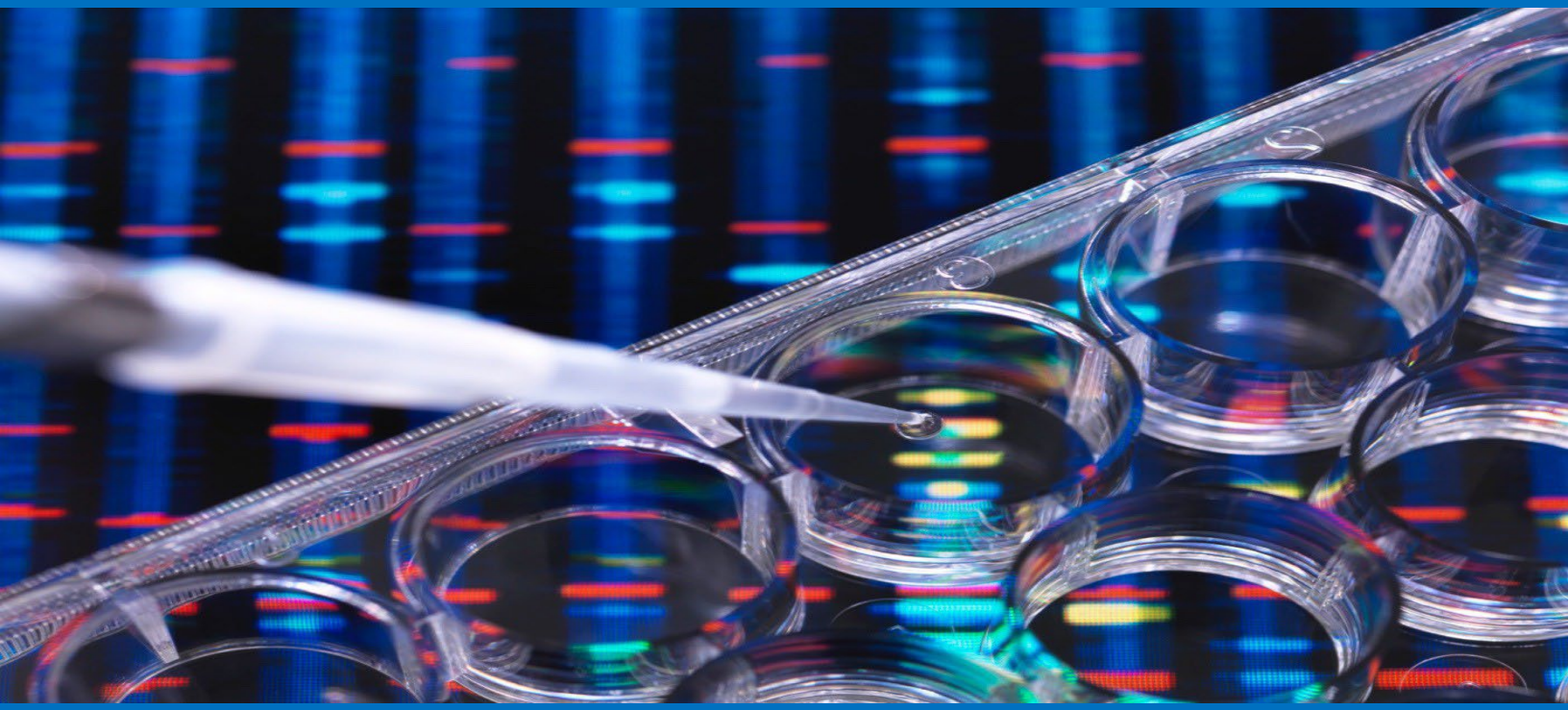
Medical and Molecular Sciences



Compendium of Graduate Student Abstracts (2017-2023)

A SYSTEMATIC APPROACH TO ASSESS THE CLINICAL SIGNIFICANCE OF THE *ABCA4* VARIANTS IN RETINAL DISEASES

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Introduction: ABCA4 encodes a transmembrane transporter that removes toxic retinal byproducts from photoreceptor cells. Missense variants (n=1489) make up a significant portion of ABCA4 variants. Nearly half of these are categorized as variants of uncertain clinical significance (VUS), and an additional twelve percent present conflicting interpretations (CI). In ClinVar, genetic variations are classified based on their clinical significance: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign.

Methodology: We reviewed seven comprehensive genetic studies that detailed ABCA4 variants of patients with ABCA4-related inherited retinal diseases. We then focused on missense ABCA4 variants listed as either VUS or CI in the ClinVar and LOVD databases. Using the ACMG/AMP guidelines, we aimed to clarify the pathogenicity of these variants.

Results: From our analysis, 92 ABCA4 variants emerged as VUS or CI. Notably, eleven of these were reclassified as "Likely Pathogenic" following the ACMG/AMP standards. Additionally, we detected five previously unlisted variants in the databases.

Conclusion: This study highlights the significance of adhering to established ACMG/AMP guidelines and the value of comprehensive databases in refining variant interpretation. This approach not only enhances our understanding of variant pathogenicity but also streamlines the reclassification process.

BIOINFORMATIC PREDICTION AND VALIDATION OF DOMAINS WITH cADPR HYDROLYTIC ACTIVITY

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Recent research has shown that NAD+ and ADP-ribose derivatives are at the interface of a network of macromolecular modifications and second messenger signaling, which is central to virus-host biological conflicts. Signal-generating ADP-ribosyl cyclases process NAD+ to generate soluble cyclic ADP-ribose (cADPR) second messenger in plants. The cADPR signals are then sensed by sensor domains that often play a role in setting the activation threshold for the induction of a diverse array of effector proteins. Thoeris System is a novel antiphage system found in bacteria. It relies on two proteins, ThsB and ThsA, to combat phage infections. Upon detection of phage infection, Toll/interleukin-1 receptor (TIR) domains in ThsB have recently been discovered in bacteria to function as ADP-ribosyl cyclases that process NAD+ in bacteria to produce "variant" cADPR second messenger signals that have unique 1″-2′ or 1″-3′ linkages. These 1″-2′ or 1″-3′ cADPRs (called 2' and 3' gcADPRs, respectively) are known to execute death of infected bacterial cells in communities via depleting NAD+ levels in the cells by ThsA effector proteins. Here, we bioinformatically analyze Macro, NADAR, SLOG, and Nudix domains from phage genomes previously predicted to bind low molecular weight ADP-ribose derivatives for their potential interaction with gcADPRs. Our blastp searches identified conserved binding residues that were analyzed in their potential ligand binding pockets. Using PyMOL and JGI we also studied the gene neighborhood of proteins containing these domains. Finally, we used HPLC to purify 3'cADPR to its interaction with MACRO domains and ThsC as bioinformatically predicted to be potential cADPR binders.

POLARIZED CANCER-ASSOCIATED MACROPHAGES M2 PROMOTE CHEMORESISTANCE IN PANCREATIC CANCER

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General Topic: Pancreatic Ductal Adenocarcinoma is a difficult cancer to treat and has a dismal prognosis. PDAC prognosis is characterized by lack of: early diagnosis, treatment options; and chemoresistance to current treatment options such as gemcitabine.

Background: PDAC tumor microenvironment has tumor associated macrophages (TAMs) that comprise a decent amount of the microenvironment, and can alter their behavior based on different signals they receive. The M2 macrophage is a subtype of TAM that plays important roles in promoting neoplastic characteristics including chemoresistance.

Trends: Tumor associated macrophages (TAMs) polarize into M2 macrophages by a variety of mechanisms involving KrasG12D, non-coding RNAs, and secretion of factors that promote polarization in downstream pathways. Additionally, once macrophages are polarized to M2, they can establish chemoresistance within the cancer.

Comparative Analysis: Growing body of evidence identified that multiple factors contribute to the polarization and chemoresistance of PDAC. Among them, mTOR/AKT/PI3K pathway may be a key pathway in conferring chemoresistance to PDAC.

