the Apoptosis at different extents. While PLA2G6-GFP overexpression induced cell death, the addition of SKF96365 increased these numbers eight times and potentiated the apoptosis induced by the overexpression alone. The GFP signal was found in vesicular structures around the nucleus that did not colocalized with LC3a and b, two markers of autophagy. The addition of SKF96365 did not induce an increase in the protein content.

**Discussion:** These results suggest that the internal content and the activity of PLA2G6 is tightly regulated by the RPE cell. The internal content of PLA2G6 is regulated independently of autophagy. The dysregulation of activity has deleterious effect on RPE cell survival. The possibility of local factor that induce dysregulation in either in quantity or activity of this specific phospholipase may cause an unbalanced production in arachidonic acid and other lipid mediators that affects the survival of the RPE cells. Promising therapeutically approaches may emerge from the study of the activity of PLA2G6 in retinal degeneration diseases.

**Marco A. Acosta**

Undergraduate

University of Louisiana-Lafayette, Lafayette, LA

Mentor: Minolfa Prieto, M.D., PhD  
Tulane University, School of MedicineMentor: Jason Gardner, PhD  
Louisiana State University Health Science Center, School of Medicine**“Effects of Prorenin Receptor on the Epithelial Sodium Channel in M-1 Cells”**

The systemic renin-angiotensin system (RAS) is the major regulator of blood pressure (BP) and water and electrolytes balance in the human body. In the RAS, angiotensinogen secreted from the liver is then converted into angiotensin (Ang) I by renin, which is secreted by the juxtaglomerular cells (JG) of the kidney. Ang I is converted to Ang II by angiotensin converting enzyme (ACE), secreted primarily by the pulmonary and renal epithelium. Therefore, Ang II, the most important hormone of the RAS, stimulates sympathetic activity, sodium reabsorption, water retention, and cell proliferation. The kidney expresses all of the RAS components; thus the intrarenal RAS regulates sodium reabsorption and BP.

In the kidney, the epithelial sodium channel (ENaC) located on the plasma membrane of the principal cells in the distal nephron segment, allows the reabsorption of sodium in response to Ang II. Excess stimulation of ENaC can lead to sodium retention and hypertension. The ProRenin Receptor (PRR), a component of the RAS, is a receptor for renin and prorenin that enhances renin activity and fully activates prorenin by a non-proteolytic mechanism. This receptor is expressed in various tissues, including brain, lung, placenta, pancreas, fat, and kidney. In the kidney, PRR is primarily expressed in the apical cell side of the collecting duct cells. In a recent study (Prieto MC *et al.*, *AJP Renal* 2017), mice with PRR deficiency in the collecting ducts showed an attenuated blood pressure response to chronic Ang II infusion along with decreased in the levels of intrarenal Ang II and renin activity, as well as diminished sodium reabsorption. These mice showed reduced ENaC activity and open probability ( $P_o$ ) measured in fresh isolated collecting ducts using path clamp. However, whether in this mouse models ENaC alterations are due to solely the decreased intrarenal Ang II levels or are a result of a direct action of PRR remain unclear.

To clarify this question, here we test the **hypothesis** that the PRR upregulates the expression of ENaC in M-1 cells. To address this hypothesis, we culture of mouse collecting duct cells (M-1 cell line) and activate PRR using mouse recombinant ProRenin (mRPro), as agonist of the PRR. The cells were separated into four different groups. M-1 cells were treated with mRPro with a concentration of  $10^{-7}$  M for different time durations, 6 hours, 12 hours, 24 hours, and the control group was left untreated. Two independent experiments were performed. The cells were harvested in the presence different inhibiting enzymes and protein extraction and quantification of protein concentration was done using the Bradford Assay. According to their different protein concentrations, dilutions were prepared, so they would have the same protein concentration in each sample. The protein levels of the different ENaC isoforms (alpha, beta and gamma) and PRR will be quantified using Western blot and the following antibodies: rabbit anti-alpha ENaC (generously provided by Dr. Mark Knepper from the NIH-NHLBI); rabbit anti-beta ENaC (StressMarq # spc 404); rabbit anti-gamma ENaC (StressMarq Cat # spc 405); and rabbit anti-PRR (Sigma, Cat # hpa 003156), respectively. It is expected that the result from these experiments will allow evaluating the direct effect of PRR activation on ENaC expression in collecting duct cells.

**Nkhilesh V. Alahari**

High School

Athens Drive High School, Raleigh, North Carolina

Mentors: Lin Yuan, MD, Frank H. Lau, MD; PhD

Department of Surgery, LSUHSC School of Medicine, New Orleans LA 70112

**“Glucose Uptake in Primary Cultured Human Adipose Tissue”**

**Objective:** *Ex vivo*, the glucose uptake capability of primary white adipose tissue (WAT) diminishes rapidly. Since storage of excess calories is a primary function of WAT, this loss of function has limited the utility of cultured primary WAT as a physiologic model. Our group recently developed a novel approach to culturing primary human WAT called Sandwiched White Adipose Tissue (SWAT). This experiment tested our hypothesis that adipocytes cultured as SWAT would demonstrate no significant loss of glucose uptake activity even after several days in culture.

**Research Design and Methods:** Subcutaneous abdominal WAT from 3 human subjects undergoing elective plastic surgical procedures were procured and cultured for 4 days as SWAT. Culture media was: Dulbecco's Modified Eagle Media. Prior to assay, the SWAT was insulin and glucose starved for 12 hours. Individual adipocytes were isolated using standard collagenase digestion after 1 and 4 days in culture. Glucose uptake capability of the isolated adipocytes was quantified using the Glucose Uptake-Glo Assay Kit (Promega). Assay was performed in triplicate per sample. One-tailed t-test was used for statistical analysis.

**Results:** Subcutaneous abdominal WAT was procured from 3 adult women (47.667 +/- 12.115 years old; BMI 35.133 +/- 2.849 kg/m<sup>2</sup>). Glucose uptake on day 1 averaged 1.58 +/- .34 following insulin treatment (vs. controls) and 1.53 +/- .31 on day 4 ( $p = .0009$ ). Induction of glucose uptake on day 1 vs. day 4 was not statistically significant ( $p = 0.0062$ ).

**Conclusions:** Insulin-stimulated samples yielded a higher glucose uptake rate than the control samples at both day 1 and day 4 in SWAT. There was no significant loss of glucose uptake capability between day 1 and day 4, indicating that SWAT preserves primary human white adipose tissue glucose uptake capability *ex vivo*.

**Zaynah F. Alam**  
Undergraduate  
Emory University, Atlanta, Georgia

Mentor: Sijiong June Liu  
LSUHSC

**“The expression of PPAR $\alpha$  in the cerebellum”**

Endocannabinoids are lipid-based molecules that function as neuromodulators. The major endocannabinoids are anandamide and 2-arachidonoylglycerol (2-AG), which target cannabinoid receptors throughout the central nervous system. Specifically, high levels of 2-AG are observed in the cerebellum, a brain structure associated with the regulation of emotions. 2-AG is primarily hydrolyzed by monoacylglycerol lipase (MAGL) and therefore, the regulation of MAGL gene expression has an impact on 2-AG levels. One mechanism of promoting MAGL gene expression is through stimulation by peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ), a transcriptional factor belonging to a group of nuclear receptor proteins. PPARs affect the expression of target genes involved in cell proliferation, cell differentiation, and immune responses. However, little is known about the role of PPAR $\alpha$  in the central nervous system. In this study, we aimed to characterize the expression of PPAR $\alpha$  in the cerebellum. Immunohistochemistry was used to identify the level and localization of PPAR $\alpha$  in the cerebellar vermis. We specifically observed immuno-staining in the Bergmann glia and granule cells. Interestingly, these cells are known to express MAGL. Notably, the cerebellar vermis participates in the processing of fear memory and endocannabinoid signaling has been associated with fear memory consolidation. We are currently testing whether fear conditioning alters PPAR $\alpha$  levels/distribution in the cerebellar cells.

**Omar A. Alnajjar**

Undergraduate

Xavier University of Louisiana, New Orleans, Louisiana

Mentor: Arnold H. Zea, PhD  
LSUHSC**“The Effects of Arginase Inhibition on L- Arginine-induced Renal Cell Carcinoma”**

Renal Cell Carcinoma (RCC) is one of the most chemically resistant tumors known to the medical world characterized by a suppressed T-cell function caused by L-arginine depletion. Many different medical trials using L-arginine to suppress the harmful effects of RCC have had minor success in humans, but have shown some encouraging results in rodents. L-arginine is metabolized intracellularly by nitric oxide synthase (NOS2) and arginase2 to produce nitric oxide and polyamine synthesis. Therefore, targeting arginine metabolism can modulate key aspects of these tumors that can result in a better disease control. We hypothesize that inhibition of arginase2 highly expressed in RCC had a direct effect in tumor growth by decreasing polyamine synthesis.

To test our hypothesis, we used a RCC cell lines expressing high (CL19) and moderate (Renca) arginase2 activity. The cells were treated with 50 and 100  $\mu$ M of the specific arginase 2 inhibitor boronoethyl-L-cysteine (BEC) for 24 and 48 hours. At each time point, tissue culture supernatants and cell lysates were collected and stored for future use. Supernatants were tested for amino acid contents (L-arginine, L-citrulline, putrescine) and assayed for nitric oxide (Greiss assay). Cell lysates were also tested for arginase activity (enzymatic assay) and arginase 2 protein (Western Blot).

The results show a significant decrease ( $p=0.04$ ) in arginase activity at 48 hours when compared to untreated cells. However, arginase 2 protein expression was abolished by the inhibitor. Interestingly, the inhibitor appears to be more effective in CL-19 than in Renca cells as seen by their different arginase2 protein expression. The inhibitory effect of BEC in CL-19, parallels with an increment in L-arginine and L-citrulline ( $p=0.02$ ). No changes in polyamine (putrescine) levels were seen when compared to untreated cells. We did not performed proliferation assays, but we could observe macroscopically a 60-70% cell confluence in the treated cells as compared to untreated cells (100%). No increments in nitric oxide (nitrite production) were seen in both cell lines.

Based in this preliminary data we can conclude that: **1.** RCC expressing high levels of arginase can better benefit from treatments with arginase inhibitors. **2.** It is possible that the elevated production of L-citrulline by arginase inhibition could be an additional benefit to increase L-arginine levels since L-citrulline can be used as substrate for the novo production of L-arginine. Also, increased levels of L-arginine can restore T-cell function. **3.** A better understanding of the role of L-arginine in cancer may lead to novel antineoplastic and chemo preventive strategies. The, functional consequences and how these mechanisms can be harnessed, need more in depth studies.

**Joussette I. Alvarado**

Undergraduate

Loyola University New Orleans, New Orleans, LA

Mentor: Jovanny Zabaleta, M.S., Ph.D.

Louisiana State University Health Sciences Center (LSUHSC), Department of Pediatrics, Stanley S. Scott Cancer Center

**“Differential Gene Expression according to Ancestry in Hispanic/Latina Women with Luminal B Breast Cancer”**

Breast cancer is the second-leading cause of cancer-related death in women. There is a lack of breast cancer studies showing the differential gene expression in minorities. Previous studies from the group have shown differences in gene expression according to ancestry in Hispanic/Latina women with breast cancer. The goal of our study was to determine the differential gene expression between high and low European, African, and Native ancestries of Hispanic/Latina women with luminal B breast cancer.

The breast tissue samples were collected from the Moffitt Cancer Center (MCC) in Tampa, FL. The tissue samples had been analyzed for ancestry informative markers (AIMs) to find the ancestry fractions of each sample as part of a collaboration between MCC and the University of Puerto Rico. We found a significant inverse correlation between European and both African and Native American ancestries. The RNA was quantified and used to prepare genomic libraries using regents and protocols from Illumina. We performed RNAseq at the Translational Genomics Core, Stanley S. Scott Cancer Center, LSUHSC. Quality assessment showed close to 50% alignment in coding regions. Raw counts were normalized and used to perform differential gene expression analysis ( $p \leq 0.05$ ) using DESeq2 in R-Studio between individuals with high and low European, African, and Native ancestral fractions (based on the average). DESeq2 retrieved the most statistically significant genes in each of the fractions in each ancestral group, clearly separating the two fractions as shown by heatmaps. We then performed subsequent filtering based on log<sub>2</sub> fold change. Using the online tool Venny, we identified 18 genes shared between European and Native American ancestries, the expression of which suggests an ethnicity-associated expression. Furthermore, one gene *SPAG6* was commonly expressed among all ancestries with a positive correlation in Native ancestry and negative correlation in European ancestry. In addition, we used online tools to determine the type of cellular infiltration in our samples and found that African ancestry was associated with the infiltration on lymphocyte progenitors and type 2-macrophages. Meanwhile, European and Native ancestries correlated with type 2-lymphocytes and monocytes, respectively. Pathway analysis revealed possible activation of immune responses driven by genes significant in European and Native ancestries. Interestingly, cBioportal analysis showed that *ANO1*, *EPN3*, *PLAT*, and *TRPA1* genes are frequently amplified in breast cancer. We validated one of those genes, *EPN3*, and are in the process of validating others using real-time PCR analysis.

Overall, our data suggest that patients with luminal B breast cancer may have differential gene expression associated to ancestry and to gene abnormalities and differential immune responses.

**Sara I. Beaulieu**  
High School  
Lusher Charter School, New Orleans, LA

Mentor: Matthew Whim, PhD  
Department of Cell Biology and Anatomy, LSUHSC

**“Locating Tissue Resident B Cells in the Murine Adrenal Gland”**

The adrenal glands are (neuro)endocrine glands that secrete hormones that maintain homeostasis. Adrenal functions include the control of blood glucose, cardiovascular function and the response to stress. The adrenal glands are composed of two regions, the adrenal cortex and the adrenal medulla. The adrenal cortex, the outer layer, secretes steroid hormones such as aldosterone and corticosterone. In adult mice, the adrenal cortex has two zones, the zona glomerulosa and the zona fasciculata. The zona glomerulosa produces aldosterone which controls blood pressure. The zona fasciculata produces glucocorticoids, including corticosterone, which increases blood glucose, suppresses inflammation and regulates blood pressure. The adrenal medulla releases the catecholamines, epinephrine and norepinephrine, into the bloodstream. These hormones are secreted during the fight-or-flight response to stress, particularly in response to a fall in blood glucose.

Recent studies show that the adrenal gland also contains a diverse population of immune cells, including B cells. The function of these cells in the adrenal is not known but since they are located in both the zona fasciculata and the medulla it is likely that they modulate the release of adrenal hormones. The aim of this project was to test the hypothesis that adrenal activation would lead to a change in the zonal distribution of adrenal-resident B cells.

Because hypoglycemia leads to the secretion of both corticosterone and epinephrine we compared the distribution of B cells in mice exposed to two different metabolic stressors (fasting and insulin-induced hypoglycemia). In the first protocol, the experimental mouse was fasted for 24 hrs while the control animal was free to eat ad lib. In the second protocol, the experimental animal was injected with 2.5 U/kg insulin and the control animal was injected with saline. All mice were sacrificed after 24 hours, the adrenal glands were removed and cryosections were prepared. Adrenal B cells were localized using an immunohistochemical approach by staining sections with an anti-CD45R/B220 antibody which recognizes an antigen present on all B lymphocytes.

