April 18-19, 2018

Presented by
John A. Burns School of Medicine
University of Hawaii Cancer Center

Venue
Sullivan Conference Center
701 Ilalo St.
Honolulu, HI  96813

Divisions
• Undergraduates • Graduates • Medical Students • Residents • Post-Doctoral Research Associates • Faculty

2018 Biomedical Sciences and Health Disparities Symposium

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Day 1 April 18, 2018

8:45-9:00 Setup for poster Session 1-Sullivan Conference Center

9:00-11:30 Poster Session 1
Divisions: Graduate Students, Technicians, Postdoctoral Fellows, Research Associates

12:00-1:00 KEYNOTE ADDRESS MEB 202 (Access Grid Room)
Keshav K. Singh, PhD
Joy and Bill Harbert Endowed Chair
Director, Cancer Genetics Program
UAB Comprehensive Cancer Center
School of Medicine
University of Alabama at Birmingham

“Mitochondria in Health and Health Disparities”

1:00-1:15 Setup for poster Session 2

1:30-4:00 Poster Session #2
Divisions: Graduate Students, Technicians, Postdoctoral Fellows, Research Associates

Day 2 April 19, 2018

8:45-9:00 Setup for Poster Session 3-Sullivan Conference Center

9:00-11:30 Poster Session 3
Divisions: Faculty, Residents, Medical Students, Medical Fellows, Postdoctoral Fellows, Research Associates, Undergraduates

12:15-12:30 Setup for Poster Session 4 Section 1

12:30-2:20 Poster Session 4 Section 1
Division: Undergraduates

2:20-2:30 Setup for Poster Session 4 Section 2

2:30-4:30 Poster Session 4 Section 2
Division: Undergraduates
Dr. Singh did his undergraduate degree in India, Ph.D. in Australia and the postdoctoral studies at Harvard, USA. After completing his postdoctoral studies, he joined John Hopkins as Assistant Professor of Oncology and Environmental Health at Johns Hopkins School of Public Health. In 2003, he moved to Roswell Park Cancer Institute in Buffalo NY as Associate Professor of Oncology. At Roswell he rose through the ranks to Professor and then in 2010 to Distinguish Professor of Oncology.

At present, he is Joy and Bill Harbert Endowed Chair and Director of Cancer Genetics at UAB Comprehensive Cancer Center. He is also a Professor of Environmental Health at UAB School of Public Health. Dr. Singh is the author of more than 100 research publications, 3 books. He serves on various expert panels in the United States, Italy, UK, Poland, Singapore and other countries. He has won numerous awards.

He founded the Society for Mitochondrial Research and Medicine in India (www.mitoindia.org) and United States (www.mito.us). These are registered professional nonprofit organizations. SMRM, India organizes symposium, conferences and workshop and create awareness about the emerging mitochondrial diseases in India. SMRM, India also help patients suffering with mitochondrial diseases.

Besides several research grants from National Institute of Health, he was also funded from Center for Disease Control (USA) to carry out studies related to arsenic contamination in drinking water and development of cancer in Kolkata, India.

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PRESENTERS

Alphabetical Order

Sessions 1, 2, and 3

Faculty, Medical Fellows, Residents, Medical Students, Postdoctoral Fellows, Graduate Students, Research Associates, Technicians, Undergraduates

Posters 1 - 108

UNDERGRADUATE DIVISION PRESENTERS

Alphabetical Order

Session 4

Undergraduate Students

Posters 109 - 179
CREATIVE ART THERAPY: IMPORTANCE OF DANCE THERAPY

In this study, the definition, history, purpose, method and used fields of creative art therapy, how the dance movement therapy is explained in psychosocial theories, and the relationship of psychology with the field of art is examined.

The purpose of this study is to highlight the status of creative art therapy in the psychosocial field as well as the status and importance in movement gradient. It is also examined how the creative art therapy is applied in the treatment of mental disorders, personal development processes, and movement gradients. Human beings have used art as a way of expression of the internal experiences and emotions since their existence.

Art, creativity and therapy have an important place to self-understanding, self-explanation, self-expression and the effort to self-prove for the individual. In order to heal, improve and rehabilitate their spiritual, mental, emotional and physical being, this therapy is a field where art is used. The creative art therapy is considered to be the merge of psychology and the other art fields (drama, dance, music, sculpture, etc.).

Accordingly this kind of therapy is used for the treatment of mental disorders of children, adults and elderly people, to maintain quality of life in elderly people, and for individuals with disabilities as well as cancer patients. Creative art therapy is now popular in several areas across the world including Turkey.

In accordance with our study, the importance of creative art therapy is high in movement gradient. It is suggested for physical educators, trainers and executives to use creative art therapy activities in movement gradients according to the fields of interests for this kind of therapy as it will contribute to their cognitive, mental, physical and personal development.
ULTRASOUND-MEDIATED THERAPEUTIC GENE TRANSFER

Ultrasound Targeted Microbubble Destruction (UTMD) is a platform technology that can deliver gene-expression vectors bound to the shells of lipid microbubbles to organs accessible to ultrasound. In UTMD, the DNA-loaded microbubbles are injected intravenously and are deposited at the target organ by acoustic cavitation at a resonant frequency of the bubbles. We used UTMD to direct the delivery of plasmid and transposase-based vectors encoding human factor IX (hFIX) to the livers of hemophilia B (FIX-/-) mice. Ultrasound parameters were identified that produced transfection of hepatocytes in vivo without substantial damage or bleeding in the livers of the FIX-deficient mice. Exogenous hFIX levels were evaluated in the plasma and livers of FIX-/- treated mice at multiple time points after UTMD. We detected hFIX in the plasma by western blotting from mice treated with conventional or transposon-based plasmids during the 12 days after UTMD, and in the hepatocytes of treated livers by immunofluorescence. Reductions in clotting time and improvements in the percentage of FIX activity were observed for both plasmids 4 to 5 days after UTMD compared with untreated FIX-/- control mice ($P=0.001$ and $P=0.012$ for conventional and transposon plasmids, respectively). Reduced clotting times persisted for both plasmids 12 days after treatment (reflecting percentage FIX activity of $3.12\pm1.56\%$, $P=0.02$ and $3.08\pm1.0\%$, $P=0.001$, respectively). Clotting times from an additional set of treated mice were evaluated for long-lasting effects and demonstrated a persistent reduction in average clotting time 160 days after a single treatment ($P=0.044$).

To advance these studies, we are currently evaluating high intensity focused ultrasound (HIFU) as an approach for improving ultrasound-based gene transfer efficiency. HIFU uses an acoustic lens to focus multiple beams of ultrasound at a single point. We hypothesize that HIFU-based gene transfer may enhance vascular permeability in the liver permitting the delivery of a more concentrated dose of a therapeutic gene. We are currently evaluating the bioeffects of varying HIFU parameters and ultrasound exposures on reporter gene transfer in the livers of C57Bl/6J mice. This new optimized HIFU-based approach will be used to direct the delivery of human FIX to the livers of FIX-/- mice and these findings will be compared to our previous unfocused UTMD gene therapy studies. This work will advance the development of an anatomically targeted, minimally invasive gene therapy strategy for treating the monogenic blood disease Hemophilia B and may be applied to treating other hepatic gene deficiency disorders such as Familial Hypercholesterolemia.

(Acknowledgements: Chad B. Walton, PhD (UH Manoa) was a co-principal investigator for the unfocused UTMD hFIX studies, developed HIFU technology at the CCR, and provides support for the HIFU studies. Stefan Moisyadi, PhD (Manoa Biosciences and UH Department of Anatomy, Biochemistry and Physiology) provided the piggyBac transposase and pmGENIE™ plasmids. Abigail Avelar (CCR) and Aaron Tuia (JABSOM Mouse Phenotyping Core), research and animal husbandry support. Miyoko Bellinger and Kris Ewell (JABSOM Histology Core), histological sample preparation.)
CHARACTERIZING THE LINK BETWEEN α7-NICOTINIC RECEPTOR-G-PROTEIN REGULATION OF CALCIUM SIGNALING AND BETA AMYLOID-INDUCED NEUROTOXICITY

The homomeric α7 nicotinic acetylcholine receptor (α7-nAChR) has been identified as one of the high-affinity functional targets for soluble oligomeric beta amyloid (Aβ), the latter having been shown to trigger synaptic and memory deficits in Alzheimer’s disease (AD). α7 nAChRs have a unique ability to operate in both ionotropic and metabotropic modes. It has recently been shown that α7 nAChRs associate with G proteins via a G protein-binding cluster (GPBC) in the M3-M4 loop of the receptor, activating a downstream calcium signaling response that leads to calcium-induced calcium release (CICR) and G protein-associated inositol trisphosphate (IP₃)-induced calcium release. A mutation of the GPBC in the α7 nAChR (α7345-348A) abolishes interaction with Gαq as well as Gβγ, thereby attenuating the α7 nAChR-induced Gαq calcium signaling.

In this study, we used the model neuroblastoma cell line NG108-15, transfected with wild-type (WT) α7 nAChRs or mutant α7345-348A nAChRs, and treated or not (control) with full-length beta amyloid (Aβ1-42), to gain insights into the link between α7 nAChR-G-protein-associated calcium signaling and Aβ-induced neurotoxicity. As an early measure of Aβ toxicity, we assessed mitochondrial membrane depolarization as triggered by calcium overload. The mitochondrial membrane potential was measured using a TMRE (tetramethyl rhodamine ethyl ester)-mitochondrial membrane potential assay kit. The TRME-stained cells were imaged live using an Olympus IX71 epifluorescence microscope at excitation/emission of 549/575 nm, respectively. Changes in intracellular Ca²⁺ levels in individual varicosities of differentiated NG108-15 cells were separately monitored using the Ca²⁺-selective fluorescent dye Fluo-4 via a rapid exchange Warner perfusion chamber. Changes in fluorescent intensity in response to Aβ1-42 treatment were visualized by a Nikon PCM 2000 Chameleon confocal imaging system. Lastly, the expression levels of molecules involved in α7-mediated calcium signaling and potentially Aβ toxicity, specifically CaMKII, PI3-kinase/Akt, PKC and CREB, were determined by Western immunoblot. Our results demonstrated a significant reduction in mitochondrial membrane potential in the cells transfected with α7345-348A compared to the WTα7, as evident from much decreased levels of TMRE staining in the mutant, which was further reduced on exposure of Aβ1-42 for 3 days. We also expect to see altered presynaptic Ca²⁺ responses elicited by acute stimulation of Aβ1-42 in cells transfected with α7345-348A versus the WT counterpart.

In conclusion, this study is the first to characterize a putative link between α7 nAChR-G-protein associated calcium signaling and the underlying beta amyloid-induced neurotoxicity. The results from this study will help unravel the mechanisms by which beta amyloid regulates α7 nAChR-G-protein-associated calcium signaling during early stages of AD.

(Co-authors: Ruth Taketa, Justin R. King, Nadine Kabbani and Robert A Nichols)
Acknowledgment: UHF
THE ROLE OF SELENOPROTEIN K AS A NOVEL COENZYME DURING STORE-OPERATED CALCIUM ENTRY (SOCE)

Selenoprotein K (SELENOK) is a dietary selenium-sensitive, selenocysteine (Sec)-containing protein localized in the endoplasmic reticulum (ER) membrane. SELENOK interacts with the DHHC6 (single letter symbols represent Asp-His-His-Cys amino acids) enzyme and increases efficiency of DHHC6 catalyzed protein palmitoylation by stabilizing the acyl-DHHC6 intermediate, thus promoting protein acyl transferase (PAT) reactions. SELENOK is required for palmitoylation of the Ca^{2+} channel protein, inositol-1, 4, 5-trisphosphate receptor (InsP_{3}R) in the ER membrane. Because SELENOK has been implicated in Ca^{2+} flux within immune cells during activation and migration, we hypothesized that SELENOK plays a similar role in melanoma cells. To test this hypothesis, we developed an in vitro human melanoma cell model using the NCI-60 validated human melanoma cell line, SK-Mel28. CRISPR/Cas9 technology was used to generate a SELENOK-null clone and effective mutation of SELENOK was confirmed by genomic sequencing, as well as western blot analyses showing truncation of the functional domain. A calcium flux experiment utilizing caged inositol 1,4,5-trisphosphate (InsP_{3}) and the calcium dye, Fura Red, was performed to compare w.t. and SELENOK-null SK-Mel28 Ca^{2+} flux levels using two-photon excitation microscopy. Caged InsP_{3} was released intracellularly by a 2-second pulse of UV light and subsequent calcium flux was measured. The fluorescence-based assay was repeated in triplicate for Ca^{2+} flux induced by InsP_{3}, as well as treatment with thapsigargin as a control. Compared to w.t. cells, SELENOK-null SK-Mel28 cells exhibited impaired InsP_{3}-induced Ca^{2+} flux and were similar to w.t. cells when treated with thapsigargin, indicating SELENOK is necessary for proper Ca^{2+} flux by enhancing InsP_{3}R expression as opposed to modulating alternative calcium flux pathways. Ca^{2+}-dependent calcineurin activity was also examined in w.t. and SELENOK-null SK-Mel28 cells along with the subsequent translocation of NFAT to the nucleus. Our results indicate that genomic deletion of SELENOK reduces calcineurin activity and inhibits NFAT translocation to the nucleus in SK-MEL28 cells. Lastly, Ca^{2+}-dependent functions including proliferation and migration were also hindered in SELENOK-null SK-Mel28 cells compared to w.t. cells. Collectively, these data show that SELENOK is an important protein for mediating proper Ca^{2+} flux responses within human melanoma cells required for proliferation and migration, thereby representing a possible therapeutic target for treating melanoma.

(Co-authors: Michael Marciel, Katie Lee, FuKun W. Hoffmann, Peter R. Hoffmann)
Francine F. Azouz, Graduate Student, (Advisor: Mukesh Kumar, PhD)  
Department of Tropical Medicine, Medical Microbiology and Pharmacology

FUNCTION OF SCHLAFEN4 IN THE PATHOGENESIS OF FLAVIVIRUS ENCEPHALITIS

Members of flavivirus genus are the most important arthropod-borne viruses causing disease in humans. This genus includes West Nile virus (WNV) and Japanese encephalitis virus (JEV), which are the leading cause of arboviral encephalitis in the United States and worldwide. No effective therapies exist for treating individuals with encephalitic flavivirus infections, and pathogenesis of flavivirus encephalitis is not completely understood. Schlafen4 (SLFN4) is a poorly characterized but important member of the Schlafen family that includes several mouse and human genes. Recently we demonstrated that SLFN4 is required for WNV replication in primary mouse embryonic fibroblasts. Herein, we used newly developed SLFN4-deficient mice (SLFN4−/−) and an established murine model of WNV and JEV to determine the in vivo role of SLFN4 in flavivirus pathogenesis. After subcutaneous inoculation with 100 plaque forming units (PFU) of WNV, SLFN4−/− mice exhibited significantly higher survival percentage than wild-type (WT) mice. Increased survival in SLFN4−/− mice was associated with significantly reduced viral burden in serum, spleen, kidney and brain compared to WT mice. Thus, the absence of SLFN4 caused decreased WNV replication and dissemination after subcutaneous inoculation. Moreover, levels of pro-inflammatory cytokines and chemokines in the serum, and brain were significantly reduced in SLFN4−/− mice compared to WT mice. Similarly, after subcutaneous inoculation with 10,000 PFU of JEV, SLFN4−/− mice exhibited significantly higher survival percentage than WT mice. Collectively our data for the first time indicate the novel role of SLFN4 in flavivirus replication and pathogenesis. Our studies provide new insight into a novel host factor for flavivirus replication and dissemination, and thus will have a significant impact on the development of much-needed therapeutic interventions that will reduce virus spread and improve disease outcome.

(Co-authors: Keeton K. Krause, Eileen Nakano, Vivek R. Nerurkar and Mukesh Kumar)

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POSTER #16

Chantell Balaan, Graduate Student, (Advisor: Masato Yoshizawa, PhD)
Department of Anatomy, Biochemistry and Physiology

ASTYANAX MEXICANUS AUTISM SPECTRUM DISORDER (ASD)-LIKE PHENOTYPIC BEHAVIORS INFLUENCED BY KETOGENIC DIET

Autism spectrum disorder (ASD) is a pervasive, multifactorial neurodevelopmental disorder discernible in the earlier stages of child development. Recent studies suggested that the brain-gut reciprocal pathway, gut microbiota and metabolic process can play a vital role in the expression of ASD symptoms. In our latest study, the ketogenic diet, which induces a shift from glycolytic to ketogenic metabolic processes, mitigates symptoms in children with ASD (Lee et al., 2018). However, we are far from the full understanding of ASD systemic etiology. Here we utilize Astyanax mexicanus, a species of teleost composed of a surface and cave morphs, to determine the molecular pathways that connect gut and metabolic systems to ASD-like symptoms. The cave morph exhibits a battery of ASD-like symptoms: less social, adherence to a particular stimulus, hyperactivity, loss of sleep and repetitive behavior, and significantly reduced gut firmicutes. We then tested whether a ketogenic diet mitigates ASD-like symptoms in the cave morph. Both young and adult morphs (N=15 each) were treated with standard or ketogenic diets for ≥ 4 weeks and assayed for adherence behavior, hyperactivity and sleep duration. Our results showed that the ketogenic diet significantly reduced hyperactivity and increased sleep in the cave morph, however did not change adherence behavior. These are similar responses to those of ASD patients. We have recently developed assay systems for repetitive turning and social interaction. We will discuss how we plan to integrate these behavioral data with multiple -omics to acquire a full-view of systemic etiology in ASD-like symptoms.

(Co-authors: Motoko Iwashita, Ryan Lee and Masato Yoshizawa)

[Funded by Hawaii Community Foundation]
PRODUCTION OF CHIKUNGUNYA VIRUS STRUCTURAL PROTEINS USING A DROSOPHILA S2 CELL EXPRESSION SYSTEM

Objective: Chikungunya virus (CHIKV) is a positive sense, single-stranded RNA virus in the family Alphaviridae that causes chikungunya fever and, in some cases, debilitating joint pain that can last several months to years. CHIKV is a mosquito-borne virus that is spread through Aedes aegypti and Aedes albopictus, both of which are found in Hawaii. CHIKV causes outbreaks in Africa, Asia, Europe, and the Indian and Pacific Oceans and, as of 2013, local transmission has been reported in the Americas, including the United States. Due to the expansion of mosquito vector range, the lack of licensed vaccines and therapeutics has become a growing concern. We aim to produce Chikungunya structural protein subunits using a Drosophila S2 expression system. Because S2 cells have been shown to produce glycosylated protein similar to mammalian cells and are easy to maintain in suspension cultures, S2 cells provide an efficient way to produce large amounts of antigen that can be used as diagnostic and vaccine candidates. We hope to produce the structural proteins and virus-like particles (VLPs) using S2 cells as well as determine which structural proteins (or combinations of protein) are the most effective for use in diagnostic and vaccine development.

Methods: To express the Chikungunya structural proteins, the polyprotein gene encoding Capsid, E3, E2, 6k, and E1 was generated using synthetic genes. Three pieces were obtained, cut using restriction enzymes and ligated together into a single vector that was transformed into He5α bacterial cell lines. Different combinations of subunit proteins were generated using PCR cloning, inserted into pMT-Bip, and cotransfected with a pCoHygro selection vector into S2 cells. Currently, we have S2 cell lines transfected with E3, E3 with Capsid, E2, E3 combined with E2, E1, and the entire structural polyprotein to produce VLPs. So far, we have been able to successfully express E1. Deletion of the C-terminal transmembrane domain and addition of an N-terminal Bip secretion signal and C-terminal poly-histidine tag allowed for secretion of E1 subunits that could be purified from S2 cell culture fluid using metal affinity chromatography. Western blots using anti-histidine-tag antibody or anti-Alphavirus antibody SKL42 were both able to detect purified E1 protein.

Conclusion: Insect cell line expression systems can produce high levels of fully processed Chikungunya structural proteins that can be used for developing much needed diagnostics and additional vaccine candidates. Production of structural protein combinations and virus-like particles is ongoing. As we produce more antigen, we will begin testing their potential as vaccine and diagnostic tools.

Co-authors: Kenji Mfuh, Albert To, Caitlin Flores, Madhuri Namekar, Axel Lehrer
This project was funded by a contract with the United States Army Medical Research and Acquisition Activity (USAMRAA), contract number W81XWH-15-C-0106, and by institutional funds. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders.
Bjarne R. Bartlett, Graduate Student, (Advisor: Youping Deng, PhD)
Bioinformatics Core, Department of Complementary and Integrative Medicine (Youping Deng)

DEVELOPMENT OF AN RNA-Seq-BASED PROGNOSTIC SIGNATURE FOR COLON CANCER

RNA-Seq data has recently been used to successfully develop prognostic signatures to predict cancer patients who will have a worse prognosis. We designed a study to ascertain whether such a prognostic model would have clinical utility for predicting survival in patients with colon adenocarcinomas (COAD). Data from 468 COAD patients from The Cancer Genome Atlas (TCGA) were obtained and divided into training (n=312) and validation (n=156) datasets. The training cohort was used to develop a prognostic signature by using univariate cox analysis to assess the prognostic potential of each gene and subsequently building a prognostic model using multivariate cox analysis.

In the training cohort, multivariate cox analysis generated a 5 gene signature (p<0.05) that included 2 long noncoding RNAs (lncRNAs). A threshold for high-risk patients was chosen by looking at the top 25% of risk scores, setting a threshold of 0.432. High-risk patients predicted by our 5-gene, RNA-Seq signature had significantly shorter survival in both the training (p=0.00) and test (p=0.003) cohorts. Additionally, early-stage patients predicted to be high-risk had significantly shorter survival (p=0.006 in both cohorts).

Here we present the first RNA-Seq prognostic signature that can identify high-risk COAD patients predicted to have shorter overall survival. This signature would have clinical utility as part of an RNA-seq screening program for cancer patients. The ability of our prognostic signature to identify early-stage COAD patients at high-risk for poor clinical outcomes is particularly encouraging – the ability for oncologists to identify early-stage patients who would benefit from more aggressive adjuvant therapy has great potential to impact patient survival.

(Co-authors: Mark Menor, Ting Gong, Tianying Zhao, Vedbar Khadka)
August Boeglin, MD, Resident, (Advisor: Natascha Ching, MD)
Hawai‘i Pediatric Residency Program, Kapi‘olani Medical Center for Women and Children

IMPACT OF HIGHER PEDIATRIC VANCOMYCIN DOSING ON SERUM TROUGH LEVELS

Objectives: Vancomycin is a medication frequently used to treat bacterial infections. To ensure that the dose of Vancomycin is appropriate, serum trough levels are measured frequently. Several studies have suggested that the standard dose of Vancomycin in children frequently produces serum trough levels that are below goal. Our institution began using a higher initial dose of Vancomycin in 2016. The main aim of this study is to evaluate whether this change in dose has been effective in raising serum trough levels. Secondary aims include assessing whether this increase in dose has led to increased side effects from Vancomycin.

Methods: A retrospective review was conducted of all pediatric patients who received Vancomycin in our institution for a 17-month period in 2016 and 2017. Data was abstracted from the electronic medical records and by chart review. Findings were compared with data collected prior to the dosing policy change when available; statistical comparisons were performed using Chi-squared and Fisher’s Exact tests.

Conclusion: The use of a higher initial dose of Vancomycin substantially increased the percentage of serum trough levels at goal. After the adoption of the higher dose protocol, the number of initially therapeutic troughs increased (28% vs. 6%, p<0.01). The number of supratherapeutic troughs decreased, though the change was not statistically significant (10% vs 23%, p>0.05). The number of initially subtherapeutic troughs decreased, though the change was not statistically significant (61% vs. 71%, p>0.05).

(Acknowledgements: Len Yonemura, PharmD; Marian Melish, MD; Bessie Lau; Andrea Siu)
MARKERS OF T CELL EXHAUSTION PREDICT CORONARY ARTERY CALCIUM PROGRESSION TO HIV

Objectives: Cardiovascular disease (CVD) is a leading co-morbidity in HIV. Chronic exposure to HIV antigens increase the expression of negative checkpoint receptors (NCR) on T cells. Coronary artery calcium (CAC) is a surrogate marker for subclinical coronary artery atherosclerosis and the ability to quantify CAC has established it as a predictor of myocardial infarction. We assessed the relationship between baseline expression of PD-1 (programmed cell death protein 1), TIGIT (T cell immunoreceptor with Ig and ITIM domains) and TIM-3 (T cell immunoglobulin and mucin-domain containing-3) expression on CD4+ and CD8+ T cells and 2-year change in coronary artery calcium (CAC). We further examined the relationship between these T cell populations and monocyte [classical (CD14++CD16-), intermediate (CD14++CD16+), and non-classical (CD14+CD16++)] subsets.

Methods: Flow cytometry was used to quantify the frequency of NCR expression on CD4+ and CD8+ T cells and monocyte subsets from banked peripheral blood mononuclear cells of HIV-infected individuals on stable ART enrolled in the Hawaii Ageing with HIV-Cardiovascular Disease (HAHC-CVD) Cohort Study. Framingham Risk Score (FRS) was calculated using the National Cholesterol Education Program website (http://hp2010.nhlbihin.net/atpiii/calculator.asp). Computer tomography (CT) examinations for CAC were performed locally using a dual source CT scanner (Siemens 64-slice Somatom) and analyzed at the Los Angeles Biomedical Research Institute (M Budoff). Analyses were performed by Pearson correlation and binary logistic regression.

Results: 43 HIV-infected participants who were predominantly male (88%) and Caucasian (62%) with a median age of 52 years, median CD4 count of 518 cells/µL, self-reported median nadir CD4 count of 93.5 cells/µL, and median CD4:CD8 T cell ratio of 0.68 at baseline. The median duration on ART was 14 years and 83.7% had plasma HIV RNA levels < 50 copies/mL. CAC was present in 51% (n=22) of the population and 25.5% (n=11) showed an increase in CAC after 2 years. As anticipated, NCR-expressing CD4+ and CD8+ T cells negatively correlated with CD4 count, activated CD8+ T cells, and CD4:CD8 ratio. PD-1+, TIGIT+, and PD-1+TIGIT+ CD4+ T cells both correlated with absolute numbers of intermediate monocytes with Pearson correlation coefficients of r=0.326 (p=0.040) and r=0.357 (p=0.024), respectively. PD-1+, PD-1+TIGIT+, and PD-1+TIM3+CD8+ T cells were associated with absolute non-classical monocyte numbers with r=0.422 (p=0.007), r=0.331 (p=0.037), and r=0.29 (p=0.038), respectively. PD-1+, TIGIT+, and PD-1+TIGIT+CD4+ T cells (odds ratio=1.110 to 1.261, all p<0.05) at baseline was associated with a 2 year increase in CAC after adjusting for FRS (See Figure). We found no association between NCR-expressing CD8+ T cells and 2 year increase in CAC.

Conclusion: Our study found PD-1 and TIGIT-expressing CD4+ T cells predict a 2 year increase in CAC, as well as an association between increased frequency of exhausted CD4+ T cells and higher levels of intermediate monocytes in HIV-infected individuals on stable ART. In HIV-infection, PD-1 expression levels are up-regulated on CD4+ T cells. Our data suggests that this may result in an increased risk of CVD in this population.

(Glen M Chew, Matt Budoff, Dominic Chow, Brooks I Mitchell, Michelle D’Antoni, Lishomwa Ndlovu, Cecilia Shikuma)
ASSESSING STRENGTH AND BALANCE IN HIV

Objective: People living with HIV/AIDS (PLWHA) are suffering age-related declines in health. HIV-associated non-AIDS (HANA) conditions include cardiovascular disease, diabetes mellitus, metabolic syndrome, and neurocognitive disorder. Published literature suggests that HIV-infected (HIV+) patients on controlled antiretroviral therapy (ART) demonstrate decreased muscle strength and problems with balance. This may lead to risk of falls, frailty and morbidity. The purpose of this study was to construct an easily administered test battery to evaluate objective measures of strength and balance, and to obtain local data among HIV+ patients controlled on ART in preparation for testing exercise regimens.

Methods: This was a cross-sectional study of HIV+ patients on ART. Entry criteria included adults aged 18 or older and verified HIV+ status. Patients completed 3 trials of each of the following tests including isometric body strength, stork balance, grip strength using a hand dynamometer and the Timed Up and Go walk. Thirty cc of blood was drawn from subjects and processed. Plasma and viably preserved peripheral blood mononuclear cells (PBMC) were banked for future analyses of immunologic factors.

Results: To date, 15 HIV+ males (79.9% Caucasian, 6.7% Asian, 6.7% African American, and 6.7% Hispanic) ranging in age from 51-79 years, 80% with plasma HIV RNA <50 copies/mL, averaging CD4 count of 634, weight of 81.5 (kg), and Body Mass Index of 27.7. The test battery took ≤ 30 min to complete and was well tolerated by all subjects. Table 1 includes objective measures of strength and balance. Table 2 includes grip strength compared to age matched NHANES normative data values. One-sample t-test results indicated that HIV+ patient grip strength mean values were significantly lower than age matched general population NHANES mean values \(t(14) = 3.635, p=0.003\).

Conclusion: Objective strength and balance tests were well tolerated and successfully implemented. Preliminary analysis revealed that local HIV+ patients have poorer hand grip strength values compared to normative values. Published research indicates that grip strength correlates well with other total body and limb specific strength measures. Our data, NHANES and other published data revealed well controlled individuals with chronic HIV on potent ART have lower strength and balance values than uninfected HIV individuals of older and similar ages. Therefore, HIV+ patients are predisposed to falls, frailty, mobility deficiencies and morbidity. Future investigations will involve strength and balance exercise regimens with HIV+ patients having
NOVEL STAT3 INHIBITOR SUPPRESSES GROWTH OF HUMAN BREAST CANCER CELLS IN VITRO

Signal transducer and activator of transcription 3 (STAT3) belongs to a family of proteins that function to propagate extracellular signals to the nucleus to regulate gene transcription. The STAT3 signaling pathway is traditionally activated by cytokines and growth factors. Following binding by their cognate ligands, these receptors undergo conformational changes to allow for tyrosine kinases, such as Janus kinases, bound to their cytoplasmic domain to be brought together to undergo cross-autophosphorylation. Activated JAKs can then phosphorylate STAT3 at a critical tyrosine residue (pTyr) that then permits dimerization of STAT3 via pTyr peptide interactions with the SH2 domain. Phosphorylated STAT3 can then translocate to the nucleus and function as a transcription factor for regulating gene expression. Constitutively-active STAT3 has been implicated in many solid and hematological malignancies, including triple negative breast cancer. Hyperactive STAT3 promotes oncogenesis through upregulating genes involved in cellular processes such as cell growth and proliferation, cell survival, angiogenesis, and invasion. Given the causal role of hyperactive STAT3 in human cancers, research efforts are focused on developing STAT3 inhibitors as novel anticancer therapeutics. A series of small molecule inhibitors derived from the earlier lead salicylic acid-based compounds were evaluated. Here we show that the analog H-174 inhibits STAT3 activation with nanomolar potency. Treatment with H-174 decreases cell proliferation of human breast cancer, MDA-MB-231 cells, with a preference over normal human breast epithelial, MCF-10A cells. Immunoblots of MDA-MB-231 cells treated with 3µM H-174 show a decline in phosphorylated STAT3 levels in a time-dependent manner. Phosphokinase array analysis shows that the inhibition of STAT3 activity leads to a downregulation of mitogen- and stress-activated protein kinase 1/2 (MSK1/2), cAMP response element binding (CREB), hematopoietic cell kinase (Hck), and focal adhesion kinase (FAK) in MDA-MB-231 cells following H-174 treatment. The potential that H-174 is inhibiting these kinases in part through STAT3 cell signaling is being investigated. These results suggest that the direct inhibition of STAT3 activity and the subsequent downregulation of the aforementioned kinases are part of the mechanism leading to the overall anti-proliferative effect of H-174.

(Co-authors: Peibin Yue, Francisco Lopez-Tapia, Christine Brotherton-Pleiss, Marc Tius, and James Turkson)

(This work was supported by the NIH/NCI Grant CA161931 (JT), CA208851 (JT), and University of Hawaii start-up funds.)
POSTER #72

Monica Cheung Katz MD, Medical Fellow, (Advisor: Kamal Masaki, PhD)
Department of Geriatric Medicine
Kuakini Medical Center

HEARING LOSS AND RISK OF COGNITIVE DECLINE IN ELDERLY JAPANESE-AMERICAN MEN: The Kuakini Honolulu-Asia Aging Study

Background: Hearing loss in the elderly is a known risk factor for falls, hospitalization, social isolation, and depression. Longitudinal studies of hearing loss and cognitive decline in older adults has not been well studied in Asian populations.

Objectives: To investigate whether hearing loss predicted cognitive decline over 8 years of follow-up in an elderly Japanese-American male population

Methods: The Kuakini Honolulu Heart Program (HHP) is a longitudinal cohort study in 8,006 Japanese American men in Hawaii that began in 1965. The Kuakini Honolulu-Asia Aging Study began in 1991-93 of the HHP in 3,741 men ages 71 to 93 years. The Cognitive Abilities Screening Instrument (CASI) was used to assess global cognitive function, with maximum score of 100 (higher is better). CASI scores were followed from exams 3, 6 and 8 years later, and cognitive decline was defined as a drop in CASI score of > 1 SD (>10 points; 3 years and > 14; 6 and 8 years). Hearing was measured by handheld pure tone audiometry in a sub-sample of participants. Moderate-severe hearing loss was defined as inability to hear at 40 dB any frequencies of 500, 1000, 2000 and 4000 hertz in both ears after 2 trials. After excluding men with dementia or cognitive impairment (CASI<74) at baseline, we had data on audiometry and cognitive decline on 283 men.

Results: Cognitive decline was more common in those with hearing loss at 3 years (42.9% vs. 18.3%, p<0.0001) and 8 years (59.3% vs. 30.5%, p=0.005) of follow-up, respectively. Using logistic regression models adjusted for age, education, ApoE4 allele positive, prevalent stroke, baseline CASI, time of follow-up and cardiovascular risk factors (BMI, physical activity index, hypertension, diabetes, smoking, and cholesterol), the odds of 3 year cognitive decline for moderate-severe hearing loss was significantly increased (OR=2.88, 95% CI=1.41-5.91, p=0.004). Although positive trends shown, this association was not significant for 6, or 8 year decline after adjustment for covariates, maybe partly due to loss of power from lower numbers of participants.

Conclusions: Moderate-severe hearing loss was a significant independent predictor of 3 year cognitive decline in elderly Japanese men, but not for longer term follow-up. Future longitudinal studies on multiethnic elderly cohorts should use both peripheral and central auditory function to predict cognitive decline or incident dementia to determine at-risk populations.