Conclusion: This systematic review with comparative analysis aimed to study how TAMs polarize to M2 and confer chemoresistance to PDAC. One of our findings show that KrasG12D may polarize TAMs and provide chemoresistance to PDAC. Studying these research objectives enables identifying key areas for novel treatment development.

IMPROVED CLINICAL OUTCOMES IN PATIENTS WITH BACTEREMIA THROUGH MOLECULAR METHODS: MALDI-TOF MASS SPECTROMETRY AND MULTIPLEX POLYMERASE CHAIN REACTION (PCR)

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General Topic: Bacteremia and sepsis are leading causes of morbidity and mortality posing a worldwide public health problem.

Background: The early and timely identification of bloodstream infections along with susceptibilities is crucial in patient outcomes. The BioFire® FilmArray® Blood Culture Identification 2 Panel (BCID2) and Bruker SepsiTyper® Kit use molecular technology to identify pathogens in a timely, sensitive, and specific manner.

Trends: BioFire® FilmArray® BCID2 Panel and Bruker SepsiTyper® Kit reduce turnaround times by their ability to test directly from a positive blood culture. BioFire® FilmArray® BCID 2 Panel does this via polymerase chain reactions. The SepsiTyper® Kit does this through matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF).

Comparative Analysis: The BioFire® FilmArray® BCID2 Panel and Bruker SepsiTyper® Kit are sensitive and specific molecular bacterial identification methods for positive blood cultures. The BCID2 panel has the ability to provide identification and detection of antimicrobial-resistance genes in a timely manner. The SepsiTyper® Kit does not have the ability to detect resistance genes but is the most cost-effective method and has the ability to identify far more pathogens due to the extensive MALDI database.

Conclusion: The BioFire® FilmArray® BCID2 Panel appears to be the molecular method that provides the best identification while maintaining high specificity and sensitivity, even if it is the more expensive option.

SETTING THE CONDITIONS FOR STERILITY AND ENDOTOXIN TESTING FOR THE PROTOCOL OF INJECTING BONE MARROW CONCENTRATION FOR KNEE OSTEOARTHRITIS

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This research investigates the preliminary efficacy of a bone marrow concentrate injection for adults with knee osteoarthritis. This requires insurance of a sterile and bacteria-free sample. Hence, the purpose of this study is to establish conditions for a rapid sterility test, culture sterility test, and FDA-mandated endotoxin assay. A 0.5 McFarland standard of E. coli was used to inoculate spent specimens, test the endotoxin limit of detection and correlate it to bacterial counts. Gram stained slides were checked for the presence of overt bacteria. The Pierce™ Chromogenic Endotoxin Quant Kit (Rockford, IL) was utilized. Sample 7 was inoculated as a 1/10 dilution of a 0.5 McFarland standard of E. coli equivalent in plasma. A 1/50 dilution was created for Gram staining, plating to nutrient agar and the endotoxin assay. There were approximately 3 colonies of growth on the nutrient agar plate and the Gram stained slide was negative for the presence of bacteria. Using the endotoxin assay, we defined the limit of detection as approximately 50 CFU/mL, or 3.7 x 10-2 UE/mL. The conditions were attempted to be set for the sterility testing and endotoxin assay, however, due to the circumstances of the research funding it is suggested more experiments are conducted to confirm the endotoxin limit of detection and correlate it to the bacterial counts.

ORAL MANIFESTATIONS OF BACTERIAL/FUNGAL INFECTIONS AND VIRAL REACTIVATION FOLLOWING COVID-19 INFECTION

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General Topic This case series correlates oral manifestations of COVID-19 with specific bacterial/ fungal coinfections and/ or viral reactivation and provides a report of oral manifestations to aid healthcare providers recognize these disease markers.

Background The SARS-CoV-2 Virus is a single-stranded RNA virus containing spike proteins that attach to human angiotensin-converting enzyme 2 receptors expressed in salivary glands of the oral cavity and on epithelial cells of the tongue.

Methods An online literature search was completed to find current case reports that include the appearance of any oral manifestations due to COVID-19 infection; cases from December 2019- January 2022. Key terms such as "Oral" or "Oral Manifestations" and "COVID-19" or "SARS-CoV-2"and "case reports" were used in various combinations in order to aid in retrieval of the most specific case study reports available. Each included publication is summarized to include the demographics of the patients, the specific oral manifestations, pre-existing health conditions, any treatment or medications that were administered, and the final outcome of the oral manifestation. Oral manifestations stemming from bacterial/ fungal coinfection and viral reactivation are listed. 14 cases were found with bacterial/fungal coinfection or viral reactivation. 6 cases were due to bacterial/fungal coinfections, 2 cases presented with a mix of viral and fungal manifestations, and 6 cases were due to viral reactivations.

Results Patients with Bacterial/ fungal coinfection presented with hemorrhagic ulcers, focal necrosis of tissue, halitosis, plaques, xerostomia, angular cheilitis, and gingival changes. Patients with viral reactivation presented with mucopurulent/ hemorrhagic ulcers, desquamative gingivitis, and stomatitis. These oral manifestations sometimes present alongside common initial symptoms of COVID-19. Other times oral manifestations come about weeks after the original systemic symptoms.

Conclusion: Further research must be completed to determine if oral symptoms result directly from viral attachment, or caused by SARS-CoV-2's impact on another biological pathway. This case series can serve as groundwork for medical professionals to treat patients affected by COVID-19 and its oral manifestations.

ASSOCIATION OF ACE2 GENETIC POLYMORPHISMS WITH SUSCEPTIBILITY OF GETTING INFECTED WITH COVID-19

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In late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in the Wuhan provenance of China. Due to its fast transmission, this virus spread widely, and on March 11th 2020, the coronavirus disease (COVID-19) was officially announced as a global pandemic by the WHO. It has been demonstrated that the incidence and mortality rates between countries varied significantly. These defined differences could be due to genetic factors, political guidance, and behavioral differences among nations. To achieve the objectives mentioned above, this study was conducted as a systematic review with a comparative analysis of relevant studies that explored the effect of polymorphism on the susceptibility of severe COVID-19 patients. Due to the existence of different variants of the ACE2 gene, there are few articles on each specific polymorphism. However, there are articles on the rs2285666 variant due to its possible role in the severity of COVID-19. Numerous studies have suggested the possible role of ACE1 gene polymorphism and COVID-19 outcome, but it could not be concluded decisively which genotype could increase or lower the risk of COVID-19. Overall, it was concluded that ACE1 I/D polymorphism and ACE2 polymorphism (rs2285666) are related to COVID-19 infection, risk of hospitalization, the severity of illness, and mortality rate, however additional studies are needed to assess the specific genotype and allele in these variations.

THE EFFECT OF COVID-19 mRNA VACCINES ON IgA NEPHROPATHY

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This study examines the relationship between COVID-19 mRNA vaccines and IgA Nephropathy (IgAN). IgAN represents a kidney disease emerging from the accumulation of IgA in the kidney resulting in inflammation and damage to renal tissues. IgAN can cause severe renal failure identified by hypertension, ankle edema, and facial puffiness in victims. The study also elaborates on whether the mRNA vaccines escalate the risk of IgAN in patients of all ages. The researcher conducted a systematic literature review to identify articles with primary data aligned with the study objectives. The research was performed by utilizing the articles from PubMed and other academic search engines like google scholar on the subject covered in the past ten years. The findings indicate cogent trends of mRNA vaccines on patients with pre existing IgAN and new cases with regard to clinical manifestations. The most common clinical manifestation patients exhibited after receiving the Pfizer or Moderna vaccine were hematuria and proteinuria; typically after the second dose; serum creatinine levels were variable This was evident in both relapsed IgAN cases and new cases among adults and pediatric cases. The study indicates that the COVID-19 mRNA vaccines significantly impacted patient healthcare and prompted clinicians to more carefully consider IgAN in a differential diagnosis.