Preliminary results indicate that neither insulin-induced hypoglycemia nor fasting led to a marked change in the number of adrenal-resident B cells. In all conditions, the normalized density of CD45R/B220-ir cells was higher in the cortex than the medulla but the relative distribution did not change after metabolic stress. These results are inconsistent with our original hypothesis. In future experiments, directly measuring adrenal function in the absence of B cells may provide a more direct way to assess the role of these tissue resident B-cells.

**Melissa E. Berner**  
High School  
Loyola University, New Orleans, Louisiana

Mentor: Dr. Scott Edwards:  
LSUHSC, Department of Physiology

### **"Neuroadaptations in PAG Endocannabinoid Signaling in Pain and Addiction"**

**Background:** The endocannabinoid system consists of cannabinoid receptors and corresponding neurotransmitters called endocannabinoids. This system's tasks include maintenance of neuronal homeostasis and provision of an endogenous analgesic response. The two main endocannabinoids are 2-AG and anandamide. The two major types of cannabinoid receptors are CB<sub>1</sub> and CB<sub>2</sub>, with CB<sub>1</sub> receptors being the predominant receptor subtype found in the brain. In the present study, we measured expression of proteins responsible for the biosynthesis and degradation of endocannabinoids in the periaqueductal grey (PAG) region, a major regulator in descending antinociceptive signaling.

**Methods:** Adult male Wistar rats received daily subcutaneous (s.c.) injections of either sterile saline or morphine in an escalating dose regimen (from 10-20 mg/kg) for three weeks. Pain avoidance behavior was measured using the mechanical conflict avoidance task, where rats must exit a brightly lit start chamber and cross nociceptive probes to reach a dark goal box. Latency to exit onto probes was measured as an indicator of pain avoidance-like behavior. Western blot analysis was used to quantify protein levels of cannabinoid receptor type 1 (CB<sub>1</sub>R) and 2-AG synthetic and degradative enzymes diacylglycerol lipase-*a* (DAGL-*a*) and monoacylglycerol lipase (MAGL). Protein expression was normalized to GAPDH.

**Results:** Chronic morphine exposure increased pain avoidance behavior, and pain avoidance behavior was negatively correlated with CB<sub>1</sub>R levels across both morphine- and saline-treated animals. Pain avoidance was also negatively correlated with DAGL-*a* levels only in morphine-treated animals.

**Conclusions:** Finding different brain changes can develop potential pharmaceuticals to treat opioid-induced hyperalgesia. Our results indicate that decreased endocannabinoid activity plays a role in opioid-induced hyperalgesia and suggest that exogenous cannabinoids would be a potential pharmacotherapy for hyperalgesia associated with opioid withdrawal.

**Fernando Blank**

Undergraduate

The Ohio State University, Columbus, OH

Mentor: Dr. Hamilton Farris, Ph.D.

Louisiana State University Health Sciences Center, New Orleans, LA

**“Morphological variation in retinal architecture of diurnal and nocturnal frogs”**

Photon availability under different light regimes selects for differences in the optical and neural components of the visual system. With respect to animal models of research of visual pathologies, nocturnal and fossorial murine models do not mimic the optical, cellular, biochemical, or physiological processes of the diurnal human eye. Previous findings in our lab have shown that nocturnal and diurnal frog species differ in their optical anatomy, photoreceptor outer segment dimensions, and physiological sensitivity. Herein, we examined the inner nuclear layer (INL) and outer nuclear layer (ONL) to determine if these differences are reflected in the cellular architecture of the retina. We used 1  $\mu\text{m}$  thick plastic sections of retina to count and calculate the density of the INL and ONL cells for four species of frogs (two diurnal species and two nocturnal species). We found that, on average, the INL of diurnal species have a significantly higher number of cells than those of nocturnal species. Ultimately, our results indicate that visual circuitry in the retina of diurnal species is approximately 2.5x greater than in nocturnal species.

In the future, we plan to characterize the various cells of the INL to see if the observed hypertrophy is limited to a specific cell type, and if it is associated with a specific class of retinal photoreceptor. Most biomedical research on the visual system is performed on nocturnal rodent species; Thus, these data are critical for providing a framework for nocturnal versus diurnal retinal differences that could affect clinically relevant research with humans.

**Ann M. Byerley**  
High School  
Mandeville High School, Mandeville, Louisiana

Mentor: Dr. Martin J. Ronis  
LSUHSC, Department of Pharmacology

**“Antioxidants Ability to Protect Against Binge Alcohol-Induced Liver Damage”**

According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), binge alcohol consumption contributes to more than 200 reported diseases and injury-related health conditions including liver cirrhosis. The initial stage of alcoholic liver injury is steatosis, also known as fatty liver disease. Although research supports that alcohol leads to hepatic steatosis, the mechanism by which alcohol does this is not well understood. Therefore, this study asked: does ethanol produce hepatic steatosis acutely after binge drinking and can this be protected against? Theories suggest that alcohol promotes oxidative stress pathways in the liver, so consuming a diet high in antioxidants should inhibit alcohol's effect on the liver. In this study, the mitochondrial targeted antioxidant Mito-Tetramethylpiperidine (MitoTEMPO) was tested and possible interactions with delta 9-tetrahydrocannabinol (THC) were examined since ethanol and THC are often consumed together. THC is a cannabidiol with known neuroprotective actions through oxidative pathways and, therefore, might also act as an antioxidant. To test the effects of binge drinking and oxidative effects, mice were used to create a binge drinking model. Control male C57 BL/6 mice aged 6 weeks were gavaged with saline or with an ethanol gavage over a four day period (day one and two 3g/kg, day three 4g/kg, and day four 4.5g/kg). Ethanol treated mice were given intraperitoneal (i.p.) injections of MitoTEMPO (2mg/kg) or THC (10mg/kg) one hour prior to gavage. Liver and serum samples were isolated and used to assess liver necrosis and steatosis using two assays: alanine transaminase assay (ALT) and a colorimetric triglyceride assay. ALT results were negative demonstrating that mice have a high tolerance for necrotic injury produced by acute alcohol exposure. ALT enzyme levels were not elevated enough to determine liver damage when compared to control groups. However, the ethanol treated groups showed a statistical difference in hepatic triglyceride content compared to the control models ( $P < 0.05$ ). However, the MitoTEMPO + ethanol and THC + ethanol groups exhibited even higher levels of triglyceride than the ethanol group ( $P < 0.05$ ). This research suggested that the steatotic effects of binge alcohol consumption are actually worsened by antioxidants, such as MitoTEMPO, and THC, suggesting that development of steatosis is not linked to an oxidative stress pathway in the liver. To further develop the idea that hepatic steatosis follows a mechanism other than an oxidative stress pathway, a protein carbonylation assay was used on the liver samples to measure oxidative protein damage. Results suggest that alcohol increases protein carbonylation; however, MitoTEMPO and THC did not worsen hepatic oxidative protein carbonylation after ethanol gavage, suggesting that the steatosis-promoting effects of MitoTEMPO and TCH are not mediated by elevated oxidative stress. Supported in part by R37 AA018282 (M.R).

**Meija M. Caldwell**  
Undergraduate  
Xavier University of Louisiana, New Orleans, LA

Mentor: Donna L. Williams, DrPH  
Louisiana State University Health Sciences Center School of Public Health, Louisiana Cancer  
Prevention and Control Programs

### **“Quitline Calls to Lung Cancer Screening”**

Lung cancer is the leading cause of cancer death in the US, and it is estimated that 154,050 deaths from lung cancer will occur in the US in 2018. Louisiana’s lung cancer incidence and mortality rates are statistically significantly higher than those of the rest of the country. Lung cancer patients tend to have lower 5-year survival rates, due largely to the fact that lung cancer is often diagnosed at a late stage when it is less treatable. However, with lung cancer screening, more abnormalities may be detectable before late stages, which could increase 5-year survival rates for patients. The screening guideline from the National Comprehensive Cancer Network (NCCN) and the U.S. Preventive Services Task Force was updated in 2018 and recommends low-dose computer tomography (LDCT) scan to screen for lung cancer in individuals that are at high risk for developing lung cancer. Quitlines are free telephone helplines which can provide or refer smokers who want to quit smoking to treatments including individual counseling, group counseling, or cessation medications. This will aim to build upon the precedent set by HIV and suicide-prevention helplines at directing callers to treatment. Callers to a tobacco cessation quitline will be directed to discuss lung cancer screening with their healthcare provider if they are at a high risk for developing lung cancer.

We will introduce a set of questions to the quitline to be integrated into the regular quitline script to determine which patients should be referred to lung cancer screening. Some of the NCCN criteria such as whether a patient displays signs and symptoms of lung cancer, can only be determined by a physician, quitline counselors cannot refer patients directly to LDCT lung cancer screening, but will direct patients to discuss screening with their healthcare provider. The healthcare provider and patient will have a shared decision-making visit, and the healthcare provider may then refer the patient to screening.

So far, we have:

- Contacted the quitline;
- Gotten a list of providers in the quitline’s Fax-to-Quit (F2Q) program;
- Requested data on caller demographics for analysis;
- Created provider education materials for distribution;
- Created the rubric or question tree to determine caller eligibility; and
- Introduced the eligibility question tree to quitline.

Education materials will be mailed to existing F2Q providers and added to the quitline’s webpage. Mailed education materials will be created with providers and patients in mind, while materials posted to the quitline’s website will be directed specifically toward providers.

**Laura C. Carrasquilla**

Undergraduate

University of Alabama, Tuscaloosa, AL

Mentor: Jovanny Zabaleta, M.S., Ph.D.

Louisiana State University Health Sciences Center, Louisiana Cancer Research Center

**“The relationship between ancestry fractions and gene expression in triple negative breast cancer in Hispanic/Latina women”**

Breast cancer is the most frequently diagnosed form of invasive cancer in women and is the second leading cause of all cancer deaths in women. Triple negative breast cancer (TNBC) accounts for 10-20% of all breast cancers. This aggressive subtype lacks receptors for estrogen, progesterone, and HER2, drastically reducing treatment options. Hispanic/Latinas derive ancestry from European, African, and Native origins and are at least 30% more likely to be diagnosed with TNBC compared to non-Hispanic White women. However, little is known about the genomic architecture of TNBCs in Hispanic/Latinas. The goal of our study was to find the correlation between differential gene expression and different ancestral backgrounds within TNBC samples in Hispanics/Latinas to help identify potential targets for future therapies.

We obtained 41 TNBC tissues through collaboration with the National Cancer Institute in Colombia along with demographic and clinical information. Ancestral fractions were determined by examining the frequency of 106 Ancestry Informative Markers (AIMs). RNA was extracted from FFPE tissues, qualified using Agilent chips, and used for RNA sequencing at the Translational Genomics Core at the Stanley S. Scott Cancer Center. Normalized read counts used in online tools validated the TNBC status for 33 of the 41 samples, and quality control ensured that the samples were of acceptable quality for further analysis. Differential gene expression between high and low ancestral fractions was found utilizing DESeq2 in R-Studio. We also determined the cell type infiltration based on gene expression using online tools.

We found a significant inverse correlation between European and Native ancestries. 91 genes were differentially expressed in the European ancestry, along with 21 for the Native and 12 for the African. Interestingly, 5 genes were common between the European and Native ancestries. Of the 5, *LGALS9C* and *MESP1* had significant differences in normalized counts between high and low ancestry for both the Europeans and Natives, though the subgroups displayed opposite correlation patterns. These results were validated by real-time PCR. Using cBioPortal, we found that *MESP1* and *LGALS9C* were associated with amplification in 2.8% and 1.5% of breast cancer cases respectively. Remarkably, *LGALS9C* expression displays an inverse correlation with disease outcome in several cancers including breast. Cell infiltration analysis showed that African ancestry is associated with infiltration of various types of B-cells and CD4+ T-cells. Meanwhile, European and Native ancestries correlated with monocytes and immature dendritic cells. Interestingly, the subgroups once again showed opposite correlation patterns.

Our data suggests that several genes, most notably *MESP1* and *LGALS9C*, may be related to ancestry in TNBC patients. Targeting these genes, studying the immune landscape, and considering ancestry may improve diagnosis and provide options for future interventions.

**Anna E. Champ**  
High School  
Saint Scholastica Academy, Covington, Louisiana

Mentor: Stefany D. Primeaux, Ph.D  
Department of Physiology, LSU Health Sciences Center, New Orleans, Louisiana; Joint Diabetes, Endocrinology & Metabolism Center, Pennington Biomedical Research Center, Baton Rouge, Louisiana

**“Effects of Chronic Ethanol Consumption on CD36 mRNA Expression in Mouse Gastrointestinal Tract”**

The chronic consumption of ethanol has been linked to an increased risk of metabolic dysfunctions and their comorbidities such as fatty liver disease, type II diabetes, and hypertension. Preliminary data from our laboratory suggests that acute administration of ethanol (1g/kg, intraperitoneal) increases the preference for high fat diet, which may promote metabolic dysregulation. The fat taste receptor, CD36, plays a significant role in sensing and regulating the intake and preference for long chain fatty acids contained in high fat foods. Furthermore, CD36 is expressed throughout the gastrointestinal (GI) tract, specifically in the circumvallate papillae of the tongue and in the duodenum. The goal of the current study is to determine whether chronic ethanol consumption alters CD36 mRNA expression in mouse circumvallate papillae and the duodenum.

Forty C57BL/6 male mice were divided into two groups ( $n=20$ ) and fed a high fat (35% fat) Lieber DeCarli liquid diet for sixteen weeks. The experimental group received increasing amount of ethanol in their diet, with a final concentration of 28% ethanol. Body weight and kilocalorie intake were assessed. Based on their average daily kilocalorie intake, mice were divided into high drinkers and low drinkers using a median split analysis. At sacrifice, the circumvallate papillae and enterocytes from the duodenum were harvested. Expression of CD36 mRNA was determined by Real Time PCR.

Chronic consumption of ethanol led to a decrease in the average body weight and caloric intake in both high and low drinkers. CD36 mRNA expression was upregulated in the duodenum of high and low ethanol drinkers, suggesting that chronic consumption of ethanol regulates CD36 mRNA levels in the small intestine of the GI tract. Chronic ethanol consumption decreased CD36 mRNA expression on the circumvallate papillae in low drinkers but increased CD36 mRNA expression in high drinkers. Increased CD36 mRNA levels in the duodenum are suggestive of increased fat transport from the small intestine into the blood and lymph, which may lead to increased fat accumulation. In addition, increased fat sensing in the duodenum may lead to an increase in the release of satiety hormones, which may explain the decrease in kilocalories consumed. Decreased CD36 mRNA expression in the circumvallate papillae of the low drinkers may indicate a decrease in preference for high fat foods, thus explaining the decrease in kilocalorie intake. Increased CD36 mRNA expression in the circumvallate papillae of the high drinkers may suggest an increase in preference for high fat foods and thus an increase in kilocalorie intake. More studies are needed to determine ethanol's role on fat transport and fat sensing in the GI tract.