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POSTER #12

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THE IMPACT OF METFORMIN ON IMMUNOLOGICAL AND VIROLOGIC PARAMETERS IN ART SUPPRESSED HIV-INFECTED ADULTS

Objective: HIV infection is characterized by high levels of general immune activation, T cell exhaustion, and viral persistence within T cells and tissues. These changes occur even among individuals on antiretroviral therapy (ART) who are virologically suppressed and may be responsible for the higher risk of non-infectious age-related complications. Because HIV remains a significant health disparity in Hawaii, effective strategies to reverse these complications are actively being investigated. Emerging data from clinical studies show that the FDA-approved oral drug Metformin (Met) may have beneficial effects, beyond its anti-hyperglycemic effects in the treatment and prevention of Type 2 diabetes. Here we evaluated if adjunctive Met therapy in HIV-infected adults on ART could improve anti-HIV immunity and impact viral persistence.

Methods: We utilized cryopreserved peripheral blood mononuclear cells from a completed open-label, 8-week pilot study of eight euglycemic HIV-infected individuals on ART, stable for >1 year with plasma HIV RNA <50 copies/ml, median age of 58 years and all male. Met extended release dosing was given at 500mg at entry, increasing to 1000mg at Week 4 onwards to week 8. Utilizing flow cytometry we assessed changes in the frequencies of CD8 T cell differentiation subsets (CD45RA, CCR7, CD28) and exhaustion (TIGIT, PD-1, TIM-3) including HIV-specific CD8 T cell responses using an ex vivo stimulation assay measuring T cell function via CD107a, IFN-γ, TNF-α responses. CD4 T cell integrated HIV DNA levels were quantified by qPCR. Non-parametric Wilcoxon rank-sum test and Spearman’s rho test were used for analyses.

Results: Over the 8-week course of Met, we observed a significant increase in double expressing TIGIT+PD-1+ CD8 T cells from baseline (Entry; Median Frequency 10.9% (IQR 5.8, 12.1) to Week 8 (11.9% (8.4, 14.4); p=0.03). There was also a significant increase in the central memory CD8 T cells population (CD45RA-CCR7+CD28+, Entry; 8.1% (5.3, 11.7) to Week 8 12.2% (6, 13.8) p=0.03). Met resulted in a dynamic range of both CD8 T cell effector functions in response to HIV Gag peptide stimulation and CD4 T cell integrated HIV levels from Entry to Week 8. In further analyses we found that the fold change of integrated HIV DNA in CD4 T cells inversely correlated with the fold change of effector CD8 T cell functions [CD107a (rho=-0.88, p=0.03), IFN-γ (rho=-0.82, p=0.05), TNF-α (rho=-0.82, p=0.05), CD107a+IFN-γ+ (rho=-0.88, p=0.03)].

Conclusion: Met increased TIGIT+PD-1+ CD8 T cells along with the effector central memory CD8 T cell compartment and may destabilized the CD4 T cell HIV reservoir. The potential link between lower anti-HIV CD8 T cells responses and viral persistence highlights the importance of appropriate CD8 T cell effector capacity. This study argues for adjunctive treatment to ART, including a combination of Met and immunotherapies targeting both TIGIT and PD-1 pathways, which are currently being investigated in “Shock and Kill” type HIV cure strategies.

(Co-authors: Shikuma CM, Chow DC, Souza SA, SahBandar I, Park EY, Corley MJ, Pang AP, Clements DM, Ogata-Arakaki D, Hanks N, Gerschenson M and Ndhlovu LC) (This study was funded by DHHS/NIH grant U54MD00760131)
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SERODIAGNOSTIC ASSAY FOR IDENTIFICATION OF ZIKA VIRUS ANTIBODIES IN CORD BLOOD

Background: The re-emergence of Zika Virus (ZIKV) has caused global concern due to recent outbreaks and links to the development of severe congenital defects, including hydrocephaly and microcephaly, and aberrant autoimmune responses. Currently there are no licensed vaccines or treatments for ZIKV disease and assessment of ZIKV serology in cord blood is absent from the literature. Similarities between flaviviruses, such as dengue virus (DENV) and ZIKV, results in cross-reactivity and poses challenges for definitive ZIKV laboratory diagnosis.

Objective: We aim at evaluating commercially available ZIKV serological kits and developing in house ZIKV serodiagnostic assays to detect anti-ZIKV antibody in archived matched mother and cord blood samples collected at the Kapiolani Medical Center for Women and Children in Honolulu, Hawaii (UH IRB CHS#23889).

Methods: In this study, we investigated archived cord blood samples collected between 2009 and 2015 from mothers previously evaluated for ZIKV infection (PLoS Negl Trop Dis. 2016 Dec 20; 10(12):e0005262. PMC5215948) and gave birth in Hawaii to neonates with microcephaly. We evaluated the diagnostic performance of anti-ZIKV IgM and IgG ELISA based on recombinant ZIKV non-structural protein 1 (NS1). The serology for ZIKV IgM and IgG assays was assessed in cord blood and mothers’ plasma by the commercially available Euroimmun anti-ZIKV NS1 IgM and IgG ELISA, InBios anti-ZIKV Env IgM, and a in house developed fluorescent microsphere multiplex microsphere immunoassay for anti-ZIKV NS1 and Env, IgM and IgG. These serological tests were compared to ZIKV, DENV-1 and DENV-2 plaque reduction neutralization test 80 (PRNT 80) antibody titers.

Conclusions: Euroimmun anti-ZIKV NS1 IgG assay was highly sensitive and specific for the detection of anti-ZIKV IgG in the cord blood of neonates born to mothers with ZIKV infection, which was further confirmed by ZIKV PRNT. IgM for either ZIKV NS1 or Env proteins was not detected in cord blood samples. This is the first study to investigate the use of a commercially available ZIKV ELISA kit using cord blood samples. This data suggest that Euroimmun anti-ZIKV NS1 IgG assay could be used to confirm cases of ZIKV-associated microcephaly in the context of a maternal primary ZIKV infection at time of birth in the neonate’s cord blood. Additionally, this data demonstrating the detection of ZIKV antibody in cord blood of neonates born with microcephaly combined with previously published data (PubMed - PMC5215948) on presence of ZIKV antibodies in their mothers, attests to the presence of ZIKV positive cases associated with microcephaly in the United States as early as 2009.

(Co-authors: Madhuri Namekar, Jasmine Tyson, Wen-Yang Tsai, Axel Lehrer, Wei-Kung Wang, Mukesh Kumar, Joshua Astern, Alex Stokes, Marian Melish)

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**POSTER #54**

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**DYNAMIC CHANGES IN INFLAMMATORY AND CARDIC MARKERS DURING KAWASAKI DISEASE**

Objectives: Kawasaki disease (KD) is the leading cause of acquired heart disease in the developed world, presenting in healthy infants and young children as an acute febrile, self-limiting, systemic vasculitis disease of unknown etiology. We analyzed dynamic changes in the amount of circulating immune factors and tissue degradation markers in the development and resolution of inflammation to understand the factors central to KD pathogenesis.

Methods: Children with acute KD were followed to normalization of inflammatory markers, ESR and CRP. Echocardiograms were conducted and sera were collected at acute (KD diagnosis), sub-acute (5-10 days after initial IVIG treatment), and early (4-6 weeks) and late (8-10 weeks) convalescent phases. We evaluated 58 factors including cytokines, chemokines, and cardiovascular biomarkers using ELISA and Luminex assay.

Results: A variety of cytokines (G-CSF, GRO, IFN-alpha2, IL-6, IL-10, TGF-alpha, VEGF), chemokines (IP-10/CXCL10, ITAC/CXCL11, MCP-1/CCL2, MDC/CCL22), matrix metalloproteinases (MMP-3, MMP-9, TIMP-1), and cardiovascular markers (pentraxin-3[PTX-3], thrombomodulin[TH]) were significantly elevated at the acute phase and either decreased or normalized at either the sub-acute or convalescent phases. MMP-2 and MDC/CCL22, levels in the acute phase were significantly higher than at sub-acute and convalescent phases. Several cytokines (GRO, TNF-beta), chemokine (MCP-3/CCL7), vascular markers (MMP-1, calponin 1[CNN1], soluble elastin fragments [sELAF], sE-selectin) increased during the sub-acute KD phase, and decreased by convalescent phase, which correlated to peak coronary artery lesions (CAL) in most KD patients. We confirm the involvement of IL-6, MMP-9, TIMP-1, VEGF, and IP-10/CXCL10 in KD pathogenesis. Further we identified novel factors, including ITAC/CXCL11, MCP-1/ CCL2, PTX3, Calponin, sELAF, with peaks in the early KD phases.

Conclusions: These data suggest prognostic value of several key protein markers for coronary artery involvement at the acute or subacute phases of KD. We confirm involvement of IL-6, MMP-9, TIMP-1, VEGF, and IP-10/CXCL10 in KD pathogenesis. We identified novel factors, ITAC/CXCL11, MCP-1/ CCL2, PTX3, Calponin 1, sELAF, with peaks in the early KD phases. MMP-9 and IL-6 are attractive targets for therapeutic investigation with already available drugs. Biomarkers with differential levels at acute, sub-acute, and convalescent phases are potential targets for therapy, coronary risk scoring and development of a diagnostic profile for early identification of KD.

(Co-authors: Eunjung Lim, Andras Bratinesak, Marian Melish)

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CONSTRUCTION AND EXPRESSION OF CHIMERIC ClyA AND WEST NILE VIRUS PROTEINS IN E.coli OUTER MEMBRANE VESICLES

Background: West Nile Virus (WNV) encephalitis is one of the leading causes of arboviral diseases in the continental United States. Human infection can result in acute inflammation of the central nervous system that can cause permanent brain damage as well as death. WNV was introduced to North America in 1999 and there is no vaccine to prevent infection in humans. ClyA is a bacterial hemolysin found in the outer membrane vesicles (OMV) in all E. coli strains and has been found to have high immunogenic properties. ClyA has been shown in previous studies to be a protein suitable for making functional chimeric proteins due to its ability to remain biologically active when fused to another protein.

Objective: To fuse ClyA from E. coli with WNV NS1 or NS4B protein so they can be expressed on E. coli outer membrane vesicles to explore its potential as an OMV vaccine candidate for WNV.

Methods: Genes for WNV proteins NS1, NS4B and E. coli protein ClyA were PCR amplified with synthetic oligonucleotides designed to create complementary overlaps for the ligation with pET-15b at the NdeI and BamHI sites. pET-15b plasmid was digested with NdeI and BamHI and the linearized vector backbone was purified. The vector was ligated with ClyA and NS1 or NS4B genes using the NEBuilder HiFi Gibson Assembly mix (New England Biolabs). Two recombinant plasmids: pClyA-NS1 and pClyA-NS4B were cloned into E. coli DH5α cells and sequenced. After the size, sequence and reading frame of the fusion constructs were confirmed, they were transformed into E. coli BL21 (DE3) cells and production of fusion proteins were induced. Expression of ClyA-NS1 and ClyA-NS4b proteins were confirmed by western blot (WB) assay to verify proteins.

Results and Conclusions: The two constructs: pClyA-NS1 and pClyA-NS4b were successfully constructed. DNA sequencing confirmed that the two genes were properly in frame. Fusion-protein production was verified by WB. Further downstream experiments will include OMVs from E. coli being isolated, purified and assayed. OMVs containing the ClyA-WNV proteins will be tested in-vitro for immune cell activation. Protection will be assayed in an animal model.

(Co-authors: Matthew Hamilton-Cave, Kabi Neupane, Vivek R. Nerurkar, and Pakieli H. Kaufusi).

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Urinary tract infections (UTIs) comprise a significant portion of disease burden in elderly populations both in terms of complications and healthcare costs. The symptoms of UTIs in the elderly are usually less specific and can be masked by other comorbid conditions like dementia that affect the patient’s ability to communicate. The purpose of this project was to determine whether NGAL and KIM-1, two proteins recently discovered to be secreted by kidney cells into the urine in response to injury or UTIs, could be used as biomarkers for early detection of UTIs in incontinent patients. Using these human biomarkers to detect UTIs is an advantage over the current methods of measuring nitrites and leukocyte esterases because there is no risk of fecal contamination giving false positives. After thorough consideration of different prototype designs, a lateral flow assay strip system utilizing monoclonal antibodies specific to NGAL and KIM-1 was constructed and integrated into diapers. First, we screened various commercial antibodies for sensitivity and threshold limits using standard dot blot immunoassays. After identifying the best antibodies for both proteins, we generated lateral flow strips made of porous nitrocellulose membrane coated with immobilized capture antibodies against NGAL and KIM-1. Conjugating a separate detection antibody to colloidal gold allowed a color change after the lateral flow when the detection antibody-biomarker complex was bound to the capture antibody. The future steps of the project include increasing the detection threshold of the prototype and moving onto sensitivity and specificity testing in adult diapers.

(Co-authors: Tanaka B, Fogelgren B.)
A CASE OF CUTANEOUS COLON CANCER

Introduction
Carcinoma metastatic to the skin is rare and occurs in varying instances of 0.7-10% of patients with visceral cancers. In patients with advanced disease, incidence has been reported to be 10.4%. Breast cancer is the most common primary cancer with skin metastasis, followed by lung, colorectal, renal, ovarian, and bladder cancer. The estimated incidence of skin metastases in colorectal cancer is less than 5% and usually suggests advanced disease and is associated with a poor prognosis. We present a case of cutaneous colon adenocarcinoma that presented as an erythematous and occasionally pruritic rash.

Case Presentation
The patient is a 43 year old male with past medical history significant for stage IV colon adenocarcinoma with metastasis to the liver, lung, bone, and non-regional lymph nodes who presented with a rash on his torso, abdomen, and right thigh. His cancer was unrespectable, and he had undergone chemotherapy with multiple regimens including capecitabine, oxaliplatin, and bevacizumab (CAPOX-Bev), cetuximab and Imprime PGG, 5-fluorouracil and oxaliplatin (FOLFOX), cetuximab and irinotecan (XELIRI), and most recently regorafenib. Imaging demonstrated progression in hepatic, musculoskeletal and nodal metastasis. Two months prior to consult, he developed an erythematous occasionally pruritic but non-tender mass on his left chest. The rash subsequently spread to his lower abdomen and right thigh. On physical examination, the patient was found to have firm, non-tender, erythematous papules coalescing into plaques over his left chest, lower abdomen, and anterior right thigh. Shave biopsies were consistent with metastatic colon adenocarcinoma.

Discussion
Colon cancer is often thought to metastasize via the bloodstream and typically invades the liver and lung. Cutaneous metastases are rare, with the most frequent site being the abdomen, followed by the extremities, perineum, head, neck, and penis. These metastases often occur with visceral metastases. Some investigators suggest that cutaneous metastases occur through an immunohistologic spread through lymphatic vessels and capillaries, but they may also occur through direct extension of tumor, surgical implantation and spread via embryonal remnants. We present a rare case of cutaneous colorectal cancer to emphasize the importance of recognizing that new cutaneous manifestations may be evidence of progressive malignant disease.

THE ROLE OF IRON-CARDIOMYOCYTE CELL DEATH IN EX VIVO ISCHEMIA-REPERFUSION INJURY

INTRODUCTION: The mortality due to acute myocardial infarction (MI) has declined due to enhancements in treatments such as percutaneous coronary intervention (PCI). However, a risk for heart failure (HF) in patients following acute MI remains high. Rapid reperfusion during PCI leads to ischemia/reperfusion (I/R) injury, which is linked to adverse left ventricular (LV) remodeling that is the major pathogenesis of HF post-MI. Although many groups have studied the mechanism of I/R injury, no effective therapy to control I/R injury has been developed.

OBJECTIVES: We recently reported that ferroptosis, an iron-mediated non-apoptotic cell death, is a significant form of cell death in cardiomyocytes and is mediated by mTOR. Based on this report and clinical evidence suggesting that myocardial iron is a risk factor for LV remodeling, we hypothesized that ferroptosis plays a significant role in I/R injury.

METHODS: To examine the role of ferroptosis in I/R injury, we investigated the role of iron and ferroptosis in an ex vivo Langendorff model using iron chelators, deferoxamine (DFO) and 2, 2-Bipyridyl (2, 2-BP), and a ferroptosis inhibitor, ferrostatin-1. Adult male wild-type mice were subjected to ex vivo global I/R (20 min ischemia, 40 min reperfusion) as done previously. The hearts were perfused with 10μM of ferrostatin-1 (n=4), 80μM of DFO (n=3), 80μM of 2, 2-BP (n=3), or control buffer (n=5) during the initial equilibration period, and assessed recovery of LV left ventricular developed pressure at reperfusion.

RESULTS: Hearts perfused with ferrostatin-1 recovered better than control animals (58.22% ± 11.26 vs. 31.19% ± 7.72, p<0.05), suggesting that ferroptosis plays a significant role in I/R injury. Interestingly, groups perfused with 2,2-BP 80μM recovered worse than control animals (1.60% ± 0.75 vs 31.19% ± 7.72, p<0.05).

CONCLUSION: The decreased recovery of hearts perfused with iron chelator 2,2-BP may indicate that some iron is needed for cell recovery, and that complete absence of iron may exacerbate cardiomyocyte injury. In conclusion, understanding of the role of ferroptosis in I/R injury will lead to the development of new therapies for patients with MI.

(Co-authors: Motoi Kobayashi, MD, PhD, Takashi Matsui, MD, PhD)
A SURVEY OF THE USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE THERAPIES (CAM) BY INTERCOLLEGIATE ATHLETES AT A NCAA DIVISION I UNIVERSITY

PURPOSE
To determine the prevalence and types of complementary and alternative medicine (CAM) therapies utilized by collegiate student-athletes attending a Division I NCAA University.

METHODS AND STUDY DESIGN
A 22-item web-based survey instrument was delivered via email link to incoming and returning collegiate student-athletes representing 21 different sport teams at the University of Hawai‘i at Mānoa (UHM). Data collected included subject demographics and the usage of allopathic medical care and CAM therapies, including those with Hawaiian origins (La’au Lapa’au, Ho’oponopono, Lomilomi, La’au Kahea). Questions regarding identification of a primary care provider (PCP) and referral patterns to specialists were also included. Data were summarized using descriptive statistics.

RESULTS
Response rate was 32% (162/514; 111 women; 51 men). Sixteen percent of respondents identify with Hawaiian ethnicity. CAM utilization practices during the past 12 months and lifetime were reported at 64% and 79%, respectively. The most common types of CAM utilized were vitamins & minerals (48%), massage (33%), yoga (27%), kinesiology tape (22%), chiropractic (20%), probiotics (16%), herbs/botanicals (14%), relaxation (14%), and Lomilomi (9%). CAM usage did not differ significantly by sex ($\chi^2=0.29; df=1; P=0.59$), race/ethnicity ($\chi^2=12.09; df=9; P=0.29$), college year ($\chi^2=2.11; df=4; P=0.72$) or sport ($\chi^2=16.88; df=20; P=0.61$). Overall, 69% identified having a PCP, with no significant difference by sex ($\chi^2=1.63; df=1; P=0.21$). Referral rates to specialists within the last 3 years also did not differ significantly by sex ($\chi^2=2.60; df=1; P=0.11$).

CONCLUSIONS
CAM usage is common among collegiate student-athletes with rates similar to those previously reported in this population (56%, Nichols 2006) and higher than adults nationwide (33%, NIH 2012). This study can increase allopathic/osteopathic physicians’ awareness of CAM utilization patterns by collegiate athletes and enhance the ability to optimize athletic health care.

SIGNIFICANCE
CAM is commonly used by collegiate athletes. Allopathic/osteopathic PCPs and specialist physicians are often unaware of these practices.

ACKNOWLEDGEMENTS
The project was partially supported by U54MD000X7584 grant from National Health Institute (NIH). The content is solely the responsibility of the authors and does not necessarily represent official views of NIH.
ABSTRACT: The purpose of the University of Hawaii (UH) Histopathology Support Facility is to provide education, training, service and access to equipment and consultation for histopathological techniques to users at UH and the broader research community within the State of Hawaii, as well as interested users from other institutions.

METHODS: Training and advice for general histopathology and imaging techniques. Service and training in histology processing, including paraffin embedding, frozen or floating sections, staining and other conventional histopathology techniques. Assistance with optimization of immunohistochemical or in situ hybridization approaches. Equipment includes automated tissue processors, cryostats, sliding microtomes, and a rotary microtome.

RESULTS: Users include over 100 faculty and staff from the John A. Burns School of Medicine (JABSOM), 5 UH departments or research units outside of JABSOM, and three institutions outside of UH - Hawaii Pacific University, Chaminade University of Hawaii, and Pacific Health Research Institute. Histopathology was acknowledged in over 50 peer-reviewed publications since 2011, over 40 presentations, and multiple R01s, F, K, P and U awards. User satisfaction ranged from 4.8 to 4.95 out of 5.0 for the past 5 years.

DISCUSSION/CONCLUSIONS: The Histopathology Support Facility is a unique and valuable resource for the biomedical research community at the University of Hawaii and throughout the state.
POSTER #23

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EPIGENETIC REGULATION OF HUMAN POLYOMAVIRUS JC

Background: Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease caused by the lytic infection of oligodendrocytes by the human polyomavirus JC (JCV). In healthy individuals, JCV exists as a persistent latent infection, however during immunosuppression the host mechanisms that maintain JCV latency are disrupted, leading to a more pathogenic, rearranged JCV. The rearranged JCV also contains deletions and/or duplications in the non-coding control region (NCCR) of the genome. It has recently been demonstrated that certain histone modifications impact viral replication in a transfection model, however the effects on infection in vitro and in vivo are unknown. The proposed study will address the gap in our knowledge on the mechanisms that maintain JCV in a latent state.

Long-term goal: To further understand the molecular mechanisms involved in the reactivation of archetype JCV that occurs during immunosuppression. The

Objective: This study is to determine the effect that histone deacetylase inhibitors (HDACi) have on the replication kinetics of archetype JCV infection in vitro. Our

Hypothesis: The archetype JCV replication is affected by histone modification events that occur during JCV replication under the control of the NCCR. The proposed study will use an in vitro primary cell culture model system previously developed in our laboratory to determine the effects of HDACi on archetype JCPyV replication. Cytotoxicity of HDACi TSA and SAHA were assessed in primary human brain cortical astrocytes (HBCA), renal proximal tubule epithelial cells (RPTE), and transformed Cos7 cell types over 20 days. SAHA showed cytotoxicity at all concentrations tested whereas TSA was not cytotoxic. Subsequently TSA was used to treat HBCA cells infected with archetype JCPyV and JCPyV DNA and mRNA were assessed by qPCR over 20 days. Data analysis is in progress. PML remains a cause of morbidity and mortality affecting immunocompromised patients with no preventative or therapeutic options available. These findings will open new therapeutic strategies for PML aimed at preventing viral expression and containing JCV in a latent state.

[Co-authors: Nelson Lazaga, Priscilla Seabourn and Vivek R. Nerurkar]

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DEVELOPMENT OF A PRODUCTION SYSTEM FOR NUCLEOPROTEINS OF THE HANTAAN AND PUUMALA VIRUSES, UTILIZING THE Drosophila S2 CELl EXPRESSION SYSTEM

OBJECTIVES: Hantaviruses cause severe disease including hemorrhagic fever with renal syndrome (Old World hantaviruses) and hantavirus pulmonary syndrome (New World hantaviruses). These viruses are transmitted via the inhalation of aerosolized rodent excreta. There is currently no FDA approved cure, or vaccine for hantaviruses and therapy is supportive only. Production of high quality purified protein is key for use in development of diagnostic tools, therapies, and primary preventative interventions such as vaccines. The nucleoprotein of Hantavirus is a multifunctional protein which recognizes and binds to the viral single stranded RNA genome as well as viral mRNA. It serves to protect viral mRNA from degradation and facilitates binding of an RNA cap to the viral mRNA. This allows the Hantavirus RNA dependent RNA polymerase to recognize and transcribe viral mRNA. The hantavirus nucleoprotein oligomerizes into trimers which form a polymer protein-nucleic acid complex with the viral RNA genome, encapsulating each of the three Hantavirus RNA segments that make up the virus genome. Antibody titers against nucleoprotein may be an indicator of exposure to replicating virus, this allows purified nucleoprotein to serve as a useful diagnostic tool both during the acute phase and for retrospective investigations. Due to the multiple roles in the virus lifecycle, the protein may also be useful in development of an antiviral therapy specifically targeting this protein.

METHODS: His tagged or untagged nucleoprotein sequences of Hantaan virus (HTNV) or Puumala virus (PUUV) was cloned into the pMT/BiP expression vector and transfected into Drosophila S2 cells. The nucleoprotein is then detected with either an anti-His-tag antibody or an anti-Hantavirus antibody EC01-AG12 via western blot. His-tagged antigens are purified using Ni-affinity chromatography while immunoaffinity chromatography utilizes NP-specific antibodies to purify nucleoprotein from tissue culture supernatant via antibody bound to sepharose.

CONCLUSION: Hantavirus nucleoproteins can be expressed using the S2 cell expression system and purified using conventional or immunoaffinity purification methods.

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(Albert To, Aquena Ball, Axel T. Lehrer)
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Department of Cell & Molecular Biology

NEUROPROTECTION OF THE ACTIVE CORE SEQUENCE OF AMYLOID BETA AGAINST AMYLOID BETA SYNAPTIC TOXICITY

OBJECTIVE: Alzheimer’s disease (AD) is the most common cause of dementia in the aging population. As the disease progresses, AD leads to cognitive deficits affecting memory, changes in personality, and language dysfunction. AD is characterized by the pathological extracellular accumulation of fibril β-amyloid (Aβ) into senile plaques and the intraneuronal accumulation of the microtubule-associated protein tau as neurofibrillary tangles. Initially, insoluble, fibrillary Aβ was believed to be central to disease pathogenesis, but more recent evidence implicates soluble oligomeric Aβ as the trigger behind the earliest cognitive deficits in AD. Under normal conditions, Aβ at low, physiological levels (pM) functions as a positive neuromodulator, enhancing synaptic plasticity and function. In addition, models lacking Aβ show deficits in cognitive function and loss of synapses, suggesting, therefore, a vital role of Aβ in the maintenance of synaptic activity. On the other hand, pathological levels of Aβ (high nM-μM) cause irreversible degeneration of neuronal processes and the loss of synaptic function and connections in select areas of the brain. Previously, we reported that an endogenous N-terminal fragment derived from full-length Aβ retains the latter’s positive neuromodulatory activity and, notably, protects against Aβ-induced synaptic and memory deficits. Furthermore, through subsequent mutational analysis, we found a core sequence (YEVHHQ: N-Aβcore) within the N-terminal fragment accounting for its activity. Here, we aimed to characterize the neuroprotective potential of the N-Aβcore against Aβ-induced neuronal and synaptic damage, while elucidating the neuroprotective mechanism(s) of the N-Aβcore.

METHODS: To study synaptic plasticity, we utilized a model mouse system that overexpresses human Aβ to a level where both synaptic deficits and behavioral deficits that are indicative of AD (5xFAD) were present. Electrophysiology was performed to assess the effects of long-term potentiation (LTP) and long-term depression (LTD) with treatment of the N-Aβcore. Additionally, western blot analysis was performed to assess the changes in signaling molecules implicated in synaptic plasticity.

CONCLUSIONS: In accordance with previous findings, we found that pathological levels of endogenous soluble Aβ significantly inhibits LTP induction, but treatment with the N-Aβcore reverses this deficit. Although not significant, treatment with the N-Aβcore in wild-type slices showed a trend towards LTP enhancement. Additionally, we found that high concentrations of endogenous soluble Aβ shown to be present in the brains of 5xFAD mice enhances LTD in isolated hippocampal slices, and treatment with the N-Aβcore prior to and during the LFS induction of LTD reverses this enhancement. Taken together, these findings suggest that the N-Aβcore is neuroprotective against Aβ-induced neuronal and synaptic toxicity by partially inhibiting Aβ binding to target receptors and subsequently activating an Aβ-independent neuroprotective pathway.
TEXT MESSAGE LINK TO ONLINE SURVEY: A NEW HIGHLEY EFFECTIVE METHOD OF LONGITUDINAL DATE COLLECTION

OBJECTIVE: To evaluate use of a text message link to an online survey as a method of data collection over the course of a medical abortion.

METHODS: We conducted a randomized, double-blind, placebo-controlled trial of women initiating a medical abortion up to 70 days gestation. For data collection during this trial, electronic surveys were sent to participants via text message link at six specified time points over 72 hours (baseline, 2-, 6-, 12-, 24-, and 72-hours). Messages were automated through the Android application “SMS Scheduler,” sent from a phone dedicated to the trial, and held by an investigator. The text provided a link to a secure online survey where participants could enter information without storing any data on their phones. Participants were remunerated with electronic gift card for every survey response, with a bonus for completing all surveys.

RESULTS: From June 2015 to October 2016, 110 women were randomized. Three women (2.7%) were lost to follow-up after enrollment. Participants had a mean age of 27 years. Out of 241 women screened for inclusion, three (1.2%) were excluded due to lack of access to a cellular phone or the Internet. All surveys were completed by 93.6% of participants. All women who responded gave a response at the 24-hour mark. Over three-quarters of all responses were received within two hours of the requested time.

CONCLUSION: In this population of young women seeking medical abortions, repeated text message link to online survey response appears to be an effective mode of data collection.

(Acknowledgement: Bliss Kaneshiro MD, MPH)
THE LONGITUDINAL EXPERIENCE OF PAIN DURING MEDICAL ABORTION

OBJECTIVE: Pain with the evidence-based, mifepristone-misoprostol medical abortion regimen has been described using retrospective data collection. We present the first study where real-time pain scores were collected to describe the pain experience.

METHODS: As part of a randomized, double-blind, placebo-controlled trial of women using pregabalin at the time of a medical abortion up to 70 days gestation (described separately), we collected real-time data on pain (11-point numerical rating scale) experienced in the placebo group. All participants were dispensed ibuprofen and oxycodone with acetaminophen for analgesia. Electronic surveys were sent via text message link at six specified points over 72 hours to assess pain, analgesic use, and adverse effects in real-time.

RESULTS: From June 2015 to October 2016, 110 women were randomized and 54 were assigned to the placebo group. Two women were lost to follow-up. Participants anticipated a maximum pain score of 6.8 +/- 2.0 but experienced a mean maximum pain score of 5.5 +/- 2.2. The mean time of maximum pain was 3.7 +/- 2.4 hours after misoprostol. By hour 12 after misoprostol, 61% reported no pain, which increased to 77% at 24 hours and 82% at 72 hours. Median ibuprofen usage was two 800mg tablets (IQR 1-3) and 0.5 oxycodone/acetaminophen 5/325mg tablets (IQR 0-1). No ibuprofen was used by 11% of participants, and 50% did not use a narcotic.

CONCLUSION: Using real-time data collection, pain scores were lower than previously reported for medical abortion, and the duration of pain was shorter. Analgesic use was lower than previously described.

(Acknowledgement: Bliss Kaneshiro MD, MPH)
POSTER #7

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THE EXOCYST COMPLEX IN INSULIN STIMULATED GLUCOSE UPTAKE IN SKELETAL MUSCLE CELLS

Objectives: The Centers for Disease Control and Prevention lists Diabetes as the 7th leading cause of death in America. People who have type 2 diabetes mellitus (T2DM) are at a greater risk for numerous sequelae, such as cardiovascular disease, renal failure, and limb amputation. Many of the related illnesses that are linked to T2DM are a result of the excess glucose in the blood. Skeletal muscle cells are responsible for 80-90% of the insulin-induced glucose uptake. When stimulated by insulin, skeletal muscle increases the amount of glucose transporter type 4 (GLUT4) on the cell surface. An eight-protein complex called the exocyst has been recognized to be necessary for the insulin-induced exocytosis of GLUT4 vesicles in cultured adipocytes. However, it remains to be seen if the exocyst has a similar function skeletal muscle. We hypothesize that the exocyst complex is essential for insulin-stimulated glucose uptake in skeletal muscle and also glucose homeostasis on the organismal level.

Methods/Results: To analyze exocyst-mediated intracellular trafficking in skeletal muscle in vitro, we utilized CRISPR/Cas9 to create Sec10 knockout clones from L6 GLUT4-myc myoblasts. Sec10 is a central part of the exocyst where shRNA knockdown results in the down regulation of other exocyst components as well as a mitigation of the function of the exocyst complex (Zuo et al., 2009). L6 Sec10 knockout myoblasts fail to increase the uptake of 2DG in response to 100nM insulin. Moreover, Sec10 knockout clones uptake ~50% less than wild type cells given the same treatment. Cellular fractionation reveals that Sec10 knockout cells are unable to traffic GLUT4 to the plasma membrane in response to insulin. For in vivo studies, we generated a tamoxifen-activated skeletal muscle-specific Sec10 knockout mouse. Four weeks after tamoxifen treatment, Sec10 skeletal knockout mice demonstrate impaired glucose tolerance.

Conclusion: Based on our findings, the absence of Sec10 in mouse skeletal muscle cells disrupted glucose homeostasis due to defective GLUT4 trafficking. Ongoing work on skeletal muscle-specific Sec10 knockout will provide valuable insights regarding the intracellular mechanisms that occur in skeletal muscle cells in response to insulin. Moreover, our research will help to assess the likelihood of the exocyst complex as a potential therapeutic target for type 2 diabetes.

(Co-authors: Amanda Lee, Adrian Wong, Lamar Carter, Ben Fogelgren, Noemi Polgar)
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UTILIZING FARMERS MARKETS AS A MEANS OF DISTRIBUTING ACCURATE INFORMATION ABOUT THE ACQUISITION OF RAT LUNGWORM DISEASE (ANGIOSTRONGYLIASIS) IN HAWAII, AND CLINICALLY QUANTIFYING NEMATODE PRESENCE

2,800 people globally were admitted into local hospitals with symptoms of nausea, vomiting, neck stiffness, and headaches. These are the normal symptoms of meningitis, but what quickly differentiates these 2,800 cases from the other reported cases of meningitis are the presence of Eosinophils. Eosinophils are what are known as granulitic white blood cells. This type of white blood cell is typically only present during a parasitic infection. Compared to the other types of meningitis, eosinophilic meningitis is relatively time—patients that come in with eosinophilic symptoms are discharged after inspection with medical professionals incorrectly diagnosing the patient with a particularly nasty case of the flu. According to the Centers for Disease Control and Prevention, and supported by studies, these eosinophilic meningitis cases are caused by Angiostrongylus cantonensis, a parasitic nematode that found on gastropods such as snails, and carried in the lungs of rats, where it is also known as the rat lungworm. The basics of Aniostrongylus infection are known, but what is to be tested and monitored are the distribution patterns of the parasitic nematode, which is the primary and longtime goal of this study. The methods and partners of this project come from the Department of Tropical Medicine and Microbiology and its partners. Angiostrongylus cantonensis is what is known as an emerging infectious disease, and its prevention comes from understanding both its distribution patterns globally and through the human body.