HPV INFECTION AND CERVICAL CANCER: THE INTERSECTION OF EPIDEMIOLOGY, MOLECULAR BIOLOGY, AND TESTING METHODS

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Human Papillomavirus (HPV) infection is the most common Sexually Transmitted Infection (STI) observed in sites like the lower genital tract. HPV infections are caused by HPV types classified into low-risk HPV, which causes anogenital warts and benign epithelial lesions, while high-risk HPV can cause cancer-like cervical cancer. HPV types can be detected and typed by conventional PCR and hybrid capture methods. However, conventional methods can have limitations, including being sensitive to multiple infections, misidentification, and the inability to identify HPV types and new variants, subtypes, or mutations. This limitation is a major barrier to complete and unbiased HPV detection and typing and has led to global HPV prevalence being underestimated and distribution misestimated.

The prevalence and distribution of HPV types can be geographically or ethnically specific. In sub-Saharan Africa, HPV detection and typing have always shown how conventional methods can impact the variability and distribution of HPV types. However, I have tested the hypothesis that Next Generation Sequencing (NGS) high sensitivity and specificity have an unbiased HPV detection and typing and validated the results by ts-PCR, which will characterize the pre-vaccination HPV types and prevalence in Nigeria. For comparative analysis, we reviewed the prevalent HPV types in sub-Saharan west African countries surrounding Nigeria. Also, we evaluated the behavioral and demographic risk factors for exposure to HPV infection and cervical cancer development among Nigerian women. Additionally, we examined the HPV DNA LCR prevalent HPV types found in the population to know if they contain genomic nucleotide variation that does not match their epidemiological classification and evolutionary pattern of the HPV types.

Our findings indicate that HPV types 71, 82, and 16 as the top three prevalent HPV types and are unique to Nigeria. We effectively established that there is geographical specificity of HPV types in the countries close to Nigeria, where they share similarities and differences unique to their country. Also, we showed that certain behavioral and demographic risk factors influence the odds of exposure to HPV infection with certain risk types and multiple infections in women. Finally, we showed that nucleotide variation is characteristic of cancer- causing HPV type, and molecular re-classification is needed.

Conclusively, our study identified the baseline pre-vaccination prevalence of HPV types, elucidated the specific behavioral or demographic risk factors for exposure to HPV infection and cervical cancer development, and identified if nucleotide variations specific to the frequent HPV DNA types found in the population to predict the risk for cancer-causing HPV types.

ROLE OF TART CHERRY IN THE PREVENTION OF HYPERTENSION AND THE MODULATION OF INFLAMMATORY SIGNALING

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Hypertension increases the risk for vascular damage, atherosclerosis, and subsequent cardiovascular disease (CVD) related morbidity and mortalities. The development and progression of several chronic conditions, including hypertension, is influenced by inflammation. Chronic low-grade inflammation and hypertension are both prevalent in the aging population.

Blood pressure (BP) can be modulated by a number of factors including diet and lifestyle choices. Our laboratory has previously shown that 12 weeks of tart cherry (TC) juice consumption can reduce systolic BP and markers of inflammation and oxidative stress in older adults. Several bioactive compounds are present in TC, and there is evidence suggesting that these compounds in isolation are able to influence inflammatory signaling pathways such as the NF-κB signaling pathway which can contribute to the pathogenesis of hypertension.

To first understand the impact of diet on BP, we conducted a cross-sectional study in 128 adults aged 65–80 years. Multiple linear regressions were conducted to examine the influence of major dietary factors on systolic and diastolic BP. We also wanted to understand the role of TC in reducing BP. To study this, human coronary artery endothelial cells (HCAEC) were exposed to 0-500μg/mL of TC extracts in the presence or absence of Angiotensin-II (Ang-II), which is known to increase BP and inflammation. Western blots were used to examine the effects of TC and/or Ang-II on the protein expression of nitric oxide synthases and inflammatory molecules associated with the NF-κB signaling pathway.

Results of the cross-sectional study showed a significant association between intake of added sugar and systolic and diastolic BP in females after controlling for physical activity, socioeconomic factors, and BP medication use. Whole fruit consumption was associated with a reduction in diastolic BP in both males and females. The regression model predicted that for every 0.71 cup increase in whole fruit consumption, there would be a 2.8 mmHg reduction in diastolic BP.

In the absence of Ang-II, TC exposure did not influence eNOS expression. Expression of iNOS was reduced by TC at all doses in the absence of Ang-II. Levels of p65 were significantly reduced at 62.5 and 125μg/mL compared to the control. Phosphorylated p65 was upregulated at the 62.5 μg/mL dose and ICAM-1 was similar between groups. In the presence of Ang-II, the 62.5 Ang and 125 Ang exposures resulted in a 0.75 fold and 0.71 fold reduction in iNOS respectively. Ang-II did not significantly affect NOS or inflammatory markers compared to the control. This could be due to metabolism of Ang-II or loss of Ang-II type 1 receptor in cell culture.

Our findings support increased fruit consumption for the reduction of BP in older adults.
Additionally, TC can potentially reduce levels of iNOS at low dose.

ESTABLISHING A CONSENSUS MODEL FOR THE ROLE OF GENOMIC CONTEXT IN CRISPR-DIRECTED GENE EDITING

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CRISPR-mediated gene editing systems have grown rapidly throughout all biological disciplines, due to their ability to facilitate robust gene editing and its ease of use and design. As more research was performed it quickly became apparent that many of the so-called “low-hanging fruit” of clinical gene editing targets, such as sickle cell anemia, would be much more difficult to address than previously believed. Because CRISPR/Cas gene editing systems rely on the DNA repair activity of the cell to introduce genetic changes, variations in activity and interplay among the three main DNA repair pathways (NHEJ, MMEJ, and HDR) lead to variation and on-target disruptions when attempting to target genes for clinically-relevant disease targets. Previously, These complex repair outcomes were not deeply interrogated, due to the high effort and comparatively low efficiency of pre-CRISPR gene editing tools. However, with the increasing applications of CRISPR-based gene editing research, these complex repair outcomes are being seen in more spaces, and at rates that warrant a deeper look and elucidation.

In the work presented in this dissertation, I examined the mechanisms of DNA repair in order to determine the role individual gene sequence and genetic context plays in CRISPR/Cas-mediated gene repair. To accomplish this, I developed a new analysis software that allowed for precise interrogation of complex CRISPR editing reactions, called DECODR. Utilizing DECODR, I could rapidly analyze and iterate on the findings of the gene editing outcomes in both in vitro and live-cell gene editing reactions. I proposed a model for how CRISPR/Cas12a can influence editing outcomes and indel directionality, called traumatic dissociation, and synthesized it with a model that I had previously described, called the ExACT model, to create a new, highly comprehensive model for DNA repair that offers much deeper insight into the complex mechanisms that can influence gene editing reactions across a wide variety of organisms and gene targets.

COMPREHENSIVE ANALYSIS OF ACTIN BINDING PROTEINS AND ACTIN NUCLEATION FACTORS IN ERYTHROPOIESIS REVEALS UPREGULATION OF TNS1 DURING ERYTHROID DIFFERENTIATION

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Objective: This study seeks to characterize the tensin-1 (TNS1) identified through data mining of datasets for actin binding proteins and actin nucleation factors integral to definitive erythropoiesis in humans.

Design: The study is experimental in design utilizing methods for analyzing gene expression, protein levels as well as architecture within cells to provide a baseline for statistical evaluation.

Methods: Data mining strategies, TaqMan gene expression assays, western blotting, alternative splicing assays, and immunostaining with confocal microscopy were employed for this study.

Results: TNS1 mRNA and protein levels were highly up-regulated. Western blotting also identified a truncated form of TNS1 (designated as e-TNS1). Analysis showed that all exons of the TNS1 gene are present, but the C-terminal domain expressing a 350 fold-change compared to the N-terminal actin binding domain in the mRNA terminally differentiating erythroblast. Immunostaining demonstrated that e-TNS1 did not colocalize with F-actin.