**Hsiao-Man (Schumann) Chang**

Undergraduate  
Emory University, Atlanta, GA

Mentor: Dr. Lauri O. Byerley PhD  
Department of Physiology, LSUHSC, New Orleans

**“Gut microbiome alterations as a potential mechanism for walnut-induced weight preservation amongst cancer cachectic rats”**

Characterized as the involuntary loss of body weight, cachexia is observed with other malignant diseases. Since cachectic development is independent of conventional nutritional support, there are no medical interventions to completely reverse the effects of cachexia. Experienced by about 50% of cancer patients, cachexia detrimentally impacts cancer prognosis and therapy. Thus, it is imperative to alleviate cachectic syndromes for cancer patients to yield favorable outcomes.

In a pilot study, walnuts were shown to preserve weight during the pre-cachectic tumor-bearing phase in rats. Additionally, walnuts are known to have anti-cancerous effects in genetically programmed mice. A possible area to observe the beneficial effects of walnuts is within the gut microbiome, the most diverse of the human microbiome. Since our lab previously observed significant gut microbiota changes of the non-tumor bearing rats on walnut diets, the aim of this investigation was to determine the effect of walnuts on the gut microbiome of tumor-bearing cachectic rats. We hypothesized that walnuts would significantly and favorably change the gut microbiome amongst cachectic rat, providing a potential mechanism for the weight gain.

To test our hypothesis, 18 male Fischer 344 rats were implanted with the Ward colon carcinoma subcutaneously on the left hind flank. This model developed tumor-driven cachexia. The rats were placed on a walnut diet, control diet, or a walnut/control diet. Essentially, the control diet replaced the fat, protein, carbohydrate, and fiber contents of the walnut diet with corn oil, casein, corn starch, and alphacel fiber, respectively, allowing the presence of walnuts to be the only testing factor. Fecal matter was collected from the rats upon sacrifice, and 16srDNA was sequenced to elucidate specific microbial communities.

Results revealed that rats on the walnut diet had the greatest weight preservation. Changes in the gut microbiome via the consumption of walnuts were evident through measures of diversity, relative abundance, and metabolic pathways at the family level. Compared to the control diet, the walnut diets led to a greater diversity and separation of the gut microbial communities. Walnut diets also significantly altered the relative abundances of *Prevotellaceae* and *Desulfovibrionaceae* (p-value < 0.05) at the family taxa level. Lastly, the functional metagenomic data revealed a greater prevalence of metabolic pathways for plant-pathogen interaction, flagellar assembly, bacterial chemotaxis, and ABC transports in rats on the walnut diet.

In summary, we conclude that walnut consumption alters the gut microbial community, suggesting a potential mechanism by which walnuts may preserve weight. This warrants further investigation to unravel the role gut microbes may play in cancer cachexia.

**Taylor K. Collins**

High school

Isidore Newman School, New Orleans, Louisiana

Mentor: Alix D'Angelo, MGC, CGC:

Department of Genetics, LSU Health Sciences Center

**“Experience of a New Hereditary Cancer Practice in an Underserved Population”**

Individuals who have hereditary forms of cancer have significantly increased lifetime risks of developing various cancers. Identifying at-risk individuals is crucial, so treatment plans can be personalized and/or interventions can be offered before a cancer is diagnosed. Until recently, access to genetic testing in Louisiana has been difficult due to a number of reasons, including a shortage of genetic professionals, prior lack of insurance coverage, and high test costs. However, over the last two and a half years, routine genetic counseling and genetic testing for hereditary cancer have been offered to patients at the LSU Healthcare Network and University Medical Center by a certified genetic counselor. Louisiana has a unique patient population, consisting of under-served individuals from a variety of ethnic backgrounds, for which there is little data regarding mutation frequencies. Therefore, we aimed to characterize the hereditary forms of cancer present in the under-served populations of Louisiana in comparison to other US and world populations.

Of the 293 individuals who underwent panel genetic testing, 13.4% tested positive for a (likely) pathogenic mutation, 57.5% tested negative, and a VUS (variant of unknown significance) was identified in 30.5%. The most commonly mutated genes in the breast/gynecologic cancer population were: *BRCA1*, *BRCA2*, and *FANCC*. The positive rate was lower (11.1%) in the African American population compared to other ethnicities such as Caucasian (19.6%) and Hispanic (18.8%). However, there were lower numbers of patients in the later groups, making comparison difficult. The most commonly mutated genes in the colorectal cancer population were *MSH6* and *APC*, however, testing numbers were small in this group. Three individuals (~1%) tested positive for pathogenic mutations in two separate hereditary cancer genes. Overall, our data is similar to previous studies published in other patient populations, aside from the *FANCC* mutation rate. Continued genetic testing of greater numbers of Louisiana's unique patient population may be able to elucidate additional differences in hereditary cancer genes.

**Garth W. Cook**

Undergraduate

Johns Hopkins University, Baltimore, Maryland

Mentor: Dr. Liz Simon

LSUHSC Department of Physiology

**“Effects of Alcohol and Gonadal Hormone Loss on Regeneration after Muscle Disuse Atrophy”**

Immobilization or disuse causes detrimental skeletal muscle alterations including atrophy and decreased functional capacity. Aging and alcohol consumption both increase the incidence of fractures, leading to immobilization. Chronic alcohol use exacerbates disuse atrophy and impairs recovery by lowering the myogenic potential of satellite cells, as well as increases the risk for hyperalgesia. Skeletal muscle regeneration is integral to recovery and is tightly regulated by muscle regulatory factors including genes and microRNAs. Estrogen also activates transcription factors necessary for myogenesis, resulting in impaired muscle recovery in postmenopausal women. Loss of estrogen also increases the risk for osteoporotic fractures. 1 in 2 women over 50 will have fractures and immobilization, leading to muscle disuse atrophy. Estrogen has anti-inflammatory effects, while alcohol increases inflammation. The aim of this study was to elucidate the mechanisms of alcohol and gonadal hormone loss-induced impairment in recovery after unilateral hind limb immobilization. Additionally, we will assess the potential effects of alcohol and gonadal hormone loss on inflammation and peripheral pain sensitivity. Using three-month-old female Fischer-344 rats as a model, the rats (N=35) were split into either ovariectomy or sham groups. After ovariectomies were performed, the rats were administered either a Leiber DiCarli control or alcohol diet (average blood ethanol concentration 0.1g/dl) for 10 weeks and subjected to unilateral hind limb immobilization for 1 week. The cast was then removed, and the rats were allowed to recover for either 3 or 14 days. The quadriceps muscle was collected and the non-immobilized limb served as a control. Preliminary von Frey data showed an increase in pain sensitivity due to alcohol and immobilization. There was a significant decrease in overall body weight due to alcohol in the 3-day recovery group; however, there was no significant differences in the body weight in the 14-day recovery group. In the 3-day group, there was a statistically significant decrease in the quadriceps muscle weight due to alcohol, ovariectomy, and immobilization independently. However, in the 14-day group, there was a significant decrease in quadriceps weight due to immobilization. qPCR was used to measure expression levels of markers for myogenesis (Mef2C, Myh1, MyoD, Myogenin); inflammation (MCP1, TNF $\alpha$ , IL1 $\beta$ ); and peripheral pain sensitivity (P2RX3, TRPV1). Hematoxylin and Eosin staining was performed on the quad tissue to determine cell size and regenerating muscle-fibers. Initial results show a significant decrease in Mef2C expression due to alcohol and ovariectomy in the 3-day recovery group, indicating an impairment in muscle regeneration.

**Marissa A. Duckett**

Undergraduate

University of Kansas, Lawrence, KS

Mentor: Dr. Edward Peters DMD, SM, ScD

LSUHSC School of Public Health, Professor and Chair Epidemiology

**“Rural and Urban Oral Health Disparities of Children in Louisiana”**

**Purpose:** The purpose of this study is to identify and compare the burden of oral health disparities in elementary aged students in rural and urban areas of Southeast Louisiana. To achieve this goal, dental sealant data from Eat Move Grow (EMG) was analyzed.

**Methods:** To achieve this goal, de-identified dental screening data from 2007-2014 provided by EMG Southeast Louisiana sealant program were entered into a REDCap database, extracted and analyzed in SAS 9.4. Screening was conducted throughout 46 elementary schools in 12 parishes. Screening forms recorded caries, presence of sealants; and decayed, missing, and filled teeth (DMFT). When "urgent" need was identified, the child was referred for emergent care. The DMFT score was calculated by summing the numbers of permanent teeth with those conditions per child. Summary and descriptive statistics for children's demographic characteristics and oral health conditions were estimated. Based on US census, school addresses were classified as either rural or urban. Health Professional Shortage Area (HPSA) Dental Full-Time Equivalent practitioners (FTE) were examined. Parish fluoridation was also identified through data provided by LA Dept. of Health. Finally, this work was annotated through field work in partnership with EMG to identify and comprehend current need in the community for oral health education.

**Results:** The children ages ranged between 6-12 years (mean=8.3 years) old and their self-reported race was 54% Black, 43% White, and 3% missing. 58% of the schools were located in urban parishes and 42% in rural parishes. At least 29% of the children have some dental disease experience (DMFT>0). The children's DMFT scores varied by race (White=0.94, Black=0.56, Other=0.32). There was a notable difference in mean DMFT score between the urban (0.58) and rural (0.82) schools ( $p<.001$ ). The urban HPSA FTE score was 16.64 and 66% of the parishes were fluoridated. The rural HPSA FTE score was 2.65 and 33% of the parishes were fluoridated.

**Discussion:** We observed a significant difference in DMFT score between rural and urban parishes, with rural communities having a higher burden of oral health disease. The rural parishes display a greater dental workforce shortage and less access to community water fluoridation systems compared to urban parishes. The combination of fewer providers and less fluoridation may result in a greater burden of disease in rural compared to urban parishes.

**Tommy Giang**

Undergraduate

Xavier University, New Orleans, Louisiana

Mentor: Dr. Lisa Moreno-Walton MD, MS, MSCR, FAAEM  
UMCNO Emergency Department, LSUHSC School of Medicine

**“Characteristics and Demographics of Patients Who Tested Positive for HIV in the University Medical Center New Orleans Emergency Department”**

Human immunodeficiency virus (HIV) is transmitted through the exchange of bodily fluids, generally through sexual activity or sharing needles with someone who is infected. Acute HIV infection can be defined as the time interval from HIV acquisition to seroconversion.<sup>3</sup> Over time, the virus will deplete the host's CD4+ T cells, which serve as an important component of the human immune system.<sup>1</sup> Once this occurs, the ability of the host immune system to fight off infections and diseases is reduced.<sup>1</sup>

According to the Centers for Disease Control and Prevention (CDC), there are approximately 37,600 new infections each year and an estimated 1.1 million individuals living in the United States with HIV.<sup>1</sup> Of these individuals, 15% of them are unaware of their infection.<sup>1</sup> In 2016, southern states accounted for more than half of new HIV diagnoses in the United States with Louisiana ranked the 2<sup>nd</sup> highest with an incidence of 29.7 per 100,000 people.<sup>1</sup> Although the incidence and prevalence have slowly decreased over the years due to advancements in technology and medicine, problems remain. Currently, the most common and effective treatment for HIV is antiretroviral therapy (ART), which is designed to slow the progression of HIV and protect the body's immune system. ART reduces viral load, which then minimizes the risk of transmitting the disease to others.<sup>2</sup> Despite the effectiveness of ART, there is no cure for HIV at this time. Acutely infected individuals have particularly high viral loads, which makes them highly infectious. One of the best ways to reduce the prevalence of HIV is by identifying these individuals as early as possible to begin treatment immediately to lower their viral load.

This study is a retrospective review of the records of patients who tested positive for HIV in the University Medical Center New Orleans Emergency Department (UMCNO-ED) from March 2013 - June 2018 for quality improvement purposes. We identify clinical characteristics and demographic features of patients who test positive for HIV, both acutely and chronically. This study looks at variables that correlate with the prevalence of acute HIV infections among patient groups. The study identifies correlations between characteristics or demographics and linkage to care. Our conclusions can be utilized to improve the opt-out HIV patient testing program here at UMCNO-ED and improve on the current protocols for patient linkage to care.

**Hannah D. Guichet**

Undergraduate

University of Southern Mississippi, Hattiesburg, MS

Mentor: Michael Celestin, Jr., MA, CHES, CTTS

LSU Health Sciences Center: School of Public Health, Tobacco Control Initiative

**“Characteristics of Smokers Who Would Use E-Cigarettes to Quit Compared To Traditional Cessation Treatments”**

Though tobacco use reduced by half in the last 50 years since the first Surgeon General’s Report on Smoking and Health, it remains prevalent in society today. In 2016, 38 million (15.5%) adults in the US reported current smoking, and 3.2% of adults reported e-cigarette use. However, in 2015 almost 60% of traditional cigarette users reported using e-cigarettes and over one-third (35%) reported using e-cigarettes to help them quit. While guidelines recommend using counseling and medication for smoking cessation, studies are needed to evaluate the use and intent to use other cessation aids, such as e-cigarettes, especially among vulnerable populations. The Louisiana Tobacco Control Initiative (LA-TCI), which integrates cessation interventions in health systems, administers an annual patient survey to assess provider treatment, patient tobacco use, quit attempts, and cessation treatment preferences. The purpose of this study is to examine sociodemographic differences among current smokers who intend to use e-cigarettes as a smoking cessation aid, compared to traditional cessation treatments.

We conducted a cross-sectional data analysis using patient survey data collected between February and May 2018 by the LA-TCI. The LA-TCI administered the survey to all patients  $\geq 18$  years old seen in primary care clinics ( $n = 610$ ) operated by the University Health System in Shreveport and Monroe, LA. Among respondents reporting smoking at least 100 cigarettes in their lifetime, we defined those who also reported smoking every day or some days in the past 30 days as current smokers ( $n=188$ ). Differences in intent to use e-cigarettes to quit compared with traditional treatments (counseling, medication, self-help material) were assessed. Summary statistics and Chi-square statistics using Excel determined differences between groups in age, gender, race, education, and income at the  $P < .05$  level.

Patient survey respondents were mainly African American females aged 45-64 with a high school diploma/GED and an annual income of less than \$20,000, who were less likely to report intent to use e-cigarettes as a cessation aid. However, more white females with the same sociodemographic characteristics reported intention to use e-cigarettes as a cessation aid. In addition, Chi-square analysis showed a significant ( $p < 0.05$ ) difference by income ( $< \$20,000$  vs.  $\geq \$20,000$ ). Those who intend to use e-cigarettes as a cessation aid were more likely to report making less than \$20,000 a year compared to those who did not intend to use e-cigarettes as a cessation aid. Since little is known about e-cigarette use as a smoking cessation aid in vulnerable populations, further research is needed with larger sample sizes.