(Co-authors: Blane Garcia, Jessica Cabusog, Jourdan Posner, William Gosnell, Kenton Kramer)
R-RAS: A KEY REGULATOR OF SEPSIS-MEDIATED VASCULAR PERMEABILITY

Increased vascular permeability is a hallmark of several life-threatening diseases including atherosclerosis, cancer and sepsis. Sepsis, characterized by an extreme inflammatory response to infection, induces permeability leading to edema, organ failure and death. Although changes in permeability are necessary for facilitating immune responses, prolonged leakiness is a driving factor in the progression of sepsis to septic shock and is directly associated with increased mortality rates. Currently there are no sepsis-specific therapies and treatment remains supportive. We recently reported that Native Hawaiians have a 2-fold increased risk of dying from sepsis compared to other ethnicities, pointing to the need for developing sepsis-specific therapies for Hawaii. The endothelium, which lines the inner walls of the vascular system, functions as a selective barrier for the movement of solutes and leukocytes to and from the bloodstream, and is regulated by intracellular signaling, the microenvironment and blood flow-induced shear stress. Nevertheless, the precise mechanisms regulating permeability remain to be elucidated. Although shear stress is an important regulator of endothelial signaling, most work investigating permeability regulatory mechanisms has been done in static conditions. Therefore, we sought to investigate the signaling pathways regulating barrier function in more physiologically relevant conditions. We previously reported that the small GTPase R-Ras, in its active form, is required for maintaining endothelial barrier function. Loss of R-Ras activity promotes leakiness by activating Src (Y416), a known inducer of permeability, leading to VE-cadherin (Y731) phosphorylation-induced permeability in static conditions. However, the molecular mechanisms by which R-Ras regulates vascular permeability in the presence of shear stress are unknown. Therefore, the purpose of this study was to elucidate the signaling pathway by which active R-Ras regulates leakiness in physiologically relevant conditions to aid in the development of sepsis therapies. To identify the set of proteins involved in R-Ras signaling, we used active, patient active and dominant negative R-Ras constructs and inhibitors of well-established vascular regulator proteins to determine permeability changes in the presence of sepsis-mediated permeability cytokine, TNF-α and patient sepsis serum. Protein expression and activity following transfection or inhibitor treatment in the presence of TNF-α was detected using biochemical techniques. Protein-protein interactions were identified using proximity ligation assays and co-immunoprecipitation. Permeability was followed using Electric Cell-substrate Impedance Sensing (ECIS), which measures real-time changes in barrier tightness in the presence of fluid-induced shear stress. The experiments were done in human microvascular endothelial cells. Each experiment was done three times and statistically analyzed using student’s t-tests or one-way ANOVAs followed by appropriate post-hoc comparisons. P values of <0.05 were used as a minimum for statistical significance. Under flow-induced shear stress we found that upon TNF-α treatment, R-Ras activity was suppressed inducing phosphorylation of Src (Y416), FAK (Y295,Y576/577), and VE-Cadherin (Y731) thereby promoting vascular leakiness. In contrast, active R-Ras blocked phosphorylation of this pathway to block TNF-α-induced leakiness. Additionally, specific chemical inhibitors for Src or FAK blocked leakiness in cells expressing dominant negative R-Ras or treated with TNF-α. Most importantly, active R-Ras expression blocked patient sepsis-induced permeability. Taken together, our data points to an R-Ras regulated Src-FAK-VE-Cadherin signal transduction pathway in maintaining endothelial barrier integrity, which is affected by TNF-α signaling to induce permeability. In conclusion, we have demonstrated that R-Ras is a key regulator of vascular permeability, controlling the Src-FAK-VE-Cadherin signal transduction pathway. We are currently determining whether R-Ras may be a potential therapeutic target in blocking sepsis-mediated leak.

(Natalija Glibetic1, 2, Geng-Xian Shi1, Pavlos Anastasiadis1, 2, John Allen3, and Michelle Matter1. 1University of Hawaii Cancer Center. 2Department of Molecular Biosciences and Bioengineering. 3Department of Mechanical Engineering)
OBJECTIVES: Selenoprotein M (SELENOM) is an endoplasmic reticulum (ER)-resident selenoprotein that is highly expressed in hypothalamic regions implicated in energy metabolism and leptin signaling. One of the key features of obesity is hypothalamic leptin resistance, of which ER stress is a known causative factor. Our group previously reported that constitutive knockout of SELENOM in mice resulted in elevated body weight, adiposity, and serum leptin levels, symptoms indicative of leptin resistance. The primary purpose of this study was to investigate the relationship between SELENOM and leptin signaling in hypothalamic neurons.

METHODS: To determine whether hypothalamic leptin resistance is the root cause of metabolic abnormalities in SELENOM KO mice, we first assessed hypothalamic leptin signaling in young adult (10 week old) SELENOM KO mice prior to the onset of overt changes in body composition. Further leptin signaling studies were carried out in vitro using an immortalized hypothalamic cell, mHypoE-44, where SELENOM expression was knocked out, knocked down, or overexpressed. In addition, to identify genes and pathways regulated by SELENOM, microarray analysis was conducted on both hypothalamic samples and mHypoE-44 cells where SELENOM was genetically ablated.

CONCLUSIONS: Our results indicate that SELENOM is a leptin-responsive selenoprotein involved in the promotion of leptin signaling. In young adult SELENOM KO mice, we found that hypothalamic leptin resistance preceded the development of evident metabolic alterations. Microarray analysis on hypothalamic tissue and mHypoE-44 cells revealed that SELENOM-deficiency significantly downregulated expression of thioredoxin-interacting protein (TXNIP), a negative regulator of thioredoxin (TXN) signaling linked to energy metabolism and ER stress. Further studies determined that SELENOM-deficiency attenuated TXN activity in samples from both tissue and cell lines. In summary, these findings suggest that SELENOM may augment leptin responsiveness by means of TXN-mediated signaling.

(Co-authors: Matthew W. Pitts, Ann C. Hashimoto, Vedbar Khadka, Marla J. Berry)
RELATIONSHIP BETWEEN SELENOPROTEIN P AND SELENOCYSTEINE LYASE: INSIGHTS INTO SELENIUM METABOLISM

Objectives: Selenoprotein P (SelenoP) is a selenium (Se) transport protein with multiple selenocysteine (Sec) residues synthesized in the liver and exported to the tissues of the body. Selenocysteine lyase (Scly), also highly produced in the liver, decomposes Sec into selenide, providing recycled Se for the synthesis of new selenoproteins. Previous studies have demonstrated that male mice lacking SelenoP (SelenoP KO) or Scly (Scly KO) have decreased total hepatic Se. While SelenoP regulation by Se is well-studied, Scly regulation by Se is unclear. We provide here evidence of tissue-specific Se regulation of Scly expression. We hypothesize that Scly is negatively regulated by Se levels. This absence of SelenoP furthermore jeopardizes Scly-dependent Se recycling due to diminished Sec availability and either activates either the transsulfuration pathway, whereby available selenocompounds can be alternatively decomposed by the actions of either cystathionine γ-lyase (CTH) or cystathionine β-synthase (CBS), or activates selenite reduction via thioredoxin reductase 1 (Txnrd1), to provide selenide for selenoprotein production.

Methods: To assess the relationship between SelenoP and Scly affecting Se metabolism, we first evaluated the expression of Scly in mice and cells treated with varying Se concentrations. We then measured Scly mRNA, protein expression, and activity in tissues of the SelenoP KO mouse and SelenoP-/- Hepa1-6 cells. Levels of CTH, CBS and Txnrd1 were also assessed in both models to gauge for alternative pathways for the metabolism of Se compounds in these models.

Conclusion: We found a negative correlation between Se levels and Scly expression in hepatocytes but not in neuronal cells. We observed decreased Scly expression and activity in SelenoP-/- Hepa1-6 cells but not in tissues of SelenoP KO mouse model. CBS was unaffected in the SelenoP KO mice and in SelenoP-/- Hepa1-6 cells, while CTH was increased in these cells but not in liver tissue. In cells, we found an approximately 30% increase in Txnrd1 protein expression in the hepatic cells, while compared to a ~40% decrease in the SelenoP mouse liver. Our results suggest the transsulfuration pathway is possibly metabolizing the excess Se observed in the SelenoP KO mouse.

(Lucia A. Seale, Ann C. Hashimoto, Marla J. Berry)
CONSTRUCTION AND EXPRESSION OF ZIKA VIRUS PROTEINS IN LOAD IN THE E. COLI MICROVESICLES

Background: Commencing in early 2015, the rapid emergence and dissemination of Zika virus (ZIKV) to epidemic levels throughout the Americas has created a persistent public health concern. Though often characterized by asymptomatic or mild infections, the associated increase in risk of Guillain-Barré syndrome and microcephaly has necessitated the exploration of prophylactic measures against ZIKV infection.

Objective: To produce fusion proteins containing the E. coli Cytolysin A (ClyA) and ZIKV proteins, envelope (Env) or NS1 in a bacterial microvesicles. Two constructs consisting of ClyA-ZIKV Env, and ClyA-ZIKV NS1 will be constructed and expressed, then subsequently analyzed qualitatively.

Methods: ZIKV Env, ZIKV NS1 and ClyA genes were PCR amplified utilizing overlapping primers to create sequence homology for cloning into pET-15b vectors using a Gibson Assembly (NEBuilder HiFi Assembly mix, New England Biolabs). Recombinant plasmids were cloned into E. coli DH5α cells and verified through sequencing. Desired plasmids with correct size and reading frame were transformed into E. coli BL21 (DE3) competent cells for protein expression. Fusion protein expression was verified using western blot (WB) assay and appropriate monoclonal antibodies against ClyA and Env proteins.

Results and Conclusion: DNA sequences for the constructs ClyA-ZIKV Env and ClyA-ZIKV NS1 were verified using sequencing. Expression of the aforementioned constructs resulted in fusion protein expression that was confirmed using WB. Downstream applications involving bacterial outer membrane vesicles (OMV) will be employed to test the efficacy of a vaccine strategy that combines these fusion proteins with the innate immunogenicity of OMV.

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REIRRADIATION OF RECURRENT HIGH GRADE GLIOMAS: OUTCOMES AND PROGNOSTIC FACTORS

Objective(s): Identify prognostic factors for progression-free survival (PFS) and overall survival (OS) after reirradiation (re-RT) for recurrent high grade glioma.

Methods: An institutional database was queried for patients with high grade glioma (HGG) who received re-RT for progressive disease from 2010 to present. PFS and OS after re-RT were estimated using the Kaplan-Meier method, and prognostic variables were examined using univariate and multivariate Cox models. Receiver operative curve (ROC) analysis was used to determine best predictive thresholds for continuous variables.

Results: 58 eligible patients received surgery and adjuvant radiation ± temozolomide for initial diagnosis of HGG (51 grade IV, 7 grade III). The median time to first progression after initial radiation was 11 months. Prior salvage therapy before re-RT included chemotherapy (60%) and surgery (45%). The median number of separate progression events before re-RT was 1 (range 0 – 5). The median time from first progression to re-RT was 2.7 months, and from initial radiation to re-RT was 18 months. 36% received single fraction stereotactic re-RT (SRS) (median 18 Gy, range 14 – 19 Gy) and 64% received fractionated re-RT (median 35 Gy in 10 fractions, range 10 – 60 Gy in 2 – 30 fractions). The median biologically effective dose (BED10) of re-RT was 47 Gy (range 15 – 72). The median planning target volume (PTV) was 16.8 mL (range 0.4 – 783.6). 50% received concurrent chemotherapy and 36% received bevacizumab concurrent and/or adjuvant to re-RT. Acute (≤ 3 months) toxicity ≥ grade 3 was 7%.

The median PFS after re-RT was 4.7 months (1 year PFS 18%), and the median OS was 11 months (2 year OS 21%). By univariate analysis, lower PFS was significantly (p<0.05) associated with shorter time to first progression after initial radiation, lower KPS, and lower re-RT dose (BED10). Lower OS was associated with shorter time to first progression after initial radiation, lower KPS, and larger PTV. Time from initial RT to re-RT, time from first progression to re-RT, use of SRS, and chemotherapy or bevacizumab at re-RT were not significantly associated with PFS or OS. ROC analysis of time to first progression and re-RT dose showed best predictive thresholds at time > 12 months and BED10 > 42 Gy. All significant univariate factors retained significance on multivariate analysis except re-RT dose (p = 0.07).

Conclusion: Reirradiation was well tolerated with infrequent high grade acute toxicity. PFS and OS after re-RT for high grade glioma were both predicted by time to progression after initial radiation. Published prognostic scores have used total time from first to second radiation courses; however in our series the period from initial progression to re-RT did not add prognostic information. There was evidence for a dose threshold of BED10 > 42 Gy (> 16 Gy in 1 fraction, 27.5 Gy in 5 fractions, or 32 Gy in 10 fractions) irrespective of radiotherapy technique. Use of chemotherapy and bevacizumab with re-RT were not associated with improved PFS or OS.

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**POSTER #37**

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DEVELOPING A METHOD TO ISOLATE ZIKA-E REACTIVE B-CELLS FROM NON-HUMAN PRIMATES AND DETERMINING THEIR VDJ SEQUENCES

Objectives: Currently Zika virus (ZIKV) is classified as a global health threat and is considered an emerging infectious disease (EID) by the Center for Disease Control. ZIKV causes newborn and fetal microcephaly, along with serious neurological complications such as Guillain-Barré syndrome. Concerningly, a previous West-Nile virus (WNV) or Dengue virus (DENV) infection may increase the virulence of ZIKV through antibody-dependent enhancement (ADE). Collectively, these three flaviviruses pose a major global health threat. While the ZIKV envelope (E) glycoprotein is well established as the cell adherence and viral entry mediator as well as the main target of neutralizing antibodies, no clinically approved therapeutics or vaccines are currently available to prevent ZIKV infection and spread. Given this target, a broadly neutralizing monoclonal antibody (mAb) may serve as an attractive prophylactic or treatment. Thus, we developed a method to retrieve antigen reactive B cells from the non-human primates (NHP), *Macaca fascicularis*, that have been vaccinated with a recombinant subunit vaccine and subsequently challenged with ZIKV. Furthermore, a novel primer set was developed to rescue mAb heavy chain VDJ sequences from this emerging model organism. We plan to recombinantly express these mAb to recover potential broad neutralizers. The goal of this study is to eventually produce potential immunotherapeutics to protect against flavivirus infections.

Methods: A cohort of NHPs were vaccinated with a ZIKV-E vaccine candidate developed in our laboratory and subsequently challenged with live virus. Sera collected indicated broadly neutralizing antibodies to ZIKV, DENV, and WNV were present in two of the NHPs. Therefore, a flow cytometry antibody panel was developed for isolation of antigen reactive B cells from vaccinated NHP. Concurrently, a negative selection custom antibody cocktail for NHP (Stem Cell Technologies) was used to obtain B cells from NHP. Total RNA from collected B cells were extracted and reverse transcribed into cDNA. A primer set for *Macaca fascicularis* heavy chain variable domain isolation was developed and tested on the cDNA. In the future, we plan to develop a recombinant expression pipeline to develop potentially therapeutically relevant mAb.

Conclusions: With the detection of broadly neutralizing antibodies in recombinant ZIKV E vaccinated NHPs, we have developed a strategy to rescue mAb genetic information from these animals. Recombinant expression of mAbs may facilitate development of a novel immunotherapeutic against these harmful viruses.

Co-Authors: Albert To, Liana Medina, Thomas Premeaux, Chih-Yun Lai, Axel Lehrer

Acknowledgements: (This project was supported by grants from the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS))
THE DEVELOPMENTAL PROGRESSION OF THE HO’OUNA PONO DRUG PREVENTION CURRICULUM

Objective. The Ho’ouna Pono Drug Prevention Curriculum is a culturally grounded, school-based intervention that is being tested currently in a clinical trial. This presentation will focus on the progression of the drug prevention curriculum from health science to health practice: 1) intervention development & refinement, 2) testing for efficacy via clinical trial, and 3) future implementation, adoption, and sustainability (IAS). With a focus on Native Hawaiian and rural health, the Ho’ouna Pono program of research may be used as a framework to develop culturally grounded interventions for other minority groups.

Methods – Phase 1. Phase 1 of Ho’ouna Pono was a mixed-methods, intervention development study. Native Hawaiian youth from Hawai’i schools were interviewed in focus groups to find ecological determinants of drug use. These results then were used to develop culturally grounded videos and prevention curriculum. A set of pilot studies established the curriculum’s validity among students and community stakeholders (i.e. parents, teachers), and refinements to the intervention were made. These Phase 1 results segued to Ho’ouna Pono’s clinical trial. Methods - Phase 2. Instead of a conventional experimental design using treatment and control groups, the Phase 2 clinical trial was designed in order for each cohort to receive the curriculum. The waitlist design accommodated community members’ request that all schools receive the curriculum. The 13 public middle schools participating in the Ho’ouna Pono study were randomly grouped into 1 of 4 cohorts. Each cohort completed six waves of data collection for a measurement at baseline, pre-intervention, post-intervention, and follow-up.

Methods – Phase 3. Finally, a future study will use mixed-methods to identify the most influential and challenging barriers to the IAS of the Ho’ouna Pono curriculum in rural Hawai’i, and to establish the most effective solutions to address these barriers. Ultimately, the research team will produce an action plan for implementation, adoption, and sustainability of this culturally grounded drug prevention curriculum for use with middle school students.

Conclusion. For communities that are small and with a majority population being Native Hawaiian, drug prevention intervention curricula tend to be effective when researchers 1) understand Native Hawaiian and rural health disparities 2) develop an intervention with a culturally grounded approach, and 3) find community-derived barriers, challenges, and solutions to sustainable implementation.

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PSYCHOSOCIAL FACTORS PREDICTIVE OF AGITATION IN ETHNICALLY DIVERSE PATIENTS WITH DEMENTIA

INTRODUCTION: Currently, 6.9% of North Americans over the age of 60 have dementia—a neurodegenerative syndrome deteriorating cognitive processes severe enough to hinder activities of daily living—however, the proportion of dementia patients will grow by 150% over the next forty years. During the course of the disease, 60% of dementia patients will exhibit agitation, defined as verbal or motor disturbances resulting from unmet needs or confusion of the agitated individual. Dementia patients who exhibit agitation are more likely to experience earlier progression to severe dementia and poorer quality of life. There are currently no FDA approved treatments for agitation in patients with dementia. The American Psychiatric Association advises judicious use of antipsychotics may be appropriate for the management of agitation in dementia patients. In order to facilitate treatment discovery, better understanding of why some patients experience agitation is required. Few studies have examined what factors make a dementia patient more likely to experience agitation. For example, potential risk-factors of agitation have been identified in predominately white nursing home patients with dementia. Cognitive status was an independent predictor of agitation. Additionally, sleep disturbances in nursing home residents with dementia can explain agitation independently of cognitive function. Comorbidities correlated to agitation in dementia patients include depression and anxiety. While some minority groups, such as African Americans and Latinos, may experience more dementia-related behavioral disturbances, no studies have examined predictors of agitation in ethnically diverse dementia patients. Hawaii is a unique state with ethnic diversity where non-Hispanic whites do not form a majority of the population. Given the growing heterogeneity of the mainland United States, a sample investigating ethnic differences is warranted since studies done with predominantly white patients may or may not hold for other ethnicities. This study examines factors predicting agitation in an ethnically diverse sample of dementia patients as a step toward developing preventive strategies and therapies for use in the unique patient population of Hawaii.

OBJECTIVES: This study aims to analyze factors predicting agitation in Hawaii’s ethnically diverse dementia.

METHODS: A systematic retrospective review was performed on patients referred to Hawaii Pacific Neuroscience between January 2010 and July 2017. Data was extracted from patient charts using ICD-10 codes for dementia and statistical analysis was performed in SPSS.

RESULTS: Of 350 patients, 135 were male (38.6%), 215 were female (61.4%). Of 350 patients, 107 were Asian (30.6%), 95 were Caucasian (27.1%), and 55 were Pacific Islander (15.7%). 109 patients were agitated, and 241 were not agitated. MMSE scores (-.170), measuring level of cognitive impairment, portrayed statistical significance in dementia patients with agitation. All other hypothesized factors revealed statistical significance: sleep disturbances (.176), depression (309), and anxiety (.452). CONCLUSIONS: Previous studies have shown associations between agitation and cognitive and psychosocial factors in samples of predominantly white dementia patients. In agreement with previous literature, we have shown that decreased cognitive function is a strong predictor of agitation. Cognitive impairment strongly predicts agitation because it underlies and influences the psychosocial factors predicting agitation, including sleep disorders, depression, and anxiety. Significant psychosocial factors, such as depression and anxiety, are clinically important because they also predict accelerated cognitive decline, institutionalization, and increased cost of care (Beaudreau & O’Hara, 2008; Gibbons, Teri & Logsdon, 2002).

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PATENT DUCTUS AETERIOSUS OUTCOMES IN EXTREMELY LOW BIRTH WEIGHT INFANTS ADMITTED TO THE KMCWC NICU FROM 2016-2017

Background: Patent ductus arteriosus (PDA) is commonly present in preterm infants. It has been associated with an short and long-term complications such as bronchopulmonary dysplasia (BPD) and necrotizing enterocolitis (NEC). An emerging co-morbidity of BPD is pulmonary arterial hypertension (PAH) suggesting that there could be a similar association with PDA, however, the pathophysiology of PAH is complex and the causality of relationship in between PDA and PAH is unclear.

There is no national standard for screening or treatment of PDA, which leads to variety of practices in PDA management among institutions, ranging widely from universal prophylactic treatment to no treatment at all. In our unit, there is lack of consensus on screening, defining hemodynamically significant (hs) PDA and management of the PDA in preterm infants, which results in frustration and dissatisfaction among physicians and other medical staff.

Although the effect of the PDA on outcomes is unclear, the standardization of practice has been shown to improve clinical outcomes. Therefore a guideline was created in our unit to screen all ELBW infants for PDA by 1 month of age in order to determine natural history of PDA and to start the process of standardizing practice with regard to PDA.

Objective: To further define natural history of PDA by comparing outcomes of ELBW infants with and without PDA, treated and spontaneously closed.

Methods: Retrospective chart review of ELBW infants born and admitted to the Kapi'olani Medical Center for Women and Children Neonatal Intensive Care Unit from January 2016 through December 2017. Demographics, PDA status, and major morbidities were collected. Infant groups were defined as spontaneous closure, treated, and open at discharge. Infants still hospitalized at the end of December 2017 were excluded. Groups were compared by chi-square analysis.

Results: A total number of 94 ELBW infants were born within the study period. 80 ELBW infants were free of congenital malformations or heart defects and survived to hospital discharge. An echocardiogram was performed in the first 30 days of life, 4 infants did not have an echocardiogram. 58.9% of infants had no PDA on the first screening echocardiogram or PDA was spontaneously closed [spontaneous closed group] prior to discharge. 13.7% of infants had PDA still open [open group] at the time of discharge and 27.4% of infant received medical (Ibuprofen, Acetaminophen, or both), surgical ligation, or both treatments [treated group] prior to discharge. There was an increased incidence of BPD and PAH found in treated group compared to spontaneous closed and the open at discharge group (85% vs. 44.2% vs 60%; 55 % vs. 18.6% vs. 0% respectively). The infants who underwent treatment for PDA had a statistically higher rate of BPD (p=0.0094) and PAH (p=0.0015) compared to those not treated. No statistically significant relationships were found between PDA and other neonatal morbidities. There was also no statistically significant difference in gestational age or birth weight among the three groups.

Conclusion: The likelihood of spontaneous closure of PDA was high in ELBW infants. There was a significantly higher incidence of BPD and PAH in the treated group compared to those not treated. Further investigation into the definition of hemodynamically significant PDA and factors determining decisions to treat are needed to be able to develop guidelines that will distinguish those infants who should be treated and determine optimal timing and method of treatment.

(Co-author: Lynn Iwamoto, MD)
DISSEMINATED CAT SCRATCH DISEASE (CSD) IN PEDIATRIC PATIENTS IN HAWAI’I

Background: Cat Scratch Disease (CSD) is caused by the gram negative bacteria *Bartonella henselae*, which is primarily spread to humans from cat scratches or bites. While seen as a somewhat rare cause of fever of unknown origin (FUO) on the mainland, Hawaii appears to have an anecdotally larger than expected share of inpatient admissions for CSD. CSD usually presents as a benign illness with fever and lymphadenopathy that self resolves. A CDC study estimated the inpatient incidence of CSD to be 0.19/100,000. Traditional treatment approaches for reported complicated diseases have included azithromycin, rifampin, ciprofloxacin, bactrim or gentamicin.

Objectives: To investigate the incidence of disseminated CSD admissions at KMCWC, characterize the clinical presentation, diagnosis and management of CSD, and provide clinical guidance in the care of these patients.

Methods: A retrospective review of patients hospitalized at KMCWC from 2009-2017 was performed using ICD9 and ICD10 codes corresponding to CSD, Bartonellosis, liver abscess, spleen abscess, fever of unknown origin and vertebral osteomyelitis. Cases were then narrowed based on radiologic and lab documentation suggestive of CSD (i.e. Bartonella PCR, serology, and/or imaging indicative of microabscesses or osteomyelitis).

Results: 25 patients with CSD were identified, with 4 patients excluded due to lack of diagnostic workup. Disseminated CSD, defined as patients with disease involving more than lymphadenopathy, was demonstrated in 15 of the 21 patients. 13 patients had liver or splenic lesions, 3 had osteomyelitis and 1 had encephalitis. The median patient age was 7 years (range 1.16 to 15 years). 12 of the 15 patients had fever prior to admission, with a median duration of 20 days (range 3-35). Excluding one patient who was readmitted, 10 patients defervesced during hospital stay. Of the disseminated cases, 50% had both *B. henselae* IgG and IgM positive. None of the cases had positive serum Bartonella PCR, whereas two patients had a positive Bartonella PCR on lymph node fluid tissue or aspirate. IgG was the only positive titer in 19% of the disseminated cases. Median white blood cell count on presentation was 11.6 (range 6.7 - 20.2) with median absolute neutrophil count 7,314 (range 3,034 to 12,322). Median length of hospitalization was 8 days (range 0-15 days). Treatment consisted of azithromycin in 14/15 cases with 1 case of azithromycin monotherapy. Combination antimicrobial therapy included azithromycin & rifampin (7/15), azithromycin, gentamicin & rifampin (5/15), azithromycin, gentamicin, & bactrim (1/15) and gentamicin/rifampin (1/15).

Conclusion: Based on our results, Hawaii has a higher incidence of inpatient CSD than the mainland, approximately 0.54/100,000 per year (based on 2016 US census of 308,568 children in Hawaii). While frequently categorized as an insidious and benign process, CSD can be disseminated and thus should be considered early in patients with prolonged fever without a source, even prior to reaching 14 days of FUO criteria. Combination antimicrobial therapy was used in all but one patient with resolution of fever and disseminated disease. Azithromycin with rifampin was used in 50% of disseminated cases. Early consideration of disseminated CSD with serological, molecular and radiological diagnosis will help clinicians diagnose this clinical disease. If there is a high clinical suspicion for CSD in patients with prolonged fever without a source, serological testing for *B. henselae* as well as blood Bartonella PCR should be sent with initiation of empiric treatment, as PCR may be limited later in the disease process.

(Co-authors: Jessica Kosut, Natascha Ching)
FINE NEEDLE ASPIRATION/CORE NEEDLE BIOPSY OF EXTRANODAL LYMPHOMAS: A 25 YEAR RETROSPECTIVE

Objectives: Extranodal lymphomas may affect almost any organ and manifest in a variety of presentations, thus necessitating distinction from other disorders. We sought to determine the incidence, sites of occurrence, and subtypes of extranodal lymphomas diagnosed by fine needle aspiration (FNA)/ core needle biopsy (CNB), including the use of ancillary tests, such as immunohistochemistry (IHC), flow cytometry (FC), and cytogenetic/molecular testing. We also aimed to characterize the cytomorphologic features of the lesions encountered in our study.

Methods: A Natural Language search was undertaken in our CoPath database for all extranodal lymphomas diagnosed by FNA/CNB cytology during our 25 year study period from 1992 to 2017.

Conclusion: One hundred fourteen patients, including 62 (54%) males and 52 (46%) females ranging in age from 19 to 93 (average 66) were diagnosed with extranodal lymphomas by FNA/CNB. Fifty-two (45.6%) patients had a prior diagnosis of lymphoma. Thirty-five (30%) cases were diagnosed in the liver, followed by 24 (21.0%) in the lung, 10 (9%) each in the pancreas, bone and salivary gland, 9 (8%) in the breast, 8 (7%) in the thyroid, 6 (5%) in the kidney, and 2 (2%) in the stomach. Most were diffuse large B cell lymphomas (51.8%, n=59), followed by follicular lymphoma (13.2%, n=15). Twelve (10.5%) cases were extranodal marginal zone (mucosal associated lymphoid tissue) lymphomas, including 8 (66.6%) from the lung, and 2 (16.7%) each from the thyroid and breast. Less frequently encountered lymphomas included chronic lymphocytic leukemia/small lymphocytic lymphoma (n=5), mature/peripheral T cell lymphoma (n=2), Hodgkin's lymphoma (n=2), and pre B cell lymphoblastic lymphoma/ALL. Both immunohistochemistry (IHC) and flow cytometry (FC) were employed in 44 (38.5%) cases, FC alone in 34 (30%), and IHC alone in 22 (15.3%). The diagnosis was rendered by only cytomorphology in 14 (12.2%) of cases. Cytogenetic/molecular testing was employed in 3 (2.6%) cases. FNA/CNB in conjunction with FC, IHC, cytogenetic/molecular testing was an effective diagnostic modality, precluding the need for more invasive procedures.
B. F. Kaleionaia K-aloha, Graduate Student, (Advisor: Keawe Kaholokula, PhD)
Department of Native Hawaiian Health

THE MāHINA PROGRAM: A MULTIFACETED HEALTH TRAINING PROGRAM TO INCREASE INDIGENOUS HEALTH RESEARCH

The Māhina International Indigenous Health Research Training Program is an intensive summer research endeavor that partners the University of Washington, Department of Native Hawaiian Health at John A. Burns School of Medicine at the University of Hawai‘i, and the University of Auckland New Zealand. This unique program is geared towards Indigenous undergraduates and graduates who are interested in biomedical, behavioral science, public health, and social science health research careers. Māhina is a part of the nation-wide Minority Health and Health Disparities International Research Training initiative sponsored by the U.S. National Institutes of Health. This poster provides an overview from the lens of one Native Hawaiian trainee who was a part of the first Māhina Cohort. It showcases successful educational strategies to enhance the academic quality of Indigenous students in Hawai‘i. Trainees completed orientation in Washington and Hawaii to learn about the social, cultural and historical determinants of health. This preliminary focus provided the cohort foundation to relate the history of health among Native Hawaiians, Pacifica and Maori. Coursework and research internships were then completed in Aotearoa. Supervision was conducted by an international network of mentors with expertise in indigenous health methodologies. A culturally-grounded 360 degree learning experience created space for mentoring one trainee across multiple levels throughout the program. Trainees received experiential research training opportunities focused on theoretical, methodological, and substantive issues concerning Indigenous health in native communities. Rigorous learning in culturally grounded conceptual models and community based participatory/tribal participatory research allowed the trainees to learn within their own cultural framework and shared belief systems. This included values and principals in Indigenous research ethics and protocols in decolonized methodologies. The Māhina Program is designed to increase research capacity of indigenous health research for the next generation and greatly contributed to the rich learning experience from the described student perspective.
INVESTIGATION OF ZIKV INFECTION IN HUMAN LEYDIG CELLS

Background: Zika virus (ZIKV) is a positive sense single strand RNA flavivirus transmitted by mosquitos. In the most recent 2015-16 outbreak, almost 40,000 ZIKV cases were reported in the US alone. This outbreak was also associated with two new routes of virus transmission- in utero and sexual route of transmission. A recent study showed that 56 % of ZIKV serum positive men were also positive for the virus in the seminal fluid for almost 6 months after infection. This suggests that ZIKV can hide and persist in the immune privileged site of the testis even when the virus is cleared from other parts of the body. However, little is known about cellular targets of ZIKV in human testes and underlying mechanisms. Previous studies from our lab demonstrated Sertoli cells as one of the targets for ZIKV infection. Sertoli cells are the nursing cells of the seminiferous tubules that support maturation of the germ cells. Another very important testicular cell type includes Leydig cells (LC) that reside in the interstitial compartment outside the seminiferous tubules. The LC are the testosterone producing cells of the testis. To further our understanding of ZIKV testicular infection, the objective of this study was to investigate the infection kinetics of ZIKV in human LC and determine its effect on testosterone production and key host defense response.

Methods: Human primary LC were purchased from ScienCell and cultured according to company protocol. The cells grown on a 6-well plate were infected with ZIKV at MOI 1 and MOI 5. Supernatant and cell lysate was collected at different time points after infection. Virus titers were measured and RNA extracted was used to determine changes in the expression of genes associated with key host defense response and testosterone production by qRT-PCR.

Results: Plaque assay and qRT-PCR confirmed productive infection of ZIKV with peak virus titers at 96h post infection. ZIKV did not induce any cytotoxicity in LC, however ZIKV did not significantly affect the gene expression of enzymes required for testosterone production HSD3B1 and HSD17B3. Ongoing experiments will characterize the changes in the key host defense response genes.

Conclusion: This study provides first evidence that LC can support ZIKV replication, however the robustness of the infection is significantly lower as compared to Sertoli cells. We speculate that by supporting limited replication of ZIKV, LC may act as a reservoir for virus in the interstitial space before the virus can infect other cells in the seminiferous tubules including Sertoli cells. Further studies are needed to compare replication kinetics, persistence and host response between other testicular cells to ultimately understand the underlying mechanisms of ZIKV infection and persistence in the testes.