Conclusion: TNS1 is highly upregulated during erythropoiesis, with e-TNS1, predominantly expressed in terminal erythroid stages. Moreover, e-TNS1 mRNA was found to preferentially express C-terminal, suggestive of a non-canonical expression with selective translation start sites independent of alternative splicing. The lack of colocalization with F-actin indicates a novel fun5ion for e- TNS1 in erythroblasts, distinct from the canonical TNS1 function.

IMPACT OF AGE AND TESTOSTERONE ON ETB RECEPTOR EXPRESSION IN MEN

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Objective: The purpose of this study is twofold: 1) to test the hypothesis that endothelial cell ETBR (Endothelin B Receptor) expression is attenuated in older men (OM) compared to younger men (YM), and 2) to examine the effects of serum testosterone on ETBR expression in men.

Design: A cross-sectional study in human subjects at an academic university.

Methods: We recruited OM between the ages of 50-70 yrs, and YM aged 18-35 yrs. Brachial artery flow mediated dilation (FMD) was measured to assess endothelial function. We harvested primary endothelial cells from the vein of OM and YM using a J-wire, and quantified ETBR using immunocytochemistry. We performed an ELISA to measure total testosterone concentration ([T]) and correlated serum [T] with ETBR expression and FMD.

Results: The ETBR expression was not different between OM (0.39 ± 0.16 a.u) and YM (0.39 ± 0.19 a.u) ($P > 0.05$). OM ($5 \pm 3\%$) had a lower FMD compared to YM ($7 \pm 2\%$). FMD was negatively correlated with ETBR expression. [T] did not have a correlation with either FMD or ETBR expression.

Conclusion: These data suggest that ETBR expression does not decline in men with aging. These findings are in contrast to previous data showing that ETBR expression is lower in postmenopausal women compared to younger women, reinforcing the important sex difference in the endothelin pathway.

EVALUATION OF ANGIOTENSIN-CONVERTING ENZYME 2 (ACE2) BLOCKING ANTIBODY LEVELS IN COVID-19 VACCINATED PATIENTS

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Objective: This study's aim is to track the ACE2 neutralizing antibodies that are formed after a person has received a full COVID-19 vaccine regime.

Design: The design of this exploratory research study is to conduct a clinical study in order to collect semi-quantitative and qualitative data.

Method: To achieve this goal of this exploratory research, whole blood samples from vaccinated patients will be taken and tested using NIDS® COVID-19 ACE2 Blocking Antibody Test which is a lateral flow immunoassay. The sample will then be read using a Stand Alone Reader 4 (SAR4) to determine the approximate amount of antibodies present in patients' blood over time. The NAb levels against the wild type (WA1/2020), Delta (B.1.617.2), and Kappa (B.1.167.1) will be evaluated.

Results: Depending on the current health and medical history of each individual and vaccination status, the level of NAb for a majority of fully vaccinated people showed signs of decreasing within 2-6 months.

Conclusion: This study provides evidence and information on the evaluation of Angiotensin-Converting Enzyme 2 (ACE2) Blocking Antibody Levels in COVID-19 vaccinated Patients. After the introductory vaccinations, most individuals experienced a rise in NAbs that persisted for months.

THE USE OF ACE II LATERAL FLOW IMMUNOASSAY IN THE EVALUATION OF ANTIBODY LEVELS IN COVID-19 VACCINATED INDIVIDUALS 65 AND OLDER

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Objective: The purpose of this study was to further understand the efficacy of vaccines against SARS-CoV-2 using lateral flow immunoassays to test for antibody levels. The specific focus of this research was to determine the efficacy of the COVID-19 vaccines in high-risk older populations, 65 and older. The research objective was to gather data through a clinical trial, testing individual antibody levels over time to develop a timeline of efficacy in high-risk groups.

Design: The design of this exploratory research study gathered qualitative and semi-quantitative data through a clinical study.

Data Identification and Analysis: The clinical aspect of the study was completed by testing neutralizing antibody levels in fingerstick blood samples using the NIDS® COVID-19 ACE II Blocking Antibody Test. The research plan was to determine vaccine efficacy by monitoring individual antibody levels over a span of 12 months.

Results: The high-risk older population should monitor their antibody levels every two months.

Conclusion: Adults 65 and older should take precaution during the on-going pandemic due to age, health and medications affecting vaccine effectiveness. Consulting with a doctor about receiving a booster vaccine is recommended for high-risk groups. Antibody testing can be a major factor in deciding when it is a healthy time to receive a booster shot.

UNDERSTANDING MOLECULAR CUES UNDERLYING POLARIZATION OF TUMOR-ASSOCIATED MACROPHAGES AND CLINICAL IMPACT ON PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDAC) has an average 5-year survival rate of nearly10%, making it an extremely deadly cancer. The immunosuppressive tumor microenvironment (TME) allows tumor cells to escape the immune surveillance pressure, largely by evolving mechanisms related to immune evasions. An aspect of the TME is tumor-associated macrophages (TAM), which are classified based on their polarized state. M1 macrophages are marked by CD80 and 86; while M2 macrophages are identified by the expression of CD163, CD204, and CD206. TAMs in PDAC are polarized to the M2 characteristic by the TME with subsequent roles in promoting tumorigenesis, aggravating immune checkpoints, accelerating growth and metastasis, and inducing resistance to chemotherapy. This literary review was conducted by examining published research and review articles available online through databases, such as PubMed. M2 macrophages are induced by cytokines, colony–stimulating factors, and metabolic factors. The most common cytokines that polarize TAMs to the M2 phenotype and are produced by M2 are IL–4 and IL–13. IL–10, IL–1β, IL–6, and TGFβ. CSFs activate TAM polarization by interacting with PI3K/Akt and MAPK pathways. They also influence the stroma of PDAC to promote tumorigenesis.

A COMPARATIVE ANALYSIS OF CRPS SYMPTOM QUANTITATION AMONGST TYPE I AND TYPE II PATIENTS

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General Topic: Complex Regional Pain Syndrome (CRPS) is a condition that develops following a physically traumatic event or nerve injury.

Background: There are two types of CRPS diagnosis, type I and type II. There are current treatment options, but no cure.

Trends: This study aims to quantify the occurrence of each diagnostic section and subsections extrapolated from the Budapest Criteria: sensory, vasomotor, sudomotor, and motor/trophic. A comparative literary analysis of the presenting and reported symptoms amongst CRPS type I and type II patients was performed.

Comparative Analysis: Accounting for demographics, presentation, and recollection of symptoms were factored into the quantitative comparison. To classify and quantify reported and presenting symptoms, each category was broken down into its signs, and then further sectioned off into specific symptoms.

Conclusion: Data was compiled on a scoring basis, if the sign or symptom was mentioned in the patient's case description, a score of 1 was documented for that specific criteria, if they were not mentioned, a score of 0 was documented. At the summation of the data collection, each sign and symptom documentation scores were totaled to assess the overall quantity. The most common symptom experienced based on this study is some form of extreme pain from commonly non-pain inducing sensations. The second most commonly reported symptoms were edema, a sudomotor symptom resulting in swelling of the affected area, and issues surrounding range of motion.

DIAGNOSIS AND TREATMENT OF GLIOBLASTOMA USING FLOW CYTOMETRY ANALYSIS

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General Topic: Glioblastomas are aggressive and fast-growing cancerous cell of the brain and central nervous system. Background: Patients with glioblastoma have a very poor prognosis. The general symptoms of these malignant tumors are headaches, blurred vision, onset of seizures, speech difficulty and difficulty learning. These can lead to the need for frequent Magnetic Resonance Imaging (MRI) for detection and treatment monitoring. Hypothesis: The use of Flow Cytometry can also be used to monitor, diagnose, and help find treatments for patients with glioblastomas.