**Thor B. Henderson**

Undergraduate

St. Olaf College, Northfield, Minnesota

Mentor: Tiffany Wills, PhD Neurobiology  
LSUHSC, Department of Cell Biology and Anatomy**“Exploring the effects of adolescent chronic intermittent ethanol exposure: Identifying altered BNST afferents and evaluating impact on stress- and anxiety-linked behaviors”**

Alcohol is one of the most well-known and widely-used drugs of abuse. One of the strongest predictors of future alcohol abuse is adolescent alcohol exposure. A greater understanding of the effects of early exposure is critical to advance current knowledge of addiction biology. The bed nucleus of the stria terminalis (BNST) is a brain region within the extended amygdala and has been implicated in addiction behaviors such as stress-induced drug reinstatement and withdrawal-induced negative affect (anxiety and depression), both of which are main contributors to relapse. The Wills lab has demonstrated that adolescent chronic intermittent ethanol exposure (AIE) increases glutamate release and synaptic plasticity in the BNST. This work indicated sex differences in the mechanisms of glutamatergic plasticity in the BNST in response to AIE. These changes in plasticity may contribute to a propensity for future stress-induced relapse.

The goals of this project were twofold. First, we intended to investigate glutamatergic projections to the BNST for further insight into the increased glutamate release previously observed. First, fluorescent retrobeads were infused via cranial surgery into the BNST of adolescent mice. Retrobeads were taken up into local terminals, thus labeling neurons that project to the BNST. Mice underwent AIE treatment, which was two 4-day ethanol vapor chamber exposures separated by a 3-day withdrawal period while control mice were exposed to water vapor chambers. Mice were perfused during acute withdrawal (5hrs) and brains are currently being processed via immunohistochemistry for Fos, a marker of neuronal activation. Co-labeling of retrobeads and Fos expression will allow us to locate brain regions which project to the BNST and are activated by acute withdrawal. This will hopefully allow us to determine the neurocircuitry of increased glutamate release in the BNST during AIE withdrawal.

The second goal of this project was to explore effects of AIE on stress- and anxiety-associated behaviors. To do this, mice were exposed to AIE, as described above. Five hours into withdrawal, we performed three experiments which are analogs of stress and negative affect. The first was an elevated plus maze, which examines the drive to explore an open arm compared to remaining “safe” in a closed arm. In males and females, AIE mice made significantly fewer partial open arm entries than controls. Only male AIE mice made significantly fewer full entries and spent less time on the open arms of the maze versus controls. The greater changes found in male behavior may be a result of different mechanisms by which withdrawal affects males versus females. The second test was an electronic Von Frey nociception assay, which records response thresholds to mechanical stimulus and may be influenced by withdrawal-induced negative affect. The final test was a marble-burying task, which is a simple measure of OCD/anxiety-attributed burying or digging behaviors which can be attenuated by anxiolytic drugs. Neither the nociception assay nor the marble-burying task exhibited significant differences between the AIE and control groups. Future work will assess these behaviors in adulthood in relation to stress. A greater understanding of mechanistic differences in addiction between different age groups and sexes may improve the methods by which we treat addiction in human patients.

**Ryan D. Hoffman**

Undergraduate

Louisiana State University, Baton Rouge, LA

Mentor: Dr. Patricia Molina M.D. Ph.D.

LSUHSC Department of Physiology

**“Effects of Chronic Binge Alcohol Administration on Adipose Tissue Phenotype in SIV Infected Female Rhesus macaques”**

Approximately one million people are living with Human immunodeficiency virus (PLWH) in the United States, and hazardous alcohol use is frequent in this population. The advent of antiretroviral therapy (ART) has shifted the course of HIV to a chronic illness and is associated with increased incidence of comorbid conditions. HIV infection, ART, and chronic binge alcohol (CBA) consumption all independently associate with metabolic dysregulation, and PLWH who misuse alcohol are at an increased risk for developing insulin resistance and type 2 diabetes mellitus. Metabolic dysregulation can be attributed to pathophysiological changes in the adipose tissue phenotype including increased fibrosis, oxidative stress, and inflammation which are potentially exacerbated by alcohol use. Previous studies have shown that CBA administration causes dysregulation of adipose tissue phenotype in male simian immunodeficiency virus (SIV) infected rhesus macaques, irrespective of ART treatment. However, the effects of CBA on adipose tissue in SIV-infected female macaques are not known. Therefore, the objective of this study was to determine whether CBA administration results in alterations in adipose tissue phenotype that potentially leads to metabolic dysregulation in ART-treated SIV-infected female macaques. Female macaques were administered either alcohol (n=4) or water (n=3) via intragastric catheter for 58 weeks during which time all animals were infected with simian immunodeficiency virus (SIV) and administered ART. Both subcutaneous (SQ) and omental (OmAT) adipose tissue was collected at necropsy. Quantitative PCR was used to measure gene expression for markers of fibrosis, inflammation, and oxidative stress in both adipose tissue depots. Picrosirius red staining was performed to visualize collagen expression and cell size in sections of formalin fixed, paraffin embedded adipose tissue. These parameters were then quantified using ImageJ. Crown like structures were detected and counted with hematoxylin and eosin staining. Preliminary data suggests that there were no statistically significant differences in the mRNA expression of the pro-fibrotic cytokine TGF- $\beta$ , the protective adipokine, adiponectin, or the adipose specific transcription factor PPAR- $\gamma$  in the CBA female *macaques* compared to control animals. mRNA expression of collagen I, III, and IV were undetectable in all groups. CBA did not cause a statistically significant increase in collagen expression or change in cell size. There were no statistically significant differences in the number of crown like structures present within the adipose tissue. Taken together, the preliminary data suggest that there are no significant changes in the adipose tissue parameters that were measured in female SIV-infected *rhesus macaques*. Ongoing studies will determine whether CBA and or gonadal hormone loss exacerbates metabolic dysregulation in SIV-infected females.

**Marvin Hudson, Jr.**

Undergraduate

University of Alabama, Tuscaloosa, AL

Mentor: Dr. Yaguang Xi, MD, PhD, MBA

Department of Genetics, Stanley S. Scott Cancer Center,  
Louisiana State University Health Sciences Center, New Orleans, LA**“Metabolism Shifts Through Inhibition of KSHV MicroRNAs by CRISPR/Cas9”**

Kaposi's sarcoma-associated herpesvirus (KSHV/ HHV8) can establish a latent infection in human cells, and cause several cancers in immunocompromised patients, including Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castlemann disease (MCD). The metabolic forms of KSHV-infected cells resemble that of the metabolic forms of cancer cells. The KSHV microRNAs cluster can induce metabolic transformations in infected cells. However, the dominant KSHV microRNAs that play a role in altering host cell metabolism remaining poorly understood. In this project, we aim at in finding the key viral microRNAs in the host cells metabolism shifts.

In this study, we used CRISPR (clustered regularly interspaced short palindromic repeats)/ Cas9 (CRISPR-associated gene 9) to target KSHV microRNAs and its' transcriptional promoter in latently KSHV-infected PEL cell (BCBL-1). Genome targeting efficiency was confirmed using a T7 Endonuclease I (T7EN1) assay which showed successful KSHV DNA cleavage by CRISPR/Cas9. QRT-PCR results showed a significant inhibition of two KSHV microRNAs (KSHV-miR-K12-1/9) expression by targeting single-guide (sgRNAs) to corresponding viral genes. Furthermore, two sgRNAs were simultaneously used to direct a targeted deletion of 230bp in the promoter region of the KSHV microRNA cluster, which encodes 10 different viral microRNAs (KSHV-miR-K12-1 through -9 and KSHV-miR-K12-11). By having this promoter deletion, the KSHV microRNAs derived from this cluster were all down-regulated. Mitochondrial stress was evaluated in these cells, with single KSHV microRNA inhibition or promoter deletion. The loss of viral KSHV-miR-K12-9 expression and promoter showed that microRNAs inhibition can significantly increase mitochondrial respiratory capacity. While there was no change for the knock-down of KSHV-miR-K12-1.

Our study indicated that the CRISPR/Cas9 system can adequately target KSHV microRNAs and change the metabolic status in KSHV-infected cells. CRISPR/Cas9 may be a potent therapeutic antiviral strategy that can be used to impair viral replication and eliminate latent KSHV infection.

**Jamal N. Jordan**  
Undergraduate  
Howard University, Washington, DC

Mentor: Jason D. Gardner, Ph.D.  
Louisiana State University Health Sciences Center, Department of Physiology

### **“Effects of Inhaled Nicotine on Cardiac Collagen Expression”**

According to a 2016 study conducted by the World Health Organization, cardiovascular and associated pulmonary diseases (CVPD) account for more than 59.2% of global deaths, and that number is still rising. Approximately 38.5% of CVPD are attributed to smoking. This equates to about 7 million of the 18.2 million people who die from CVPD die from the harmful effects of smoking. Over the past decade, e-cigarettes have presented a potentially less harmful alternative to smoking. However, the number of e-cigarette users has steadily increased among adolescents. E-cigarettes deliver similar or even greater amounts of nicotine as regular cigarettes, albeit without the harmful aldehydes, tars, and oxidants found in a traditional tobacco cigarettes. The general public regards vaping as a healthy alternative because the aerosol produced by an e-cigarette is thought to have little to no effect on the heart and lungs. However, the reality is that almost nothing is known about the chronic effects of inhaled nicotine. Our goal is to determine whether inhaling nicotine vapor adversely affects the heart.

In particular, we are interested in the effects of chronic nicotine inhalation on cardiac fibroblast activation. The cardiac fibroblasts are the most numerous cell type in the heart and are essential for production of the extracellular matrix (ECM), in addition to regulating cardiac hypertrophy, and other signaling processes. Collagen is a structural protein and makes up the majority of the ECM. Collagen provides tensile strength to the heart; however, an overabundance of collagen results in fibrosis and dysfunction. Because smoking is a known risk factor for cardiac fibrosis, we hypothesize that inhaled nicotine causes an increase in expression and deposition of cardiac collagen, leading to fibrosis. To test this hypothesis, two control groups and two experimental groups were set up: air, air+Ang-II, nicotine, and nicotine+Ang-II. The control groups and experimental groups were exposed to air and nicotine, respectively, for 12 hours per day corresponding with the night cycle which they are active. After 4 weeks of exposure, osmotic minipumps containing angiotensin II (Ang-II) were installed in the cages.

To properly measure collagen in cardiac tissue samples, we used three main procedures: the western blot, the qRT-PCR, and PicroSirius Red staining. The western blot allows us to detect specific proteins, namely collagen, in a homogenized tissue sample. We calculate the collagen concentration in each sample by measuring the densitometry of the bands. The second method we used was the qRT-PCR. qRT-PCRs are commonly used for detecting RNA expression in a specific sample. After obtaining the results from the qRT-PCR, we compiled all the information into an Excel spreadsheet and created a chart, which allowed us to compare the values of each group in the experiment. Finally, we used PicroSirius Red (PSR) staining to quantify the amount of fibrosis in a given tissue section. Cardiac sections were taken from the heart, fixed in paraformaldehyde, and blocked in paraffin. The PSR staining allowed us view collagen present in the sections and collect images using fluorescent microscopy. We were then able to quantify fibrosis using image analysis.

**Kelsey A. Lain**

High School

Benjamin Franklin High School, New Orleans, LA

Mentor: Michael D. Celestin, Jr., MA, CHES, CTTS

LSU Health Sciences Center: School of Public Health, Louisiana Tobacco Control Initiative

**“A Review of Literature on E-Cigarette Use among Pregnant Women”**

Traditional cigarette sales have declined in recent years, but sales of e-cigarettes have increased. While public support exists for decreasing use of e-cigarettes, more information is needed to understand the use of e-cigarettes as an alternative product and as a method of smoking cessation, especially among vulnerable groups such as pregnant women. The purpose of this study was to synthesize the current literature on e-cigarette use during pregnancy.

Pubmed, Medline, Embase, PsycINFO, and Cochrane databases were searched for relevant records published between June 2008 and June 2018, and references for each article were also reviewed for inclusion. Key terms searched included “pregnancy” and “ENDS.” Only cross-sectional surveys, longitudinal surveys, and qualitative focus groups measuring 1) e-cigarette use among pregnant women or 2) perception of e-cigarettes among pregnant women that took place in the US, were considered. The participants in the reviewed studies also had to be adults aged 18 or older who lived in the United States. Out of 33 studies identified, 12 fit the inclusion criteria.

The 12 studies had a total of 7,424 participants. Participants were predominantly white and reported low education levels. Many participants believed e-cigarettes were healthier and safer than traditional cigarettes; pregnant women that had previously used e-cigarettes were more inclined to believe e-smoking is safe. E-smokers were likely to smoke less as the pregnancy progressed, which may suggest use as a cessation aid. Finally, the taste of e-cigarettes was identified as an appealing factor for use.

Three themes were identified: most study participants were of low socioeconomic status, e-cigarettes may have been used as a method of smoking cessation, and there was an appeal of e-cigarettes as safer and more flavorful compared to traditional cigarettes. The recommended intervention would be an educational campaign to inform expectant mothers and their clinicians on the risks of e-cigarettes. Future research should further examine these themes and adverse health outcomes associated with e-cigarette use by pregnant women and the effects on their children.

**Carrie E. Lloyd**

High School

Mandeville High School, Mandeville, Louisiana

Mentors: Nicholas W Gilpin, PhD; Marcus M Weera, PhD  
Department of Physiology, LSUHSC-NO

**“Effects of CeA CRFR1 blockade on stress-induced escalation of alcohol drinking and avoidance of stress-paired context”**

Alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD) are highly comorbid in humans and studies suggest that PTSD may facilitate AUD development in some individuals. The current lack of understanding of mechanisms underlying PTSD and AUD, as well as in treatment options demand more basic science studies using appropriate animal models. Using a rat model, our lab showed that a subset of subjects (termed Avoider rats) exposed to traumatic (predator odor) stress developed avoidance of the stress-paired context and escalated alcohol drinking, thereby recapitulating the individual differences seen in the clinical setting. Given that the central amygdala (CeA) and CRF type 1 receptors (CRFR1), respectively, have been shown to be important neural and pharmacological mediators of stress effects, we hypothesized that CeA CRFR1 blockade will attenuate stress-induced escalation of drinking in Avoider rats. Rats were trained to self-administer alcohol (10% ethanol) and were exposed to a four day stress conditioned place aversion (CPA procedure). On Day 1 (Pre-test), rats were allowed to freely explore two chambers with distinct visual and tactile cues for 5 minutes. On Day 2 (Neutral), rats were placed in one of the chambers for 15 minutes. On Day 3 (Odor), rats were placed in the opposite chamber in the presence of predator odor (or no odor for control) for 15 minutes. On Day 4 (Post-test), rats were again allowed to freely explore the two chambers for 5 minutes. Rats with a greater than or equal to 10 second decrease in their time spent in the odor-paired chamber were classified as Avoiders. Subsequently, rats were given alcohol self-administration sessions that were immediately preceded by intra-CeA infusions of either R121919 (CRFR1 antagonist) or vehicle. Preliminary results show that intra-CeA R121919 infusions may attenuate post-stress alcohol self-administration in Avoider rats.