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(Co-author: Maile Amine1, Boonyanudh Jiyarom1, Goral Trivedi1, Daniel P. Strange1, Saguna Verma1)
POSTER #9

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Department of Anatomy, Biochemistry and Physiology (Yukiko Yamazaki)

NON-CANONIC ACTIVITY OF RETINOIC ACID REGULATES MEIOTIC INITIATION IN PRIMORDIAL GERM CELLS (PGC)

Introduction: Meiosis is a germ cell-specific cell division system to produce haploid gametes. In mammals, retinoic acid (RA) is an extrinsic cue for meiotic initiation in both male and female germ cells. RA stimulates the expression of stimulated by retinoic acid gene 8 (Stra8), which is a meiotic gatekeeper gene by controlling the switch from mitosis to meiosis. Recently it has been demonstrated that RA controls two different activities in the cells. Canonically, RA is translocated into the nucleus and binds to the nuclear RA receptors (RARs), which are ligand-dependent transcriptional regulators to control the expression of RA target genes (canonical activity of RA). Recently multiple studies have proposed that RA quickly stimulates various signal transduction pathways in the cytoplasm (non-canonical activity of RA). In germ cells, the molecular connection between RA and the regulation of Stra8 expression still remains unclear. Our question was whether the non-canonical activity of RA controls Stra8 expression in primordial germ cells (PGCs) or not. Using a germ cell culture system, we discovered for the first time that RA treatment predominantly stimulates the extracellular signal regulated kinase1/2 (ERK1/2) pathway to control Stra8 expression in cultured murine PGCs. The next question we addressed was what is the molecular connection between RA signaling and the ERK1/2 activity. In diverse cell contexts, extrinsic RA rapidly stimulates the MAPK cascade (Raf-MEK-ERK), which contributes to the transcriptional activities of RA target genes. In this study, we examined whether the signal transduction cascade of Src-Ras-Raf-MEK-ERK kinetically transmit RA signals, which eventually stimulates the ERK1/2 activity to initiate Stra8 expression in PGCs.

Results: Isolated XX and XY PGCs at 12.5 dpc were incubated with or without RA for 30 min, 1 and 2 h for western blot analysis. In the presence of RA, the ERK1/2 pathway was quickly enhanced within 2 h in XX PGCs. We also determined that the activity of MEK1/2, a direct upstream kinase of ERK1/2, was promoted by RA treatment in cultured PGCs. To investigate the relationship between the MEK/ERK activity and the Src tyrosine kinase, XX PGCs were cultured with or without RA and/or the Src inhibitor (PP2). RA treatment quickly simulates both Src kinase and MEK1/2 within 30 min~1 h in XX and XY PGCs. Interestingly, the MEK1/2 activities were strongly suppressed under the RA+PP2 condition.

Conclusions: Our results demonstrate that (1) The non-canonical activity of RA stimulates MEK1/2 to induce the ERK1/2 activity in PGCs regardless of their sex, (2) RA primarily stimulates the non-receptor type Src signaling, then the activated Src eventually phosphorylates MEK1/2 and ERK1/2 cascade to regulate the Stra8 transcriptional expression in cultured PGCs.

(Co-Investigators: Yukiko Yamazaki and Dylan Ng. Research supported by NIH R01HD078679 to YY.)
ALPH-MACROGLOBULINS DEFICIENT MICE ARE RESISTANT TO LETHAL FLAVIVIRUS ENCEPHALITIS

West Nile virus (WNV) and Japanese encephalitis virus (JEV) are the leading causes of arboviral encephalitis in humans. No effective therapies exist for treating individuals with encephalitic flavivirus infections. \(\alpha\)--macroglobulins are physiological proteinase inhibitors with important roles in inflammation and immune modulation. In mice, two main \(\alpha\)-macroglobulins are present as plasma proteins, pregnancy zone protein (PZP) and murinoglobulin-1 (MUG). We have previously reported that WNV infection induced upregulation of \(\alpha\)-macroglobulins in mice. To define the role of \(\alpha\)-macroglobulins in flavivirus infection \textit{in vivo}, we investigated the susceptibility of mice deficient in \(\alpha\)-macroglobulins (PZP and MUG-1 double knockout; DKO) against lethal dose of WNV or JEV. We found that DKO mice were completely resistant to lethal WNV and JEV encephalitis, suggesting that \(\alpha\)-macroglobulins play a deleterious role in flavivirus infection. Increased survival in WNV-infected DKO mice was associated with significantly reduced viral burden in serum, spleen, kidney and brain compared to wild-type (WT) mice. Moreover, levels of cytokines and chemokines in the serum, spleen and brain were significantly reduced in WNV-infected DKO mice compared to WT mice. Collectively our data demonstrate that \(\alpha\)-macroglobulins contribute to the pathogenesis of flavivirus encephalitis in mice.

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POSTER #32

Kimberly Lactaoen, Undergraduate, (Advisor: Masato Yoshizawa, PhD)
Department of Biology

GENETIC CORRELATION BETWEEN CORE AUTISM-LIKE BEHAVIORS IN AN EMERGING TELEOST GENETIC MODEL

Autism spectrum disorder (ASD) is diagnosed in 1-2% of the population, yet its complex mechanisms obstruct effective therapy. Good approaches to unravel ASD is to have a proxy genetic model, which (i) overlaps the molecular and neural pathways of humans and (ii) shows a set of behaviors similar to core ASD symptoms. Such a model could highlight ASD’s core etiology. The Mexican tetra, Astyanax mexicanus, which is composed of cave-dwelling and surface-dwelling forms, substantially fill these aspects: cave-forms exhibit less-socialness, imbalanced attention, sleep-loss, hyperactivity, repetitive behavior and higher anxiety hormone levels than surface forms. Our former bioinformatics, pharmacological and diet-treatment studies, showed that these two forms are useful to provide experimental approaches that link the nervous system, immune system, metabolism, and gut microbiota as ‘the core mechanisms’ for ASD-like symptoms shared between cavefish and humans. Here we are determining the genetic correlation among ASD-like behaviors. Since cave and surface forms are interfertile, we are rearing ~670 F2 intercross individuals to gain enough statistical power to detect $\geq 2.8\%$ of phenotypic variance. We are currently assaying imbalanced attention (i.e. attraction to a particular frequency of water oscillation), sleep-loss, hyperactivity, and repetitive turning behaviors in F2 hybrids derived from a single pair of surface and cavefish. We have assayed 570 of 670 F2 for imbalanced attention, and 200 of 670 have been assayed for sleep-loss/hyperactivity. We are also including repetitive turning scores and are aiming to resolve genetic correlation among these behaviors.

(Acknowledgements: Chantell Balaan, Sabrina Podhorzer, Jimmy Nguyen, Masato Yoshizawa)
EBOLA VIRUS GLYCOPROTEIN ENHANCES IMMUNE RESPONSES BY ACTIVATION OF DENDRITIC CELLS

Background and Objective: Ebola virus (EBOV), a member in the Filoviridae family, causes the most severe form of viral hemorrhagic fever. No FDA licensed vaccine or treatment against Ebola virus disease (EVD) is currently available. EBOV glycoprotein (GP), the major antigen used in all vaccine candidates has shown to induce protective immune responses in mice, guinea pigs and nonhuman primates (NHPs). However, the mechanisms by which GP confers protection is not clear. Early initiation of innate immune responses has been correlated with survival of EBOV-infected humans. Recent reports of protection by the most advanced rVSV-based vaccine expressing EBOV GP before full adaptive immunity was generated suggests that the innate immune responses elicited during the early stage of vaccination may contribute to the protection. However, the underlying mechanisms, particularly innate immune responses induced by GP are not fully characterized. We have developed a recombinant protein-based subunit vaccine using a platform in which antigen is expressed from stably transformed Drosophila S2 cells and demonstrated that our vaccines induce protective immunity in mice and NHPs. Our previous study showed that three immunizations with purified recombinant EBOV GP in the absence of other viral components or adjuvants resulted in 70% of protection in mice, suggesting that GP alone is capable of inducing protective immune responses against EBOV infection. Dendritic cells (DCs) are the central for both activation of innate immune response and initiation of adaptive immunity. The objective of this study was to characterize how GP initiates innate activation in DCs and modulates innate-adaptive interface, and whether GP-associated innate responses are critical for induction of adaptive immunity.

Methods and Results: We demonstrated that EBOV GP was efficiently phagocytosed by mouse bone marrow-derived dendritic cells (BMDMs) and human monocyte derived dendritic cells (MoDCs) independent of adjuvants. We further tested the ability of EBOV GP to trigger maturation of DCs and found that exposure of EBOV GP led to elevated expression of surface costimulatory molecules CD40, CD80, CD86 in mouse BMDCs and CD40, CD80, and CCR7 in human MoDCs. Moreover, treatment of EBOV GP1 induced significantly higher levels of expression of costimulatory molecules than GP2 treatment in mouse BMDCs, indicating that GP1 domain has stronger immunostimulatory effect. Future studies will aim to analyze the cytokine secretion by EBOV GP-stimulated DCs and the ability of EBOV GP-stimulated DCs to support T cell proliferation. Finally, we investigate the splenic germinal center (GC) responses elicited by EBOV GP in the absence and presence of an adjuvant in mice by measuring the frequencies of GC B cells and Tfh cells using flow cytometry. We observed a higher proportion of Tfh cells after two immunizations of EBOV GP compared to naïve mice. However, in the presence of an adjuvant (CoVaccineHT), we observed significantly higher proportions of mouse splenic GC B cells and Tfh cells, suggesting that an adjuvant may be required to enhance GC responses if only one or two doses are administered.

Conclusion: This study provides mechanistic evidence that unlike EBOV-associated or derived GP, recombinant EBOV GP is an effective stimulator of DCs and has potential in enhancing innate and adaptive immune responses. The new data obtained may help improve key features in EBOV vaccine development including dosing and support testing of GP for pre- or post-exposure prophylaxis.

(Co-authors: Teri-Ann S. Wong, Madhuri Namekar, Axel T. Lehrer; This project was funded in part by Grant Number R01AI119185 from the National Institutes of Allergy and Infectious Diseases (NIAID), and by Grant Number P30GM114737 from the Centers of Biomedical Research Excellence (COBRE))
INVESTIGATION OF THE NEUROPROTECTIVE ROLE OF N-TERMINAL BETA AMYLOID FRAGMENTS AGAINST BETA AMYLOID-INDUCED TOXICITY IN GLIA

A chief hallmark of Alzheimer’s disease (AD) is the accumulation of soluble, oligomeric beta amyloid (Aβ) peptide, promoting neurodegeneration and chronic neuroinflammation. Normally, microglia and astrocytes provide diverse neuromodulatory functions to maintain a healthy neuronal environment. However, in AD, chronically elevated Aβ levels induce persistent glial cell activation that exacerbates neuronal death by limiting synaptogenesis, upregulating phagocytic activity to remove synapses, and increasing the secretion of pro-inflammatory cytokines, chemokines and ROS species. Currently, there is no effective cure for AD, but controlling neuroinflammation and maintaining the neurosupportive environment may be critical for developing a successful anti-inflammatory AD treatment.

Our previous studies have shown that the endogenous N-terminal fragment of Aβ1-42, termed Aβ1-15, and its critical hexapeptide core sequence, Aβ10-15 (Aβcore), protect against full-length Aβ1-42-induced cellular neurotoxicity and synaptic dysfunction in neurons and behavioral dysfunction in whole animals. Our objective was to investigate whether the neuroprotective functions of these N-terminal Aβ fragments extend beyond neurons to glia cells. In this study, we examined the neuroprotective potential of the N-terminal Aβ fragments against Aβ1-42-induced toxicity in primary astrocytes and microglia. Primary glia, cultured from mouse cortex, were treated with media (control) or 2.5 µM Aβ1-42, Aβ1-15 or Aβcore, alone or in combination, over the course of several days prior to examining alterations in cellular toxicity (oxidative stress: ROS assay and cell viability: direct cell counts), calcium homeostasis (live cell calcium imaging) and mitochondrial membrane potential (TMRE staining) due to calcium overload. Our results demonstrate that treatment of primary glial cells with 2.5 µM Aβ1-42 induced ROS production and reduced cellular viability, both of which were mitigated via co-treatment with either 2.5 µM Aβ1-15 or Aβcore. Elevated levels of Aβ1-42 have been shown to disrupt calcium homeostasis in glia, producing a prolonged increase in intracellular calcium concentrations ([Ca2+]i) that evoke persistent activation of many cellular pathways. We have shown that co-treatment with the N-terminal Aβ fragments prevent the pronounced Aβ-triggered increase in [Ca2+]i in neurons. However, experiments are ongoing to delineate the means by which Aβ1-15 and Aβcore attenuate calcium dysregulation induced by Aβ1-42 in primary glia. We have postulated that the N-terminal Aβ fragments compete with Aβ1-42, by producing transient increases in intracellular calcium concentrations in primary microglia and astrocytes while remaining bound to target Aβ receptors, similar to that seen in neurons.

In conclusion, the findings of this study provide insight into the protective function of the N-terminal Aβ fragments in primary glial cells. These results provide a basis for the development of novel approaches for maintaining the neurosupportive role of astrocytes and microglia in AD by reducing or reversing Aβ1-42-induced glial toxicity.

(Acknowledgments: UHF, Robert Nichols)
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Department of Psychiatry

REDUCING MENTAL HEALTH DISPARITIES AMONG NATIVE HAWAIIAN YOUTH THROUGH PATIENT NAVIGATOR MODELS: A REVIEW

Objective. While mental illnesses begin to arise during childhood and adolescence, almost half of these youth do not receive mental health care. Additionally, youth living in rural areas are less likely to have access to an outpatient mental health facility. Native Hawaiian children and adolescents are at-risk as they experience depressive disorders at higher rates compared to non-Hawaiian youth as well as higher rates of disruptive behavioral disorders compared to Caucasian youth. Patient navigator models are designed to reduce barriers to healthcare. Most navigator models have been implemented among cancer, HIV, or diabetic patients; few have investigated navigator models relevant to mental health. To reduce mental health disparities among Native Hawaiian youth, patient navigator models were reviewed to outline implementation feasibility at federally qualified community health centers.

Methods. To identify relevant articles for review, PubMed was searched using the keyword “patient navigation”. This search method yielded 48 results. For articles to be considered for review they needed to be review articles written in English over the past 5 years with full text available. Articles were also excluded if patient navigation was not a main focus of the review. Based on these criteria, only 20 articles were selected for review. An additional two articles were also included for a final total of 22 review articles.

Preliminary Results. Health navigator models address two types of healthcare barriers: 1) system-level barriers and 2) individual-level barriers. System-level barriers are due to the structure of the healthcare system. Individual-level barriers are obstacles a patient must overcome to receive care. Navigators include trained lay persons or healthcare professionals, such as a social workers, nurses, or physicians. Additionally, models may team sets of healthcare professionals or lay persons and professionals as navigators.

Navigators can provide two types of services to patients: 1) logistical services and 2) relational services. Logistical services can involve identifying healthcare facilities, making appointments, and/or finding transportation for patients. Relational services involve providing emotional and social support, building a trusting relationship with patients, and continuity of contact.

Outcomes for patient navigator models are divided into three categories: 1) patient outcomes, 2) provider/navigator outcomes, and 3) health system outcomes. Patient outcomes include improved health and well-being, increased patient satisfaction, increased access to care, and increased uptake and follow-up for screening. Provider/navigator outcomes include improved care coordination, increased communication between primary care and community services, and increased trust between navigators and physicians. The most common health system outcome is reduced emergency room and hospital use.

Conclusion. Patient navigator models address vital issues to reduce barriers to healthcare and report positive outcomes for patients, providers/navigators, and health systems. Findings from this review will be presented to academic researchers and physicians as well as federally qualified community health centers to determine implementation feasibility of patient navigator models in the community to reduce mental health disparities among Native Hawaiian youth.

(Co-authors: Susana Helm, PhD, Joy Andrade, MD, Helen Kekalia.)
POSTER #22

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HIV DNA COPIES IN PBMCS PRE- AND POST MARAVIROC TREATMENT:  
EFFECTS ON COGNITIVE IMPAIRMENT

Background: Neurological deficits among individuals with HIV remain prevalent even with highly active anti-retroviral therapy (HAART). The blood brain barrier (BBB) plays a vital role in the selection of substances, such as retroviral pathogens trafficking into the central nervous system. Transmigration across the BBB of monocytes infected with HIV leads to the production of pro-inflammatory cytokines, neuronal degradation, and the disruption of homeostasis. Viral surface proteins, such as gp120, enable HIV binding to monocytes for host cell entry. In vitro BBB models are used to demonstrate the mechanism of actions between monocytes and brain endothelial cells. Maraviroc, an anti-retroviral drug, allosterically binds to CCR5 receptors to inhibit the entry of HIV into monocytes. Activated monocytes have been thought to be the main cause of inflammation in the brain; recently, Maraviroc has been shown to decrease the amount of activated monocytes that preferentially transmigrate through the BBB.

Objective: This study looked at the effects of therapeutic intervention with Maraviroc on HIV DNA concentration and cognitive impairment status.  
Hypothesis: Post-Maraviroc treatment will show decreased transmigration of HIV infected PBMCs across the BBB, which will limit neuronal inflammation and impairment.

Methods: An in-vitro BBB was model was set up with astrocytes and endothelial cells on inserts within growth medium. Peripheral blood mononuclear cells (PBMC) were drawn from patient volunteers in accordance with UH IRB protocol and analyzed pre- and post- Maraviroc intervention. PBMC were allowed to transmigrate in an in-vitro BBB. HIV DNA copies were quantified via ddPCR.

Results/Conclusion: Clinical applications for this model can be used to determine HIV DNA copies pre- and post- anti-retroviral therapies within PBMC and their neurological impact. Preliminary results may provide insight into mechanism of cognitive impairment progression in patients that are compliant with ART.

(Acknowledgements: Bruce Shiramizu, Robert Oda)
POSTER #88

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BARRIERS TO BEATING SEPSIS: ADHERENCE TO FLUID RESUSCITATION IN CONGESTIVE HEART FAILURE/RENAL FAILURE

Following implementation of the Surviving Sepsis Campaign care bundle for management of sepsis patients in 2013, bundle-compliant intravenous fluid (IVF) resuscitation (30 cc/kg in the first 3 hours) continues to be a challenge for fluid-sensitive patients such as those with congestive heart failure (CHF) and/or renal failure (RF).

Our objective was to evaluate hemodynamic, respiratory, and mortality outcomes of fluid-sensitive adult sepsis patients who received bundle-compliant fluid resuscitation for sepsis. We sought to provide clinicians data to aid with clinical decision making in this special sepsis patient population.

A retrospective chart review was performed to compare adult severe sepsis patients with previously diagnosed CHF and/or RF who received bundle-compliant fluid resuscitation versus those who did not receive bundle compliant fluid resuscitation. Patient data from July 2013 to February 2017 was collected from three major hospitals in Hawaii. Student’s 2-sample t-test was used to compare inpatient mortality, logistic regression was used to obtain odd ratios, and multivariate logistic regression was used to adjust for BMI, sex, length of stay, continuous veno-venous hemodialysis (CVVH), intubation, and BIPAP.

Of 228 patients with CHF/RF and severe sepsis/shock who received fluids, 121 (53%) required diuretic support, 7 (3%) required CVVH, and <20% required respiratory support (39 (17%) BIPAP, 25 (11%) intubation). The mortality adjusted odds ratio (aOR) in patients who received fluid was 0.62 (95% CI=0.42-0.91), which adjusted for Age, BMI, Sex, Length of stay, CVVH, Intubation, and BIPAP.

A statistically significant decline in mortality rate was observed in patients with CHF and/or RF with severe sepsis who received bundle-compliant fluid resuscitation. Among CHF patients, a statistically significant decline in mortality rate is observed independent of ejection fraction. Thus, these fluid-sensitive patients may benefit from bundle-compliant fluid resuscitation.

(Co-authors: Jonathan D. Woo, Ryan T. Yanagihara, Melinda Ashton, Jaclynn Ruiz, Chieko Kimata, Shilpa J. Patel)
TREATMENT WITH SMALL MOLECULE INHIBITORS MIMIC THE EFFECTS OF Sec 10 KNOCKOUT IN AN ORGAN CULTURE MODEL OF URETER OBSTRUCTION

Congenital malformations that obstruct the urinary tract are the leading cause of kidney disease in children, and these obstructions most commonly occur in the upper ureter. We have recently characterized a novel Sec10 conditional knockout mouse (Sec10-CKO) that is the first animal model of the prenatal ureter obstructions that are commonly seen in humans. The Sec10 gene is one of the central subunits of the exocyst, a highly conserved protein complex that mediates the trafficking and docking of certain intracellular vesicles. The ureter obstruction in these mice is caused by the failure of differentiation of the epithelial cells that line the ureter, called urothelial cells. This suggests that Sec10 and the exocyst may regulate key developmental pathways that are involved in urothelial differentiation. To better investigate the role of Sec10 in ureter development, we have established an ex vivo ureter explant organ culture model where we can follow differentiation of the mono-layered urothelial progenitors until maturity. Using this ex vivo model also allows us to target various signaling pathways using small molecule inhibitors and then measure for potential defects in urothelial differentiation. Embryonic mouse ureters at E15.5 were dissected and placed onto 0.1 µm filters at the air-liquid interface and cultured with or without various small molecule inhibitors. After 72 hours, ureters were either collected for histology or collected for RNA extraction. Real time qPCR and immunostaining was used to measure the expression of uroplakin genes, which are markers of urothelial terminal differentiation. Results confirmed Sec10 knockout in the urothelial cells caused failure to differentiate and stratify, leading to cell detachment and death. Treatment with a retinoic acid inhibitor also led to a similar phenotype with an absence of uroplakin gene expression in explanted ureters, and we are currently investigating several additional intersecting signaling pathways. These data suggest the exocyst may be a mechanism regulated by retinoic acid signaling required for urothelial stratification. This novel ureter organ culture model can be an innovative technique to investigate ureter development and the signaling pathways that may contribute to congenital ureter obstructions in pediatric patients.
Laterality consists in dominance of one side of the body or brain over the other. Asymmetrical distribution of internal organs (heart, liver, stomach) and handedness are examples of laterality. Abnormal brain asymmetry has been frequently reported in various mental disorders such as schizophrenia and depression. Many animal species exhibit laterality in sensation and behavioral responses, namely the preference for using either the left or right side of the sensory system. However, it is largely unknown whether such laterality in sensory-behavior coupling evolves during rapid adaptation processes. A cave form of the Mexican tetra, Astyanax mexicanus, evolved from surface-dwelling ancestors and rapidly adapted to cave by enhancing traits including vibration attraction behavior (VAB), an adaptive foraging behavior in darkness, and its underlying mechanosensor, superficial neuromasts (SN). The SN enhancement is promoted by the budding of neuromasts, which is, in zebrafish and medaka, encouraged by the underneath dermal bone formation. In the present study, we inhibited the endothelin signaling, which is a major known regulator for dermal bone formation, in juvenile fish (from 1 to 3 months-old) via endothelin inhibitors. The endothelin-signaling inhibition increased the cranial SN in both surface and cavefish and also increased the dermal bone formation in cavefish, however, bone enhancement was observed in the much later stage than that of SN, suggesting that the endothelin signaling may independently regulate the SN increase and the bone formation in cavefish. Surprisingly, the left side-SN increase regardless of by the normal development or by inhibitor treatment, was positively correlated with VAB in cavefish. the association with right side-SN, or laterality in surface fish was not observed, suggesting the laterality between left SN and VAB may have evolved during adaptation to cave. Accordingly, cavefish emerged as a useful model to investigate the evolution of laterality.

(Co-authors: Christian Macaspac, Louise Lu)
DETERMINING BIOPSYCHOSOCIAL PREDICTORS OF PSYCHOGENIC NON-EPILEPTIC SEIZURES IN ETHNICALLY DIVERSE PATIENTS TO SPECIFY AMBIGUOUS DIAGNOSES FOR PATIENTS WITH PNES

INTRODUCTION: Psychogenic non-epileptic seizures (PNES) are seizures that encompass any combination of motor, autonomic, cognitive, or sensory disturbances. They occur without warning and have no definite cause. PNES is not to be confused with epilepsy, a neurological condition with physical manifestations of seizures with corresponding abnormal electrical discharges in the brain. About 20% of patients who were first suspected of having epilepsy were confirmed to have PNES instead. This is due to the similarities in physical manifestations that make PNES hardly distinguishable from epilepsy.

OBJECTIVES: The primary objective of this project is to describe their biopsychosocial characteristics to better specify future diagnoses made for patients with complex seizures. These factors included personal history, family history, and clinical characteristics, including biological, psychological, and social characteristics.

METHODS: In this study, patients are categorized into clusters of clinically established PNES, probable PNES, and not PNES based on the physician’s impression and the history of present illness from physician’s notes. This study was a systematic retrospective review of patients referred to Hawaii Pacific Neuroscience. Data was extracted from patient charts using ICD-10 codes (ICD R56.9 and ICD F44.5) and video-electroencephalogram (VEEG) referral and monitoring.

RESULTS: Of the 133 patients evaluated for complex seizures, 20 were determined to have clinically established and/or documented PNES, 33 had probable or possible PNES, and 79 did not have PNES but rather an onset of seizure-like events due to other medical conditions. Post-traumatic stress disorder, anxiety, personality disorders, migraines, and traumatic history (sexual, physical, or psychological abuse) were variables found to be statistically significant when using the chi-squared statistical analytical test for association.

CONCLUSION: The different clusters studied offers insight into the factors that may actually influence the onset of PNES and those that may be attested to coincidence. This study hopes to clarify some of the ambiguity surrounding PNES and help doctors make more accurate diagnoses.

(Co-authors: Jasen Ocol, Richard Ho, Michael Yang DO, Paul Adapon, MD, Enrique Carrazana, MD)
POSTER #69

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X-LINKED INHIBITOR OF APOPTOSIS PROTEIN AS A TARGET FOR IMAGING THE LATENT HIV-1 RESERVOIR

Objectives: Despite virologic suppression, Human Immunodeficiency Virus (HIV) can persist in resting memory CD4+ T cells, monocytes, macrophages and potentially other cells by way of latent infection. Since latent virus is transcriptionally silent, it is not expected to produce viral products, making latently infected cells difficult to identify. Moreover, complex viral and host mechanisms which favor cell survival may prevent the detection and elimination of the reservoir. Specifically, anti-apoptotic host factors are thought to play an important role in maintaining the HIV latent reservoir. In this project, we focus on X-linked inhibitor of apoptosis protein (XIAP) as a potential biomarker that can serve as a non-viral target for imaging or treatment of the latent reservoir. Our objectives are 1) to assess the suitability of XIAP as a marker for latent HIV infection, and 2) to identify small molecule ligands for XIAP with potential for novel therapeutics or diagnostics.

Methods: To assess whether XIAP can serve as a marker for latent HIV infection, we are correlating XIAP levels with the presence of HIV DNA. CD4+ T cells are sorted by intracellular XIAP level using fluorescence-activated cell sorting (FACS) and the HIV DNA levels from sorting subsets determined by quantitative PCR. To identify small molecule ligands for XIAP, we are developing an enzymatic caspase derepression assay and a cell based Caspase detection assay using the Hamamatsu functional drug screening system (FDSS)

Conclusions: Preliminary results indicate a positive correlation between XIAP and HIV DNA levels, suggesting that XIAP may indeed serve as a biomarker for latent HIV infection. To our knowledge, this represents the first such study from patient-derived CD4+ T-cells. Additionally, we have successfully developed a caspase derepression assay for the high-throughput screening of small molecule libraries (synthetic and natural). Future studies will further explore the correlation between XIAP and latent infection as well as implementation of the developed assay for small molecule screening.

(This project is supported by grant number U54MD007584 from the National Institute on Minority Health and Health Disparities (NIMHD), a component of the National Institutes of Health (NIH))
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DEVELOPMENT OF A MULTIPLEX MICROSPHERE IMMUNOASSAY TO DISTINGUISH ZIKA VIRUS AND OTHER FLAVIVIRUS INFECTIONS

Background and Objectives: Zika (ZIKV) is an arbovirus belonging to the Flaviviridae family, which also include other viruses causing human diseases such as dengue virus (DENV), yellow fever virus (YFV) and West-Nile virus (WNV). After the explosive outbreak of ZIKV in the Central and South Americas during 2015 and 2016, major concerns on ZIKV infection are its link to Guillian-Barre syndrome and microcephaly and other birth defects, known as congenital Zika syndrome. Sensitive and specific diagnostic tests to distinguish ZIKV and other flaviviruses are urgently needed. Nucleic acid tests can distinguish ZIKV and other flaviviral infection only during the acute stage; serological tests are important after acute infection and for most asymptomatic infections. Traditional envelope-protein based flaviviral serological tests are hampered by cross-reactivities among various flaviviral infections. We reported previously that NS-based ELISA can distinguish ZIKV and different DENV infections. In this study, we develop a multiplex IgG microsphere immunoassay (MIA) based on NS1 proteins and Luminex2000. The multiplex Luminex can read several analytes at the same time and reduce the sera used.

Methods: Recombinant NS1 proteins from ZIKV, DENV, YFV and WNV (Native Antigen) were coupled to luminescent microsphere beads with separate luminescent tags specific for each type of NS1. Beads coupled with PBS and BSA were used as negative control. Mixtures of 7 beads were added into 96-well plate, incubated with positive controls (different panels of serum or plasma from subjects with lab-confirmed ZIKV, DENV, WNV infection or YFV vaccinations) and negative controls (serum or plasma from flavivirus-naïve subjects), followed by adding secondary antibody (phycoerytrin-conjugated anti-human IgG), and read with Luminex 2000. For each NS1 protein, the mean fluorescent intensities (MFI) of negative controls plus 3 standard deviation were used as cut-off.

Results and Discussion: For each panel of samples with primary DENV, ZIKV or WNV infections, they recognize NS1 protein of DENV, ZIKV or WNV, respectively, but not those of other serocomplexes. For samples with secondary DENV infection or ZIKV infection with previous DENV infection, some cross-reactivities were found. Further analysis of the ratio of MFI to different NS1 proteins are ongoing to distinguish different flaviviral infections.

(Acknowledgements: Jasmine Tyson, Wen-Yang Tsai)
POSTER #18

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A RECOMBINANT SUBUNIT BASED ZIKA VACCINE IS EFFICACIOUS IN NONHUMAN PRIMATES

Objectives: Zika virus (ZIKV) is a positive sense RNA virus belonging to the flavivirus family. Few human cases were reported until 2007, when an outbreak on Yap Island, Federated States of Micronesia resulted in the infection of an estimated 5,000 people. Since then ZIKV infections have been reported in 61 countries, with 27 countries reporting ongoing transmission as of February 2018. Despite global initiative to develop an efficacious ZIKV vaccine, none are currently FDA approved. Our goal was to develop a safe and efficacious ZIKV vaccine that is based on recombinantly expressed ZIKV E protein. Previous work in our lab has demonstrated the safety and efficacy of this vaccine candidate in a murine model. Our objective here is to demonstrate that this vaccine candidate is also efficacious in a nonhuman primate (NHP) model.

Methods: Nonhuman primates were immunized with vaccines containing 25ug ZIKV E adjuvanted with either Alum or CoVaccine or one containing 75ug of unrelated glycoprotein as a control. Two immunizations were given 3 weeks apart, and challenge with ZIKV was done 3 weeks after the final vaccination. To assess the immunogenicity of the vaccine candidates, ZIKV E specific IgG was assessed using a Luminex based microsphere immunoassay (MIA), and plaque reduction neutralization tests (PRNTs) were used to determine the functional potential of the elicited antibodies. Viremia was looked at using RT-PCR for 7 days following viral challenge, to assess the protective efficacy of the vaccine. Due to the well established role that neutralizing antibodies play in flavivirus infection we transferred plasma from vaccinated NHPs with known antibody titers into BALB/c mice and challenged the mice with ZIKV. Viremia, NHP IgG, and mouse IgG were analyzed to determine the ability of the passively transferred antibodies to protect in naïve mice.

Conclusion: Nonhuman primates receiving two doses of our CoVaccine adjuvanted ZIKV E vaccine developed high ZIKV E specific IgG titers, demonstrating the immunogenicity of our vaccine. These animals were partially protected against viral challenge with 3 of the 4 vaccinated animals developing no viremia. Neutralizing titers showed that the 1 animal that did develop viremia had very low neutralizing titers compared to the other vaccine recipients. These results were further confirmed in our passive transfer study, where mice receiving plasma from the unprotected vaccinee were only partially protected, while those that received plasma from a high neutralizing titer donor were completely protected against viral challenge.

(Co-authors: Madhuri Namekar, Teri Ann Wong, Michael Lieberman, Eileen Nakano, Albert To, Hanne Anderson, Stephen Higgs, Jake Yalley-Ogunro, Axel Lhrer)
Background: The Hawai‘i Homeless Outreach and Medical Education (H.O.M.E.) Project was established in August 2005 at the John A. Burns School of Medicine to provide opportunity for medical students to serve Hawai‘i’s homeless community through a mobile student-run free medical clinic. The H.O.M.E. clinic currently provides services at 7 sites across the island of O‘ahu using a mobile RV. In 2016, the H.O.M.E. Project switched from paper charts to an electronic health record (EHR) platform, Practice Fusion, in order to better coordinate services at a completely mobile, multi-site clinic and to prepare medical students for the current standard of patient documentation. This study investigated the impact of implementing a cloud-based EHR platform on clinic operations and communication.

Methods: We created three preliminary surveys for medical students, pre-clinical volunteers, and adult patients. Surveys were conducted either on paper or electronically and collected anonymous responses. These surveys asked questions regarding paper and EHR charting preferences, the comparison between EHR systems, and the use of an EHR during a medical student (provider) and patient interaction.

Results: Survey results demonstrate that the majority of medical students prefer to document notes on an EHR over paper charts. The most common alternative EHR system of reported use was the client-based EHR system EPIC, which is used in many medical institutions in Hawai‘i. While some students have indicated the challenge of navigating Practice Fusion for clinical measurements, the majority of the comments have evaluated the cloud-based system as comparable or better than other client-server based systems. The introduction of EHR charting has also affected the one-on-one communication between provider and patient, where most providers use a laptop and EHR almost all the time or all the time during the encounter. This does not appear to negatively affect the provider’s focus on the patient or the patient’s satisfaction. The majority of providers are aware of the patient even while using the EHR and prioritize listening and talking to the patient during a patient encounter.

Conclusions: The switch to mobile devices and a cloud-based EHR has challenged our clinic’s standard operating procedures (SOP) in all aspects. The benefits of accessibility and information management are clear. Yet, the efficiency and effectiveness of clinic has become more sensitive to the speed and function of our electronic tools. New challenges include troubleshooting slow or malfunctioning electronic devices and training new users of the cloud-based EHR. Further training and new SOP in information technology (IT) and HER etiquette is recommended to ensure smooth clinic operation and communication.

In conclusion, implementing a cloud-based EHR system was valuable in pursuit of our clinic’s mission to improve quality and access to healthcare for Hawai‘i’s homeless while providing valuable training for our future physicians.