Trends: During the literature review, various clusters of differentiation (CD) markers were discussed. Markers including: CD3, CD4, CD14, CD25, CD31, CD34, CD45, CD83, CD133, CD146, and CD163 can be used to gain a better understanding of cell populations in glioblastoma patients. Comparative Analysis: Many of the articles use peripheral blood or peripheral blood mononuclear cells as the sample to be analyzed by a flow cytometer. Differences between the studies are the utilization of flow cytometry.

Conclusion/Summary: Glioblastoma patients will benefit from continued research on Flow cytometry analysis for diagnosis, monitoring, and treatment of glioblastomas. Future studies should employ larger numbers and a diverse array of cell markers thought to characterize glioblastoma.

PROPHYLACTIC LOW MOLECULAR WEIGHT HEPARIN REDUCES MORTALITY RATE AND RISKS OF THROMBOTIC EVENTS IN HOSPITALIZED COVID-19 PATIENTS

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Severe COVID-19 patients are frequently complicated with an incidence of thrombotic events such as deep vein thrombosis (DVT) and pulmonary embolism (PE) due to inflammatory driven processes. A high proportion of COVID-19 patients have changes in coagulation tests especially the D-dimer levels, which can be used for the prognosis of COVID-19. The ISTH guidelines recommend the use of low molecular weight heparin (LMWH) as soon as possible after hospital admission for thromboprophylaxis. This literature review compared some studies to show the efficacy of LMWH in prevention and treatment of thrombotic events. By comparing the outcomes of using LMWH prophylaxis with different dosages, this review supported the ISTH recommendation about LMWH thromboprophylaxis. However, the optimal LMWH dosage is still uncertain, requiring more research and clinical trials. Until the optimal dosage is determined, the standard prophylactic-dose LMWH can be employed in non-critically ill COVID-19 patients. The critically ill patients in ICU should be administered with unfractionated heparin or therapeutic-dose LMWH, which showed more benefits than prophylactic-dose LMWH in that patient group.

THE CONTRIBUTION OF THE ABCA4 VARIANTS TO THE SEVERITY OF AGE-RELATED MACULAR DEGENERATION

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Numerous genetic loci have been identified, but some genetic variations that lead to complex traits and diseases have yet to be recognized. Recent advancements in genetic sequencing technology allow for the exploration of the contribution of rare and low-frequency variants to human traits. Our focus was to analyze exclusively the ABCA4 gene, "using primary data derived from the European EYERISK study conducted by de Bruek and colleagues." The study aims to analyze an association between clinical phenotype or disease severity caused by the nonsynonymous variation (SNV) in the ABCA4 gene. Through the statistical analysis and current knowledge from the literature, we analyzed the contribution of these minor allele frequencies of genetic variants to risk factors for complicated diseases such as age- related macular degeneration (AMD). For that reason, we conducted a two-way analysis of variance to explore differences among the MAC control, MAC early AMD, and minor allele count (MAC). The results of the two-way analysis of variance (ANOVA) showed a statistically significant intersection between group and variants on the MAC for an early AMD, late AMD, and control group. Due to a single nucleotide change or non-synonymous variation (SNPs) in the DNA sequence, many of the genes contribute to causing risk factors for AMD. The study highlighted the need for understanding genetic factors in the early and late stages of AMD disease. In the future, to design new therapies it is essential to uncover more details about individuals who are genetically at risk of carrying minor allele variants of the disease.

CHARACTERIZATION OF THE FUNCTIONAL ROLES OF *ABCA4* IN THE PATHOLOGY OF INHERITED VISUAL DISEASES

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The retina-specific ABC transporter, ABCA4, is localized in the rod and cone photoreceptor outer segment discs and is essential for the proper functioning of the visual cycle. ABCA4 is a key player in the continuous recycling of retinoid substrates required for vision through its transport of vitamin A derivatives across the rod outer segment disc membranes. Mutations in the ABCA4 gene lead to a wide variety of blinding inherited visual diseases, including Stargardt disease, Cone-Rod Dystrophy, autosomal recessive Retinitis Pigmentosa, and Age-Related Macular Degeneration. More than 1,000 variants have been identified in the ABCA4 gene, yet there is no clear correlation between specific genetic variants and the wide ranges in age of onset and degree of progression of ABCA4-linked diseases. This is in part due to the lack of a facile approach to evaluate the association of a given genetic variation, and the consequences in terms of patient phenotype and protein function. ☐ Recombinant full- length ABCA4 protein is difficult to analyze due to its complex transmembrane nature and instability upon purification. Thus, a stable, uniform, and high-throughput expression platform in a biological membrane-like setting is needed to holistically understand the function of ABCA4 and its role in the pathophysiology of visual disease. In the work presented in this dissertation, I have tested the hypothesis that expression of human ABCA4 protein in virus-like particles (VLPs) will lead to the production of stable recombinant protein of uniform topology. Using the baculovirus expression vector system (BEVS), I have developed a novel platform for efficient expression and characterization of the full-length ABCA4 protein and its disease- associated variants in virus-like particles. We have physically, functionally, and topologically characterized ABCA4 VLPs and, similarly, investigated variant VLPs for their enzymatic function. For a comparative analysis, the recombinant NBD2 polypeptide and its variants purified from E. coli were assessed for ATP binding, ATP hydrolysis, and subdomain interactions. Our key findings indicate that expression of ABCA4 in VLPs produces proteins that are biologically active, stable and of uniform membrane topology. Furthermore, I have demonstrated that VLPs are an efficient and robust platform to functionally characterize ABCA4 disease-associated variants. Using this platform, I have interrogated the functional significance of the C-terminal domain in ABCA4. We have demonstrated that the C-terminal VFVNFA motif is essential for both ATP hydrolysis and retinal binding, thereby elucidating the significance of this domain in ABCA4 associated retinopathies. Conclusively, our established platform is ideal for the high-throughput investigation of various ABCA4 disease-causing mutations of unknown significance, which may aid in patient prognoses and the delivery of novel therapies.

MOLECULAR ANALYSES OF CRISPR-DIRECTED GENE EDITING ON THE *NRF2* GENE

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Lung cancer remains the leading cause of cancer-related death worldwide. As a result, the prognosis of patients diagnosed with Non-small cell lung carcinoma (NSCLC), particularly, is dismal indicating the need for continued improvement in prevention, diagnosis, and treatment. Despite this, treatment options and regimens are similar to what was originally established many years ago. Recent studies point to the evolution of drug resistance in lung cancer as being centered, in part, on the upregulation of various genes involved in controlling efflux or drug inactivation. Among these genes is Nuclear Factor Erythroid 2-related Factor 2 (NRF2), which is considered a master regulator of 100-200 target genes involved in cellular responses to oxidative and/or electrophilic stress. There is a subset of NSCLC patients who carry mutations in NRF2, which cause the transcription factor to act like an oncogene, favoring cell survival and growth in cancerous cells; these mutations also create new recognition sites for cleavage and gene disruption by CRISPR/Cas9, making NRF2 a good molecular target. While the oncogenic role of NRF2 continues to be investigated, there is a gap in knowledge of the molecular mechanism involved during and after CRISPR-directed knockout of NRF2 in solid tumor cells. To address this, I proposed establishing a clinically relevant model system to study the site-specific efficacy and fidelity of CRISPR/Cas9 for targeting NRF2. With this approach, I identified the global gene expression profile after CRISPR-directed gene disruption which helps to establish the structure-function relationship of CRISPR-induced mutations in NRF2. These data begin to define the molecular framework upon which safe and efficacious therapeutic strategies can be built.

CHARACTERIZATION OF *NICOTIANA BENTHAMIANA*-PRODUCED *ALFALFA MOSAIC VIRUS* (ALMV) VIRUS-LIKE PARTICLE (VLP) ASSEMBLY

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Objective*:* The objective of this research project was to identify assembly conditions promoting the most extensive polymerization of *Alfalfa mosaic virus (AlMV)* coat proteins (CP) into icosahedral virus-like particles (VLPs). Effects of protein concentration, buffer ionic strength, assembly time, and protein sequence variations on extent of VLP formation were investigated. Ability of coat protein-antigen genetic fusions to form VLPs was assessed within identified optimal assembly conditions.