**Camille R. Loy**

Undergraduate

Louisiana Tech University, Ruston, LA

Mentors: Xinping Yue, MD, PhD; Maureen Basha, PhD  
Department of Physiology, LSUHSC New Orleans, LA

**“Effects of Chronic Nicotine Inhalation on Lung Structure and Function”**

According to the CDC, more than nine million U.S. adults regularly use electronic nicotine delivery systems. Vaporizers are increasingly popular among adults because they are perceived to be safe although there is very little basic and clinical research on the topic. Nicotine is extremely addictive, and the increasing popularity of electronic nicotine delivery systems emphasizes the need for further research on the effect on the lungs. Preliminary data from our lab showed that right ventricular systolic pressure (used to estimate pulmonary arteriole pressure) was increased in mice exposed to nicotine with or without angiotensin-II (Ang-II) infusion compared to mice exposed to air control. We hypothesize that chronic nicotine inhalation leads to vascular remodeling and the development of pulmonary hypertension.

Mice were divided into four groups: air, nicotine, air plus Ang-II, and nicotine plus Ang-II. After four weeks of air or nicotine exposure, a subset of mice were exposed to Ang-II infusion through subcutaneous osmotic mini-pumps for an additional four weeks before sacrifice. Immunohistochemistry (IHC) was then performed on paraffin embedded cross sections of lung tissue. The antibody  $\alpha$ -smooth muscle actin (SMA) was used as a marker for vascular smooth muscle cells and Ki67 was used as a marker for cell proliferation.

IHC using the  $\alpha$ -SMA antibody showed trend of an increase in medial wall thickness in fully muscularized arterioles when comparing the groups exposed to nicotine with or without Ang-II to the air control. Analysis of the Ki67 shows there was a trend of increase in cell proliferation in the bronchiole epithelial cells and macrophages when comparing the mice exposed to nicotine with or without Ang-II to the air control. However, we did not see and Ki67<sup>+</sup> staining in the smooth muscle cells.

We conclude that chronic nicotine exposure results in vascular remodeling leading to pulmonary hypertension.

**Manal S. Malik**

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Biostatistics<sup>1</sup>, Behavioral and Community Health Sciences<sup>2</sup>, School of Public Health, LSU Health Sciences Center

### **“Gender Disparity of Smoking and Obesity on Asthma”**

Asthma contributes a large burden on the individual and on health care systems. Researchers have estimated that total asthma related healthcare costs around \$56 billion per year. This burden will continuous to increase due to the asthma rate in adults is growing the United States in recent years. There is no true cure for asthma; current medication can only relieve symptoms. Because of the current inability to cure asthma and its growing health care burden on society, it is important to identify high-risk groups of asthma. Obesity and smoking are well-known risk factors for asthma. Obese people have been shown to have a higher asthma rate than people with normal weight while smokers are more likely to have asthma than non-smokers. Moreover, studies have shown that obese smokers face higher asthma rates than the normal weight smokers. In regards to gender disparity, female adults have a higher risk of asthma than male adults. However, the gender disparity impact on these risk factors associated with asthma is under studied. The study objective is to evaluate the impact of smoking and obesity on asthma risk by gender.

This study analyzed the gender disparity for various asthma risk factors, including obesity status, smoking status, race, and age. The data consisted of 11,689 subjects compiled from the 2013-2016 National Health and Nutrition Examination Survey (NHANES) data of individuals over the age of 18 with complete data for asthma, smoking status, and body mass index (BMI). In our study population about equal amounts of males and females were surveyed. About one-third of the population defined themselves as White. The Black race constituted 21.1% of the study population and the Hispanics made up 25.6%, while the others race made up 15.4% of the study population. There was approximately an equal distribution of 33% for Obesity Status, yet the average BMI was still relatively high at 28.3 with a standard deviation of 6.7.

Descriptive statistics were used to summarize participants' selected characteristics overall and by gender. Mean and standard deviation were calculated for continuous variables, and frequency and percentage were generated for categorical variables. For testing gender differences for the selected factors, the t-test was applied for the continuous factors (such as age and BMI) and the chi-square test was applied for the categorical factors (such as race, smoking and obesity status). Our findings of identifying high risk groups of asthma by considering gender disparity will provide more valuable and accurate information of asthma risk, and will be beneficial to health policy makers, healthcare professionals, and individuals. Custom intervention for asthma awareness and control can be designed for the asthma high-risk groups. Furthermore, this increased awareness may lower the asthma rate, which in turn, would lower asthma related health care expenditures overall.

**Eleanor C. McCall**  
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Mentor: Dr. Martin Ronis  
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**“Binge EtOH effect on bone turnover and calcium homeostasis in mice”**

Chronic alcohol use can affect bone development and lead to diseases such as osteoporosis. Alcohol consumption increases bone resorption and decreases bone formation. Oxidative stress is one mechanism behind this increase in turnover. Reactive oxygen species affect several pathways that control formation of osteoblasts and differentiation of osteoclasts. Previous studies have suggested that antioxidants prevent ethanol-induced changes in bone turnover by decreasing oxidative stress in bone cells caused by alcohol and by blocking alcohol disruption of the calcium-vitamin D3-PTH axis. The first part of this study examined the effect of ethanol on bone turnover in mice as well as the potential for the mitochondrial antioxidant mitoTEMPO and counteract ethanol's effect and if there was any interaction between ethanol and THC which are commonly consumed together. CTX-1 serum concentrations were measured as an indicator of bone resorption and osteocalcin levels were measured as indicators of bone formation in addition to serum calcium concentrations to determine the rate of bone turnover. It was hypothesized that mice consuming EtOH would have increased markers of bone turnover in serum compared to mice receiving the saline control, and that mitoTEMPO and THC would counteract these effects of EtOH. Mice were randomly assigned to groups receiving EtOH or a saline control via oral gavage over four days; doses started at 3 g/kg, increasing to 4 g/kg on day 3 and 4.5 g/kg on day 4. These mice were further divided into groups receiving a saline control or mitoTEMPO (2 mg/kg via intraperitoneal injection 1hr prior to gavage). An additional group of EtOH-administered mice received THC (10 mg/kg) via IP injection. Osteocalcin and CTX-1 serum concentrations were measured by ELISA and the serum calcium concentration was measured with a colorimetric assay. Results were consistent with the hypothesis that mice receiving EtOH would show greater bone turnover than saline controls ( $P < 0.05$ ). However, mitoTEMPO did not alter ethanol's effect on markers of bone turnover. Results suggest that THC exacerbated ethanol's effect on osteocalcin ( $P < 0.05$ ), but THC had no significant effect on CTX-1 or calcium concentrations. The second part of this study looked into the effect of alcohol on calcium homeostasis by examining alcohol's effect on the relationship between PTH and calcium concentrations in mice. Previous studies have suggested that alcohol causes a disruption in calcium homeostasis by impairing PTH secretion in response to decreased serum calcium. In this study, PTH and calcium serum concentrations were compared in mice receiving ethanol or saline via oral gavage (as above) while also receiving EGTA (500  $\mu\text{mol/kg}$  via IP) to stimulate hypocalcemia. It was hypothesized that the alcohol would prevent an increase in circulating PTH in response to EGTA-induced hypocalcemia. Twelve mice were randomly assigned to two groups, one receiving alcohol and one saline. In addition to a blood sample collected prior to the first gavage (baseline), three blood samples were taken from mice an hour apart on the final day of the experiment after injection of EGTA. The concentrations of PTH and calcium were measured with an ELISA and colorimetric assay, respectively. Supported in part by R37 AA018282 (M.R.).

**Michael Milo**

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***“In Vivo Deep Calcium Imaging of the Central Amygdala of a Freely Behaving *Mus musculus* during External Stimuli”***

*In vivo* multi-channel fiber photometry is a sensitive and useful tool for real-time imaging of neural circuit processes in moving mice at single cell levels. Circuit-level understanding of brain rhythms and activity dynamics is fulfilled by genetically encoded protein calcium indicators, such as GCaMP, which convert action potential information during heightened neuronal activity into a fluorescent signal that is then sent through the optical fiber. The following procedure is the easiest way to identify patterns within specific pathways in mouse behavior. A virus is injected into the mouse's amygdala expressing GCaMP, then the mouse rest for 2-3 weeks, and after undergo optical fiber surgery. The mice are exposed to either a control state or different stressors such as restraint or placing a piece of gauge soaked in predator urine inside the mouse's cage. The neuronal population due to the stimulus is imaged, the fluorescence is graphed as a waveform, and the power spectrum density as well as spectrogram are plotted. The raw signal, relative signal change, number of events, recovery period, amplitude, and standard deviation are compared between trials to judge the metrics during normal control states and quantify characteristic changes due to external-evoked states.

The data shows that the different stimuli results in various frequencies of the trace signal, proving that specific brain rhythms are enabled during fear. Neurons in distinct brain areas are found to respond in coordination, integrating the overall signal to produce a response with adaptive information processing during spontaneous and stimuli conditions. Neural synchrony is a product of the external stimuli, leading to big changes in calcium transients. Neuronal responses are correlated with the mouse's behavior to show that the brain acts as a dynamical system with organization of genetically defined neuronal classes and subclasses.

The experiment evinces that GCaMP-based fiber photometry is an effective technique that reveals functional and behavior neural activity *in vivo*, ultimately allowing one to predict important features of threat response and the effect of neuropsychiatric disorders. Future implications include optimizing behavior and imaging to record faster detection spike times with increased temporal resolution. For example, new methods that use red genetically encoded indicators have higher sensitivity, greater brightness, and deeper tissue application. Lastly, different locations of the brain can be imaged to identify the patterns of motor functions, neurotransmitter reward systems, or hypothalamus metabolism circuits.

**Olivia L. Morrison**

Undergraduate

Xavier University of Louisiana, New Orleans, LA

Mentor: Rakesh K. Srivastava, PhD

Louisiana State University Health Sciences Center. School of Medicine, Department of Genetics,  
Stanley S. Scott Cancer Center**“Anticancer Activity of Mirabegron Repurposing Drug”**

Hepatocellular Carcinoma (HCC) is one of the most common types of liver cancer and is the third leading cause of cancer deaths worldwide. HCC is most commonly found in individuals with chronic liver diseases such as cirrhosis but can be caused by several other factors such as genetics, lifestyle, and environmental factors. Therefore, novel treatment of HCC is urgently needed. *Mirabegron* is an FDA approved drug for the treatment of overactive bladder. The drug targets the pathway to relax the smooth muscle and increases the bladder's ability to store urine. The anticancer activity of Mirabegron has never been demonstrated. Therefore, the purpose of this study is to examine the anticancer activity of Mirabegron.

**Objective:** The objective of the study was to examine the anticancer activity of Mirabegron against Hepatocellular Carcinoma.

**Methods:** Liver cancer cells were purchased from ATCC and grown in RPMI-1640 cell culture media. With a significant amount of cells, we were able to test cell viability and colony formation. We measured intracellular adenosine triphosphate (ATP) with a cell viability assay. We also tested colony formation with crystal violet staining.

**Results:** From the cell viability assay, there was a linear correlation between the doses of Mirabegron on the Hepatocellular Carcinoma cells. As the concentration of the drug increased, data showed the luminescent outputs, which indicated the ATP level of active cells, were decreased. Furthermore, the colony formation without the drug showed the highest ATP levels. From the colony formation assay, cell growth also reduced due to the elevated concentration of Mirabegron. As expected, the control group showed the highest number of colony. Mirabegron inhibited colony formation by HCC.

**Conclusion:** The cell growth of HCC was tested with Mirabegron and the higher the dose, the more effective the drug is for treatment. The ATP levels decreased as drug concentration increased and the number of colonies also reduced due to elevated concentration of the drug. Mirabegron can be used for the treatment of Hepatocellular Carcinoma.

**Riley T. Nguyen**

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**“Effect of Gonadal Hormone Loss and High Fat Diet – Induced Lymphatic Leakage on Perilymphatic Adipose Tissue (PLAT) Insulin Signaling”**

Insulin resistance, defined as a diminished response to a given concentration of insulin, is one of the hallmarks of metabolic syndrome, which encompasses a group of risk factors that contribute to cardiovascular disease, type II diabetes, and other metabolic comorbidities. The contributing risk factors, specifically increased visceral fat and obesity, are significantly prevalent in postmenopausal women and result in predisposal to insulin signaling impairment, especially in insulin sensitive tissues such as adipose tissue. Similarly, mesenteric lymphatic vessel leakage has also been associated with mesenteric adipose tissue expansion and metabolic dysregulation. Whether these postmenopausal alterations are a consequence of leakage from lymphatic vessels into perilymphatic adipose tissue (PLAT) is completely unknown. We hypothesized that gonadal hormone loss and high fat diet (HFD)-induced lymphatic leakage leads to impaired insulin signaling in perilymphatic adipose tissue. To test this hypothesis, female Fisher 344 rats underwent ovariectomies; one week later the ovariectomized (O) and sham (S) rats were fed either high fat (H) or low fat (L) diets for 10 weeks. We had four final groups, OL, OH, SL, and SH. After 10 weeks, the rats were given Evans blue dye intragastrically, sacrificed 30 minutes later, and PLAT was isolated for lymphatic leakage measurement, reflected by Evans blue concentration in the PLAT. Another group of animals were sacrificed and the PLAT isolated and stimulated with insulin for 60 minutes. The PLAT was then homogenized for protein extraction. Western blot analysis was performed on the homogenized PLAT. Antibodies that recognize AKT, phospho-serine/threonine kinase 1 (pAKT) (ser 473), phosphatase and tensin homolog (PTEN), and alpha serine/threonine kinase substrate 160 (AS160) were used to detect concentrations of these proteins in PLAT. We found that HFD and ovariectomy significantly increase body weight, adiposity, and lymphatic leakage. In addition, we found that ovariectomized animals had significantly high blood glucose levels compared with sham animals. We predict that there will be an effect from both HFD and gonadal hormone loss, shown through decreased PLAT AKT phosphorylation and consequently decreased AS160 phosphorylation, suggesting an insulin signaling impairment in PLAT. We speculate that lymphatic leakage into PLAT plays a role on the metabolic consequences induced by ovariectomy and HFD.