(Co-authors: Jason T. Huynh, Taylor Sinn, Chloe Liu, Jill S.M. Omori, MD)
PURIFICATION OF UNTAGGED AND HISTIDINE-TAGGED MARBURG VIRUS NUCLEOPROTEIN PRODUCED USING THE DROSOPHILA S2 EXPRESSION SYSTEM

PURPOSE: Marburg virus (MARV), a member of the Filoviridae, causes severe, and usually fatal hemorrhagic fever in humans and non-human primates. Recent outbreaks with hundreds of cases of MARV infection in the Democratic Republic of the Congo and Angola with case fatalities approaching 90% dramatically highlight its lethal potential. FDA approved antiviral treatments or vaccines are currently not available leaving only conventional options of intensive supportive care. Filovirus nucleoprotein (NP) has been used as one of the promising vaccine candidate along with other viral proteins such as virus glycoprotein (GP). In addition, it can also be used for the development of serological assays, for example in multiplex diagnostic assays along with other filovirus proteins for rapid differential Filovirus diagnosis during severe outbreaks. Herein, we purified the untagged and histidine-tagged MARV NP protein produced using the Drosophila S2 cell expression system.

METHODS: We used the AKTA pure system for protein purification, which is an automated chromatography system and allows fast, efficient, and multi-step purification. For purification of histidine-tagged proteins to which a short stretch of six histidines (His-tag) is added to the C-terminus of the target protein, which is then exploited to enable purification of the "tagged" protein by Immobilized Metal Affinity Chromatography (IMAC). Since the IMAC purification resulted in leaching of nickel ions from His-Trap column, anion exchange purification was first done for MARV NP (his-tagged version) using an anion exchange (HI Trap Capto Q) column. The combined fractions from anion exchange purification were subsequently purified using the His-Trap column using linear gradient elution using PBS, 300mM NaCl and 500mM Imidazole, pH 7.4. To increase purity, His-trap purification was repeated using a modified gradient elution protocol. To remove other non-specific proteins, fractions from His-trap purification were run on the SEC (Size exclusion chromatography) column using PBS. Yield was about 5.6 mg from about 1 liter of supernatant. To purify untagged MARV NP using Immunoaffinity purification (IAC), anti-MARV NP antibody was conjugated to HI Trap NHS column. Yield was about 1.3 mg from about 1 liter of supernatant. RESULTS: We have demonstrated that purified tagged and untagged MARV NP protein can be detected by SDS-PAGE and western blot analysis, suggesting that we can efficiently express and purify untagged as well as histidine-tagged MARV NP protein using the Drosophila S2 expression system. Compared to single step IAC purification of untagged protein, multistep purification of histidine-tagged protein is time consuming, as it requires multiple purification steps. Results of Western blotting showed that both Histidine tagged and untagged MARV NP proteins were detected as protein bands with of molecular weight of around 97 kDa and reacted strongly with anti-MARV NP antibody. However, more non-specific proteins were observed in the fractions of histidine-tagged protein compared to untagged MARV NP protein, not surprising given the specificity of protein-specific immunoaffinity purification. CONCLUSIONS: 1) We conclude here that untagged MARV NP protein is more pure compared to histidine-tagged MARV NP protein. 2) Therefore, untagged MARV NP protein can be more effectively used for vaccine studies, as it will generate more specific immune response compared to histidine-tagged MARV NP protein. 3) Similarly, untagged MARV NP protein will be better for use in diagnostic assays as it will prevent non-specific binding or false-positive results.

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(Coauthors: Axel T. Lehrer, Albert To, Teri Wong)
THE RA-STIMULATED ERK1/2 PATHWAY REGULATES THE EXPRESSION OF MULTIPLE RETINOIC ACID TARGET GENES IN CULTURED PRIMORDIAL GERM CELLS

Objective: Retinoic acid (RA) stimulates the expression of Stimulated by retinoic acid gene (Strα8), a RA-target gene, which leads to meiotic initiation in both male and female germ cells. Recently, it has been demonstrated that RA controls two different activities in somatic cells. Canonically, RA moves into the nucleus and binds to nuclear RA receptors (RARs), which are ligand-inducible transcription activators. RA-activated RARs induce transcription of RA target genes through direct binding to RA response elements (RAREs) in the promoter region (canonical activity of RA). Recent studies have proposed that RA also quickly activates multiple signal transduction pathways in the cytoplasm to regulate various cellular events (non-canonical activity of RA).

Previously, we investigated whether this non-canonical pathway of RA is involved in Strα8 gene expression. Using a murine germ cell culture, we discovered that RA treatment predominantly stimulates the extracellular signal regulated kinase1/2 (ERK1/2) pathway to control Strα8 expression in cultured primordial germ cells (PGCs). With this finding, our focus shifted to whether the ERK1/2 pathway regulates other RA-target genes (RARα, RARβ, RARγ, Rec8, and Cellular retinoic acid binding protein 1(Crabp1)) that contain RAREs in their promoter regions. These genes, along with Strα8, also play critical roles in commencing meiosis in PGCs, while Crabp1 is suspected to be involved in RA-mediated differentiation. In this study, we examined (1) whether RA treatment promotes the transcriptional expression of these genes, and (2) whether the RA-stimulated ERK1/2 pathway regulates the transcriptional activity of these genes.

Methods: XX and XY PGCs at 12.5 days post coitum were isolated from Oct4-GFP transgenic mouse fetuses. PGCs were then cultured with or without 100 nM RA and/or 50 µM ERK1/2 inhibitor (U0126) for 16 hr. Cultured PGCs were subjected to generating cDNA to perform quantitative gene expression analysis for Strα8, Rec8, Crabp1, RARα, RARβ, RARγ, Oct4 and β-actin.

Results: RA treatment significantly enhanced the transcriptional levels of Strα8, RARβ, RARγ, and Rec8 approximately 2 to 7-folds compared to the control group in both XX and XY PGCs. In XX PGCs, RA increased RARα up to 3 to 4-folds, but not in XY PGCs. Interestingly, Strα8, RARα, RARβ, RARγ, and Rec8 expressions were strongly suppressed under the RA+U0126 condition compared to the RA treatment, but Crabp1 and Oct4 had no significant change. Similarly, the transcriptional levels in both Crabp1 and Oct4 were not changed in culture with and without RA in both XX and XY PGCs.

Conclusions: Our results indicate that the expression of Strα8, RARβ, RARγ, and Rec8 are regulated by the RA-stimulated ERK1/2 pathway in both XX and XY PGCs. The RARα transcription was controlled by the RA-stimulated ERK1/2 pathway only in XX PGCs. In contrast, the transcriptional expression of Crabp1 and Oct4 are not regulated by the RA-stimulated ERK1/2 pathway in both XX and XY PGCs. Future studies include testing more RA-target genes in order to create a clearer overall picture of the mechanism of meiosis entry in PGCs.

(Co-Investigator: Sung-Min Kim, Ph.D. and Lydia McAllister. This study is supported by NIH R01HD078679 to YY.)
POSTER #24

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UTILIZING MINI CASE STUDIES TO ENHANCE USMLE MICROBIOLOGY AND IMMUNOLOGY CONCEPTS

Objective: The United States Medical Licensing Examination (USMLE) is a three-step examination that assesses a medical student’s ability to apply knowledge, concepts, and principles that demonstrate effective patient care.

Methods: Improve medical student success in microbiology and immunology through a series of 25 mini case studies reviewing the content of bacteriology, parasitology, and virology. The microbiology and immunology concepts are assessed and evaluated through student knowledge of the following concepts:

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CHARACTERISTICS OF PATIENTS EVALUATED FOR A RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED AND DELAYED-START CLINICAL TRIAL TO INVESTIGATE THE SAFETY AND EFFICACY OF AN INVESTIGATIONAL PRODUCT IN MILD ALZHEIMER’S DISEASE DEMENTIA

OBJECTIVES: The Center for Healthy Aging, Memory and Brain Health at Hawaii Pacific Neuroscience is one of the selected sites in the US currently conducting a randomized, double-blind, placebo-controlled and delayed-start study of the investigational product for the treatment of patients with Mild Alzheimer’s Disease Dementia. The investigational product is a human Beta-site amyloid precursor protein-cleaving enzyme 1, inhibiting beta secretase BACE from cleaving and preventing the production of Aβ plaque. The primary objective was to describe the patient population suited for this study.

METHODS: A systematic retrospective review was performed on patients referred to Hawaii Pacific Neuroscience between January 2010 and July 2017. The inclusion criteria were: ≥55 years old male or female patient with primary caregiver, diagnosis of probable Alzheimer’s disease, and MMSE score or MoCA converted MMSE score. Data was extracted from patient charts using ICD-10 codes for dementia. Data was analyzed via Chi-Square Test.

RESULTS: Of 156 patients, 61 were male (39.1%) and 95 were female (60.9%). Of the total sample population, 46 were Asian (29.5%), 44 were Caucasian (28.2%), and 31 were Pacific Islander (19.9%). Of the four AD medications, Donepezil (52.8%) was the most common, followed by Memantine (37.3%), Rivastigmine (8.1%), and Galantamine (1.9%). Alongside Alzheimer’s Disease, Vascular Dementia (53) was common among this patient population, followed by Mixed Dementia (48), Parkinson’s Disease (12), Fronto-temporal Dementia (7), and Lewy Body Dementia (4). From 156 patients, Asians (32) portrayed MMSE scores indicating Mild Cognitive Impairment and Severe Cognitive Impairment, followed by Pacific Islanders (27), Caucasians (24) and Other Minorities (9). Pacific Islanders (.037427) portrayed statistical significance in AD patients with MMSE scores indicating MCI. Caucasians (.005515) and Pacific Islanders (.025159) revealed statistical significance in AD patients with MMSE scores indicating MCI and SCI.

CONCLUSIONS: Statistical analysis shows that there is a significant correlation between Pacific Islanders (.037427) and MMSE scores indicative of Mild Cognitive Impairment, while there is no significant correlation to any other ethnicity within this patient population. With Hawaii’s diverse community, this correlation may be attributed to differences in lifestyle between Pacific Islanders and the other ethnicities represented. Mild and Severe Cognitive Impairments have a significant correlation with the Caucasian (.005515) and Pacific Islander (.025159) patients. The correlation between Pacific Islanders and Cognitive Impairment support the theoretical idea of lifestyle differences within this ethnically diverse population, which makes them predisposed to developing Cognitive Impairment.

(Alec Sheppard, Richard Ho, William Lew, Daniel Ota, Mitsuki Ota, Adam Schadler, Jennifer Rose del Castillo, MD, Levy Jo Manuntag, MD, Kore Kai Liow, MD, FACP, FAAN)
OBJECTIVES: Anal dysplasia is a potentially chronic disease that affects HIV-seropositive and-seronegative men who have sex with men (MSM) and transgender women. Novel approaches to AIN screening could improve healthcare through access to timely care and treatment since appropriate training and equipment are currently required for screening and follow-up. Recently, Raman spectroscopy (RESpect), a laser-based technology, has identified unique anal tissue fingerprints. We assessed anal tissue for RESpect phenotypes for differences in HIV-serodiscordant couples.

METHODS: HIV-serodiscordant couples were enrolled in a clinical study to assess anal biopsy specimens as per IRB guidelines. Anal tissue was flash-frozen and mounted onto aluminum reflective slides and subjected to RESpect point scans accumulations. RESpect information was processed using asymmetric least squares to baseline the data and subjected to principal component analysis (PCA).

CONCLUSIONS: Data from 3 couples showed that PCA distinguishes between HIV+ and HIV-individuals of the couples. In HIV+ individuals, PCA also distinguishes AIN from normal tissue. RESpect was shown to identify not only AIN amongst all individuals but also suggested there may be a unique HIV effect in the RESpect data from anal tissue. Further work on RESpect could provide groundbreaking information towards the design of a RESpect monitoring instrument to diagnose and follow patients for AIN.

(Natalie Kamada, Cris Milne, Anupam Misra, Tayro Acosta-Maeda. Supported in part by R21CA216830 and U54MD007584)
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Kapi‘olani Medical Center for Women and Children (KMCWC)

THE PARENT/CAREGIVER KNOWLEDGE AND ATTITUDES ABOUT PEDIATRIC CLINICAL TRIALS

Background: Clinical trials (CT) are the gold standard for assessing the effectiveness and safety of treatments in clinical medicine. At least 20% of US children are affected by at least one chronic medical condition, with limited CTs focused on the etiology, prevention, and/or treatment of these illnesses. Residents of rural and underserved areas, in particular, are more likely to report limited access to and/or awareness of available CT. Thus, there is a critical need to increase the opportunity for children to participate in CTs, particularly those living in rural communities.

HIPACT or Hawaii IDeA Center for Pediatric and Adolescent Clinical Trials, one of 15 centers of the IDeA States Pediatric Clinical Trials Network, provides underserved and rural populations with access to state-of-the-art pediatric clinical trials. The University of Hawaii John A. Burns School of Medicine has partnered with Kapi‘olani Medical Center for Women and Children (KMCWC), the pediatric tertiary-care referral center for Hawai‘i, and Waianae Coast Comprehensive Health Center (WCCHC), a large community health center serving the rural west Oahu. This initial study describes a community needs assessment based at these two centers.

Objective: To assess parent/caregiver knowledge about, perceptions of, and barriers to pediatric CTs

Methods: Cross sectional study. Participants were able to speak English and parents/caregivers of children, 1 day-17 years old. They completed a 46-item survey, consisting of multiple choice and Likert 5-point response questions.

Results: 198 adult participants (WCCHC=98; KMC=100). (Table 1). The majority had never participated (88%) or been invited to participate in CTs (90%). They believed: researchers hide information from participants - 26%; researchers are motivated by their interests and not participant welfare – 17%; are worried about sharing personal information – 11%. Over 50% of parent/caregivers were interested in participating in research for themselves at both sites. However, attitudes differed between the two study sites on several other issues (Table 2). Several participants speaking English as a second language (ESL) had difficulty understanding research concepts and thus, declined to complete the survey.

Conclusion: Parent/Caregiver participants from the two populations differed in demographic factors, including mean age, ethnicity, education, and income. Participants based at the hospital site had more concerns about exposure to harmful medications or procedures while those from the rural community site were less inclined to endorse participation in research involving their children. Interestingly, both groups had similar levels of interest in research for themselves and endorsed the importance of healthcare providers supporting research for themselves or their children. Attitudes and barriers related to pediatric research for ESL parent/caregivers needs further exploration.

Table 1:

<table>
<thead>
<tr>
<th></th>
<th>WCCHC</th>
<th>KMCWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>86%</td>
<td>73%</td>
</tr>
<tr>
<td>Mean Age (Years)</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>Native Hawaiian</td>
<td>62%</td>
<td>26%</td>
</tr>
<tr>
<td>Employed</td>
<td>59%</td>
<td>80%</td>
</tr>
<tr>
<td>Single</td>
<td>41%</td>
<td>15%</td>
</tr>
<tr>
<td>College or post-graduate degree</td>
<td>14%</td>
<td>46%</td>
</tr>
<tr>
<td>Receive food stamps/EBT benefits</td>
<td>58%</td>
<td>10%</td>
</tr>
</tbody>
</table>

Table 2 – Percent in Agreement

<table>
<thead>
<tr>
<th></th>
<th>WCCHC</th>
<th>KMCWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research will give them, and their family, a chance to “give back”</td>
<td>61%</td>
<td>64%</td>
</tr>
<tr>
<td>Researchers expose participants to medicines or procedures that may be harmful</td>
<td>23%</td>
<td>39%</td>
</tr>
<tr>
<td>Feel safe if healthcare provider is involved in the research study</td>
<td>73%</td>
<td>84%</td>
</tr>
<tr>
<td>Likelihood of participating in a CT themselves</td>
<td>54%</td>
<td>58%</td>
</tr>
<tr>
<td>Likelihood of allowing their child to participate in a CT</td>
<td>33%</td>
<td>52%</td>
</tr>
<tr>
<td>Likelihood of participating if a doctor found a CT for them and recommended joining</td>
<td>58%</td>
<td>70%</td>
</tr>
<tr>
<td>Likelihood of participating if a doctor found a CT for “their child” and recommended joining</td>
<td>51%</td>
<td>76%</td>
</tr>
</tbody>
</table>

(Co-authors: Kosut J., Duke L., Easa D., Shiramizu B)
IDENTIFYING AN IMMUNOMETABOLIC PROFILE OF TYPE 2 DIABETES

Objective: Type 2 Diabetes (T2D) is a major health disparity in Hawaiʻi – approximately 1 in 3 adults are diagnosed with diabetes or prediabetes and T2D related complications is the leading cause of death. T2D is a metabolic disease characterized by high blood glucose levels. Previous studies have shown that the immune system may play an important role in the development and progression of T2D, however whether metabolic mechanisms in specific immune cell populations are altered in T2D remains unclear. In this pilot study, we aimed to test the hypothesis that the immune cell metabolism and lymphocyte frequencies are altered in diabetic compared to healthy individuals.

Methods:
We obtained cryopreserved peripheral blood mononuclear cells from de-identified individuals diagnosed with T2D (n=10) with glycolated hemoglobin (A1c) levels >6 and healthy controls (n=10) from ZenBio. Metabolic profiling was performed using the Agilent Seahorse XFe96 Analyzer to measure glycolysis and mitochondrial respiration in highly viable PBMC’s. Flow cytometry was used to determine both CD4 and CD8 T cell subset frequencies. In addition to measuring T cell subset frequencies, we also assessed T cell exhaustion markers and functional T cell responses. Non-parametric Mann Whitney U test and ANOVA were used for statistical analysis.

Results/Conclusion:
We observed significant differences (p<0.05) in both mitochondrial respiration and glycolysis in PBMC cells of T2D compared to healthy controls. Basal glycolysis (p=0.003) and other measures of glycolysis were increased in T2D samples compared to healthy controls. Conversely, mitochondrial respiration was decreased in T2D PBMC’s compared to controls (p=0.03). Naïve CD8 T cell frequency was significantly decreased in individuals with T2D compared to controls (p=0.04). Interestingly, basal glycolysis significantly associated with T cell exhaustion marker TIGIT on CD8 T cells (p=0.04). Ex vivo stimulation of CD8 TIGIT+ cells indicated an increase in inflammatory response in this population of T cell (p=0.002). These preliminary results suggest a potential role for the immune system in the pathogenesis and progression of T2D, possibly through metabolic programming of T cell responses and warrant further studies.

(Acknowledgements: Glen M. Chew, Kristen L. Ewell, Lishomwa C. Ndhlovu, Michael J. Corley and Mariana Gerschenson)
POSTER #39

Jarom Pollister, Medical Fellow, (Advisor: Pamela S. Tauchi-Nishi MD)
Department of Pathology and the Queens Medical Center

URINE CYTOLOGY AND uroVysion FLORESCENCE IN-SITU HYBRIDIZATION OF BLADDER SQUAMOUS CELL CARCINOMA

Objectives: Bladder squamous cell carcinoma (SCC) has a high prevalence in the Middle East, Africa, Southeast Asia and South America, due to the endemic parasite Schistosoma haematobium. Bladder SCC in Western regions is much rarer, and is predisposed by prolonged catheterization in patients with a spinal cord injury. SCC is typically detected in urine cytology by the presence of atypical squamous cells, which can also signify either benign findings or other malignancies. UroVysion fluorescence in-situ hybridization (FISH) is a molecular-based assay designed to analyze cells in voided urine for chromosomal abnormalities characteristic of urothelial carcinoma. It is utilized to detect aneuploidy of chromosomes 3, 7 and 17, as well as homozygous loss of 9p21. With regards to bladder SCC, previous experimental investigations have used UroVysion FISH probes only on paraffin-embedded tumor tissue, rather than urinary specimens. The purpose of this study is to determine the efficacy of urinary cytology and UroVysion FISH testing in the diagnosis of bladder SCC.

Methods: We searched our laboratory database for all patients who underwent urinary cytological analysis and UroVysion FISH testing at the Queens Medical Center from January 2003 to December 2012.

Conclusion: During our 10 year study period, 26,202 urinary cytology specimens, including voided urines, bladder, ureteral, and urethral washings and brushings were found in our CoPath database. Only 2 (0.006%) cases were confirmed to be bladder SCC upon cystectomy. Urine cytology examination in both cases revealed atypical squamous cells. In one of these cases, UroVysion FISH testing demonstrated aneuploidy in 53 (33%) of 161 cells examined. Affected cells had 2 or more gains in chromosomes 3, 7 or 17 and no homozygous loss of 9p21. Our study suggests that UroVysion FISH may play a role as an ancillary test to urine cytology in the diagnosis of SCC. However, UroVysion FISH is not specific for SCC and shares chromosomal abnormalities characteristic of urothelial carcinoma. Therefore, caution should be exercised in the interpretation of a positive UroVysion FISH test in this setting.

(Knowledgements: Kanna Aoyama MS6, Abdulwahab Ewaz MD, Ricky Kaneshiro MD, Christopher Lum MD, Pamela S. Tauchi-Nishi MD)
POSTER #80

Mark Porter, MD, Resident,  (Advisor: Natascha Ching, MD)
Department of Pediatrics
Kapi‘olani Medical Center for Women and Children

THE SCOPE OF MYCOPLASMA PNEUMONIAE (MP) PNEUMONIA DIAGNOSED BY MULTIPLEX POLYMERASE CHAIN REACTION RESPIRATORY VIRAL PANEL IN PEDIATRIC PATIENTS IN HAWAII

Objectives: To understand the clinical presentation and outcomes of pediatric patients diagnosed with *Mycoplasma pneumoniae* pneumonia (MPP) by Respiratory viral panel.

Background: *Mycoplasma pneumoniae* (MP) pneumonia is classically associated with radiologic findings including bronchopneumonia, plate-like atelectasis and nodular infiltrate in school age children. The multiplex polymerase chain reaction (PCR) respiratory viral panel (RVP) test allows for rapid diagnosis of multiple viruses as well as treatable bacteria including MP, *Bordetella Pertussis* and *Chlamydia pneumoniae*.

Methods: A retrospective study was performed of patients 0-18 years old who had positive MP RVP from January 1, 2013 to June 30, 2017. Clinical cases of patients hospitalized with positive MPP testing by RVP multiplex PCR were reviewed for clinical presentation, hospital course, demographic data, clinical course, radiological imaging and laboratory data. All patients were analyzed for age, length of stay, chest x-ray (CXR) findings, available laboratory data and duration of fever/cough.

Results: A total of 4,333 respiratory viral panels were tested during the three and a half year period. Forty-nine patients were positive for MPP by RVP. In regards to age of patients, 27 of 49 (55%) positive for MPP were between 3rd month of life and 4 years old as compared to 22 of 49 (45%) between 5-18 years old. Of note, the majority of RVP obtained (73%) were in younger patients between the first month of life to 4 years of age. A clinical symptom of cough was noted to be present for a mean of 8.3 days before RVP collection (median 7 days; range 1 to 27 days). Fevers were present for a mean of 7.6 days before date of RVP collection (median 7 days; range none to 24 days). Of the 49 MPP patients, 16 were identified on admission to have history of wheezing and 21 of 49 patients (43 %) were treated with scheduled albuterol for wheezing. Of the MPP positive patients, 38 of 48 patients had radiological findings of a pulmonary infiltrate (not perihilar) with 30 of those 38 patients (79%) noted to have bilateral infiltrate. Antimicrobial therapy was noted to be as follows: 21 non-macrolide therapy on admission, 12 macrolide and non-macrolide therapy, 12 macrolide therapy and 4 patients on no antimicrobial therapy until positive MP RVP.

Conclusion: MPP is classically described as a pathogen impacting school-age children and adults. In our sample, patients diagnosed with MPP by molecular diagnostics were noted to have a wider distribution of ages, the majority under 4 years of age. Bilateral pulmonary infiltrates and new onset wheezing responsive to beta agonists were commonly noted in patients who had MPP. Earlier consideration of MPP as an infectious etiology should be considered in all patients, especially younger patients non-responsive to typical treatment of community acquired pneumonia in the presence of wheezing and bilateral pulmonary infiltrate.

(Co-authors: Mark James Porter, MD and Natascha Ching, MD)
To fully understand the adjuvant potential of pattern-recognition receptor (PRR) ligands, it is important to explore their effects on various B cell populations that contribute to development of the germinal center reaction. We investigated the capacity of PRR ligands to drive the maturation of human transitional B cells, a precursor population of both mature, marginal zone (MZ) and follicular (Fo) B cells. Ligands for several major PRR categories were evaluated: TLR3, TLR4, TLR7/8, TLR9, NOD1, and the C type lectin Mincle. However, in the presence of IL-4, TLR7/8 and TLR9 ligands were most effective in driving the in vitro maturation of cord blood transitional B cells into Fo B cells as measured by CD23 expression. In contrast, stimulation of transitional B cells with either TLR9 ligand or Mincle ligand+IL-4 did not favor Fo B cell maturation. In addition to supporting transitional B cell survival and maturation, IL-4 was capable of converting BCR ligation by anti-IgM to a positive transitional B cell differentiation signal that synergized with PRR ligand stimulation. Our studies also found that B-cell intrinsic effects of PRR ligands on transitional B cell maturation involve components of Notch-dependent and E protein signaling pathways. In summary, the dramatic interactions between IL-4, BCR, and PRR signaling in driving transitional B cell maturation illustrates the potential synergy that may be achieved when the ligands for these receptors are combined in a vaccine formulation. Further, these studies demonstrate the potential of these ligands to drive peripheral transitional B cell differentiation during infection or vaccination.
PERIPHERAL BLOOD MONONUCLEAR CLL TRANSMIGRATION ACROSS AN IN-VITRO-BRAIN BARRIER BILAYER MODEL

Background: HIV-1 entry into the central nervous system occurs early in infection, as HIV-1 infected monocytes traffic across the blood-brain barrier (BBB). Due to the difficulty of studying the BBB in human patients, in vitro BBB models using human cells can be used to provide insights to changes that occur in the context of HIV-1 infection and prevention via pharmaceutical interventions.

Objective: An in vitro BBB bilayer model has been optimized. This model will be used to analyze the effects of monocyte movement across the BBB using peripheral blood mononuclear cells (PBMCs) from healthy donors to provide a baseline for changes that may occur in the context of pharmaceutical interventions or HIV-1 infection.

Hypothesis: Human monocytes from freshly isolated PBMCs will transmigrate more efficiently across the BBB model compared to previously cryopreserved PBMC.

Methods: BBB wells were grown to confluence over six days. Subsequently, uninfected PBMCs were isolated from healthy volunteers and 5x10^5 cells per BBB were transmigrated for 24 hr using 100ng/mL MCP-1 as a chemoattractant for monocytes. Transmigrations of freshly isolated PBMCs were compared to previously cryopreserved PBMC. Post-transmigration integrity of BBB was assessed using 0.45% Evan’s Blue Albumin (EBA). Analysis of transmigrated cell counts and populations of CD14+, CD16+ monocytes and CD3+ T-cell populations were assessed using the Attune NxT flow cytometer and FlowJo Software. Select wells of BBB transmigrated with fresh PBMC were fixed with 4% PFA, sectioned, and characterized for expression of platelet cell adhesion molecule-1 (PECAM-1), occludin (OCC), zona occludens-1 (ZO-1), and intercellular adhesion molecule-1 (ICAM-1).

Conclusions: We have begun to optimize a PBMC transmigration assay across an in vitro BBB bilayer model and characterize the bilayer for important TJ proteins and adhesion molecules in the absence of HIV-infection or drug therapy, which will provide a paradigm for comparison in the context of HIV-1 infection or preventative therapies.

(Joanna Kettlewell)
Objectives: Cancer is the second leading cause of death in Hawaii after cardiovascular disease. Microbiological infections account for up to 20% of the global cancer burden. Hawaii has higher incidence and mortality rates compared to national averages of cancers attributed to infections. Outcomes in terms of incidence and mortality show significant racial disparities within Hawaii’s racial and ethnic populations. Bacteria and bacterial metabolites have been suggested to have a dual modulating role in carcinogenesis. The microbiota of each organ is distinct and varies by location within each organ driving functional relevant inter-individual variations and determinants of disease playing a major role in carcinogenesis even influencing the outcome of chemotherapies and immunotherapies. We wanted to examine microbial relative abundance across various cancer types to provide an improved understanding of patterns of microbial abundance and determine if there are any racial-related differences that could explain cancer disparities in Hawaii’s racial & ethnic groups.

Methods: To this end we have identified differential microbial relative abundances in a subset of solid tumors and non-tumor adjacent tissue matched read pairs. Eighty-eight stomach adenocarcinoma (STAD) and eight cervical squamous cell carcinoma & endocervical adenocarcinoma (CESC) TCGA cohort cases were evaluated. TCGA genomic sequencing data was processed through a bioinformatics pipeline designed to generate microbial profiles from DNA sequences (BAM file format) using PathoScope 2.0 and R shiny app package PathoStat. Racial-related differences were derived from TCGA specimen metadata files for both cancer cohorts being evaluated. Shannon Diversity Index was calculated to compare taxa between tumor and its paired non-tumor adjacent normal. Bacterial taxa with false discovery rate (FDR) adjusted P-value< 0.05 were considered significant differences at both genus and species level.

Results: H. pylori was the most abundant bacteria in STAD normal sequence reads. H. pylori was not present in STAD tumor reads. B. subtilis and C. acnes were abundant at similar proportions in both tumor and non-tumor sequences which may be indicative of core microbiota of these specimens. Among CESC cases, C. acnes was found in Native Hawaiian tumor and White normal cases with higher abundance among Native Hawaiian tumor derived sequences. Overall, Native American/Alaskan Native had the highest microbial diversity in normal tissue sequences compared to their tumor matched pairs (Shannon Diversity Index = 2.07 vs 0.55, respectively).

Conclusion: Differential microbial abundance can be derived from human DNA genomic sequences and may help discover racial-related differences that can be applied in the elimination or reduction of cancer disparities.

(Co-authors: Vedbar S. Khadka, Mark Menor and Youping Deng)
UNDERSTANDING FACTORS AFFECTING HEALTH PROVIDERS’ PERCEPTIONS OF PHARMACISTS’ ADOLESCENT VACCINE ADMINISTRATION

Objectives: Human papillomavirus (HPV) is a viral infection that both females and males will be exposed to in their lifetime. It is highly recommended that children ages 11 to 12 years old be vaccinated to protect them before their anticipated exposure to HPV, however vaccination uptake in Hawai‘i remains low. Due to recent legislation, pharmacies are a possible venue for adolescent vaccine administration with physician referral. This study aims to examine physicians’ awareness of this law, willingness to send adolescent patients to pharmacies, and perceptions of the role of pharmacists as this would be a key determinant for patient access to vaccines and implications for changes in pharmacist training.

Methods: In 2017, a survey was mailed to 454 physicians in Hawai‘i to examine physicians’ awareness of this law and perceptions of the role of pharmacists. Frequencies and percentages were used to compare among medical specialties of the respondents.

Conclusion: Results show potential for more physician-pharmacist collaborations such as raising awareness of the recent law and trainings for pharmacists to increase physician referrals for adolescent vaccine services in pharmacies.

(Co-investigators: Reni Soon, MD, MPH, and Carolyn Ma, PharmD)

(This project was supported by the RMATRIX II grant)
XO MALES WITH LIMITED Y CHROMOSOME GENE CONTRIBUTION DISPLAY INCOMPLETE FETAL GONADAL MASCUINIZATION

Y chromosome is required for testicular development and male fertility. Previously, we demonstrated that in the mouse only two Y chromosome genes are needed to obtain a male able to reproduce with help of assisted reproduction technologies (ART): testis determinant \textit{Sry} and spermatogonial proliferation factor \textit{Eif2s3y}. Subsequently, we showed that the function of these two genes can be replaced by transgenic overexpression of their homologues, autosomally encoded \textit{Sox9} and X-chromosome encoded \textit{Eif2s3x}. Males with Y chromosome contribution limited to 2 genes (X\textit{Eif2s3yOSry}), 1 gene (X\textit{Eif2s3yOSox9} and X\textit{Sry,Eif2s3x}) and no genes (XO\textit{Sox9,Eif2s3x}) were capable of producing haploid germ cells and father offspring after ART. However, they had decreased testis size and displayed abnormal development of seminiferous epithelium and testicular interstitium. The most severely affected were some of the XO\textit{Sox9,Eif2s3x} males; these males also had an increased expression of pro-ovary factors (\textit{FoxL2}, \textit{Wnt4}, and \textit{Rspo1}) in the testes.

We hypothesized that testicular abnormalities observed in mature males with limited Y chromosome gene contribution originate from altered pro-testis and pro-ovary factor signaling in genital ridges at the time of sex determination. To test this hypothesis, we generated timed pregnancies using crosses required to obtain X\textit{Eif2s3yOSry}, X\textit{Eif2s3yOSox9}, X\textit{Sry,Eif2s3x}, XO\textit{Sox9,Eif2s3x} (tested) and XY (control) genotypes. We collected fetuses at 12.5 days post coitum (dpc), identified their genotypes by PCR and quantitative PCR, dissected genital ridges, evaluated their morphology and anatomy, and quantified transcript expression of pro-testis and pro-ovary markers.

Morphometric analyses revealed that all XO males had feminized gonadal shape, impaired development of testis cords, and altered gonadal vasculature. \textit{Sox9} driven sex determination led to ~2-fold transcript reduction of testis development promoting factors (\textit{Amh}, \textit{Fgf9}, \textit{Cyp26b1}) and upregulation of ovarian markers (\textit{FoxL2}, \textit{Rspo1}) when compared to XY. \textit{Sry}-to-\textit{Sox9 substitution} in X\textit{Eif2s3yOSox9} and XO\textit{sox9,Eif2s3x} males resulted in ~2-4-fold alteration of \textit{Cyp26b1}, \textit{FoxL2}, and \textit{Rspo1} signaling when compared to X\textit{Eif2s3yOSry} and XO\textit{Sry,Eif2s3x} respectively. \textit{Amh} and \textit{Fgf9} expression was also negatively affected by \textit{Sox9} driven sex determination but only in X\textit{Eif2s3yOSox9} when compared to X\textit{Eif2s3yOSry} males.

This work advances the understanding of the roles of Y chromosome genes and their homologues during sex determination in the mouse. Funded by NIH HD072380 to MAW.