Design*:* Basic scientific research project aimed at characterizing and optimizing *AlMV* VLP formation.

Methods*: AlMV* coat protein variants purified from *Nicotiana benthamiana* were dialyzed into assembly buffers under varied conditions. Extent and quality of VLP formation was characterized using SEC-MALS, DLS, and TEM.

Results*:* Aggregation and VLP polydispersity was minimized under 80 mM sodium pyrophosphate, pH 5.5, CP concentration ≥10-15 mg/mL assembly conditions, while providing sufficient capsomere incorporation into VLPs of correct morphology for several non-antigenic CP variants. Supplementation of CP-antigen fusion assembly reactions with free CP improved VLP antigenicity and capsomere polymerization over CP-antigen fusion assemblies alone.

Conclusion*:* Identified assembly conditions are appropriate for initial screening of CP-antigen fusion construct assembly capability, however, optimization of clinically relevant VLP formation should be performed on an individual basis.

HIGH-FIDELITY AMPLIFIED FISH FOR THE DETECTION OF CIRCULAR RNA

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Objective: Validate and optimize the use of amp-FISH, called also amp-circFISH, for imaging circRNAs.

Design: Hairpin binary probes were designed for the detection of circZBTB44.

Methods: HCR probes were purified by HPLC and then, hairpin binary probes and HCR probes were purified further by denaturing polyacrylamide gel electrophoresis. Prior to hybridization, probes were snap cooled and 2-step hybridization was performed. Following the removal of excess probes, HCR was performed using 125 nM of each HCR probe. As a comparison to test sensitivity and specificity of the method, circFISH was also performed using 35 probes. Images were analyzed using MATLAB software.

Results: Labeled HCR probes were successfully separated from unlabeled oligonucleotides and free dye. Additionally, the desired full-length hairpin binary probes and HCR probes were separated from undesired truncated oligonucleotides. Amp-circFISH probes generated bright signals and detected around 30 circular RNAs. While circFISH detected about 10 circular RNAs.

Conclusion: amp-circFISH is a suitable method for the detection of circular RNAs that can be used for the detection of shorter circRNAs due to its high specificity and sensitivity. One pair of amp-FISH probes generates signals that are about as bright as a set of 35 sm-FISH probes.

DEVELOPMENT OF A REAL-TIME ASSAY FOR SCREENING SMALL MOLECULE MODULATORS OF SECOND MESSENGER METABOLISM

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Second messengers are signaling molecules involved in intracellular signal transduction cascades and are being studied due to their ability to modulate multiple bacterial behaviors, with cyclic-di-AMP in particular a topic of interest as an “essential poison” for many human pathogens. Cyclic-di-AMP is synthesized and degraded using diadenylate cyclases and phosphodiesterases respectively, where cyclases facilitate the conversion of ATP to cyclic-di-AMP and phosphodiesterases facilitate conversion of cyclic- di-AMP to AMP. In this paper, we demonstrate the ability to monitor cyclic- di-AMP degradation by phosphodiesterase GdpP through a coralyne dye- based fluorescence assay, as well as conduct initial high-throughput screening to determine an inhibitor for said degradation. Through the assay, twelve compounds were identified as potential inhibitors, with rose bengal being most potent and likely to be an antagonist of GdpP binding to cyclic-di-AMP.

THE USE OF CIRCULATING MICRORNAS (miRNAs) IN BLOOD AS AN EARLY MARKER FOR THE DIAGNOSIS OF CANCER. A COMPREHENSIVE ANALYSIS

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General Topic: Non-invasive and early markers are critical for timely and sensitive diagnosis of cancer.

Background: Cancer continues to be a disease that affects our society despite medical advances and diagnostic tools. A comprehensive review of literature was performed to identify the use of circulating miRNAs as biomarkers for the early detection of four leading cancers in the United States.

Comparative Analysis: In this comprehensive analysis, research articles were analyzed to establish the role of cancer derived circulating miRNAs and their application in the early diagnosis of cancer.

Trends: Upon completion of this analysis, it was determined that the circulating levels of miRNAs in the four most common types of cancer (breast, colorectal, lung, and prostate) was dysregulated as compared to healthy individuals. This dysregulation influences the levels of these miRNAs in blood circulation making them good biomarkers for the early diagnosis of cancer. It was also noted that miR-21 was a common dysregulated miRNA observed in all of the four cancers discussed. Further indicating its diagnostic value for the early detection of cancer.

Conclusion: Circulating levels of miRNAs are dysregulated during cancer development. As cancer cells release miRNAs, that influences the proliferation, invasiveness, and pathogenesis of cancerous cells. Identification of these circulating miRNAs from blood provides an early non-invasive and accurate diagnosis of cancer.

A REVIEW OF HEMATOLOGICAL & COAGULATION ABNORMALITIES ASSOCIATED WITH COVID-19

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General Topic: Hematological Abnormalities & Complications of COVID-19.

Background: Since the discovery of COVID-19, the disease continues to spread and infect over millions of people, resulting in the ongoing pandemic. While COVID-19 is well known as a respiratory tract infection, emerging studies suggest that it is a systemic disease affecting multiple organ systems. The major systems impacted is the hematopoietic system and hemostasis where procoagulant patterns have been identified in critically ill patients.

Trends: Common laboratory findings in COVID-19-associated coagulopathy includes elevated results in fibrinogen, D-dimer, IL-6, viscoelastic test results, and coagulation factor activity. Incidences of VTE and mortality were also remarkedly high even with thromboprophylaxis.

Comparative Analysis: Similarities in current studies indicate that the most common hematological findings include increased fibrinogen, D-dimer, and IL-6. Viscoelastic test results and coagulation factor studies are also elevated further demonstrating that a procoagulant pattern is present. Currently, coagulation factor analysis appears to be a potential candidate for specific biomarker testing to diagnose COVID-19-associated VTE.

Conclusion: Reports on thromboembolic complications in COVID-19 are still scarce, but data suggests that a procoagulant pattern is present and does have some clinical significance. Therefore, further research into the hematological abnormalities associated with COVID-19 would be beneficial in developing better VTE prevention and management strategies.

LONGITUDINAL ANALYSIS OF ASPECTS OF DIETARY DIVERSITY & THEIR ASSOCIATION WITH RACE, SEX, POVERTY STATUS AND BODY MASS INDEX IN HEALTHY AGING IN NEIGHBORHOOD OF DIVERSITY ACROSS THE LIFE SPAN STUDY PARTICIPANTS