**Ethan J. Nicklow**  
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Mentor: Dr. Chindo Hicks  
LSUHSC, Director of Bioinformatics Program

**“Genomic Classification and Prediction of Outcomes in Pediatric ALL Patients using Machine Learning”**

**Background:** Pediatric acute lymphoblastic leukemia (pALL) is the most common childhood cancer and the leading cause of cancer related deaths in children under 20 years of age. Advances in treatment have increased overall survival rates to 95%, yet almost 30% of pALL patients who initially respond to treatment still relapse. White blood cell count (WBC) at diagnosis is a prognostic marker of relapse and has historically been used to stratify patients and guide treatment. Currently, an unmet medical issue is the lack of a more comprehensive and effective method for stratifying patients into risk groups to guide treatment decisions. Machine learning (ML) algorithms can help rectify this issue, as they provide useful tools for classifying patients using large-scale genomics data. However, to date, the use of ML to classify pALL patients using genomic data and stratify them in terms of WBC and prognosis has not been reported. The objective of this study was to investigate the ability of ML algorithms to successfully stratify pALL patients through risk prediction based on WBC. We hypothesized that genomic alterations in pALL patients related to changes in WBC could lead to measurable effects impacting risk of relapse and treatment plans, and that systematic application of ML algorithms to genomic data could accurately classify the two patient groups based on WBC and potentially identify patients at high risk of relapse. We addressed this hypothesis as follows:

**Methods:** We used public gene expression data of 207 children diagnosed with pALL, generated by the Children’s Oncology Group Trial P9906, for our study; 108 had high WBC and 99 had low WBC. Using statistical analyses, we identified 271 significant differentially expressed genes ( $p < 0.05$ ,  $|LFC| > 0.75$ ) between patients with high and low WBC. These genes were used as inputs for the classification algorithms, after log-transformation and standardization of the corresponding genomic data. Four classification algorithms were compared to determine the most effective algorithm: support vector machine (SVM), logistic regression, random forest, and naïve Bayes. Each algorithm was tested using 10 iterations of 10-fold cross validation, then evaluated for performance using several metrics. The highest performing algorithm underwent further evaluation to explore its classification capabilities.

**Results:** The four evaluated models all performed well and correctly classified at least 80% of patients. SVM performed best and classified 91.3% of samples correctly. The models achieved accuracies over 80%, sensitivities and specificities over 0.84, and areas under the ROC of over 0.9. Further evaluation of SVM yielded an average accuracy of 90.97%, with a Matthew’s correlation coefficient of 0.822.

**Conclusion:** This study applies ML to pALL and validates ML as a powerful method for effectively stratifying pALL patients. If successfully validated using an independent data set, this framework could be used for identifying patients at high risk and for guiding treatment decisions in clinical settings. Additional studies are recommended to validate these findings and for the development of models designed to identify patients at high risk of relapse, perhaps through risk prediction based on other prognostic markers.

**Nghi T. Pham**

Undergraduate

Xavier University of Louisiana, New Orleans

Mentor: Rakesh K. Srivastava, PhD

Louisiana University Health Sciences Center. School of Medicine, Department of Genetics,  
Stanley S. Scott Cancer Center**“The effect of *Riluzole* on pancreatic cancerous cells”**

Pancreatic cancer is one of the deadly type of cancers in the world with a low survival rate for 5 years with only 6 percent. Several factors may cause this deadly disease are genetic, lifestyle and diet, or environmental factors. There are many research projects working to study this disease and testing drugs to find the treatment. *Riluzole* is a drug belongs to the class of benzothiazole. Its chemical name is 2-amino-6-(trifluoromethoxy)benzothiazole. There was study showed how *Riluzole* can inhibit the signaling of glutamate during neuronal transmission, which can influence the tumor proliferation. There was also clinical study for using *Riluzole* as a treatment for ALS (amyotrophic lateral sclerosis), but the effect of *Riluzole* specifically on pancreatic cancer is unknown. Therefore, the purpose of this study is to examine the anticancer activity of *Riluzole* on pancreatic cancer.

**Hypothesis:** *Riluzole* is effectively reduced the proliferation of pancreatic cancerous cells.

**Methods:** Pancreatic cancerous cells were purchased from ATCC and cultured in RPMI-1640 media. The cells then were treated with different *Riluzole* concentrations. To test for the effect of the drug, cell viability assay (ATP assay) was performed by measuring the intracellular ATP using luminescent as the signal reagent. The quantification of ATP as luminescent outputs indicated the presence of active cells due to the drug. Secondly, colony formation assay was carried out using crystal violet. The purpose of colony formation is to test the cell growth and in vitro outgrowth cells, which were tested with *Riluzole*.

**Results:** From the cell viability assay, there was a linear correlation between different concentration of *Riluzole* and luminescent outputs (RLU). As the concentration of the drug increased, data showed the luminescent outputs, which indicated the ATP level of active cells, were decreased. Furthermore, the control cell growth without the drug showed the highest ATP levels. From the colony formation assay, colony growth also reduced due to elevated concentration of *Riluzole*. The crystal violet stains indicated the presence of cell colonies on each well plates. The control plates showed highest crystal violet stains while 5 $\mu$ M and 10 $\mu$ M had lesser colony formation. Lastly, there was negative sign of crystal violet stains on the last well which contained the highest concentration of *Riluzole*, 25 $\mu$ M.

**Conclusion:** The cell growth of pancreatic cancer were tested with *Riluzole* was consistent with the hypothesis that *Riluzole* was effectively reduced the proliferation of cancerous cells. The ATP levels were dropped as *Riluzole* concentration increased, and the colony growth also reduced due to elevated concentration of the drug. *Riluzole* was effective in inhibiting cell viability and colony formation of pancreatic cancer cells. Therefore, it can be used for the treatment of pancreatic cancer.

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Mentor: Rinku Majumder, PhD  
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**“Adjunct Therapy of Hemophilia B by Protein S, a Natural Anticoagulant”**

Defects in hemostasis cause bleeding disorders such as Hemophilia. Hemophilia B is an inherited, X-linked, recessive bleeding disorder stemming from a deficiency in functional plasma coagulation factor IX (FIX). Without FIX, blood cannot clot properly to control bleeding. FIX deficiency increases the propensity for hemorrhage from mild trauma or even spontaneous bleeding within joints and muscles. The plasma glycoprotein Protein S (PS) is a critical, negative regulator of blood coagulation. The importance of PS is demonstrated dramatically by the catastrophic purpura fulminans that develops in rare newborns homozygous for PS mutations. Heterozygous individuals have an elevated risk for deep vein thrombosis and other life-threatening thrombotic events.

We discovered a novel regulatory function of PS as an inhibitor of FIXa. Protein S-mediated inhibition of FIXa inhibits FXa formation by FIXa, implying a powerful new function of PS. This discovery of a novel regulatory function of PS towards FIX led us to test whether inhibiting PS would mitigate Hemophilia B caused by a deficiency or misregulation of FIX. The primary goal of this research project was to investigate the application of anti-protein S (PS) antibody (a PS inhibitor) in augmenting hemostasis in Hemophilia B patients.

We used a modified aPTT assay and a thrombin generation assay (TGA) to determine the effect of PS antibody on clotting time and peak thrombin formation in Hemophilia B (FIX-deficient) plasma. To demonstrate that PS antibody decreases the clotting times of the Hemophilia B plasmas, we measured clotting times of FIX-deficient plasma (commercial and patient) in the presence of FIXa and in the presence or absence of PS antibody. More importantly, a TGA showed that peak thrombin formed was ~3 fold higher in the presence 90 nM FIX (physiological concentration) and 450 nM PS antibody compared with the absence of antibody. To determine whether the effect of PS antibody was specific, we used the antibody against tissue factor pathway inhibitor (TFPI), another natural anticoagulant in the TGA assays. Anti-TFPI antibody had little effect on peak thrombin formation, thus confirming our hypothesis that PS antibody is specific and could be used as an adjunct therapy of Hemophilia B along with FIX supplementation.

Our preliminary results are vitally important because the effects of FIX deficiency include an increased risk of spontaneous, life-threatening bleeding in severely and moderately affected individuals. Factor IX replacement therapy to achieve hemostasis is the mainstay of management for this disorder. Our findings imply that anti-PS antibody could be an adjunct therapy of Hemophilia B to increase the effectiveness of FIX replacement therapy.

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**“Effects of Gonadal Hormone Loss on Skeletal Muscle Insulin Signaling”**

Weight gain after menopause is common due to lower gonadal hormone levels and a shifting fat distribution. Weight gain is further exacerbated when postmenopausal women consume a Standard American Diet, which is high in fatty foods and is correlated with increased rates of obesity, diabetes, and insulin resistance. 1 in 2 women over 50 years old will have fractures associated with significant morbidity and increased risk of immobilizations, which result in decreased protein synthesis and muscle atrophy of the fixed limbs. Studies indicate that both muscle atrophy and consuming a high fat diet can potentially lead to insulin resistance by hindering insulin-mediated glucose uptake. We hypothesize that gonadal hormone loss dysregulates skeletal muscle insulin signaling following disuse atrophy or in the presence of a high fat diet in female rodents. Fisher-344 female rats were separated into two groups, one undergoing an ovariectomy (OVX) to simulate menopause and the other acting as a control (Non-OVX). In experiment one, 10-weeks post-ovariectomy, pair-fed OVX and Non-OVX groups underwent a unilateral hindlimb immobilization for 7 days between the hip and knee joint to achieve quadriceps atrophy. They recovered for either 3 days or 14 days then were sacrificed, and quadriceps muscle tissue was collected from both casted and non-casted hind limbs of each rat. In experiment two, female Fisher-344 were divided into OVX and Non-OVX groups. These groups were fed *ad libitum*, with half of the rats receiving a low-fat diet (10% fat) while the other half received a high-fat diet (45% fat) to mimic a typical American diet. The rats were sacrificed after ten weeks. Quadriceps muscle tissue samples were collected and insulin stimulated for an hour. Western blot analysis was performed on homogenized muscle tissue samples from both experiments to detect the ratio of phospho-serine/threonine kinase 1 (pAKT) to total AKT. Beta-Actin was used as a loading control. Our results show that in experiment one, there was no significant body weight difference between the control group and the ovariectomized group. The weight of the quadriceps decreased significantly between the non-casted and casted limbs in both the OVX and control groups, indicating that casted rats experienced quadriceps atrophy. In experiment two, though the average weekly food consumption across the four groups was roughly the same, the rats consuming a high-fat diet weighed significantly more than their low-fat diet counterparts. Additionally, both groups of OVX rats weighed significantly more than the Non-OVX rats who were consuming the same food, which possibly indicates insulin resistance in OVX rats. The effects of disuse atrophy, diet, and ovariectomy on AKT phosphorylation are yet to be determined through Western Blot analysis.

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**“Protective Effects of Immunoglobulin G and Low Dose Amphotericin B on Invasive *Candida auris* infections in Mice”**

Disseminated candidiasis is most commonly caused by *Candida albicans* (41%-65%). However, in the past decade, non-*albicans Candida* (NAC) species resistant to conventional treatments have emerged as prevalent causes of candidiasis. Among NAC, *Candida auris* is classified as a “superbug” because it is resistant to most anti-fungal drugs that normally treat *Candida* infections. About 30-60% of patients diagnosed with *C. auris* infections have died. As of June 22, 2018, *C. auris* has been identified in 340 clinical cases in the United States. *C. auris* poses a serious threat to many healthcare environments, and there is no effective treatment available for human use. Therefore, effective means of prevention and treatment feasible for human use must be established.

The ultimate goal of this study was to investigate the prophylactic efficacy of intravenous immunoglobulin (IVIG) to protect A/J mice against *C. auris* disseminated infections. IVIG is approved as standard treatment for patients with primary immunodeficiency (PID). In this study, we evaluated the new use of IVIG for prophylaxis of invasive fungal infections in mice. We first tested several IVIG samples and compared the endpoint titers of protective IgG's specific for a panel of *Candida* cell surface peptides. The ELISA results suggest that high titers of some protective IgG's in those IVIG samples can promote resistance and prevent *C. auris* infections. Next, we established an A/J mouse model of disseminated candidiasis by *C. auris* which closely mimics human invasive candidiasis. Finally, we performed intraperitoneal injections on the mice with selected IVIG samples. We then challenged the mice with a lethal dose of *C. auris*. Our data showed that a single dose of IVIG given to mice prior to the lethal dose challenge reduced fungal burden in the target organs in mice, which was shown with a lower colony forming unit (CFU) count. IVIG's protective efficacy was also demonstrated by prolonged survival of mice that were treated with human IVIG alone or Amphotericin B combined with IVIG.

We concluded that the passive administration of IVIG containing anti-*C. auris* protective antibodies can prevent or hinder the disease process in A/J mouse model of disseminated candidiasis. IVIG therapy may hold the hope to prevent *C. auris* infections in a similar way in humans. For future studies, we will use the same mouse model to test and compare therapeutic efficacy of selected IVIG samples containing protective IgG's with different specificities and binding affinities to the corresponding peptides.

**Sara L. Saak**

High School

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Mentors: Michael Norman PhD &amp; Pinki Prasad M.D.

LSUHSC Human Development Center – Louisiana Deafblind Project & Children’s Hospital of  
New Orleans**“Negative Impact of Pediatric Cancer Sequelae on Educational Experiences”**

Deafblindness in terms of education is defined as having concurrent hearing and visual losses that inhibit communication or educational development and can be of either congenital or acquired etiologies. The Louisiana Deafblind Project (LADBP) aims to identify children from birth to 22 years of age who qualify as deafblind and to provide resources to the families of these children to aid with educational development. One way that the LADBP uses to identify qualifying children is through the Children’s Hospital of New Orleans (CHNOLA) Late Effects Clinic. This clinic manages survivors of pediatric cancer who are experiencing late effects of treatments at least two years after treatment has ceased. These late effects include a wide range of sequelae including hearing loss, vision loss, and many other physiological and psychosocial deficits. The Late Effects Clinic at CHNOLA has a database of all patients’ medical history, treatment plans, and current sequelae. The LADBP has an opportunity to use this database in a retrospective chart review to conduct research. At this point in the research process, we are still reviewing literature to formulate a significant research question(s) and a design a process to provide answers to these questions. Research questions being considered include identification of successful education accommodation usage, differentiating how various cancer treatment protocols impact the need for educational accommodation following treatment, and design of programs to facilitate coordination between the health and educational communities to serve this group of patients.

Thus far, the literature reviewed for this project suggests that some sequelae cause difficulties for patients as they reentered school systems. Adverse psychosocial effects are cited as adverse outcomes associated with survival of all types of pediatric cancers. These residual psychosocial deficits can complicate reintegration of patients’ into academic settings given the social demands of typical classrooms. Additionally, neurocognitive deficits associated with cranial irradiation and methotrexate treatments may cause patients to struggle keeping up with peers academically and the expectations of the classroom/school. Hearing and vision loss late effects are known to qualify patients for educational accommodations to support their new learning needs. Social, cognitive, and sensory late effects make up a portion of the potential late effects patients may face upon reentering school after receiving treatment for their childhood cancer. Consequently, we hypothesize a coordinated program between health and education communities will likely produce the most beneficial outcomes for these individuals.