(Co-authors: Eglê A.Ortega, Mayumi Fernandez, Monika A. Ward)
SUBCELLULAR LOCALIZATION OF SELENOCYSTEINE LYASE: AN UNRESOLVED MYSTERY

INTRODUCTION: Selenium is an essential dietary micronutrient processed into selenocysteine (Sec), an amino acid present at the core of selenoproteins. The enzyme Sec lyase (Scly) decomposes Sec into alanine and selenide, the latter being the selenium form that is utilized to synthesize Sec to be incorporated into new selenoproteins. Mice lacking Scly (Scly KO) develop hallmarks of metabolic syndrome, such as obesity and glucose intolerance, with increases in pyruvate levels when fed a high fat diet. In silico predictions localize mouse Scly in the cell cytosol, where it would perform its function in selenium metabolism and interact with factors involved in energy metabolism. However, several reports utilizing immunofluorescence techniques have demonstrate the enzyme to localize in the nucleus.

OBJECTIVE: Our goal is to resolve the subcellular localization of Scly in the context of its effect on energy metabolism.

RESULTS: Proteomics analysis of mouse livers revealed pyruvate carboxylase (Pcx), a mitochondrial enzyme, to be an interactor of Scly. Metabolomics profiling analysis of the livers of Scly KO mice revealed enriched pyruvate and metabolites for glycine metabolism. Using confocal microscopy, we observed most Scly to reside in the nucleus with a small fraction in the cytosol. Subcellular fractionation followed by Western Blotting demonstrated Scly to localize mostly in the cytosol. CONCLUSIONS: Our results are conflicting, depending on the performed technique. Potentially, Scly can be a shuttling enzyme regulated by factors of energy metabolism. However, further studies aimed at identifying specific conditions that regulate Scly shuttling between cell compartments and analyzing other involved factors may help to clarify the relationship between selenium and energy metabolism. Ultimately, our results may improve our knowledge of the complex interconnection between selenium and metabolic disorders affecting populations with imbalances in their dietary selenium intake.

Funding: This project is supported by grant numbers U54MD007601 – Ola Hawai‘i to the University of Hawai‘i - subproject 5544 to LAS, from the National Institute on Minority Health and Health Disparities (NIMHD), and R01DK47320 to MJB from the National Institute of Diabetes and Digestive and Kidney Diseases, components of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIMHD, NIDDK or NIH.

(Co-authors: Ann C. Hashimoto, Suguru Kurokawa, Herena Ha, Wei Jia, Guoxiang Xie, Ashley Ogawa-Wong, Marla J. Berry)
POSTER #31

Alex Settle, Graduate Student, (Advisor: William Gosnell, PhD)
Department of Tropical Medicine, Medical Microbiology & Pharmacology, (Dr. William Gosnell).
Shriners Hospitals for Children, Honolulu

CHRONIC OSTEOMYELITIS IN THE PACIFIC: NEW WORLD SOLUTIONS TO AN OLD WORLD PROBLEM?

Chronic osteomyelitis, a long-term infection of the bone, persists in low and middle income countries (LMIC) as a significant contributor to morbidity and mortality rates and increased disability-adjusted life years (DALYs). Many barriers exist to prevent the management of chronic osteomyelitis in LMIC, such as cost to patient, access to trained surgeons, and distance to treatment centers. Aid agencies and philanthropic groups can help LMICs manage the burden of chronic osteomyelitis by investigating cost-effective practices for use in low resourced regions and training local health care professionals in modern surgical techniques and appropriate use of medication. Some retrospective work has reviewed modern management of osteomyelitis in the Pacific; however, enough controversy exists as to type and duration of therapy and long-term outcomes to warrant exploration of an improved approach utilizing a prospective study. An opportunity to develop such a prospective, registry-style patient database exists with the work done in the Outreach program of the Shriners Hospitals for Children Honolulu (SHCH), where long-term follow-up data of chronic osteomyelitis patients from the Pacific region can be gathered. We wish to analyze the retrospective work already published by SHCH on chronic osteomyelitis and examine the benefits of developing a prospective, ongoing patient database for chronic osteomyelitis for children in the Pacific region.

(Paul Moroz, MD, Shriners Hospitals for Children Honolulu)
POSTER #77

William J. Sherman, DO, Medical Fellow (Advisor: Catherine Uyehara, MD)
Neonatal-Perinatal Fellowship, Tripler Army Medical Center Department of Clinical Investigation

INFLAMMATORY BIOMARKER ASSOCIATION WITH PULMONARY VASOCONSTRICTION VERSUS BLOOD OXYGENATION IN A PIG MODEL OF ENDOTOXIN-INDUCED PULMONARY HYPERTENSION

Objectives: Escherichia Coli sepsis remains the most mortal cause of early-onset neonatal sepsis and is characterized by an endotoxin lipopolysaccharide (LPS)-mediated acute inflammatory response and pulmonary edema. Cytokines and other biological markers are increasingly being evaluated as potential markers for diagnosis of sepsis, monitoring, and disease severity but the timing of inflammatory changes in relation to pulmonary hypertension and pulmonary function is unclear. We used a piglet model of endotoxin (ETX)-induced pulmonary hypertension in the absence of systemic shock to test the hypothesis that vasoconstriction versus alveolar gas exchange are mediated by different biomarkers.

Methods: Anesthetized Yorkshire cross piglets (8 kg body weight) were catheterized and after hemodynamic equilibration, baseline mean arterial pressure, cardiac output, and blood gases were assessed. E. coli endotoxin/LPS was administered intravenously (N=10) and carefully titrated (7,500-35,000 units) to achieve an acute inflammatory response, ALI criteria of PaO2/FiO2 less than 350 mmHg, and pulmonary hypertension. Control pigs received normal saline (N=5). Pigs were observed for 18 hours with hemodynamic monitoring and blood was obtained every 2-4 hours for assessment of blood gases and cytokine profiles (Milliplex MAP for Luminex, MagPix). Hemodynamic measurements and cytokine levels remained constant in the control group. Pulmonary hypertension was seen in the endotoxin group as evidenced by a mean increase in Pulmonary vascular resistance to systemic vascular resistance ratio (PVR/SVR, p<0.05) from 0.2 (+/- ) to 0.45 (+/-).

Conclusion: All 13 inflammatory biomarkers examined – GM-CSF, INFγ , interleukin (IL)-1a, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF-α increased in response to the endotoxin-induced hypertension (ANOVA, p<0.05). Inflammatory marker levels were not significantly different from baseline levels within 4 hours of ETX exposure. Multiple regression analysis was performed to determine which inflammatory markers may be related to the selective pulmonary vasoactive response to ETX as indicated by the PVR to SVR ratio, versus which markers were associated with pulmonary alveolar gas exchange PaO2/FiO2. PVR/SVR was most strongly correlated with IL-6 and TNF-α and negatively correlated with IL-1ra (r=0.92, p<0.01), suggesting that IL-6 and TNF-α may be involved in mediating pulmonary vasoconstriction whereas IL-1ra antagonizes the vasoactive response to endotoxin. PaO2/FiO2 was positively correlated with IL-1a, and negatively correlated with INFγ, TNF-α, IL-2, and IL-18 (r=0.93, p<0.01). These findings demonstrate that cytokine changes that accompany endotoxin-induced pulmonary hypertension occur independent of systemic shock. Further, there appears to be specific differential mediation of pulmonary vascular response versus pulmonary gas exchange by different cytokines.

(Collaborators: Dao H Ho, Lee-Ann M Murata, Jauchia W Blythe Jillian M Piaggione, Wayne M Ichimura, Claudia A Hernandez, Rozalia Laczko, Catherine FT Uyehara)
INVESTIGATING HOW SOCIAL NETWORKS CHANGE NATIVE HAWAIIAN AND PACIFIC ISLANDER (NHPI) COMPOSITION OF GUT MICROBIOME AND CARDIOMETABOLIC HEALTH RISKS

Social networks influence individuals' choices and behaviors that either lead to unhealthy or healthy lifestyles. These networks have an effect on a wide range of obesity-related cardiometabolic health conditions, including Type 2 diabetes mellitus (DM), cardiovascular disease (CVD), and metabolic syndrome, which are more prevalent among communities comprised of Native Hawaiian and Pacific Islanders (NHPI). Recent studies suggest a link between social networks and health that are likely mediated by biological mechanisms, influencing glucose homeostasis and gut microbiome composition. However, whether and how social networks in NHPI communities influence health has never before been explored. In partnership with MAʻO Organic Farms in Waiʻanae, we are examining the extent to which behavior/lifestyle modifications mediated through their educational ‘āina-based program impacts the health of Hawaiian youth and that of their social network. Results will help MAʻO promote social nurturance and positive environments to reduce obesity risk whilst encouraging education. This study has significant implications for a wide range of programs in our lāhui that target education, economic development, environmental protection, and cultural restoration, all of which indirectly impacts health but are not necessarily designed to address it and enables indigenous-based models to be practiced, optimized, and sustained to restore equity.

(Riley Wells, James Doherty, Christian Dye, Ruben Juarez, PhD., Alika K. Maunakea, PhD.)
ANTIGEN-LOADED MICROVESICLES: A POTENTIAL STRATEGY TO DEVELOP FLAVIVIRUS VACCINE

Background: The continuous reemergence of Zika virus (ZIKV), dengue virus and West Nile virus, members of the genus Flavivirus, warrants development of vaccines to combat flavivirus-associated diseases. Flaviviruses destroy the target cells and reaches the immature dendritic cells (DCs) to spread the virus to the periphery. Also, the infected DCs secrete microvesicles (MV) loaded with viral proteins that have the potential to stimulate robust immune response for virus clearance. Their natural function as the cell-to-cell communication vessels makes them attractive for use as therapeutic shuttles to deliver antigens to the immune cells.

Objective: To identify the viral antigenic proteins [non-structural protein-1 (NS1), precursor membrane protein (prM) and envelope protein (Env)] that are naturally assembled in the MV during virus infection to serve as a potential vaccine candidate. If these viral proteins are not found in the MV, the viral proteins will be engineered to be loaded in the MV.

Methods: In this study, the MV will be biochemically purified from human HEK293T cells supernatants collected at 24 hr post-infection or -transfection. The viral proteins contained in the purified MV will be determined using immunostaining assays such as western blotting (WB) and immunofluorescence (IF).

Results and Conclusions: IF assay of WNV infected cells showed that some NS1 co-localized with the MV markers, CD63 and CD9, on the cell surface. The presence of NS1, but not the Env and prM in the MV was confirmed with the WB assay. The oligomerization of NS1 into dimer and monomer forms was detected in the cell lysate, but only the NS1 monomer was found in the MV. Interestingly, none of these viral proteins were detected in the purified MV from ZIKV-infected cells. Based on these results, it appears that the localization of NS1, Env, and prM in the MV varies among Flavivirus species. Future research direction will focus on the engineering of the virus proteins to be loaded in the MV. Subsequent investigations include using these engineered MV for in-vitro immune cell activation assays. The potential vaccine candidates will be tested in mice for protection against Flavivirus infection.

(Co-authors: Matthew Hamilton, Derek Choi, Vivek R. Nerurkar)

[Funding: Grants from Centers of Biomedical Research Excellence, National Institute of General Medical Sciences, National Institutes of Health (P30GM114737) and Institutional Funds]
POSTER #57

Taylor Sinn, Graduate Student (Advisor: Pakieli Kaufusi, PhD)

Department of Tropical Medicine, Medical Microbiology and Pharmacology,

ANTIGEN-LOADED MICROVESICLES: A POTENTIAL STRATEGY TO DEVELOP FLAVIVIRUS VACCINE

Background: The continuous reemergence of Zika virus (ZIKV), dengue virus and West Nile virus, members of the genus Flavivirus, warrants development of vaccines to combat flavivirus-associated diseases. Flaviviruses destroy the target cells and reach the immature dendritic cells (DCs) to spread the virus to the periphery. Also, the infected DCs secrete microvesicles (MV) loaded with viral proteins that have the potential to stimulate robust immune response for virus clearance. Their natural function as the cell-to-cell communication vessels makes them attractive for use as therapeutic shuttles to deliver antigens to the immune cells.

Objective: To identify the viral antigenic proteins [non-structural protein-1 (NS1), precursor membrane protein (prM) and envelope protein (Env)] that are naturally assembled in the MV during virus infection to serve as a potential vaccine candidate. If these viral proteins are not found in the MV, the viral proteins will be engineered to be loaded in the MV.

Methods: In this study, the MV will be biochemically purified from human HEK293T cells supernatants collected at 24 hr post-infection or -transfection. The viral proteins contained in the purified MV will be determined using immunostaining assays such as western blotting (WB) and immunofluorescence (IF).

Results and Conclusions: IF assay of WNV infected cells showed that some NS1 co-localized with the MV markers, CD63 and CD9, on the cell surface. The presence of NS1, but not the Env and prM in the MV was confirmed with the WB assay. The oligomerization of NS1 into dimer and monomer forms was detected in the cell lysate, but only the NS1 monomer was found in the MV. Interestingly, none of these viral proteins were detected in the purified MV from ZIKV-infected cells. Based on these results, it appears that the localization of NS1, Env, and prM in the MV varies among Flavivirus species. Future research direction will focus on the engineering of the virus proteins to be loaded in the MV. Subsequent investigations include using these engineered MV for in-vitro immune cell activation assays. The potential vaccine candidates will be tested in mice for protection against Flavivirus infection.

(Co-authors: Matthew Hamilton, Derek Choi, Vivek R. Nerurkar)

[Funding: Grants from Centers of Biomedical Research Excellence, National Institute of General Medical Sciences, National Institutes of Health (P30GM114737) and Institutional]
MARKED INCREASE IN HYPOGONADISM IN MEN ON HEMODIALYSIS AS COMPARED TO THE GENERAL POPULATION

The prevalence of male hypogonadism in the general population is 12% (Araujo et al. 2007) and approximately 20% in men over age 60. Male hypogonadism is a syndrome characterized by the failure to produce physiological concentrations of testosterone resulting in fatigue, poor concentration, depressed mood, and decreased libido, among other symptoms (Basaria et al. 2013, Bhasin et al. 2010). Chronic kidney disease (CKD) and end stage renal disease (ESRD) can be accompanied by hypogonadism (Majzoub and Shoske, 2016). Surprisingly, there is not a significant amount of literature that explores the implications of testosterone insufficiency in ESRD patients. This study seeks to identify the incidence of low testosterone in men with ESRD currently receiving hemodialysis (HD) in Hawaii. Demographic, laboratory, and clinical data were collected from the electronic medical record for 115 ESRD men at three dialysis clinics across Oahu, Hawaii. All data were analyzed using Excel. The average age of patients was 62.3 years (range, 41-87 years old). The average testosterone level was 276.70 (range, 8-738 ng/dL) with 40.87% (n=47) of patients having a testosterone level higher than the reference level of 300 ng/d and 59.13% (n=68) of patients having a testosterone level lower than the reference level of 300 ng/dL. The average hemoglobin was 11.20 g/dL (range, 7.8-14.6 g/dL) with 4.35% (n=5) of patients within the reference range of 14.0-18.0 g/dL; 95.65% (n=110) of patients were below the reference range for hemoglobin and therefore anemic. The bone marrow of ESRD patients hypofunctions due to insufficient erythropoietin (EPO) production by the kidneys, thus resulting in anemia. ESRD patients often receive epoetin alfa—a colony-stimulating factor—to increase their hemoglobin. However, testosterone similarly increases red blood cell production by stimulating EPO and by other mechanisms. With the surprisingly high level of hypogonadism in men on hemodialysis, further study is needed to determine whether testosterone replacement therapy (TRT) will improve quality of life, improve anemia and lower the need for exogenous EPO, which adds significantly to the overall cost of hemodialysis treatments.

A REVIEW OF APPROACHES USED TO INCREASE AWARENESS OF PRE-EXPOSURE PROPHYLAXIS (PrEP) IN THE UNITED STATES

Objectives: Pre-exposure prophylaxis (PrEP) is the first biomedical method with high efficacy for HIV prevention available to at-risk individuals. PrEP consists of taking a pill daily to minimize the likelihood that exposure to the virus will result in an HIV infection among individuals of HIV-negative or unknown serostatus. Few studies have examined the specific approaches that could be used to affectively increase PrEP awareness among at-risk populations. Approaches to increase PrEP awareness may include, but are not limited to, mass media campaigns, digital media (such as social media), ehealth, mhealth, and education interventions. Research is limited as to whether these approaches, and others, increase PrEP awareness, and are appropriate for specific at-risk populations. Therefore, this systematic review examined what approaches have been and are currently being used to increase PrEP awareness among at-risk populations in the United States.

Methods: We performed a literature search using multiple databases, such as PubMed, CINHAL, Web of Science, and Academic Search Complete. We included literature published between 2012 and 2017 as Truvada™ (a composite of tenofovir and emtricitabine) was not approved by the FDA for PrEP until 2012. On-going studies were searched using Federal RePORTER and were included to examine if there is a need for additional research regarding specific approaches to increase PrEP awareness by geographic area and at-risk population. International studies were excluded as PrEP has not been legally approved for use in other countries. Studies whose target populations were health care providers, primary care providers, physicians, or residents were also excluded. Any study that measured an awareness related outcome about PrEP was included, whether that was the study’s primary outcomes(s) or not.

Results: Using PRISMA guidelines, two published articles and two on-going research studies were identified that use different approaches to increase PrEP awareness. Of the two published articles, one reported findings from a test of a clinic-based educational intervention and the other from a test of a mobile app. Of the two on-going studies, one is using a mixed methods study design to develop and pilot-test a social media-based peer-led intervention (E-PrEP) to promote PrEP uptake among young men who have sex with men (MSM) of color. The second on-going study is using formative research to develop a PrEP intervention to support PrEP use among high risk women in the U.S.

Conclusion: Despite the effectiveness of PrEP in lowering HIV risk and the lack of awareness about PrEP among at-risk populations, findings suggest that research in this area is limited. Both the educational intervention and mobile health app identified in this review target MSM, as well as one of the two on-going studies. As MSM are not the only group disproportionately affected by HIV, there is a need for research to target other at-risk populations. Also, no studies included in this review were conducted in the Southern region of the U.S, though the CDC and prior research have identified individuals living in the South to be at highest risk for acquiring HIV. More research is needed to identify ways to provide PrEP messaging to hard-to-reach populations in the South. Findings also identified technology-based HIV prevention tools, such as mhealth and ehealth, as potential approaches to help increase PrEP awareness. Overall, greater efforts are needed to increase PrEP awareness among at-risk population in the United States. Future research should consider testing technology-based and educational intervention approaches to identify whether they lead to increased awareness, accurate knowledge, and uptake of PrEP, along with examining which messaging works best for specific targeted, at-risk population(s).

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PERPECTIVES OF COLLEGE STUDENTS ABOUT SEX EDUCATION IN MIDDLE AND HIGH SCHOOL

BACKGROUND
Teens in Hawaii have higher than the national average rates of pregnancy and sexually transmitted infections while also having the lowest rates of condom use in the nation. School-based sex education (sex ed) has been recognized as a key tool in improving these indicators. Since June 2015, Hawaii’s Department of Education has required public middle and high schools to provide opt-out instead of opt-in sexual health education. Currently there is no statewide assessment of sex ed curriculum quality in Hawaii. We aimed to describe the perspectives of college students regarding the content and quality of the sex ed they received in middle and high school in Hawaii to better understand deficiencies and areas for improvement.

METHODS
The Hawaii State Department of Health partnered with the University of Hawaii (UH) John A. Burns School of Medicine Division of Family Planning and the UH Women’s Center to survey college students briefly about the sex ed they received before entering college. An anonymous online survey was sent via email to 564 students enrolled in undergraduate courses across five University of Hawaii campuses from February 2017 through October 2017. Participants could decline to answer any questions they did not feel comfortable responding to and were provided a small gift card for their time.

RESULTS
A total of 307 surveys were returned. Respondents were 70% (N=214/307) female and reflected the racial diversity of Hawaii, with about 26% (N=80/307) identifying as multi-racial. The majority of respondents were between 17 and 23 (N=219/281, 78%). Among students who attended middle and/or high school in Hawaii (N=234), 64% (N=127/199) of respondents reported receiving middle school sex ed and 64% (N=136/212) reported receiving high school sex ed. Reports of receiving sex ed were roughly 65%, regardless of the type of school (public/private) or religious affiliation of the institution. Among the respondents who received no sex ed in middle or high school, the three topics indicated that would have been most helpful to cover were sexually transmitted infections (STIs) (N=28/29, 97%), birth control methods (N=26/29, 90%), and healthy relationships (N=24/29, 83%). Among those who did receive sex ed, the three topics selected as most important were birth control methods (N=162/205, 79%), STIs (N=158/205, 77%), and healthy relationships (N=153/205, 75%).

CONCLUSIONS
Respondents reported that sex ed was not uniformly provided across middle and high schools in Hawaii, irrespective of school type (public vs. private) and religious affiliation, and were able to identify topics that would be ideal to include in sex ed curricula. Additional surveying of undergraduate college students may serve as a useful approach in providing insight on sex ed efficacy, ensuring consistency of specific content and implementation of sex ed curricula statewide, and helping policy makers and educators refine sex ed policies.

(Co-authors and Acknowledgements: Tiana Fontanilla, Mary Tschann, Betty Wood and Joanne Higashi)

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Posterior #106

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The Impact of Updated Cervical Cancer Screening Guidelines in Women >65 Years of Age: A 10-Year Perspective

Objectives: Cervical cytology screening is no longer recommended in women 65 years or older, based on the joint guidelines established by the American Cancer Society (ASC), the American Society for Colposcopy and Cervical Pathology (ASCCP), and the American Society for Clinical Pathology (ASCP) in 2012. We examined the effect of implementing these guidelines in this elder population, in order to determine the number of abnormal Pap tests, precancerous lesions, and cancers that would have been missed if screening is discontinued.

Methods: This retrospective study included all Pap tests examined at the Queens Medical Center/Hawaii Pathologists Laboratory between January 2002 and December 2011. The CoPath database was searched in order to determine the prevalence of abnormal Pap tests, their correlation with histologic diagnoses, and positive predictive values (PPV) for cervical intraepithelial neoplasia (CIN) 2 or higher. Chi-square testing was performed in order to detect statistically significant differences between findings in patients 65 years and older compared to the general population (GP). Cases of malignancy in the >65 year older age group were further analyzed to determine whether the patients exhibited criteria for exclusion from cervical cancer screening, i.e. three consecutive negative Pap tests or two consecutive negative co-tests within the past ten years with the most recent test having been performed within the past five years, no history of CIN 2 or higher, and no symptoms of abnormal bleeding at the time of Pap testing. If patients 65 years or older diagnosed with cancer had presented with abnormal bleeding, these patients would likely have been investigated for cancer regardless of whether they had an abnormal Pap test.

Conclusion: From 2002-2011, a total of 1,026,470 Pap tests were examined. 92,247 (10%) were from the >65 year old age group. There were statistically significantly more cancers and glandular lesions found in the Pap tests of >65 year old patients compared to the GP. In contrast, more squamous intraepithelial and atypical squamous lesions were found in the GP compared to the older population. The PPVs for CIN 2 or higher were statistically significantly higher in the >65 year old age group compared to the GP for glandular abnormalities, including for atypical glandular cells of undetermined significance (AGUS) and adenocarcinoma. In contrast, there were no statistically significant differences between the two populations in the PPVs for CIN 2 or higher among any of the squamous lesions. Of the 92,247 patients aged 65 years or older, a total of 171 (0.19%) histologically confirmed cases of precancerous or cancerous lesions were found. If the new 2012 guidelines had been employed, 20 (11%) of these cases would have been missed, including 5 (25%) high grade squamous intraepithelial lesions (HSIL) and 14 (70%) endometrial carcinomas. Although Pap testing is not considered an effective modality for the detection of endometrial cancer, the cessation of cervical cancer screening in patients 65 years or older may lead to the delayed detection of this malignancy.

(Sachiko Shokei MS6, Kim Nagamine MD, Raynette Kaneshiro PA, Alda Lam MS2, Pamela S. Tauchi-Nishi MD)
A CASE ON NON-IMMUNOLOGIC ANAPHYLAXIS TO N-ACETYLCYSTEINE IN A PATIENT WHO OVERDOSED ON ACETAMINOPHEN

Introduction: acetaminophen toxicity is typically managed with administration of N-acetylcysteine (NAC) in order to prevent hepatic injury. While this medication is considered a gold standard of therapy, it is also associated with risk of non-immunologic anaphylaxis. We present a case of this less commonly recognized reaction to NAC and review management of acetaminophen toxicity in patients who demonstrate such a reaction.

Case presentation: A 17-year old female with a past medical history of generalized anxiety disorder, major depression, and prior suicide attempts, presented to the emergency department with elevated liver transaminases following acetaminophen overdose three days prior. NAC was administered, and within minutes the patient reported having dyspnea, diffuse pruritus, nausea, and vital signs were concerning for shock. Epinephrine, solumedrol, diphenhydramine, and famotidine, were administered with immediate symptom resolution and normalization of vital signs. Further NAC administration was withheld, and liver transaminases trended down without any complications.

Conclusion: This case highlights an example of non-immunologic anaphylaxis to a first line treatment for acetaminophen toxicity. Given the patient experienced hypotension, and had a delayed presentation with undetectable acetaminophen levels on admission, the risks outweighed the benefits of continuing NAC therapy. However if a patient were to have a less severe reaction such as pruritus without urticaria, and the likelihood of hepatotoxicity remains high, therapy is not absolutely contraindicated and should be considered.

(Co-author: Mathew Lau, MD)
POSTER #90

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X-REALITY MODEL OF A SCIATIC NERVE VARIANT WITH AN EXAMINATION OF SPINAL NERVE CONTRIBUTIONS AND PIRIFORMIS MUSCLE MASS IN A CADAVERIC SAMPLE

INTRODUCTION. Recent advances in computer technology and visualization enables realistic depictions of anatomical structures and spatial relationships within X-reality space. Anatomical variations are particularly well suited for electronic archiving. The purpose of this study was to develop a surface rendering of a sciatic nerve variant with piriformis muscle involvement as a contribution to an anatomical model repository. In addition, piriformis muscle mass was examined in the presence and absence of sciatic nerve variation to determine whether an association exists with piriformis syndrome. METHODS. Focused dissections were conducted on the lumbosacral plexus with photo documentation and subsequent formation of Augmented Reality models imported to Sketchfab, Z-Space, and Hololens. Quantitative analysis was performed through bilateral dissection of piriformis from 37 cadavers (7 variants) and mass determined (.01 mg). Statistical analysis (SPSS 24) consisted of a paired sample T-test to assess intra-cadaver dissection reliability, and independent samples T-test to assess mass differences between variant and normal cadavers. SUMMARY. A rendering of the sciatic nerve and piriformis variant was completed and accessible on personal devices and for ease of reference and education. No significant difference was found between the mass of right piriformis in normal sciatic variants (mean: 18.83g, SD: 7.64g) compared to identified variants (mean: 18.58g, SD: 5.49), t = 0.081 (p=0.336). No significant difference was found between the mass of left piriformis in normal sciatic variants (mean: 20.45g, SD: 9.2g) and in variants (mean: 19.14g, SD: 6.06g), t=0.33, p=0.204. CONCLUSIONS. X-Reality visualization was accomplished and provided a realistic model that could be manipulated and dissected manually facilitating understanding of the segmental contributions of the sciatic nerve. However, high division of sciatic nerve did not appear to affect piriformis mass.

(Co-authors: Jesse Thompson, Scott Lozanoff)
POSTER #91

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UTILITY OF ROUTINE HEAD CT TO DEFINE RADIOLOGIC INDICATORS OF FRAILTY IN OLDER TRAUMA PATIENTS

BACKGROUND:
Older trauma patients have disproportionally worse outcomes, accounting for 47% of all trauma fatalities in 2016. Sarcopenia and brain atrophy are recognized risk factors of poor clinical outcomes, and may be detectable via head computerized tomography (CT) imaging. Given that many older trauma patients undergo CT imaging as part of their initial evaluation, we hypothesize that opportunistic assessment of sarcopenia and brain atrophy via head CT may be used to predict important clinical outcomes.

METHODS:
In this retrospective cohort study, we used masseter muscle cross-sectional area and bicaudate ratio to define sarcopenia and brain atrophy, respectively. We performed measurements on adults ≥65 years, admitted to a level 1 trauma center 2011–14. Patients who died in the hospital or had head AIS scores of 3 or greater were excluded. We divided the cohort into four groups: those with neither sarcopenia nor brain atrophy (N), those with sarcopenia only (S), those with brain atrophy only (B), and those with both sarcopenia and brain atrophy (SB). One-year mortality was assessed using survival analysis.

RESULTS:
Excluding CTs with anatomical distortions, there were a total of 294 patients with adequate imaging of both bicaudate ratio and masseter cross-sectional area CT. After adjustment for age, injury severity, comorbidity, and gender, having brain atrophy alone (HR=2.8, 95% CI=1.1-7.3, p=0.003) or brain atrophy with sarcopenia (HR=3.4, 95% CI=1.4-8.5, p=0.009) was associated with a significantly increased risk of 1-year mortality.

CONCLUSIONS:
The presence of both masseter muscle sarcopenia and brain atrophy on routine head CT was associated with one-year mortality in older trauma patients. These radiologic indicators are predictive of adverse outcomes and are easily measured through standard imaging software. The results can potentially guide conversations regarding prognosis and interventions with patients and their families.

(Co-authors: C. Tanabe, M.J. Reed, K. Penn, T.N. Pham, I. Bentov, S. J. Kaplan)
Lassa virus (LASV), a member of the family Arenaviridae, is classified as a Category A Priority Pathogen by the NIAID and NIH due to its high mortality rate, ease of dissemination, and for the lack of preventive countermeasures. Although LASV is a zoonotic disease transmitted by rodents, approximately 300,000–500,000 infections are reported annually in Western Africa. Secondary transmission through nosocomial routes is also possible. Because the symptoms of Lassa fever vary and are non-specific, clinical diagnosis is often difficult, especially early in infection. Distinguishing Lassa Fever from other viral hemorrhagic fevers such as Ebola virus disease, as well as other febrile illnesses, including malaria, remains a challenge. In endemic areas, detection of the viral nucleoprotein (NP) is both a specific and practical method for diagnosis. However, due to the hazardous nature of whole-virus extract, recombinant proteins provide a low-risk option for test validation. Previous attempts to produce the NP have been hampered by the cellular toxicity and low solubility of the protein. Here we report the use of a maltose binding protein (MBP) fusion-linkage in both bacterial and insect cells to help express and purify the LASV NP.

Briefly, the coding sequence of the LASV NP gene was cloned into both a bacterial and insect cell expression vector immediately downstream of the mal gene. Purification of the MBP-LASV NP fusion protein from bacterial cells was accomplished using an amylose resin. Addition of serine protease cleaves the LASV NP from its expression tag, and the two fragments were separated using size exclusion chromatography (SEC).

Both bacterial and insect cells yielded high concentrations of a protein approximately 97-kDa in size, that is recognized by both anti-LASV NP and MBP antibody. Affinity chromatography using the amylose resin fractionated MBP-containing proteins with a high level of purity. Partial separation is achieved through the SEC. Further method optimization is required for more complete separation.

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QUALITY ASSURANCE AND PROCESS IMPROVEMENT (QAPI) CURRICULUM FOR NURSING HOMES

Background: Nursing homes are required to participate in Quality Assurance and Process Improvement (QAPI) activities for licensing. QAPI activities are also required for graduate medical education accreditation. We developed a QAPI curriculum to meet both those needs, that was first implemented in 2016-17, and then expanded in 2017-18.

Objectives: To design and evaluate a curriculum to teach Geriatric Medicine Fellows and nursing home staff about QAPI and to train nursing home (NH) staff to assess changes in patient conditions and communicate effectively with physicians.

Methods: The curriculum was designed with three components: 1) An initial four-hour introductory seminar on QAPI was held for Geriatric Medicine Fellows; 2) Fellows provided monthly staff inservices on common medical conditions in nursing homes and use of the SBAR technique from the INTERACT program (SBAR = Situation, Background, Assessment, Recommendation); and 3) Fellows monitored weekend on-call logs for quality of communication and whether nurses were using the SBAR technique as the training unfolded. We evaluated all 3 parts of the curriculum. Fellows completed 5 Knowledge questions and 12 Attitudes/Skills questions based on Inter-Professional Collaborative Practice Core Competencies before and after the four-hour introductory QAPI seminar, and also 6 months after the seminar. At the end of the fellow-led monthly inservices, NH staff answered five questions about level of comfort in managing certain conditions with the SBAR technique using a retrospective pre-post questionnaire. Weekend on-call logs were monitored for percentage of calls where nurses used the SBAR technique. We evaluated pre-post differences in scores using paired t-tests, and percent use of SBAR over time using chi square tests.

Results: Thirteen fellows participated in the QAPI project over two years. Fellows demonstrated significant increases in knowledge immediately following the training (mean score 2.15 vs. 3.92, p=0.001), with some attenuation in knowledge at 6 months post-training (3.92 immediately after training vs. 3.33 at 6 months, p=0.07). Attitudes and skills about interprofessional care significantly improved after training (mean overall score 4.11 vs. 4.61, p=0.001). The differences in retrospective pre-post scores were even stronger at 6 months follow-up (3.61 vs. 4.56, p<0.0001). NH staff attended inservices on different topics taught by fellows, and pre-post questionnaires (N=248) showed significant improvements in their self-rated comfort level managing patients, from 3.35 before to 3.96 after training, p<0.0001. Continuing education significantly increased the percentage of calls that followed the SBAR format, from 48.8% in year 1 to 71.9% in year 2 (p<0.0001).

Conclusion: We successfully implemented a QAPI curriculum using the INTERACT program for two academic years of Geriatric Medicine Fellows, with significant improvements in knowledge and skills demonstrated. Nursing home staff also gained in knowledge and confidence in use of the SBAR technique for improved communication.

(Co-authors: Michael Tom, MD; Aida Wen, MD; Cody Takenaka, MD; Eugene Lao, MD, Rajpreet Grewal, MD; Alexandra Kovaleva, MD; Monica Cheung Katz, MD; Nicole Lum, DO; Kamal Masaki, MD)
POSTER #40

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ROLE OF SELENIUM UTILIZATION IN HYPOTHALAMIC CONTROL OF ENERGY METABOLISM

Selenium (Se) is an essential trace element that is critical for human health. Widely used as a dietary supplement, it is known mainly for its antioxidant properties and is required for proper brain function, thyroid hormone metabolism, and fertility. Dietary Se is incorporated into selenoproteins in the form of the unique amino acid selenocysteine (Sec), which requires its cognate selenocysteine-tRNA (Trsp) to be synthesized. Sec residues resulting from selenoprotein degradation are further broken down in a process catalyzed by Sec lyase (Scly) and then utilized for de novo synthesis of Sec and selenoproteins. Previous characterization of Scly knockout (KO) mice in our lab revealed an increased propensity to develop metabolic syndrome (MetS). Scly KO mice also had decreased expression of several selenoproteins in the hypothalamus, a key regulator of energy homeostasis. The purpose of this project is to elucidate the mechanisms underlying the link between disrupted hypothalamic selenium utilization and the associated metabolic disturbances.