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Dietary diversity (DD) is a universally recognized key component of a healthful diet. Traditionally counts of different foods/food groups consumed over a specific time period reflected DD. Since DD has multiple aspects, evenness and dissimilarity are additional recommended methods of measuring DD. The purpose of this study was to conduct a longitudinal analysis of three DD measures and assess their association with various demographic factors such as race, sex, poverty status, as well as body mass index (BMI). This study determined DD scores for count, evenness, and dissimilarity across adulthood in a diverse sample. Participants were from the Healthy Aging in Neighborhoods of Diversity across the Life Span Study (HANDLS), a longitudinal study, which included 3,720 African American and White adults. Dietary diversity measures were calculated for three study waves (2004-2017) using 2 days of 24-hr recalls. The count was based on consumption of ≤50% of an equivalent of food from 21 subgroups. Evenness was derived using Berry-Index adjusted by the health value of food; dissimilarity, by Mahalanobis Distance. Two sample t- test was used to compare means of DDS within each wave categorized by race, sex, and poverty status. To examine the change in DD scores over time as well as the association of DD with BMI across adulthood, multiple mixed-effects regression models were used. The model was set up with random intercept and slope for time as well as following fixed effects as predictors: race, sex, poverty status, education measured at baseline; smoking, centered age and centered energy as time-dependent variables measured at each wave. The models also included two-way interactions of time with each of the predictors. Additionally, diet quality measures- Mean Adequacy Ratio (MAR) and Dietary Approaches to Stop Hypertension (DASH) were included as predictors in the model while analyzing the association of DD with BMI. Only count and dissimilarity scores significantly differed by sex and race ($p<0.001$). All three DD measures were statistically different between income groups (>125% vs <125% poverty). White women and persons with higher incomes had better DD. The mixed model used to examine longitudinal change in DD scores as outcome across waves showed no significant interaction of time*race and time*income for count and evenness model at each study wave. However, a significant interaction was noted for time*race ($p=0.0005$), time*income ($p=0.0325$), and time*energy ($p<0.0001$) for dissimilarity mixed model at each study wave. These findings suggested a decrease in dissimilarity scores among Whites and those with self-reported income >125% poverty status compared to their counterparts. For energy across waves, dissimilarity scores increased with every unit increase in energy over time. There was no significant association noted between any DD measures (count, evenness, dissimilarity) with BMI both in models that examined the measures individually or combined as covariates. However, further inclusion of MAR and DASH as covariates improved all models with DD measures with BMI as outcome measured at each wave. Although MAR did not make any significant difference in any models, DASH had a significant main effect for all three models (count: $p=0.0291$; evenness: $p=0.0454$; dissimilarity: $p=0.0223$) suggesting an inverse association between DASH and BMI. There was a statistically significant two-way interaction of time and DASH in mixed-effects regression models that included count and/or dissimilarity as covariate and BMI as an outcome measured at each wave. The positive slope for BMI suggested an increase in BMI being associated with an increase in the DASH score over time. These findings might be attributed to the increased energy intake over time. In conclusion, our study provided unique insight into the aspects of DD and their association with selected demographic factors as well as health-related outcome BMI.

DEVELOPING AN IN VITRO SYSTEM TO ELUCIDATE THE MECHANISM AND REGULATION OF CRISPR-DIRECTED GENE EDITING AND THE RESPONDING DNA DAMAGE REPAIR PATHWAYS

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Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)- directed gene editing is a revolutionary approach to genetic manipulation and disease modeling. CRISPR-Cas systems have demonstrated utility for a wide-range of applications and biotechnological breakthroughs. Yet, challenges remain including the unpredictability of genetic outcomes generated as a result of CRISPR-directed DNA cleavage and unbalanced responses by DNA damage repair pathways. Site-specific DNA breakage introduced by Cas proteins is followed by a DNA damage repair response from one of two competing pathways, Non-Homologous End Joining (NHEJ) or Homology Directed Repair (HDR). Therefore, the influence of CRISPR- directed DNA damage as a regulating factor of these response pathways should provide a key to understanding this unpredictability. We propose to address this gap in knowledge by developing an in vitro gene editing system to study the factors affecting the mechanism and regulation of DNA repair in response to CRISPR-directed DNA disruption. The ability to assess CRISPR-Cas site-specific DNA disruption and damage responses in a simplified system will facilitate a deeper understanding of the capabilities and limitations of gene editing tools. The ability to initiate CRISPR- directed gene editing reactions with a methodical regulatory approach of strategically incorporated gene editing tools will provide a foundational system for identifying the regulatory factors affecting precise gene editing event. This will provide insight and a deeper understanding of the regulatory factors involved in CRISPR-directed gene editing and the diversity of genetic outcomes generated by competing DNA repair responses. We have been able to generate in vitro models for several unique gene editing reactions. This system enables us to gain new insights into the mechanisms of repair while providing the opportunity to assess the diversity of genetic outcomes resulting from gene editing reactions. This in vitro gene editing system will continue to provide new insight into the regulatory mechanisms of CRISPR gene editing technologies, many of which remain to be fully understood and defined.

A LITERATURE REVIEW STUDY TO ANALYZE THE RELATIONSHIP BETWEEN KETOGENIC DIET AND THE ROLE IN THE TREATMENT OF EPILEPSY

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Purpose: To analyze the relationship and role of ketogenic diet in the treatment of epilepsy.

Methods: Meta-analysis of relevant heterogenous and homogenous studies using scientific search engines were selected from all around the world. The studies were then analyzed using JMP® software to assess the effect of ketogenic diet in a given sample of the study at three months, six months and twelve months.

Results: Thirty studies were analyzed. Majority of the studies show a greater than fifty percent seizure reduction at three months, six months and a twelve month follow-up periods. Drop outs were obvious during follow-up periods due to non-compliance and common side effects of the therapy.

Conclusion: Studies tend to show that ketogenic diet has a significant reduction in the percentage of the seizures mostly seen in the age groups under eighteen years of age. Adult populations have shown an increase in non-compliance to the diet and hence a less percentage of seizure reduction. Almost all the studies concluded that the ketogenic diet therapy along with the anti-epileptic drugs must be under supervised care.

METHOD COMPARISON STUDY OF THE IL GEM PREMIER 3500 AND RADIOMETER ABL90 FLEX PLUS BLOOD GAS ANALYZERS USING VENOUS BLOOD SAMPLES

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Background: A comprehensive evaluation and comparison study was performed on two popular blood gas analyzers.

Materials and Methods: A basic method validation was performed using the IL GEM 3500 and the Radiometer ABL90 FLEX PLUS. Precision, linearity, and a method comparison using 56 venous blood gas samples were evaluated for pH, pO_2, pCO_2, ionized calcium, sodium, and potassium. Ease of use by key operators and analyzer characteristics were also evaluated.

Results: Precision passed for all analytes except for ionized calcium when compared to the established acceptable %CV. The method comparison for pH, pO_2, pCO_2, ionized calcium, sodium, and potassium were acceptable based on statistical analysis produced using the calculated bias, variance, and correlation coefficient. Bland-Altman plots showed a significant proportional bias with potassium, pO_2 and pCO_2. The ABL90 showed acceptable linearity over the analytical measurement range for all analytes. Key users felt the ABL90 FLEX PLUS was easier to use and preferred over the GEM3500.

Conclusion: Overall, most analytes showed good precision, accuracy, and linearity between the two instruments. The ABL90 FLEX PLUS was determined to be an acceptable replacement for the GEM3500 for blood gases and whole blood electrolytes.

ACCULTURATION AND DIABETES IN AFRICAN MIGRANTS TO THE UNITED STATES

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Objective: Utilizing mixed methods analysis, this study sought to analyze the association between acculturation and diabetes in African migrants to the United States (U.S.)

Methods: An analysis of cross-sectional data from the 2010-2017 National Health Interview Surveys was performed, and semi-structured interviews were conducted in both French and English with Congolese migrants (N=20) living in Minnesota and the D.M.V area (Washington, D.C., Maryland and Virginia).

Results: The prevalence of self-reported diabetes among African migrants was 6.1%, and being acculturated was associated with higher odds of diabetes diagnosis (Odds Ratio (OR) =2.2; 95% CI =1.1-4.4). BMI explained 18.9% of the total effect of acculturation on diabetes ($\mathcal{Z}Mediation$= 2.11, p=0.036). Qualitative interviewing of a specific African migrant group, Congolese, revealed that contextual characteristics that influenced acculturation included: support, gender norms, religious commitment, instability in the Congo, racism and migrant discrimination in the U.S. Findings also indicated that acculturative scales designed for Congolese migrants should include cultural identity, language, friendship, fashion sense, and knowledge of sports and politics to capture the process. Analysis of diabetes risk factors (diet, physical activity and stress) showed that even though Congolese migrants sought to maintain their pre-immigration eating habits, their diet in the U.S. was affected by accessibility and cost. Going to the gym was not particularly relevant to the Congolese culture, and the reduction in physical activity levels was accentuated by time constraints and weather conditions. Stress increased after immigration and was mainly caused by financial problems. Insight into contextual factors, acculturation, and diabetes-promoting behaviors provided potential intervention targets to reduce the risk of diabetes in the population.