**Terrell J. Shields**

Undergraduate

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Louisiana State University Health Sciences Center, Department of Pharmacology

**“Whole cell membrane proteomics of rat liver to determine cytochrome P450 cellular distribution and lipid domain localization”**

Cytochromes P450 (P450s) are a diverse group of membrane-bound heme proteins that are important in the metabolism and elimination of the majority of drugs. Individual forms of P450 are variably expressed in most tissues with liver having the highest level of expression as well as the most diverse population of different P450 forms. At least nine forms of P450 contribute to drug metabolism in the human liver, and many of these P450s are inducible. They are predominantly located in the endoplasmic reticulum (ER). However, recently, some forms have been identified in the membranes of other organelles including mitochondria, plasma, and nucleus. Our lab also has recently shown that the different forms of P450 variably localize to ordered and disordered lipid domains within the ER. Ordered lipid domains, commonly known as lipid rafts, have been extensively studied in the plasma membrane where they participate in a variety of cellular functions such as signaling and trafficking.

Our lab has demonstrated that physical interactions between different forms of P450 can stimulate or inhibit catalytic activity in a form-specific manner. This can have important consequences on drug metabolism making it important to identify which P450s have tendencies to interact. To assess P450-P450 interactions for their mutual effects on metabolism, different P450s are currently tested in purified systems in a random trial-and-error manner. To better predict specific P450-P450 interactions that potentially influence drug metabolism, the aim of this study is to identify both the cellular distribution and lipid membrane localization of rat liver P450s using whole cell quantitative proteomics. More specifically, rat liver tissue was homogenized and the membranes from plasma, mitochondria, nuclei, rough ER and smooth ER were isolated by differential centrifugation. The cellular distribution of 23 forms of rat P450 involved in drug metabolism was determined by LCMS with simultaneous proteomics on membrane extracts separately labeled with isobaric Tandem Mass Tags (TMTs) allowing for relative quantitation of over 3000 cellular proteins. Western blot densitometry of established marker proteins for cellular membranes was used to assess both the purity of membrane fractions and the reliability of the quantitative proteomic data. The results illustrate a limited number of P450s variably distribute among the organelle membranes.

To determine the lipid domain localization of P450s, membranes from mitochondria, rough ER and smooth ER were solubilized by 1% Brij 98 detergent and were subjected to high speed centrifugation to precipitate insoluble, ordered membranes. The proteins located in disordered lipid regions were solubilized following detergent treatment and were isolated in the supernatant. LCMS quantitative proteomics was again performed comparing the supernatant and pellet fractions relative to the corresponding untreated membrane suspension. These results categorized the lipid domain localization of approximately 1200 proteins including the P450s. Interestingly, all five members of the rat CYP2D family, in addition to other individual forms of P450, showed membrane-specific domain localization. In the case of the CYP2D forms, the P450s were located predominantly in lipid rafts in the smooth ER but equally distributed in the rough ER. From these data, we can predict which P450s will interact *in vivo* and in turn, more effectively test specific P450-P450 interactions for effects on catalysis.

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Mentor: Dr. Yaguang Xi MD Ph.D.  
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**“Sulindac Sulfide Downregulates EpCAM Protein expression in colon cancer cells”**

Clinical and preclinical studies provide substantial evidence that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) can reduce the risk of developing precancerous lesions and the incidence of numerous types of cancers, especially colorectal cancer. Previous clinical case studies for the treatment of precancerous conditions reported that Sulindac, a NSAID commonly used to treat pain and inflammatory conditions, could reduce colonic adenomas in patients with familial adenomatous polyposis (FAP) - an inherited disorder characterized by cancer of the colon and rectum.

A well-known hallmark of cancer is sustaining uncontrolled proliferation; the purpose of this *in vitro* study is to determine whether sulindac sulfide (SS), a metabolite of sulindac, can regulate EpCAM expression, resulting in inhibition of cell proliferation by causing cell cycle arrest in colon cancer. EpCAM (Epithelial cell adhesion molecule) is a type I transmembrane glycoprotein that has been implemented as an over-expressed oncogene in colon cancer and is known to have a role in proliferation, migration, and mitogenic signal transduction. Previous reports have determined that SS drives cleavage of the extracellular portion of EpCAM near the N-terminus resulting in decreased expression however functional and phenotypic effects have yet to be evaluated. Using western blotting, we confirmed that SS could down regulate EpCAM protein expression in Human colorectal tumor. Next, we performed quantitative real-time polymerase chain reaction (qRT-PCR), and we found that SS does not down-regulate EpCAM mRNA expression suggesting the regulation is post-translational. Additionally, we assessed if downstream targets of EpCAM were also downregulated by SS. The targets we considered were c-MYC (a gene that can promote the development of various cancers when aberrantly expressed), and CYCLINs A and E (proteins that function in regulating progression through the cell cycle). Using Western Blotting and qRT-PCR, we determined that SS inhibited both protein and RNA expression of C-MYC, CYCLINs A and E. Finally, we evaluated the biological effects of SS inhibition of Epcam. First, we evaluated the effect of SS on cell growth in HCT116 cells. We determined that cells treated with SS (at a concentration 100µM for 24 and 48hrs) had significantly reduced proliferation rates compared to control cells.

In conclusion, our results suggest a novel mechanism by which sulindac sulfide regulates cancer cell proliferation and growth through the EpCAM/c-MYC/ Cyclins pathway. These results are significant as the TCGA database suggests that EpCAM expression is selectively upregulated in patient colon tumor specimens compared to that of normal colon tissue. With further investigation, this could potentially mean SS could be used as a safe and effective cancer therapy.

**Elise M. Sterling**

Undergraduate

Xavier University of Louisiana, New Orleans, Louisiana

Mentors: Nicolas G. Bazan, MD, PhD

Surjyadipta Bhattacharjee, PhD

LSUHSC

**“Culturing Human Retinal Pigment Epithelial Cells (hRPE) and Rat primary Hippocampal Neurons to Study Role of Novel Lipid Mediators – Elovonoids (ELVs) on Neuroprotection upon uncompensated oxidative stress (UOS)”**

**Purpose:** Elovonoids (ELVs), a novel lipid mediator class, are derived from the Very-Long-Chain-Poly-Unsaturated-Fatty-Acids (VLC-PUFAs) 32:6n3 and 34:6n3. We determined ELV structure/stereochemistry and showed ELV protection of human retinal pigment epithelial cells (RPE) and neurons from uncompensated oxidative stress (UOS) (Jun B, et al. Sci Rep 2017; Bhattacharjee S, et al. Sci Adv. 2017). When there is a disruption in homeostasis in the Central Nervous System (CNS), elovonoids respond by protecting neurons and retinal cells from undergoing apoptosis. Here, we investigated the role Elovonoids in response to stress such as uncompensated oxidative stress and are trying to uncover different cell signaling pathways which may be responsible for neuroprotection.

**Methods:** 72h grown human primary RPE cultures were serum starved for 8h, stress was introduced by H<sub>2</sub>O<sub>2</sub> (1600µM) plus TNF- α (10ng/ml), and then challenged with 200nM of Elovonoids (32:6 and 34:6 Me) or NPD1 for 16h. Similarly, primary hippocampal neurons were cultured from Sprague Dawley (SD) rat embryos (E18). Mature neurons 12-14 DIV (days *in vitro*) were also challenged with UOS (200µM). Apoptotic cell deaths were detected by Hoechst staining and pictures were taken with a Zeiss LSM 510 confocal microscope. After which, the images were imported into the ImageJ, image analysis software (NIH, Bethesda, MD) and batch processed using custom macros to do an unbiased image analysis to acquire the data. Moreover, RNA and DNA from stressed/control samples were extracted using Qiagen Kits following manufacturer’s protocol which were processed and analyzed for several downstream genomic applications.

**Results:** Stress induced by UOS cause 80-90% apoptosis in hRPE. Interestingly, elovonoids and NPD1 (200nM) substantially attenuated UOS mediated apoptosis.

**Conclusions:** Our data shows that ELVs reduce UOS, providing neuroprotection by targeting several anti-apoptotic genes. We are currently defining ELV mechanisms in RPE which regulate neuro-protective functions of these genes and help in protection of photoreceptor inner segments from photo-oxidative stress. This novel signaling may be fundamental to sustain homeostasis and photoreceptor integrity.

**Kyron C. Summers**  
High School  
St. Augustine High School, New Orleans, Louisiana

Mentor: Dr. Arnold Zea, PhD  
LSUHSC MIP Department

**"The relationship between L-arginine and polyamines while inhibiting Ornithine Decarboxylase"**

According to the American Cancer Society, in 2012 there were over 60,000 new cases of Renal Cell Carcinoma and more than 10,000 of those cases were deemed untreatable. This makes RCC immunotherapies very critical to patients. L-arginine metabolism is a highly regulated mechanism for tumor growth. Arginase (ARG) metabolizes arginine to ornithine. Ornithine is then metabolized to polyamines via Ornithine Decarboxylase (ODC). Elevated levels of polyamines have been associated with various tumors. Regulation of polyamine levels have been implicated as a logical target for chemotherapies. We hypothesized that blocking ODC using Difluoromethylornithine (DFMO), therefore blocking the production of polyamines, will cause decreased arginine expression and decreased tumor cell growth.

DFMO is an enzyme-activated, irreversible inhibitor of ODC and has been used as an investigational drug in many hyperproliferative diseases. We cultured two RCC cell lines, CL-19 and Renca, and treated them with two concentrations (100 uM and 500 uM) of DFMO for 24 and 48 hours comparing them to an untreated control. We tested arginase activity, ARG protein expression by Western blot, and L-arginine, L-glutamine, and L-citrulline levels by HPLC. The Western Blot data showed an increase in ARG protein in cells treated with DFMO as compared to control. With increased ARG, we would expect decreased arginine in the treated cells as well. The HPLC data showed slightly increased levels of arginine in the treatment compared to control despite what we expected. This data implies that another mechanism of action is at play causing arginine levels to be restored. We believe that inhibiting ODC enabled L-arginine to be metabolized into citrulline via the NOS2 pathway, which can be used to produce arginine. Further investigation will be required to elaborate the implications between arginine levels and polyamine synthesis inhibition.

**Harrison J. Tanzola**  
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Mentor: Ed Grabczyk, Ph.D  
Louisiana State University Health Sciences Center, Department of Genetics

**“Expanding the Problem? Frataxin Transcription Linked to Expansion”**

Friedreich’s Ataxia (FRDA) is a relentlessly progressive and fatal neurodegenerative disorder with no available treatment or cure. It is the most common inherited ataxia and is caused by an expanded GAA·TTC trinucleotide repeat within the first intron of the Frataxin (*FXN*) gene. This repeat expansion results in decreased expression of the FXN protein, which is important in iron homeostasis and cellular metabolism. The resulting FXN deficit causes degeneration of cell function in the affected tissues, particularly cells with high-energy consumption. FXN levels decrease and disease severity increases with GAA·TTC repeat expansion length. In addition, longer repeats are more prone to further expansion. This tissue specific somatic expansion continues throughout the patient’s lifetime, contributing to disease progression.

Our working model of expansion posits that RNA·DNA hybrid structures can form during transcription of these expanded repeats. When these structures are resolved, the DNA sometimes reanneals out of alignment, resulting in loops that DNA mismatch repair must fix. These loops recruit the mismatch repair heterodimer MutS $\beta$  (MSH2-MSH3), which in turn recruits the endonuclease MutL $\gamma$  (MLH1-MLH3). It is the MutL $\gamma$  heterodimer that seems to be responsible for expanding repeat size. Some research has focused on increasing cellular production of FXN as a potential therapy. However, the possibility exists that increasing FXN transcription will worsen repeat expansion rates, thus accelerating disease progression. To explore the effects that increasing FXN transcription has on repeat expansion, we treated modified FRDA patient-derived cells with two drugs (Nutlin-3 $\alpha$  and Nicotinamide) that have been reported to increase FXN expression.

**Gabrielle H. Terranova**

Undergraduate

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Mentor: Michael C. Norman, PhD

Louisiana State University Health Sciences Center-New Orleans, School of Allied Health Professions, Human Development Center-Louisiana Deafblind Project for Children and Youth

**“Inconsistencies with Assessment and Management of Medical and Educational Related Sequelae Observed in Pediatric Cancer Survivors”**

Children living across the United States from birth to 22 years who have combined hearing and vision loss qualify to be documented educationally under their native state’s Deaf-Blind Project. The etiology of deaf-blindness can be either congenital or acquired, and our research focuses on acquired deaf-blindness as a medically related sequela of pediatric cancer treatment. Medical related sequelae, or “late effects”, of cancer related treatment can include various metabolic, physical, neurocognitive and psychosocial disorders. We explore how deaf-blindness as an observed “late effect” of cancer related treatment impacts overall health, quality of life, education, and psychosocial behavior of childhood cancer survivors.

Our research began with a review of previous studies and clinical trials concerning “late effects” of cancer related treatments observed in childhood survivors. We discovered inconsistencies in the literature concerning modes of assessment and management of medical related sequelae of cancer treatments and the resulting educational and psychosocial behavior experiences of childhood survivors. For example, in terms of assessment, for many of the studies there was a lack of standard methodical testing of neurocognitive functioning post diagnosis and treatment. Additionally, the differentiation of medical sequelae resulting from multimodal therapies was difficult to ascertain because these therapies were commonly used concurrently. In terms of management, there were often multiple forms of transition programs to help children reintegrate into school after conclusion of treatment. The standardization of assessment and management protocols concerning medical, educational, and psychosocial sequelae of cancer related treatments is essential for further research efforts regarding pediatric cancer survivors.

We began the Institutional Review Board (IRB) approval process through Louisiana State University Health Sciences Center (LSUHSC) for a retroactive chart review of patient records associated with the Children’s Hospital of New Orleans’ Late Effects Clinic. We anticipate that an analysis of these data will guide local efforts regarding assessment and management of medical, educational, and psychosocial cancer treatment related sequelae in childhood survivors. However, that process has not concluded.

**Kenneth C. Terry II**

Undergraduate

University of New Orleans, New Orleans, Louisiana

Mentor: Haydee E.P. Bazan, Ph.D

LSU Neuroscience Center of Excellence

**“To elucidate the synthesis pathway of RvD-6 in mouse cornea stimulated with Pigment Epithelium-Derived Factor (PEDF) plus Docosahexaenoic Acid (DHA).”**

The cornea is the outermost tissue of the eye, characterized by its transparency and dense innervation mainly by sensory nerves. It is also protective barrier against bacteria, viruses, and any other type of foreign object or particle from entering the ocular sphere. Inflammation is the body's natural response against trauma, barrier break or microbial infection, but delayed resolution of inflammation can damage the tissue with severe consequences for vision. Previous studies from our laboratory have found that topical treatment of PEDF plus DHA after corneal injury stimulates nerve regeneration and decreases the inflammatory response. The molecular mechanism of their action involve the synthesis of a lipid with a fragmentation pattern by LC-MS/MS similar to Resolvin D6 (RvD6), but with a retention time different that the standard of RvD6, suspecting that we are in the presence of an isomer that have bioactive properties. Specialized pro-resolving mediators (SPMs) of inflammation, such as RvD6 are important to control inflammation. The objective was to further identify the synthesis of RvD6.