Agouti-related peptide (Agrp)-positive neurons are a nutrient-sensing hypothalamic sub-population that promote positive energy balance. We generated a mouse line with Cre-driven Agrp neuron-specific Scly KO (Scly-Agrp KO mice) to determine the contribution of these neurons to the MetS phenotype observed in whole-body Scly KO mice. We also generated Agrp-neuron specific Trsp KO mice (Trsp-Agrp KO mice) to evaluate ablation of selenoprotein synthesis within these neurons. Characterization of these mouse models involved the use of metabolic chambers that monitor parameters such as food intake and oxygen consumption. Additionally, performed a glucose tolerance test and challenged mice with the anorexigenic hormone leptin, followed by tissue harvest for immunohistochemical analysis.

Scly-Agrp KO mice had increased body weight and feeding behavior, as well as reduced glucose tolerance compared to control mice, indicating a partial re-capitulation of the whole-body Scly KO metabolic phenotype. Conversely, Trsp-Agrp KO mice had reduced body weight and improved glucose sensitivity. Moreover, Trsp-Agrp KOs exhibited increased oxygen consumption and energy expenditure compared to controls, suggesting impaired Agrp neuron function. Future characterization will investigate redox balance, Agrp neuron function, and hormone signaling in these mouse models.

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POSTER #59

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HUMAN TESTICULAR ORGANOID MODEL AS AN in vitro SYSTEM TO INVESTIGATE ZIKA VIRUS PATHOGENESIS

Zika virus (ZIKV) is an arbovirus belonging to the Flavivirus genus of the Flaviviridae family. The 2015-16 ZIKV epidemic in South America resulted in more than 1.5 million symptomatic cases. Traditionally associated with mild febrile illness, the recent outbreak brought forth newly emergent features of ZIKV disease, including neonatal microcephaly and Guillain-Barre syndrome in adults. Further, sexual transmission of ZIKV emerged as a prominent threat for disease spread to non-endemic regions, a unique concern not reported for other mosquito-borne flaviviruses. ZIKV has been detected in semen for up to 188 days after symptoms onset and has been demonstrated to potentially affect fertility, indicating that the virus establishes persistence in the testes. With a lack of relevant animal models to study ZIKV pathogenesis, an in vitro human model system which incorporates multiple testicular cell types, is considered ideal to recapitulate testis function and to investigate the pathogenic features, including persistence, of ZIKV infection in the human testes. Our recently developed human testicular organoid (hTO) model, consisting of multiple testicular cell types, is shown to produce testosterone continuously and to partially support early stages of spermatogenesis. Thus, our objective here was to evaluate the hTO model as an in vitro system to study ZIKV pathogenesis. hTO were infected with an epidemic ZIKV strain PRVABC59 and then subsequently assessed for virus replication, hTO viability, and hTO function post-infection. We found that hTO supported productive ZIKV replication over the time course of infection, which resulted in reduced hTO survival and function. Collectively, our results indicate that hTO can be used as a relevant model to study ZIKV pathogenesis, including cellular targets, immune response, and potential effects on spermatogenesis.

(Co-authors: Daniel P. Strange, Nima P. Zarandi, Colin E. Bishop, Hooman Sadri-Ardekani, Saguna Verma)
PERCEIVED VALUE OF THE DAILY SAFETY BRIEFING

Background: Hospital-level Daily Safety Briefings (DSB) are important tools to facilitate communication and teamwork, but their value has not been rigorously studied.

Objective: To assess the value of the DSB in an absolute sense and in relationship to other patient safety activities performed within the hospital.

Methods: A prospective written survey of participants of the DSB at an urban academic medical center. Participants were unit managers, directors, vice presidents, and various other hospital leaders.

Results: 97 of 114 participants completed the survey (85%). Of all the activities rated, pre-procedural time-outs had the highest rating in terms of impact on patient safety (4.87 ± 0.50 on 5-point Likert scale). The DSB had a rating (4.44 ± 0.77) that was on par with The Joint Commission activities (4.47 ± 0.68) and higher than use of the Morse Falls Scale (4.10 ± 0.83). Overall, 95% of participants felt that DSB was effective use of everyone’s time. The top two benefits of the DSB was keeping patient safety a focus within the organization (4.54 ± 0.74) and increasing awareness about patient safety issues (4.52 ± 0.93). Those working within the organization before DSB started were more likely to find it effective compared to those starting afterwards (98% vs 91%, NS).

Conclusions: The DSB improves patient safety and is a valuable use of healthcare leader’s time.
POSTER #4

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Institute for Biogenesis Research

EFFECTS OF MATERNAL DIABETES ON PREGNANCY OUTCOMES

OBJECTIVE: Women with type 1 and type 2 diabetes mellitus (DM) are known to have increased risk of adverse perinatal outcomes, including carrying a fetus with congenital malformation, intrauterine fetal demise, intrauterine growth restriction, macrosomia, and postnatal metabolic disturbances. One mechanism of these adverse outcomes may be alterations in fetal epigenome, such as modifications in DNA methylation patterns. These alterations may originate from the oocyte of the diabetic mother, from her intrauterine environment, or from both. To distinguish between these possibilities we are using a mouse model of reciprocal embryo transfer. In this model embryos produced with oocytes from diabetic (DMOD) or non-diabetic (COD) oocyte donors are transferred to diabetic (DMS) or non-diabetic (CS) surrogate mothers. Using this model we will investigate the effects of DM on placental and fetal DNA methylation with the overall goal to identify modifiable interventions for diabetic mothers that can decrease the risk of adverse perinatal outcomes. In this preliminary study we focused on testing the effects DM on fertilization and pre- and postimplantation embryo development.

METHODS: Diabetes was induced by intraperitoneal injection of streptozotocin 200mg/kg into 5 weeks old CD-1 mice. Upon reaching sexual maturity diabetic females were used as oocyte donors for in vitro fertilization (IVF) or as surrogate mothers for embryo transfer.

RESULTS: Diabetic oocyte donors yielded similar number of oocytes after ovarian stimulation as non-diabetic females (16.8 ± 4.3 vs. 13.8 ± 3.7; P=0.61). These oocytes became fertilized and cleaved with similar efficiency (% 29.3 ± 12.4 vs. 52.2 ± 13.9; P=0.37) and had similar potential to develop to blastocyst in vitro (% 69.9 ± 18.1 vs. 55.6 ± 16.5; P=0.66) as oocytes from non-diabetic females. The diabetic surrogate mothers had similar ability to carry pregnancy as non-diabetic surrogates, evidenced as percentage of pups delivered from embryos transferred (% 51 ± 5 vs. 62 ± 8; P=0.33). Although placental weights were similar between two groups (g, 0.11 ± 0.00; P=0.81), fetal weights were decreased in offspring from diabetic females (g, 1.06 vs. 1.41 P<0.0000).

CONCLUSIONS: The diabetic status did not affect female ability to produce developmentally competent oocytes and did not interfere with ability to carry pregnancy. However, decreased body weight of fetuses that were derived from oocytes from non-diabetic females but were undergoing development in uterus of diabetic surrogate suggests that pregnancy environment is causative of adverse perinatal outcomes associated with diabetes.

(Co-authors: Yasuhiro Yamauchi, Jonathan M. Riel, Kialanei Geralde-Machida, Lauren Kim, Monika Ward)
West Nile Virus (WNV) reorganizes the ER membranes of infected cells to create unique intracellular compartments known as replication organelles (RO). These RO comprise the viral non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) for robust virus replication. NS3, a soluble viral protein localized in the cytoplasm, contains protease and helicase activities that are essential for virus replication. What is not clearly understood is how NS3 is recruited from the cytoplasm and stabilized in the RO. Studies have shown that NS2B, a membrane-associated viral protein, plays a central role in the functional activation of NS3. Therefore, the focus of our study is to examine the role of NS2B in the RO association of the NS3 protein. We analyzed the intracellular localization patterns of NS3 with or without NS2B in human epithelial cells, which mimics the initial target cells of WNV. Using high-resolution confocal microscopy, we demonstrated that NS3 localized exclusively at the ER when NS2B was provided in cis or trans, but showed diffuse cytoplasmic localization when it was expressed alone. Various biochemical assays also confirmed that NS3 was predominantly found in the ER fraction when NS2B was present. Using Förster resonance energy transfer combined with fluorescence lifetime imaging microscopy (FRET/FLIM), we were able to spatially resolve and quantify protein interaction between NS2B and NS3. Results from this study indicate that NS2B plays a direct role in recruiting NS3 to the RO. Future studies will examine other viral protein-protein interactions and evaluate inhibitors that disrupt the NS2B-NS3 complex as novel antiviral treatment.

(Coauthors: Nicholas James, Vivek R. Nerurkar and Pakieli Kaufusi)

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**DEVELOPMENT OF EXPRESSION AND PURIFICATION SYSTEMS FOR THE PREMEMBRANE PROTEIN OF ZIKA AND DENGUE VIRUSES**

Background and Objectives: Zika (ZIKV) and dengue viruses (DENV) belong to the family Flaviviridae and genus Flavivirus. The four serotypes of DENV cause the most significant arboviral disease in the tropics and subtropics, with over 390 million infections estimated to occur each year. Both ZIKV and DENV are transmitted by the same mosquito vector and are found in similar regions. Furthermore, ZIKV and DENV infections share similar presentations, ranging from asymptomatic to mild flu-like illnesses. DENV infection can proceed to much more severe disease such as dengue hemorrhagic fever or dengue shock syndrome, while ZIKV infections have been associated with Guillain Barre Syndrome, microcephaly, and other birth defects known as congenital Zika syndrome. Therefore, there is a need to distinguish the two infections, particularly in DENV and ZIKV endemic regions. While RT-PCR tests can distinguish them during the acute stage, serological tests remain as the major diagnostic tests after the acute stage. Traditional serological tests focus on the envelope (E) protein and nonstructural protein 1. The possibility that premembrane (prM) protein can be used in serodiagnosis remains unknown. Our lab has previously reported that DENV 4 prM protein alone expressed poorly, whereas co-expression with E protein increased its expression by maintaining stability (Tsai et al. 2012 PLOS One; e52600). Other studies have successfully expressed pr with StrepII and GST tags at the C- and N-termini, respectively, to aid purification and solubility. Here, we expressed and purified the recombinant pr protein of DENV1, West Nile (WNV), yellow fever (YFV), and ZIKV viruses in Drosophila S2 cells and HEK 293T cells and examined their antigenicity.

Methods: S2 cell-based constructs expressing pr containing a histidine tag (DENV1, WNV, YFV) or StrepII tag (ZIKV) at the C-terminus were cloned into a pMT/Bip vector and confirmed by sequencing of inserts. Stable S2 cell clones were established by hygromycin B selection and culture supernatants were examined by polyacrylamide gel electrophoresis (PAGE) and Western blot (WB). The pr proteins were purified through FPLC. The pr construct containing the genes encoding pr and the stem-anchor region of E protein were cloned into a pCB vector and confirmed by sequencing. After transfection to HEK 293T cells, the pr-virus-like particles (VLP) was isolated by ultracentrifugation of the supernatant and examined by PAGE and WB. The antigenicity of pr from both expression systems were tested by 3-layer ELISA.

Results and Discussion: PAGE and WB analysis of the culture supernatants from both S2 cells and HEK 293T cells revealed good expression of pr proteins. ELISA revealed that these pr proteins can be recognized by their respective anti-histidine mAb, anti-StrepII mAb, or anti-DENV prM mAb (2H2), as well as dengue or Zika-immune sera. Our findings suggest that these pr proteins are antigenic and could be used to develop serological tests for these viruses.

(Co-author: Wei-Kung Wang)
POSTER #41

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THE ROLE OF THE EXOCYST IN AMYLOID PROCESSING AND TRAFFICKING

Alzheimer’s disease is a chronic progressive neurodegenerative disorder and the leading cause of dementia. An important pathological feature of Alzheimer’s is the presence of extracellular aggregates of amyloid β (Aβ), which is a small peptide derived from orchestrated proteolysis of amyloid precursor protein (APP) via sequential cleavage by membrane-bound β- and γ-secretases. A recently reported RNAi screen identified the small GTPase Rab11 as a regulator of β-secretase trafficking and Aβ production. The large and diverse family of Rab GTPases act as directors of intracellular vesicle trafficking and are well conserved from yeast to humans. Rab11’s major effector is the exocyst, an octameric protein trafficking complex that we have long studied for its role in epithelial morphogenesis. Since APP and all its secretases are also expressed in the Madin-Darby Canine Kidney cell line (MDCK), and we already had stable exocyst-knockdown MDCK cell lines, we initially tested for differences in APP trafficking in these epithelial cells. With immunostaining, we found co-localization of APP and associated secretases and the exocyst members, and with immunoblotting from conditioned cell medium, we found evidence that APP trafficking was abnormal in our exocyst-knockdown cells. Based on the report of Rab11’s role in Aβ production and our preliminary data in epithelial cells, we hypothesize that the exocyst is a key mechanism that regulates the intracellular trafficking of either APP or its secretases in neurons and that blocking exocyst function could decrease pathogenic production of Aβ. Using SH-SY5Y human neuronal cells, we are performing loss-of-function experiments of the exocyst via siRNA knockdown of Sec10, a central stabilizing subunit, and biochemical inhibition of the exocyst by endosidin-2. With the exocyst perturbed, we will measure changes in overall APP protein levels, APP cleavage and secretase activity, soluble APP and Aβ release into conditioned cell medium, and subcellular localization of APP, Aβ, exocyst members, and secretases. We will also test for biochemical interactions between exocyst members and APP and associated proteins. Further characterization of the exocyst’s role in APP trafficking and processing could identify new therapeutic targets for Alzheimer’s disease.

(Co-Authors: Amanda J. Lee, Ross K. Villiger, Madison K. Williams, Robert A. Nichols, Ben Fogelgren)
Zika virus (ZIKV) has recently emerged as a new public health threat. ZIKV infections have caused a wide spectrum of neurological diseases, such as Guillain-Barré syndrome, myelitis, meningoencephalitis, and congenital microcephaly. No effective therapies currently exist for treating patients infected with ZIKV. Diagnosis of ZIKV infection remains difficult. ZIKV infection can be diagnosed by conducting qRT-PCR on serum specimens. However, the window of detection is small since viremia is short-lived, and usually undetectable by the end of first week after infection. Studies with ZIKV-infected patients have suggested that viral RNA persists in the urine and saliva for a longer period of time than in serum. ZIKV RNA has also been detected in semen and vaginal secretion. Our laboratory has previously demonstrated that guinea pigs infected with ZIKV display clinical signs of infection and have detectable viremia in whole blood and serum. The goal of this project is to evaluate the presence of ZIKV RNA in urine and saliva samples collected from ZIKV-infected pregnant guinea pigs at various time-points after infection. Viral RNA was extracted from all urine and saliva samples and converted to cDNA. Levels of viremia were assessed against standards using qRT-PCR. Results from this study will have significant impact on determining feasibility of using various bodily fluids tested via RT-PCR as a non-invasive method of screening for ZIKV RNA in pregnant females. Further, the data will enhance our understanding of the role of various bodily fluids in ZIKV transmission.

(Co-authors: Francine Azouz, Shannon Kutscher, Vivek R. Nerurkar and Mukesh Kumar. Department of Tropical Medicine, Medical Microbiology and Pharmacology; Pacific Center for Emerging Infectious Diseases, JABSOM)

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Allison L. Williams, Postdoctoral Fellow (Advisor: Ralph Shohet, MD)
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MYOCARDIAL INFARCTION INDUCES ALTERNATIVE SPLICING OF PYRUVATE KINASE

The goal of this study was to determine which genes undergo alternative splicing in the initial response to ischemia after myocardial infarction (MI). After ligation of the left anterior descending (LAD) artery in mice, we observed an abrupt loss of cardiac contractility and confirmed upregulation of hypoxic signaling by increased HIF1α and VEGF proteins. We then performed RNA sequencing on ischemic heart tissue at 1 day and 3 days post-injury to assess early transcriptional changes. The overall patterns of gene expression were similar to previous microarray findings and showed overlap between the two time points. We also examined individual transcript expression and identified 89 transcripts with altered splicing after MI. Of particular interest was the switch in Pkm isoform expression (pyruvate kinase, muscle). The usually predominant Pkm1 isoform was less abundant in ischemic hearts compared to sham-operated animals, while Pkm2 mRNA and cytoplasmic protein rapidly increased. The splicing factors that mediate the switch from Pkm1 to Pkm2 (hnRNPA1, hnRNPA2B1 and Ptbp1) were also upregulated. The action of pyruvate kinase is a key step in glycolysis that converts phosphoenolpyruvate to pyruvate. Pkm1 favors use of pyruvate in the TCA cycle while Pkm2 is less efficient and allows more pyruvate to contribute to glycolysis. Therefore, the upregulation of Pkm2 is likely to have important consequences for ATP synthesis in the infarcted cardiac muscle. Analysis by others of chromatin immunoprecipitation from embryonic mouse hearts for HIF1α shows binding to the Pkm gene and its splicing factors, indicating that HIF1 could directly regulate Pkm expression. We have used RNA sequencing to provide a detailed characterization of the early transcriptome after MI. From this analysis, we identified an alternative splicing event in the PKM gene that may influence the metabolic response to infarction.

(Co-authors: Vedbar Khadka, Mingxin Tang, Abigail Avelar, Mark Menor and Ralph V. Shohet)

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STAPHYLOCOCCAL AUREUS ANTIBOTIC RESISTANCE PATTERNS IN THE PEDIATRIC POPULATION IN HAWAI’I

Background: Methicillin-resistant Staphylococcus aureus (MRSA) infections have been a worldwide health concern that required the increase use of broad-spectrum antibiotics. Recently, there have been reports of increasing MRSA clindamycin-resistant (MRSA-CR) infections. Currently, there are clinical concerns regarding increasing methicillin-sensitive Staphylococcus aureus clindamycin-resistance (MSSA-CR).

Objectives: To evaluate antibiotic susceptibility patterns in pediatric invasive S. aureus infections.

Methods: A retrospective review of patients hospitalized at Kapi‘olani Medical Center for Women and Children (KMCWC) in Honolulu, Hawai‘i from January 2009 – May 2017 was performed using ICD9 and ICD10 codes correlated to Staphylococcus aureus, MRSA, MSSA, osteomyelitis, septic arthritis, bacteremia and staphylococcal scalded skin syndrome (SSSS). Cases were narrowed for patients based on cultures, clinical diagnosis and radiological documentation for patients treated for S. aureus infection with osteomyelitis, septic arthritis, SSSS, and bacteremia.

Results: 121 patients were identified, ages 7 days old to 17 years with 62% males, 38% females. 64% (73 of 121) patients were identified as Native Hawaiian Pacific Islanders (Native Hawaiian 34%, Samoan 14%, and Pacific Islanders 14%). There were 66 patients with osteomyelitis/septic arthritis, 18 patients with SSSS and 37 with bacteremia. Based on KMCWC antibiograms from 2009-2017, there was a decreasing trend in the amount of MSSA and MRSA isolates in inpatient setting and emergency departments. An increasing trend of clindamycin-resistance was noted in both MSSA and MRSA isolates. In 2009, MSSA-CR was 10%, MRSA-CR was 21% compared to 2017 MSSA-CR 16%, MRSA-CR was 27%. Of all 121 patients, 74% (89) were found with MSSA isolates, 18% (22) had MRSA isolates and 8% (10) patients had no identified S. aureus, negative cultures or no culture obtained. In regards to clindamycin resistance in all S. aureus infections, 21% of patient had resistance with the majority MSSA resistant: (18%) 22 MSSA-CR and 3% (3) MRSA-CR. Clindamycin resistance was seen in 9% of osteomyelitis patients (6% MSSA-CR vs. 3% MRSA-CR) and 16% bacteremia patients (13% MSSA-CR vs. 3% MRSA-CR). Interestingly in SSSS cases, 13 of 18 patients (72%) had MSSA-CR, 3 of 18 (17%) had MSSA-CS, and no patients had MRSA. Two patients had no S. aureus isolated in cultures.

Conclusion: MSSA infections occurred more frequently than MRSA in our patients with osteomyelitis, septic arthritis, SSSS, and bacteremia. MSSA with clindamycin resistance was present in the majority of SSSS cases. Community antimicrobial resistance patterns should be considered in decisions for empiric therapy to treat S. aureus due to concerns of increasing clindamycin resistance and shifts in MRSA & MSSA.

(Co-authors: Jamie Wong, BS and Natascha Ching, MD)

(Acknowledgements: Andrea Siu, MPH and Amy Onaka, Clinical Labs of Hawaii)
IMPLEMENTATION OF A HOSPITAL PROTOCOL TO PREVENT RETAINED VAGINAL PACKING IN OBSTETRICAL PATIENTS

Background: Retained vaginal packing can be associated with serious psychological and physical complications. The purpose of this study is to enhance patient safety by implementing an obstetrical vaginal packing protocol and to evaluate its efficacy. The study occurred in a tertiary maternity center with roughly 6,000 deliveries a year.

Methods: A vaginal packing protocol that incorporated adding a “vaginal packing” button in the delivery summary of the electronic health record that prompts a notification when the patient’s chart is subsequently opened and a note documenting the removal of the packing was instituted. A retrospective chart review of compliance with the protocol was performed on all deliveries that occurred during a 1-year period after it was implemented.

Results: Of the 6,118 deliveries, 91% (5625/6118) completed the vaginal packing section of the delivery summary. Vaginal packing was placed in 1% (63/5625) of the deliveries in which the delivery summary was completed. A note documenting removal of the packing occurred in 73% (46/63) of the deliveries. There were no cases of retained vaginal packing.

Conclusions: This is an effective and sustainable protocol to prevent retention of vaginal packing. This process could be adopted in the gynecologic setting and among many hospitals.

(Co-authors: Caroline Lau MD, Tracee Suetsugu, MD, Lynne Y. Saito-Ton, MD)
POSTER #97

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THERMODYNAMICS OF SURFACE PHASE TRANSITIONS

Objectives: Self-assembled phospholipid monolayer at the air-water interface is a well-defined model system for studying surface thermodynamics, membrane biophysics, thin-film materials and colloidal soft matters. The objective of this research is to comprehensively study the two-dimensional surface phase transitions in the dipalmitoylphosphatidylcholine (DPPC) monolayer at the air-water interface using a newly developed methodology called the constrained drop surfactometry (CDS).

Methods: CDS was used to study the isothermal, isobaric and isochoric phase transition processes of the DPPC monolayer. In-situ Langmuir-Blodgett (LB) transfer was used to transfer the monolayer from the air-water interface to a solid substrate for directly visualizing the lateral phase transition using atomic force microscopy (AFM) imaging.

Conclusion: We found that CDS is superior to the classical Langmuir balance in its capacity of leakage-proof environment and rigorous control of thermodynamic properties, including temperature (T), surface pressure (π), and molecular surface area (A) of the monolayer, thus making it an ideal alternative to the Langmuir balance for studying lipid polymorphism and surface thermodynamics. We have constructed the two-dimensional π-A-T phase diagram of the DPPC monolayer, analogous to the P-V-T phase diagram of a pure bulk substance. Our studies provide novel insights into the understanding of a wide range of physicochemical and biophysical phenomena, such as surface thermodynamics, critical phenomena, self-assembled monolayer, thin-film material, and biophysical study of pulmonary surfactants.

(Co-author: Yi Y. Zuo. Acknowledgement: This work was supported by NSF Grant No. CBET-1254795.)
POSTER #92

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CURRENT UTILIZATION OF SPORTS ULTRASOUND (SPORTS US) BY AMERICAN MEDICAL SOCIETY FOR SPORTS MEDICINE (AMSSM) PHYSICIANS

Purpose: To evaluate utilization patterns of SPORTS US within AMSSM.

Methods and Study Design: A seven-question web-based survey (available from August to November 2017) was distributed to members of AMSSM via email announcement. Results were analyzed with descriptive statistics.

Results: A total of 450 people completed the survey (response rate=15.60%). All levels of experience in practice were represented: 13.11% (n=59) fellowship year/less than one year, 14.67% (n=66) 1-3 years, 17.33% (n=78) 3-6 years, 13.56% (n=61) 6-9 years, and 41.33% (n=186) over 10 years. Top three primary board certifications were FM (n=326; 72.44%), PM&R (n=45; 10.00%), and Pediatrics (n=33; 7.33%). 80.44% (n=362) responded “yes” to using SPORTS US vs. 19.56% (n=88) responded “no”. Highest daily users were EM (76%; n=13), PM&R (54%; n=24), and FM (51%; n=167). Achilles tendon injuries (n=326; 91.83%) was the most used indication, while carpal tunnel syndrome (n=135; 38.03%) was the least used. Those in practice greater than ten years had the highest non-use rate (n = 50; 26.88%) vs. fellowship year/less than one year (n=3; 5.08%). Those not treating athletes had the lowest daily use rate (33.33%; n=10). Of those not currently using SPORTS US, 71.43% of respondents (n=60) were interested in future use. The primary barrier of non-use was cost of equipment (n=31; 36.90%).

Conclusions: A majority of physicians within AMSSM are utilizing SPORTS US in the treatment of athletes. The majority of those who are currently not using SPORTS US, would be interested in incorporating it in their practice.

Significance of Findings: SPORTS US is utilized frequently amongst AMSSM physicians. Future investigations may include exploring barriers to using SPORTS US.
Cherry Yamane, Undergraduate, (Advisor: Susana Helm, PhD)
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CULTURE- AS- INTERVENTION: A SYSTEMATIZED LITERATURE REVIEW TO UNDERSTAND INTERVENTIONS TO REDUCE SUBSTANCE USE AMONGST NATIVE HAWAIIAN ADOLESCENTS

Objective. Substance use is a significant health disparity amongst Native Hawaiians, who report more substance use, early use, and more offers to use in comparison to other ethnic groups in Hawai‘i. Therefore, to eliminate and reduce substance use, a systematized literature review was conducted to examine cultural interventions amongst Indigenous populations.

Methods. This review followed established methods of a systematized review. Step 1. Identified key search terms via selected article review. Step 2. Conducted search using these terms in PubMed Central (PMC) database which yielded a total of 908 articles. Step 3. Reviewed title and abstracts using inclusion and exclusion criteria. Step 4. Reviewed remaining full text using inclusion and exclusion criteria. Step 5 and Step 6. Future analysis to define what culture-as-intervention is based off the inclusion criteria of remaining articles and synthesizing this concept by application and clarification of this type of intervention to a local, case example, Puni Ke Ola.

Conclusion. The current status of this three-semester, public health undergraduate capstone project is focused on Step 3 (review of title and abstracts process). The three remaining steps will be completed in the summer (Step 4) and fall (Steps 5 and 6) of 2018. The expected results from the analysis (Step 5) is to define Culture-As-Intervention based on literature that fits the inclusion and exclusion criteria. For example, Indigenous ways of knowing, spirituality, physical, mental, and psychological wellbeing. Expected results from the synthesis (Step 6) is to apply this definition and evaluate this concept to a local, case study for a Native Hawaiian community. The findings from this literature review and concept analysis could be synthesized and generalizable to other Indigenous populations since the analysis draws upon literature stemming from the emergence of intercultural and intracultural themes across Indigenous groups.

(Co-authors: Susana Helm, PhD, Raissa Tanqueco, MD. This research was supported with grant funding: American Psychiatric Association Foundation, Minority Fellowship; and National Institute on Minority Health & Health Disparities, U54MD008149)
Katherine I. Yang, Graduate Student, (Advisor: Jane Chung-Do, DrPH)  
Department of Psychiatry  
Office of Public Health Studies  

ADVANCING COMMUNITY BASED PARTICIPATORY RESEARCH IN HAWAI'I

Objective. Community-based participatory research (CBPR) is recognized as an effective research approach in which academicians work in partnership with communities to address health disparities. Hawai‘i presents a research-rich opportunity for CBPR because of its ethnic diversity and geographic location, resulting in close-knit communities with unique histories and concerns. The purpose of this study was to understand the experiences of academic researchers who are conducting CBPR in Hawai‘i and their perceptions of its benefits and challenges as well as recommendations on how CBPR can continue to advance.

Methods. 12 CBPR academic researchers who are actively conducting CBPR in Hawai‘i were recruited for this study. Participants were first identified by searching peer-reviewed literature for CBPR articles in Hawai‘i and contacted. Of these consenting participants, snowball sampling was utilized to identify other CBPR researchers who were eligible and willing to participate in this study. A semi-structured interview guide was developed to assess the participants’ motivation for conducting CBPR, perceived benefits and challenges associated with CBPR, communities with whom they partnered with, and suggestions to advance CBPR in Hawai‘i. Interviews were conducted with participants and notes were taken to be used for content analysis. Common codes were grouped into themes using consensus coding and were shared with participants for their feedback and validation.

Conclusion. Four themes emerged from the participants’ responses: (1) Importance of prioritizing relationship-building, (2) Reciprocal learning and other community benefits of CBPR, (3) Navigating the tensions between CBPR and funding priorities, and (4) Building an academic setting that is conducive to CBPR. This study’s findings suggest that the quality of CBPR produced depends on commitment to relationship building, which leads to reciprocal learning between researchers and the community. However, barriers in funding mechanisms and the academic setting may impede its potential to eliminate health disparities in Hawai‘i. Increasing awareness of CBPR and its benefits as well as transforming the culture of research in all spaces where CBPR occurs may maximize CBPR’s potential and ultimately promote health equity.

(Co-Authors: Jane J. Chung-Do, DrPH, Loren Fujitani, Alyssa Foster, MSW, Shannon Mark, Yuito Okada PhD, Zeyana Saad-Jube, MPH, PhD, Fadi Youkhana, MS, Kathryn Braun, DrPH, Kevin Cassel, DrPH, Susana Helm, PhD, Lana Kaopua, PhD, Peter Mataira, PhD, Christy Nishita, PhD, Scott Okamoto, PhD, Claire Townsend, DrPH, Kristine Querishi, PhD, Karen Umemoto, PhD)
BIOPHYSICAL ASSESSMENT OF PULMONARY SURFACTANT PREDICTS THE LUNG TOXICITY OF NANOMATERIALS

Objectives: With the rapid development of nanotechnology and the increasing use of nano-enabled consumer products, there is an urgent need to develop a precautionary method for evaluating the acute lung toxicity of engineered nanomaterials (ENMs). The natural pulmonary surfactant (PS) film represents the initial barrier of nano-bio interactions in the lungs. The objective of this research is to develop a novel in vitro experimental method called the constrained drop surfactometry (CDS) capable of quantitatively evaluating the PS inhibition caused by ENMs.

Methods: Biophysical inhibition of ENMs on the natural PS was assessed with CDS under physiologically relevant conditions. Animal experiment was performed to test the lung toxicity of the ENMs. BALB/C mice (7-8 weeks old, male, with body weight around 20 g) were intratracheally instilled with the ENMs at 2.5 mg Kg⁻¹ body weight through a soft catheter. Lung tissue of the mice was isolated and fixed in 10% formaldehyde solution of phosphate-buffered saline for histological determination.

Conclusion: It was found that various ENMs at a very low concentration all increased the in vitro minimum surface tension of a modified natural PS, Infasurf. Along the lines of this surface tension increase, extensive alveolar collapse and inflammation were found in mice exposed to these ENMs in an intratracheal instillation model. We concluded that there may be a direct correlation between in vitro surface tension increase due to PS inhibition by ENMs and in vivo lung toxicity revealed by alveolar collapse and inflammation. Compared to commonly used animal models, the CDS holds great promise to be developed into an animal-free, easy-to-use and low-cost precautionary tool for predicting the acute lung toxicity of inhaled ENMs.

(Co-authors: Sijin Liu and Yi Y. Zuo. Acknowledgement: This work was supported by NSF Grant No. CBET-1604119, and the Hawaiian Community Foundation Leahi Fund to Treat & Prevent Pulmonary Diseases #16ADVC-78729.)
POSTER #93

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THE IMPACT OF CENICRIVIROC ON PLASMA IL-11 LEVELS IN HIV-INFECTED INDIVIDUALS VIRALLY SUPPRESSED ON ANTIRETROVIRAL THERAPY

Objectives: Lymph node fibrosis has been hypothesized to lead to poor CD4 count improvement following initiation of anti-retroviral therapy (ART) in HIV infected individuals. In a single arm, 24-week pilot study of CCR2/CCR5 antagonist drug Cenicriviroc (CVC) in individuals well controlled on ART, we have previously demonstrated that 24 weeks of CVC leads to a significant decline in the fibrotic marker TGF-β1. A recent Nature article suggested that IL-11 is a downstream effector of TGF-β1 in fibroblasts. Therefore, using banked plasma from the CVC pilot study, we examined serial plasma levels of IL-11 to study the potential changes following CVC treatment. We hypothesized that CVC would lead to a decrease in plasma IL-11 levels over the 24 weeks of the study.

Methods: We utilized banked plasma from entry (week 0), week 4, week 12 and week 24 of the CVC study and also assayed plasma from 5 age and gender-matched HIV sero-negative control individuals. Whole blood was collected into EDTA tubes at designated time points from each participant. Blood was processed within 1 hour of collection and plasma were cryopreserved until use. Cryopreserved plasma specimens were thawed and processed following the kit manufacturer's instructions: Human IL-11 Quantikine ELISA Kit (R&D systems). Differences between HIV+ and HIV- values were evaluated by Mann-Whitney Test, and comparison between different time points in the HIV+ participants were performed by Wilcoxon Signed Rank Test. Correlations were performed by Spearman Correlations.

Results: Plasma specimens from 17 HIV+ participants were available from the CVC study. Participants were all males with a median age of 55 years; 41.2% were Caucasian and 35.3% more than one race. Plasma was also available from 5 HIV- participants. At entry IL-11 levels did not correlate with TGF-β1 levels, p = .947. Entry HIV+ patients had significantly lower IL-11 levels compared to the HIV- group (median levels 36 pg/mL vs 53.7 pg/mL respectively = .019). Compared to entry levels, IL-11 levels following 24 weeks of CVC was significantly higher, (median week 0 level: 36.0 pg/mL vs week 24: 65.4 pg/mL, p < .001). At week 24, levels in the HIV+ patients on CVC were not significantly different from the levels in HIV negative controls (p=.140)

Conclusion: Contrary to our initial hypothesis, use of CVC over 24 weeks was associated with an increase in IL-11 levels. No correlation was seen between IL-11 levels and TGF-β1 levels. Further research is warranted to understand the implications of IL-11 following Cenicriviroc administration.