Conclusions: Acculturation is associated with diabetes in African migrants. To gain a better picture of the cardiometabolic health of African migrants, future research should develop better instruments to quantitatively test a comprehensive model that incorporates contextual characteristics, acculturation, diabetes, and its mediating factors. Additionally, culturally appropriate intervention programs targeting the population should be implemented to avert a diabetes crisis within the population.

CRISPR DIRECTED TARGETED GENE ALTERATION: MECHANISM TO APPLICATION

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Single base mutations can be repaired by introducing single stranded DNA oligonucleotides (ssODN) into a target cell. The frequency at which this occurs is dependent on several of factors: the length of ssODN, the position of the cell in its proliferative cycle, and the presence of double-stranded DNA breaks in the host genome. Genome editing offers a promising strategy for gene repair and correction by overcoming difficulties associated with lack of precision. CRISPR/Cas has increased the pace and lowered the cost of research, allowing the genetic manipulation even in organisms that have historically been difficult to modify. Furthermore, the combinatorial approach uniting ssODNs and CRISPR/Cas9 has emerged as a feasible therapeutic approach. In the work presented in this dissertation I focused on the mechanism and application of gene editing utilizing CRISPR systems. I tested combinatorial approach of utilizing CRISPR/Cas9 system along with ssODN to promote single base pair correction and demonstrate it is now possible to direct single nucleotide exchange in efficient manner. We find that both insertions and deletions accompany single base repair as result from allelic analysis of clonally expanded cell populations. CRISPR/Cas9 and single-stranded oligonucleotide donor DNA molecules working in tandem can lead to the precise repair of the point mutation in the eGFP gene, and led to propose a new model for the repair of point mutations, a process we have termed ExACT. The relationship between transfection efficiency and gene editing activity was tested and analyzed based on experimental and visual data and found that there is no direct correlation between efficient cellular uptake and genome modification directed by an RNP. By understanding the mechanisms by which CRISPR/Cas executes gene editing in human cells, a more efficacious and potential approach to drug development could be undertaken. The application of the CRISPR gene editing system in two different approaches to study pediatric Leukemia was explored. (1) pediatric patient specific ALL chromosomal translocation (4:11)(q21:q23) was re-created by utilizing the CRISPR/Cas9 system in HEK293 cells. This led to the development of a convenient platform for rapid modeling of cancer-related genetic mutations in vitro. (2) Implemented the use of a novel gene editing approach to create expression vectors that harbor patient specific mutations that were tested against TKI. We have developed a diagnostic system to monitor the impact of mutant FLT3 ITDs on the progression of oncogenesis and to evaluate the efficacy of novel AML drugs.

IMPACT OF INCOME AND LEVEL OF URBANIZATION ON THE HPV VACCINATION RATES

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Human Papillomavirus (HPV) infections have been a major cause of cancer in the United States. Cervical and Oropharyngeal cancers have been linked to HPV infections. Two prophylactic vaccines called Gardasil® and Cervarix have been licensed in the United States against HPV to protect adolescents from persistent infections that lead to cancer. However, according to recent published data, the rural southern part of the United States exhibits the lowest rates of HPV vaccinations in the country. Research data have also indicated that the southern regions of the United States tend to have the lowest national median income rates. Therefore, this research study examines the relationship between the national median income and the vaccination rates through conducting a correlation coefficient statistical analysis. The results of the study indicate a positive linear relationship between the two variables and explores some of the reasons behind the issue. Additionally, this study analyzes some of the potential solutions that could be implemented to improve the public health conditions in the rural south.

UNDERSTANDING THE BIOLOGY OF INFLAMMATORY BREAST CANCER (IBC) CUTANEOUS METASTASIS AND THE ROLE OF TGFβ

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Inflammatory breast cancer (IBC) is a highly aggressive form of locally advanced breast cancer with unique molecular and phenotypic properties (Dawood, Merajver et al. 2011, Dawood and Valero 2012, Joglekar and van Golen 2012). Cutaneous metastases from internal cancers are relatively rare, occurring at a rate of 0.7-9.0% (Martin 1997). IBC cutaneous metastasis is associated with chest wall recurrences, significantly decreasing the quality of life and survival (Cristofanilli, Valero et al. 2007). Although significantly different in many aspects, IBC and melanoma share a number of similarities in disease presentation and progression. Both spread via dermal lymphatics, form intralymphatic emboli and have a propensity to form cutaneous metastases (Fidler 1990, Leiter, Meier et al. 2004, Rose, Christos et al. 2011). Thus, new leads for studying cutaneous metastasis can be gathered from the melanoma literature. In melanoma, there were several studies done on radiation and TGFβ to demonstrate the role of TGFβ on the etiology of melanoma cutaneous metastasis (Schmid, Itin et al. 1995, Perrot, Javelaud et al. 2013). There were no studies done to date to understand the biology of inflammatory breast cancer cutaneous metastasis in relation to radiation and the role of TGFβ. Here my doctoral project is primarily focusing on the influence of radiation in IBC cutaneous metastasis and the role of TGFβ. I radiated normal human fibroblast cells with different doses of radiation and used the conditioned media to see the invasiveness of the IBC cells (KPL4 and SUM149) and compared them with the conventional breast cancer cells (MDA-MB-231). I observed that the IBC cell invasion is significantly higher with higher doses of radiation. Also there is higher expression of TGFβ-2 with higher (5 Gy) dose of radiation. I also used RNA sequencing to identify the molecular signature profile of IBC and non-IBC cells which may lead to the therapeutic treatment of IBC cutaneous metastasis.

ADULT BONE MARROW MESENCHYMAL STEM CELLS PRIMED FOR THE REPAIR OF DAMAGED CARDIAC TISSUE AFTER MYOCARDIAL INFARCTION

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The burden of cardiovascular disease around the world is growing, despite improvements in hospital care and time to treatment. As more people survive an initial myocardial infarction (MI), the decompensated heart tissue is strained, leading to heart failure (HF) and an increased risk for a second MI. While extensive progress has been made in treating the symptoms after MI, including HF and angina, little success has come from repairing the damaged heart tissue to alleviate the progression to these end- stage symptoms. One promising area of regenerative research has been the use of adult stem cells, particularly from the bone marrow (BMSCs). These cells can differentiate towards the cardiac cell lineage in vitro while producing trophic factors that can repair damaged tissue. When placed in the heart after MI though, BMSCs have mixed results, producing profound changes in some patients but zero or even negative effects in others. In this report, we used BMSCs as a stem cell base for a regenerative medicine system for the repair of damaged cardiac tissue. These cells are seeded on a polycaprolactone nanoscaffolding support system, which provides a growth substrate for in vitro work, as well as a housing system for protected in vivo delivery. When the nanoscaffold is pre-coated with a novel combination of a cardiac protein, thymosin β4 (Tβ4), and a small molecule effector of the WNT protein pathway, IWP-2, BMSCs differentiated towards the cardiac lineage in as little as 24hours. When injected into rat hearts that have been given an ischemic MI, the nanoscaffolding system slowly dissolves, leaving the cells in place of the damaged cardiac tissue. After two weeks of monitoring, BMSCs are present within the damaged hearts, as evidenced by immunofluorescence and nanoparticle tracking. Injections of the nanoscaffolding/cell system led to robust healing of the rat hearts that had been given small- and medium- damage heart attacks, outperforming PBS sham and cell culture media injections. Significant improvements in cardiac metrics, including ejection fraction and left ventricular end systolic volume, were seen compared to untreated animals, and were comparable to healthy controls. To our knowledge this is the first side-by-side comparison of cell culture media and stem cells to heal a predefined range of MI damage. We believe this simple, inexpensive treatment option is a new beneficial step towards healing damaged patient tissue after MI.