Using mice as our *in vivo* model allowed an efficient yet effective way of visualizing and testing the inflammatory response. Mice were handled with guidelines of the ARVO statement for the use of Animals in Ophthalmology and Vision Research. Male CD-1 mice were injured using 2.0 mm trephine to the center of the cornea that damage the corneal nerves and induce an inflammatory response. Mice were euthanized 20-hours post-injury and the corneas were incubated in the presence of inhibitors for 5-LOX (MK591 100uM), 15-LOX (ML351 200uM), Fluvoxamine (P450 200uM), or 17-ODYA (P450 50uM) with PEDF+DHA for 4 hours. The medium was then collected and the lipids were extracted by Blight and Dyer method. Each inhibitor was chosen because of the known pathways of DHA. To determine if the relative amounts of RvD6 is actually coming from the PEDF+DHA sample and not the host membrane, we used DHA Deuterium isotope(DHAd5) plus PEDF to demonstrate that the RvD6 is synthesized from the DHA we added. Lipids were analyzed by liquid chromatography-tandem mass spectroscopy (LC-MS/MS). RvD6 was identified with the full fragmentation chromatogram and UV spectrum. Results were noted and compared to the internal standard (LTB4-D4). The Fluvoxamine inhibitor showed the largest inhibition of RvD6, with further research it may be concluded that the P450 produce an isomer of RvD6.

**Kourtney D. Weaver**  
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Mentor: Dr. Angela Amedee  
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**“The Effects of Chronic Binge Alcohol on Viral Reservoir Maintenance in Gastrointestinal Tissues of SIV-infected Rhesus Macaques”**

Alcohol abuse is common among HIV-infected individuals and is associated with physiological changes that can impact disease progression and co-morbidities. The simian immunodeficiency virus (SIV)-infected rhesus macaque exposed to chronic binge alcohol (CBA) provides an ideal model to investigate the effects of alcohol misuse on virus-host interactions and decipher mechanisms by which alcohol heightens HIV disease progression and viral persistence in tissue reservoirs. In previous studies, it has been shown that CBA increases viral replication in SIV-infected rhesus macaques which is associated with accelerated progression to end-stage disease compared to control animals. Antiretroviral therapy (ART) has been shown to reduce SIV replication in the macaque model; however, like observed in HIV infection, latent virus persists in cellular reservoirs. Our overall hypothesis is that CBA influences HIV disease progression and co-morbidities by increasing viral expression in tissue reservoirs despite effective ART. The objective of this study was to further investigate the influence of CBA on SIV replication and reservoir maintenance in gastrointestinal tissues.

Female rhesus macaques were administered saline or alcohol daily via gastric catheter to achieve a blood alcohol concentration of 50-60mM for 3 months before vaginal inoculations with SIV<sub>mac251</sub>. Blood samples were collected weekly for viral load quantification and ethanol concentration. After 2.5 months of SIV infection, animals received ART (tenofovir/emtricitabine). After approximately 1 year post SIV infection/9 months of ART, the animals were euthanized and necropsy was performed for tissue collection. DNA and RNA were extracted and purified from samples of jejunum, liver, and lymph node tissues harvested at necropsy. Absolute quantification of SIV copies in RNA and DNA from tissue samples was obtained using qPCR targeting the SIV gag gene. Viral RNA levels in plasma were measured using the same qPCR assay.

Viral loads in these tissues, as well as plasma, were compared between control and CBA animals. Plasma viral loads decreased after treatment with ART in both CBA and control animals, however CBA animals maintained higher viral loads at 10 weeks SIV and after 1 month of ART compared to controls. Mesenteric lymph nodes of CBA animals also contained higher DNA and RNA viral loads compared to the control at 1 month ART, but viral loads decreased at the time of necropsy in both CBA and control animals. DNA and RNA viral loads were low in both liver and jejunum samples at necropsy.

Future studies with this model will involve similar analyses of additional gastrointestinal tissues to further characterize viral reservoirs post-ART in the presence of alcohol. These findings reinforce the need for consideration of factors, such as alcohol abuse, when treating HIV-infected individuals to better manage disease.

**LaKia M. Williams**  
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Mentor: Jennifer Cameron, PhD  
LSUHSC

**“Development of Genetic Assays for the Surveillance of HPV 90 in New Orleans”**

Human Papillomavirus (HPV) is the main etiological agent in 90% of anogenital and 70% of oropharyngeal cancers. Seventy percent of those cancers stem from HPV 16 or HPV 18 infection, with the remaining cases attributed to other high-risk(HR) HPV genotypes. The development and use of HPV vaccines such as Gardasil have directly affected the prevalence of HR HPVs in patient populations. The decline of dominant oncogenic strains may create an ecological vacuum, potentially allowing non-dominant low-risk or unknown risk genotypes to spread and evolve. HPV 90 was recently classified as an unknown etiological risk and has previously demonstrated an ability to become oncogenic from a single base pair mutation within the E6 viral gene. HPV 90 has also been seen to exhibit higher prevalence than other unknown risk HPVs in patient populations similar to those populations found in New Orleans. To allow for the real time surveillance of HPV90 during the post-vaccine era, it is our goal to establish nucleic acid-based assays that allow for sensitive and specific identification of HPV90 genes within a patient sample.

The assays utilized for the surveillance of HPV90 are based on both traditional and qualitative polymerase chain reaction (PCR) technology done with HPV90 genotype specific primers for the L1 and E6 genes. The amplification of a 155bp segment of HPV90 E6 was successfully done through touchdown qPCR. The amplification of HPV L1 was done with primers and cycle methods derived from the previously published PGMY09/11 primer set. Amplicons from each PCR method were visualized via gel electrophoresis. The L1 patient amplicon will be denatured and fixed to a nylon membrane to allow for analysis by dot blot using novel HPV 90 L1 specific biotinylated probes. The patient samples utilized in this study were collected from a longitudinal study examining the genotypes found in HIV+ women in the greater New Orleans area. The cohort of HIV+ women in New Orleans (n=100) had a HPV90 prevalence of 9% when examining L1 alone and a 10% prevalence when examining E6 alone. L1 and E6 have a preliminary correlation of 90%.

Testing the remaining samples from the longitudinal study will determine the persistence, prevalence and oncogenic contribution of HPV90 to patients in New Orleans. Future directions include confirming our findings utilizing our conceived dot blot assay. Once established, our L1 technique could be integrated into the clinically available Roche Linear Array genotyping assay for the additional surveillance of locally relevant HPV genotypes.

**Peter F. Winsauer**  
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Mentor: Dr. Patricia Molina  
LSUHSC

**“The Effects of Traumatic Brain Injury and Psychological Stress on Amygdalar Activity and Anxiety-like Behavior in Rats”**

Traumatic brain injury (TBI) is caused by external forces to the head that leads to structural damage to the brain. According to the CDC in 2013, the vast majority of TBI incidents that were documented (around 2.8 million) were caused by motor vehicle accidents, falls, or military combat. Along with the physical and mechanical damage of these injuries, TBI can produce psychological effects, such as increased anxiety, stress, and the loss of memory. TBI frequently occurs in association with traumatic stressful conditions, such as military combat, that lead to increased anxiety after the incident, but the mechanism by which TBI may increase anxiety or how stress impacts TBI-related anxiety is not understood. Previous research has found that TBI increases neuronal excitability and neuroinflammation at the site of injury, along with increasing anxiety-like behavior and excitability in the amygdala. Thus, the objective of this study was to determine if stress exacerbates TBI-related anxiety-like behavior and TBI-induced neuronal activity in the amygdala. In the study, adult male Wistar rats were given a craniotomy. After 3 days of recovery from surgery, rats were exposed to predator odor to induce stress. One day after stress exposure, rats were given a TBI using the lateral fluid percussion model. Rats were sacrificed 90 minutes after the TBI for cFos levels, a marker of neuronal activity, in the site of injury and the amygdala. A separate group of rats was tested 7-10 days after the TBI for changes in anxiety-like behavior through the elevated plus maze (EPM). We hypothesize that pre-injury traumatic stress will exacerbate both TBI-related anxiety-like behavior and amygdalar activity in the brain, leading to further psychological detrimental changes in the days following the traumatic brain injury. Understanding the neurobiological changes caused by TBI in stressful conditions may lead to the development of treatments for TBI-related anxiety disorders.

**Janusz J. Wojcik**

High School

Isidore Newman School, New Orleans, Louisiana

Mentor: Lee S. McDaniel, Ph.D.

Department of Biostatistics, School of Public Health, LSUHSC

**“New Adaptive Design for Phase I Clinical Trials”**

A phase I clinical trial is a first-in-human test of a candidate drug and its goal is to establish recommended doses for later-phase testing. However, drug development has seen increasing costs, long timelines, and high failure rates in phase II trials. This is partly due to use of the historical 3+3 algorithmic framework, which treats small groups of patients with gradually escalating doses of drug: it does not require statistical input and utilizes fixed rules to select maximum tolerated dose, but fails to incorporate dose-limiting toxicity. Here, our goal was to create a phase I trial model that would hit three marks: shorter timelines, easy implementation and improved estimation accuracy (chance of identifying the maximum tolerated dose or MTD). Model-based phase I clinical trial designs, such as CRM or TITE-CRM, typically fulfill one of these criteria well: high estimation accuracy. They allocate patients to a dose level, using a targeted toxicity rate and a statistical model describing the dose toxicity relationship between dose levels. However, model-based phase I designs are not utilized by doctors due to their statistical complexity. With this initial research and understanding, we developed our own isotonic regression model and created a new adaptive phase I clinical trial design. It combined our isotonic regression model and a time-to-event continual reassessment method (TITE-CRM) to form a new time-to-event isotonic regression method that we call TITE-IR. TITE-IR assigns doses to patients one at a time and allows patients to start their assignment at any time. It also uses isotonic regression and time-to-event information to estimate toxicity probability, recalculating the maximum tolerated dose when each new patient enrolls. We calculated predicted outcomes from our TITE-IR trial design and compared it to other clinical trial designs, using simulations in R, a coding language for statistical computing and graphics. We find that TITE-IR has a higher estimation accuracy for the maximum tolerated dose than the traditional 3+3 design and comparable accuracy to other isotonic regression designs while maintaining a shorter timeline.

**Noel S. Wolf**

High School

New Orleans Charter Science and Mathematics High School, New Orleans, LA

Mentor: Lauri O. Byerley:  
LSUHSC Department of Physiology**“Walnut’s Effect on a Gut Microbiome Influenced by Alcohol”**

The American diet contains a large amount of fat which may increase the risk of mental conditions. Omega-3 fatty acids are something the American diet is low in and needs. Walnuts contain omega-3 fatty acids and are also rich in fiber. Eating healthier foods such as walnuts can improve our overall health by cultivating certain microbiota; however the link between these microbiota and our brain remains largely unknown.

Our lab conducted an experiment that gave two groups of Wistar rats one of two diets: the walnut or replacement diet; and each of the rats were given alcohol three days a week for seven weeks to escalate their intake of alcohol. Alcohol’s effects on body weight, alcohol intake escalation, and caloric intake were measured daily. The addition of walnuts did not change body weight significantly or decrease alcohol escalation. Spatial memory/learning was measured after 7 weeks on the diet and on a day when the rats had not consumed alcohol. The rats’ spatial memory/learning was found to have increased in the walnut diet. Based off this information, we predicted that the consumption of walnut’s lead to enhanced spatial learning due to altered microbiota diversity in the gut microbiome. To test this hypothesis, we collected fecal samples, sequenced 16sRNA, and identified bacteria that were significantly different between the two diets. In order to link these bacteria to spatial learning/memory, the literature was searched to identify bacteria that may produce particular molecules that can influence the brain.

Alpha diversity (microbial richness) within each diet was greater in the walnut diet than that of the replacement diet. The data collected from the taxonomy levels showed that the genera: Bacteroides, Blautia, and Coprococcus, as well as the family Coriobacteriaceae and the species Bromii, had a greater relative abundance in the walnut diet versus the replacement diet after chronic consumption of walnuts. These were the focus of the literature review. The review of the literature found some bacteria (such as Bacteroides) produced a neurotransmitter known as serotonin, the “feel good” hormone that has been hypothesized to influence spatial memory and learning. Also, certain bacteria found significant in the analysis produced butyrate. These bacteria were from the family Lachnospiraceae and Ruminococcaceae. Ruminococcaceae had a higher relative abundance in the replacement diet at the family level; however, it had a higher relative abundance in the walnut diet at the species level, thus still making it a focus of our study. Butyrate functions as an energy metabolite for the brain, provides colon cell energy, and is an important component for G protein-coupled receptors. The link between butyrate and spatial memory/learning remains unclear, but butyrate does seem to have an effect on the brain in some way. Changing the gut microbiota by adding walnuts to the diet, may enhance spatial memory/learning due to the metabolites each microbiota produce; however, further study is required to find out the exact link between the microbiota and the brain.

**Sydney M. Youmans**  
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Mentor: Dean G. Smith  
Dean  
LSUHSC School of Public Health

**"Will the 1115B Waiver Approval Decrease the Opioid Use Disorder Epidemic among Louisiana's Medicaid Population? What to Watch"**

The Center for Disease Control labeled Opioid Use Disorder (OUD) a national epidemic, rightfully so with an opioid prescription overdose occurring every 17 minutes. Only 11% of people needing treatment receive treatment, partially due to the lack of complete OUD treatment coverage by Medicaid. Louisiana is one of eleven states recently approved for demonstration project (111B Waiver) that will inform policy-makers on the effectiveness and cost-effectiveness of inpatient treatment in the Medicaid population. Because the first month of this demonstration was June 2018, the goal of this research study was to assess which metrics will be the most important to watch during the demonstration.

The conceptual framework for this study is quite simple: prescriptions lead to overdoses, which lead to deaths. Additional inpatient admissions can disrupt this sequence. The analytical framework for this study is difference-in-difference – changes over time as compared to another state. Louisiana (a waiver state) can be compared to Mississippi (a non-waiver state), as the overall healthcare climates of each state are similar with Louisiana ranking 47<sup>th</sup> and Mississippi ranking 50<sup>th</sup>.

The statistics for LA and MS are similar for opioid-related overdose deaths per 100,000 people in 2016, 7.7 and 6.2 respectively. The number of 30-day supplies of opioids per part D enrollee in 2015 was 2.73 and 2.82, respectively. Both metrics are the most important variables to monitor during the waiver period. Because the values start around the same, it can be observed whether the change over the next 5 years is due to the waiver if Mississippi stays stagnant or some outside variable if Mississippi's statistics also improve at the same rate.

Given the small numbers of inpatient beds available for OUD patients, the waiver may not yield a substantial change in the epidemic. The Office of Behavioral Health under the Louisiana Department of Health only directly sees over 196 beds at the Central Louisiana State Hospital and 82 acute care beds through public/private partnerships.

As an interesting sub-analysis, the Medicaid prescription database permits tracking the number of prescription opioids for the state and the Louisiana State Health Report Card permits tracking overdoses for the state and individual parishes. Analysis revealed no clear factors that explain differences in overdose rates among the parishes with the highest rates. Each parish exhibits different factors that appear to explain their high rates.