(Co-authors: Shikuma, C., Mitchell, B.)
IDENTIFICATION OF DIAGNOSTIC IncRNA BIOMARKERS IN LUNG CANCER BY INTEGRATIVE CROSS-PLATFORM DATA ANALYSES

Objectives: RNA-Seq and microarray data have been extensively used to screen IncRNAs for diagnostic biomarkers in various cancer types. However, no reports thus far have used data from these two platforms together to validate each other. This study was designed to identify IncRNA biomarkers using RNA-Seq and microarray separately, then validate data from one with another platform.

Methods: Lung cancer datasets were obtained from GEO (n = 287) and TCGA (n = 216). Microarray datasets from the same platform were merged and batch effect were removed. Only common IncRNAs in TCGA, Affymetrix and Agilent microarray datasets were used. Differentially expressed IncRNAs in tumor with respect to normal were selected from Affymetrix and TCGA datasets. Then Weka feature selection correlation method was used to find top 20 IncRNAs in each Affymetrix and TCGA datasets for further analysis. Finally, Bayesian network classifier was used in top 20 Affymetrix IncRNAs and validated in TCGA and Agilent datasets. Similar procedure, except for using Voted Perceptron for classification, was applied in TCGA and validated in Affymetrix and Agilent datasets separately.

Results and Conclusion: When using TCGA dataset as training, the sensitivity was 0.991 and specificity was 0.954. And the sensitivity and specificity for Affymetrix and Agilent validation were 0.949, 0.964 and 0.600, 0.950. When using Affymetrix as training dataset, the sensitivity was 0.971 and specificity was 0.991. The result in TCGA and Agilent as validation datasets were 0.991, 0.880 and 0.850, 0.900. IncRNAs with areas under the ROC greater than 0.8 were considered to be promising biomarkers and those were further used for function and prognostic analyses.

(Co-authors: Vedbar Khadka, Youping Deng)
UNDERGRADUATE DIVISION
PRESENTERS

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**EVALUATING BORDETELLA PERTUSSIS-SPECIFIC ANTIBODY SECRETING CELLS**

Bordetella pertussis is a gram-negative bacterium that causes the respiratory infection, pertussis, more commonly known as whooping cough. Symptoms in the early stages include a low-grade fever, runny nose, and occasional coughs. In the later stages of infection, symptoms include high pitched coughing fits, exhaustion, and vomiting. Pertussis is most severe in young children and can be fatal in infants. Despite national vaccine coverage in the United States, we are in danger of a reemergence of pertussis because of the current vaccine’s inability to maintain long-lasting immunity. After vaccination with the acellular pertussis vaccine, DTaP, antibody levels rapidly wane. This may be due to the acellular pertussis antigens’ inability to generate long-lasting B cell memory. The antigens present in the acellular pertussis vaccine include: pertussis toxin (PT), pertactin (PRN), filamentous hemagglutinin (FHA), and fimbriae (FIM). The purpose of this study is to evaluate the frequency of pertussis antigen-specific B cells in vaccinated individuals.

Human peripheral blood mononuclear cells (PBMCs) and tonsil mononuclear cells (TMCs) were obtained from volunteers who have been vaccinated with DTaP and/or recently boosted with Tdap. Total PBMCs and TMCs were isolated and cryopreserved, and thawed the day before stimulation. To determine the frequency of pertussis antigen-specific cells, cell cultures were polyclonally stimulated with different combinations of TLR ligands CPG (TLR9), R848 (TLR7) and the cytokine IL-2 for six days in 37°C. Pertussis antigen-specific IgG ELISpot assays were performed. In brief, stimulated PBMCs were incubated on polyvinylidene fluoride (PVDF) plates coated with pertussis antigens (PT, PRN, FHA). The number of spots were counted to determine the frequency of pertussis-specific antibody secreting cells. Polyclonal stimulation with CpG + IL-2 and R848 + IL-2 induced the most antibody secreting cells as compared to R848 or CpG alone. Polyclonal stimulation of PBMCs led to variable frequencies of pertussis-specific antibody secreting cells per individual. This may have been due to individual variability in circulating pertussis specific memory B cells. Most antibody secreting cells were specific to FHA and PT. Future studies will include stimulating PBMCs and TMCs with TLR ligands and pertussis antigens to see if select TLR ligands synergize with antigen-stimulation will enhance the production of pertussis antigen-specific antibodies. TLR ligands that enhance pertussis antigen-specific antibody secreting cells may be effective adjuvants to include in TdaP boosters.

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(Faculty Advisor: Dr. Sandra Chang, Graduate Student Mentor: Jourdan Posner)

POSTER #109
Objectives: Hypoxia-inducible factor 1 (HIF1) is a transcription factor that regulates the expression of target genes as part of the physiological response to hypoxic, low-oxygen level environments. HIF1 acts as a dimer, composed of HIF1α and HIF1β subunits, that binds to hypoxia-response elements (HREs) to promote transcription. Several diseases including myocardial infarction, stroke, and cancer all utilize hypoxic pathways. A new strain of transgenic mice has been generated to study the role of HIF1 in hypoxia-associated diseases. These mice express a mutated HIF1α transgene, notated as HIF1α-PPN. The PPN mutation replaces two proline and one asparagine residue with alanine residues in the HIF1α protein. These amino acids normally are hydroxylated and target the protein for degradation, but the mutations allow HIF1α to remain active. The expression of this transgene is controlled by a tetracycline inducible system under the human elongation factor promoter (EF1α), which is believed to be expressed in all cells. The goal of this study was to confirm the expression of HIF1α-PPN in the various organs of these transgenic mice.

Methods: Expression of the transgene was induced with doxycycline water for 3 or 14 days in cages of HIF1α-PPN mice and tissue from multiple organs were harvested. From these tissue samples, RNA was isolated and used for reverse transcription to produce cDNA. PCR was conducted from cDNA to evaluate expression of the transgene. Western blotting was also used to visualize the HIF1α-PPN protein on the 14 day mice.

Results: Contrary to our original hypothesis, we found that RNA and protein for the HIF1α-PPN transgene were only expressed in the testes.

Conclusion: Hypoxia is known to affect spermatogenesis. Based on our results, these mice can be used to study the role of HIF1 in sperm development.

(Co-authors: Allison Williams and Abigail Avelar. This project was supported by grants from the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS), IDeA Networks of Biomedical Research Excellence (INBRE), Award number: P20GM103466. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.)
EFFECT OF AGE ON THE PREVALENCE OF SUBMICROSCOPIC PLASMODIUM FALCIPARUM INFECTIONS IN A RURAL VILLAGE IN CAMEROON, DEPARTMENT OF TROPICAL MEDICINE, MEDICAL MICROBIOLOGY AND PHARMACOLOGY

Objectives: Plasmodium falciparum (Pf) is the causative agent of the most severe form of malaria in Sub-Saharan Africa. The gold standard for diagnosing malaria is detection of infected erythrocytes in blood smears of individuals using microscopy. However, people can have low levels of infection that are not detected by microscopy, i.e., submicroscopic cases. The submicroscopic infections are important as they complete the life cycle of Pf, making it challenging to eliminate malaria. To detect low parasitemia, molecular-based PCR-assays are reliable. The traditional primers used detect the 18S rRNA gene with 4-8 copies per genome, whereas newly-reported “ultrasensitive” primers for the genes mitochondrial cytochrome c oxidase III (cox3) and var gene acidic terminal sequence (varATS) have 20-150 and 59 copies per genome, respectively. The goal of this study was to compare three primers sets for sensitivity in detecting submicroscopic infections and determine if the prevalence of submicroscopic Pf-infections correlates with age.

Methods: DNA was isolated from archival red blood cell pellets collected from Cameroonian individuals residing in a rural village. The samples (n=150) were divided according to age groups 0-13, 14-20, 21-35, 36-60, and >60 years old. DNA samples were amplified using 18S rRNA, cox3, and varATS primers. PCR products were visualized on gels using UV. The presence of a band in the expected size was reported as positive. DNA isolated from the 3D7 lab strain, US malaria-naïve individuals, and nuclease-free water were used as the positive, negative, and non-template controls.

Conclusion: The cox3 primers had the highest sensitivity in detecting Pf-infected cases. The prevalence of submicroscopic infections increased with age. An inverse correlation between the age and the number of slide-positive Pf infections was also seen. Partial immunity obtained from the constant introduction of the parasite to the body is a possible explanation of the increase in submicroscopic infections in older age groups.

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We are grateful to the Cameroonian individuals that provided the samples. Red blood cell pellets were generously provided by Dr. Isabella A. Quakyi. Thank you to Kapiolani Community College and Dr. John Berestecky for the support in conducting this project. Thank you to Kelli Ann Zane for the help in data input.)

(Co-authors: Yukie M. Lloyd, Michael Fernandez, Bradley Thomas, Dr. Rose G.F. Leke, Dr. Diane W. Taylor)

Poster #111
ASSESSMENT OF OSMOTIC STRESS TOLERANCE IN CLAUDIN DEFICIENT C. ELEGANS ROUNDWORMS

The prevalence rate of inflammatory bowel disease (IBD) has increased from 2 million to 3 million cases in the US within the past 15 years. This inflammatory disorder of the gastrointestinal (GI) tract causes diarrhea, intestinal ulceration, abdominal pain and unintended weight loss. The GI tract is lined by epithelial cells that allow for proper ion balance in the gut and block toxins and pathogens from entering the body. The epithelial cells are connected by structures called tight junctions which are composed of claudin proteins. Degradation of tight junctions allows toxins and pathogens to enter the body, which causes the symptoms of IBD. Understanding the role of claudins in gut barrier and ion balance function will aid in the identification of potential therapeutic targets aimed at alleviating IBD.

Objective: The aim of this study is to measure the function of claudin proteins in osmotoxicity in the roundworm C. elegans. Human epithelia contain many types of claudin proteins making it difficult to study tight junctions. C. elegans express claudin (clc) genes of a similar structure to human claudins based on genomic sequencing. C. elegans represent a relatively simple experimental model consisting of five claudin genes compared to 24 genes identified in the human genome, Therefore, we will address the following hypothesis: C. elegans claudin gene product CLC-1 regulates foregut ion permeability.

Methods: Wild type C. elegans and clc-1 deficient C. elegans were assayed for osmotoxicity after exposure to different sodium chloride or potassium chloride concentrations for 24 hours. The fraction of worms resistant to osmotic stress was then recorded.

Results: An increase in osmotic tolerant fractions in clc-1 deficient animals was observed when exposed to NaCl and KCl. This posits that there is a mechanism or a difference in adaptation in the barrier function of clc-1 mutants which allows it to withstand harsh ionic environments better in comparison to wild-type C. elegans.

Conclusions: Claudins in C. elegans likely play a role in permitting ion flux into the animal. Further experimentation will be required to confirm this possibility. This data supports a model whereby CLC-1 functions to facilitate ion flux into the animal. Loss of selective permeability due to loss of CLC-1 therefore protects worms from osmotic stress. Future studies will be focused on further understanding this mechanism.

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Poster #112
SYNTHETIC PATHWAYS FOR THE PREPARATION OF 2,2,2-TRIFLUORO-N-(1-TRITYL-1H-BENZO[d]IMIDAZOL-5-YL)ACETAMIDE

The development of potent drugs for use in medicinal chemistry involves the synthesis of a wide array of slightly different, yet skeletally similar molecules called analogues. Of all the molecules screened for potency and effectiveness, few will ever be considered viable candidates. As such, a priority for medicinal chemists is the ease with which a large array of analogues can be synthesized. An effective way of achieving this is through combinatorial chemistry, a technique which produces expansive compound libraries in a short period of time. This method is suitable with the right equipment and manpower, but is less useful if one is limited to conventional laboratory syntheses. In this setting, it is preferable to have a reliable method to construct an orthogonally protected “building block”. This “building block” serves as a foundational molecule that can be selectively functionalized in numerous ways to concoct more analogues in less time. Since these building block are central to the synthesis of all other analogues, it is imperative that they be produced in high yield and with minimal synthetic steps.

To this end, we investigated three routes for synthesizing 2,2,2-trifluoro-N-(1-trityl-1H-benzo[d]imidazol-5-yl)acetamide, which will be used as a building block for the synthesis of analogues that treat human cancers associated with poor survival rates. Syntheses were performed using commercially available starting materials and catalysts. Reactions were conducted with anhydrous solvents and under an inert atmosphere of argon or nitrogen. Reaction progress was monitored by thin layer chromatography and liquid chromatography/mass spectrometry. Purifications of crude products consisted of liquid-liquid extraction followed by flash column chromatography on silica gel. Product purity and structure was then assessed using proton nuclear magnetic resonance spectroscopy.

Of the three routes investigated, none were found to be adequate for the efficient preparation of the target building block. The tautomeric behavior of the benzimidazole substrate and subsequent production of isomers resulted in low conversion to the N-trityl adduct. This was the primary obstacle of our syntheses. Other sources of low yields were poor conversion of the bromo substrate to the aniline, and possible intramolecular rearrangement of the trityl protecting group. Based on our learnings, we need to explore a different synthetic strategy in order to efficiently prepare the desired building block. Utilizing reaction conditions that are both chemoselective and regioselective seem to be the most promising approaches for improvement.

(Co-Authors: Mabel Bernaldez, Dr. Christine Brotherton-Pleiss. We thank Dr. Marcus Tius and the University of Hawai’i at Mānoa Department of Chemistry for use of their lab space, NMR, and LCMS equipment. This project was supported by grants from the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS), IDeA Networks of Biomedical Research Excellence (INBRE), Award number: P20GM103466. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.)
SYNTHESIS OF ORTHOGONALLY PROTECTED INDAZOLE AND SALICYLATE DERIVATIVES

Objectives: The purpose of this study was to explore and develop syntheses of orthogonally protected Indazole and salicylate derivatives. These derivatives will be later used for the development of anti-cancer drugs which treat human cancers with poor prognoses. These intermediates allow efficiency for medicinal chemists to study Structure Activity Relationships by allowing a large variety of analogs to be synthesized from a single foundational molecule. Therefore, these analogs should be synthesized in high yield and with minimum amount of steps. Orthogonal protecting groups are the driving force behind the usefulness of these intermediates as they allow for chemoselective modification of all reactive sites. In this study, protecting groups such as triphenylmethyl, trifluoroacetamide, benzyl ether, and allyl ester were utilized in the synthesis of 2,2,2-trifluoro-N-(5-fluoro-1-trityl-1H-indazol-6-yl)acetamide and allyl 2-(benzyloxy)-4-(2,2,2-trifluoroacetamido)benzoate.

Methods: In this study, all reactions were conducted under inert atmospheres and with dry reaction solvents. The crude products were isolated from the reaction mixture via liquid-liquid extraction and later purified by flash column chromatography. The product was then characterized by Liquid Chromatography Mass Spectrometry (LCMS) and Proton Nuclear Magnetic Resonance (H1 NMR).

Conclusion: In conclusion, the synthesis of 2,2,2-trifluoro-N-(5-fluoro-1-trityl-1H-indazol-6-yl)acetamide was accomplished in four steps with 52% yield and the synthesis of allyl 2-(benzyloxy)-4-(2,2,2-trifluoroacetamido)benzoate was also accomplished in four steps but with 28% yield. Both pathways could be improved by alkylating the substrate with protecting groups in a more selective manner.

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Poster #114
FALSE POSITIVE D-DIMERS DUE TO IN-VITRO CLOTTING

Objective: In this study, we examined the pre-analytical phase of the D-dimer test. Simulating a condition in which clotting has started in a sample tube (simulating a problem during venipuncture), the time it takes for the blood specimen to generate detectable levels of D-dimers were measured. Furthermore, we examined the effectiveness of immediate separation of serum (would be plasma collected in citrate-tube in actual setting) in preventing significant in-vitro fibrinolysis.

Method: Using a commercial D-Dimer test kit, serum samples were tested for D-Dimers at 30-minute intervals from the time of blood collection until a positive result was obtained.

Conclusion: Serum harvested from the clot tube was negative for D-dimers at 90 minutes after venipuncture. However, the serum gave a positive result after remaining in the Clot Tube for 120 minutes. Serum sample that was separated from the initial clot tube tested negative for D-dimers even at 120 minutes post venipuncture.

Thus, specimen suspected of in-vitro clotting should not be tested for D-dimers, as the test kit manufacturer recommends. A clotted sample may yield a false positive result after 90 minutes of blood collection.

(Co-authors: Erin Ebisu, Kylie Haitsuka, Eric Ichinose, Michael Isidro, Que Tram Le, Paul Nam, Ray Yamaguchi, Dick Y. Teshima)

Poster #115
THE ANTICANCER PROPERTIES OF THE FUNGAL ENDOPHYTES OF PHYSALIS PERUVIANA (POHA BERRY)

Objectives: Medicinal plants utilized by various cultures have consistently been examined as sources of lead compounds for drug development. Poha Berry (Physalis peruviana) is a widely used medicinal plant, found growing naturally in Hawai‘i and many other tropical and subtropical regions across the world. The berries contain polyphenols, withanolides, and antioxidants. They are used in traditional medicine to treat a wide scope of ailments including inflammation, diabetes, hypertension, neurological problems, and cancer. The entire plant is inedible except for the fruit. It is reasonable to hypothesize that the microbial endophytes in other aerial parts of the plant may yield beneficial compounds that are different from those already consumed through the fruits. Resident foliar endophytes purified from Poha leaves and fruits presumably participate in biochemical pathways within the plant. This project will explore the role of endophytic microorganisms in the pharmacological activities of poha berry.

Methods: Endophytes are isolated and purified from leaf segments after which they will be identified via ITS sequencing. Extracts of the pure endophytes will then be tested for the inhibition of the growth of human carcinoma cell lines LU-1 (lung), LNCaP (prostate), and MCF-7 (breast) at a concentration of 20 μg/mL using the sulforhodamine B (SRB)-based colorimetric assay. The endophytes demonstrating the most cytotoxic activity will be subjected to bioassay-directed separation and identification of their active constituents using HPLC and subsequently NMR and MS. The effect of extracts, fractions and purified compounds on important cellular signaling pathways (NF-κB, Akt, MAPK, Wnt, Notch, p53) will also be explored.

Conclusion: This project will lead to the validation of ethnomedicines, and may provide promising lead molecules for drug discovery and development. Microbial endophytes also represent the next frontier for natural product drug discovery because it can be conducted in a sustainable and eco-friendly manner.

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Poster #116
IDENTIFICATION AND ANALYSIS OF NOVEL LUCIFERASE AND OPSIN GENES IN THE BIOLUMINESCENT COPEPOD GENUS, PLEUROMAMMA

Bioluminescence, the biochemical production of light by living organisms, is produced by both terrestrial and marine species. Bioluminescence can be used for courtship, predator-prey defense mechanisms, and intra-specific communication. Because bioluminescence is the result of convergent evolution, there is a diverse range of bioluminescent compounds and reactions. The bioluminescent marine copepod genus Pleuromamma consists of 11 described bioluminescent species, with many species having multiple luciferase genes. Currently, there are no published data on opsins and incomplete data on luciferases within the genus Pleuromamma. Transcriptomic analysis of Pleuromamma species can reveal the phylogeny among opsins and luciferase genes, respectively, for both intraspecific and interspecific relationships.

RNA was extracted from three individuals each of seven Pleuromamma species - P. xiphias, P. abdominalis, P. antarctica, P. robusta, P. quadrangulata, P. gracilis, and P. piseki. Confirmation of morphological identification was done using COI sequences. Known copepod luciferase and opsin genes, specifically known Pleuromamma and Metridia luciferase and known opsins from other calanoids, were used to annotate the assembled Pleuromamma transcriptomes. Replicates permitted us to quantify luciferase and opsin expression.

Transcriptome sequencing and analysis will contribute to known luciferase findings within P. xiphias and P. abdominalis and identify novel luciferases and opsins within the Pleuromamma genus. The completed data set will allow for the phylogenetic analysis of luciferase and opsins, determination of the relationships between luciferase and opsins and examination on the origins of bioluminescence in the Pleuromamma genus.

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(Tom Iwanicki, Erica Goetze, Mireille Steck, Leocadio Blanco-Bercial, and Amy E. Maas)

Poster #117
SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHORUS IN WATER AND SOIL USING THE MOLYBDENUM BLUE METHOD

Phosphorus is the P in the NPK used as fertilizer in agricultural endeavors. It is also present in human and animal waste. This project aims to test the waters that people enjoy recreationally for phosphorus runoff from agricultural use and from human and animal waste. The objective is to determine phosphorus, and phosphorus containing pesticide and herbicide levels in water and soil samples utilizing a simple, fast, and sensitive spectrophotometric method that tests water and soil. The amount of phosphate is determined using the molybdenum blue phosphorus method with a UV-visible spectrometer. Water and soils samples will be collected from the West and East side of the Big Island of Hawaii. Some of these samples were collected by EPSCoR scientists in Big Island wells. The phosphate levels are expected to correlate with fecal bacteria levels as they both derive from human and agricultural waste. Fecal bacteria in waters used for human recreation can cause infection and disease.

I would like to thank Dr. Matthew Platz, Professor of Chemistry at UH Hilo and Project Faculty Mentor, for assistance with the application of a UV-visible spectrometer with the molybdenum blue phosphorus method, and Dr. Natalie Crist, Assistant Professor of Chemistry at UH Hilo and Project Faculty Mentor, for assistance and comments that greatly improved the Phosphorus research process. I would also like to show my gratitude to my fellow lab partners that conducted and spent many afternoons with me in the laboratory over the course of this research: Heather Henning, Skyla Lee, Clara Smith, and Veronica White.

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**EFFECTS OF HIV-TAT AND METH ON CALCIUM SIGNALING IN DOPAMINERGIC NEURONS**

The purpose of this experiment is to study the pathophysiological effects of HIV-1 Tat and methamphetamine (METH) on intracellular calcium homeostasis. HIV-Tat is a trans-activator protein that is necessary for the replication of the HIV-1 virus. This protein plays a critical role in the development of HIV-associated neurocognitive disorders (HAND). Tat has been shown to significantly increase both calcium influx and firing of action potentials, leading to hyperexcitation of neurons. METH, a potent and addictive stimulant commonly abused by HIV-infected individuals, further contributes to neurological impairment due to HIV. Activation of ionotropic glutamatergic N-methyl-D-aspartate (NMDA) receptors, voltage-gated L-type calcium channels (L-channels), and transient receptor potential canonical (TRPC) channels have been implicated in contributing to the dysregulation of intracellular calcium levels, neurotoxicity and injury or death of neurons due to HIV. Blockade of calcium channels has been shown to reduce neuronal death by decreasing the excessive rise of intracellular calcium levels. Thus, calcium channel blockers should be evaluated in treating the deleterious effects of both METH abuse and HIV infection.

We used differentiated SH-SY5Y cells as a model of dopaminergic neurons. The cells were cultured and plated in a glass-bottom Petri-dishes. All-trans retinoic acid (ATRA) was used to induce cell differentiation. Then, SH-SY5Y cells were treated with 50ng/mL Tat under 37°C and 5% CO2 conditions for 24 hours. Prior to calcium imaging, cells were loaded with 1μM FURA-2AM and incubated for 10-15 minutes in the dark at room temperature. Cells were illuminated at 340nm and 380nm wavelengths of light. Emitted light was detected above 490nm. Calcium levels inside the cells were approximated by the ratio of fluorescent signals at 340nm to 380nm. Cells were imaged under different conditions: (1) 26 minutes of saline solution and (2) 3 minutes of saline solution, 20 minutes of 50μM METH in saline solution, and 3 minutes of washout.

Our preliminary data show that exposure to METH induces calcium responses in Tat-treated cells. Other findings in our lab also show that the HIV drug, lopinavir, may reduce calcium responses. These findings are significant in that they suggest that lopinavir may both target viral replication and Tat and METH-induced calcium dysfunction.

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(Alyson Shoji and Dr. Marilou A. Andres)

Poster #119
CONSTRUCTION AND EXPRESSION OF RECOMBINANT PLASMIDS CONTAINING ZIKA VIRUS NS1 AND ENVELOPE GENES FUSED WITH BACTERIAL CYTOLYSIN A (CLYA) GENE

Background: The emergence of Zika virus (ZIKV) in the Americas and Caribbean created an urgent need for vaccines to reduce the devastating neurodevelopmental defects that occur in utero.

Objective: Create a recombinant plasmid of ZIKV NS1 and Envelope (Env) genes fused with Cytolysin A (ClyA) gene and express the gene product enriched in the bacterial microvesicles (MV).

Methods: ZIKV Env, ZIKV NS1, and ClyA genes were each PCR amplified with the appropriate primers designed to create complementary overlaps for ligation with pET-15b at the NdeI and BamH1 sites using Gibson Assembly. Resulting recombinant plasmids were transformed into E. coli DH5α cells, and the purified plasmids were sequenced for DNA sequence verification, proper size, and right open reading frame.Verified plasmids were then transformed into BL21 (DE3) cells for protein expression and western blotting (WB) assays.

Results and Conclusion: DNA sequence data confirmed that the ZIKV Env and NS1 genes were each successfully fused with ClyA gene producing recombinant plasmids, pClyA-Env and pClyA-NS1. The protein expression in BL21 (DES) was confirmed using WB. Staining of the expressed proteins with the antibodies against the ClyA protein and ZIKV proteins revealed the expected size. Subsequent assays include using these engineered MV for in-vitro immune cell activation assays. The potential vaccine candidates will be tested in mice for protection against ZIKV infection.

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Poster #120
ANTIOXIDANT ACTIVITIES OF GINGERS: ISOLATION AND CHARACTERIZATION OF ISOCORONARIN D FROM HEDYCHIUM CORONARIUM

Background: Hedychium coronarium is a perennial flower and is known as butterfly ginger, butterfly lily, cinnamon jasmine, garland flower and ginger lily. H. coronarium is cultivated throughout India, Southeast Asian countries, China, Japan, Brazil and in Hawai‘i. Objective: The goal of this research was to isolate and characterize pure chemical constituents from the rhizomes of H. coronarium for further scientific study of their bioactivities. Methods: Extraction of ground rhizomes and flash column chromatography resulted in 12 major fractions. Out of the total 12 fractions collected, fractions 10, 11 and 12, eluted with 40-50% ethyl acetate in hexane, were dried and subjected to HPLC. Normal phase HPLC with 30% ethyl acetate in hexane was used to further purify the flash column fractions. Results: The HPLC fraction collected at retention time of 31 min was found to be pure Isocoronarin D based on H-NMR.

I would like to provide a special thanks to Dr. Leticia Colmenares who facilitated and mentored me through this research. I would also like Tom Null (Ho`omaluhiaies Botanical Garden) for providing the necessary ginger samples, Wesley Yoshida (University of Hawaii at Manoa) for the NMR data, and Dr. David Horgen (Hawaii Pacific University) for the MS data.

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Poster #121
ANTIBODY DEVELOPMENT OF ANTI-EBOLA VP40 RECOMBINANT HUMANIZED MONOCLONAL ANTIBODY PRODUCTION IN DROSOPHILA SCHNEIDER 2 CELLS

Objectives: Ebola virus (EBOV), of the Filoviridae family, causes acute hemorrhagic fever in human and non-human primates with a fatality rate up to 90%. In 2014, the EBOV outbreak in West Africa caused more than 11,000 human deaths and highlights the urgent need for effective vaccines and immunotherapeutic treatments. The matrix protein (VP40) is a product of one of seven genes in the EBOV genome. Not only is this protein abundantly expressed during infection, but it plays a major role in viral assembly and budding. Therefore, Ebola VP40 protein could be a potential monoclonal antibody target for diagnostics and antibody-drug conjugates. In this study, we focus on developing a large-scale tissue culture based production platform for recombinant anti-EBOV-VP40 antibody. For this, we chose Drosophila Schneider 2 (S2) Cells for their high protein production and eukaryotic post-translational modification capabilities. Collectively, S2 cells may possess the potential to be a reliable, efficient, and rapid expression system for large scale production of mAb’s in times of a need.

Methods: The murine mAb against Ebola VP40 was previously generated using hybridoma technology. Murine heavy chain (HC) and light chain (LC) variable regions were sequenced and placed upstream of human constant domains by Mapp Biopharmaceuticals. We then inserted the construct into the pMT/BiP Drosophila Expression Vector. The S2 cells were co-transfected with HC and LC plasmids for both transient production and selection of stably transformed cells lines. The supernatant of transfected S2 cells will be collected, purified, and heavy and light chains quantified. Finally, we will characterize these human mAb for diagnostic and other purposes.

Conclusion: Anti-EBOV-VP40 humanized HC and LC were successfully modified and co-transfected into S2 cells for producing recombinant mAb. Collection of mAb for detection and full characterization of secreted IgG is still ongoing.

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Co-authors: Dr. Axel Lehrer, Dr. John Berestecky, Brien Haun

Poster #122
SEMI-SYNTHESIS OF WAIXENICIN A ANALOGS & AMINOXY-TETHERED DIELS-ALDER CYCLIZATION

Two projects were undertaken. Waixenicin A, a compound previously isolated from the soft coral Sarcothelia edmondsoni, has been demonstrated to be a highly selective inhibitor on transient receptor potential melastatin 7 (TRPM7). TRPM7 is a regulated Mg2+ channel vital to cancer cell growth and proliferation. Using hydroboration-oxidation and Grignard reactions to modify functional groups on a model compound and then waixenicin A, the project ultimately aims to produce structural analogs of waixenicin A that may have improved stability, selectivity, and potency in TRPM7 inhibition.

Numerous drugs are composed of heterocyclic compounds. In this project, a click chemistry Diels-Alder methodology was investigated to potentially synthesize complex nitrogen-containing heterocyclic compounds. Cycloalkenecarboxaldehydes are condensed with activated aminooxy-tethered dienophiles. The α,β-unsaturated oxime ethers that resulted served as substrates for the Diels-Alder cyclization trials.

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(Co-authors: Gilbert Dang, Airi Ban, Jhon Baloaloa, Jason Garcia, Proebe Gensaya, Leia Hasegawa, Natalia Jimenez, Irene Molina, Gideon Berger)

Poster #123
Presenter: Tiana Elisara  
Advisor: Dr. Vivek R. Nerurkar  
Department of Tropical Medicine and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa

REVIEW: RE-EMERGING UNDER RECOGNIZED ZOONOTIC DISEASES IN HAWAI’I

Objective: Hawai’i possesses unique ecological features, which create optimal environments for various zoonotic diseases to thrive, in particular eosinophilic meningitis (Angiostrongyliasis cantonensis), Leptospirosis (Leptospira spp.), murine typhus (Rickettsia typhi), and toxoplasmosis (Toxoplasma gondii). Zoonotic diseases constitute as infectious diseases that are transmitted to humans via animal vectors. These zoonotic diseases rely on availability of competent vectors and/or reservoir hosts as well as ample interaction with human hosts. Hawai’i makes for an excellent model due to its closed geographical system. Host associations and biogeography are two important factors that can have direct effects on the patterns of infectious zoonotic disease. We aim to review the current body of literature of these four pathogens and the diseases that they cause in regard to their emergence and presence in Hawai’i. Through this review we hope to understand the sources of these infections on the islands, develop and implement preventive measures, and direct future research on these pathogens in Hawai’i.

Method: The database searches of PubMed, University of Hawaii Online OneSearch Library, Google Scholar were conducted using the following key words: “Hawai’i”, “Pacific”, “Overview”, “Transmission”, “Competent vectors”, in numerous combinations. Local newspaper articles, news stations, and journals along with CDC and WHO articles were also reviewed.

Conclusions: Upon review of the current status of these zoonotic diseases we conclude that there is a pressing need for epidemiological investigations of the prevalence of the animal reservoirs among the different islands, both infected with these pathogens and uninfected animals. Research into the interaction between these animal reservoirs and humans can provide a basis for the management of disease risks by targeting potential transmission sources and pathways.

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(Co-author: Lauren Ching)

Poster #124
PRODUCTION OF NOVEL RED FLUORESCENT PROTEIN ISOLATED FROM A SIPHONOPHORE

Objective: Many fluorescent proteins, most famously green fluorescent protein (GFP) from the jellyfish Aequorea victoria, and their homologs from diverse marine animals are widely used as universal genetically encoded fluorescent labels. Many laboratories have focused their efforts on identification and development of fluorescent proteins with novel characteristics and enhanced properties, resulting in a powerful toolkit for visualization of structural organization and dynamic processes in living cells and organisms. Currently, there is a diverse selection available of fluorescent proteins to choose from that cover nearly the entire visible spectrum, providing numerous alternative possibilities for multicolor labeling and studies of protein interactions. Genetically encoded sensors make it possible to monitor the activity of enzymes and the concentrations of various analytes. Photoactivatable fluorescent proteins enable tracking of photolabeled molecules and cells in space and time and can also be used for super-resolution imaging. Fast-maturing fluorescent proteins, cell clocks, and timers further expand the options for real-time studies in living tissues. For this project, we seek to demonstrate protein expression for two putative open reading frames (ORFs) for an unprecedented far red, near infra-red fluorescent protein (RFP) isolated from the siphonophore Physalia physalis. The selection of these ORFs for this novel RFP are based on the results from protein purification and DNA sequencing conducted by Dr. Angel Yanagihara and bioinformatic analysis by Dr. Mahdbi Belcaid. To date, these putative genes have yet to be successfully cloned or exogenously expressed. In order to accomplish this, we have generated suitable expression vectors and stable cell lines by co-transfecting Drosophila S2 cells with the expression vector and a selectable marker gene.

Methods: Two putative red fluorescent protein (RFP) ORF’s were codon-optimized for insect cell expression and synthetic genes were cloned into the pMT/BiP expression vector and co-transfected, with an antibiotic-selection marker into Drosophila S2 cells to produce stably transformed cell lines. The RFP genes also contain a sequence to express a polyhistidine-tag. Expression is induced by addition of copper sulfate to the cultures and the his-tagged proteins will be purified by nickel-affinity chromatography.

Conclusion: The current work involves confirming the presence of recombinant RFP in the induced S2 cell supernatant and lysate by western blot detection for the polyhistidine-tag. Through production and purification of these potentially expressed novel recombinant proteins followed by characterization of their biochemical and biophysical characteristics, we hope to further expand the current repertoire of currently available fluorescent proteins.

Coauthors: Angel Yanagihara, Axel Lehrer, John Berestecky

Poster #125
Monoclonal antibodies (mAbs) are unique binding proteins that are biologically important for research, disease diagnosis, and therapy. These specific proteins are created by injecting a mouse with an antigen, isolating the antibody producing B-lymphocytes, and fusing them with cancerous cells to produce hybridomas. Once generated, it is often desirable to characterize the hybridoma antibody binding site gene sequences. However, it was found to be extremely difficult to amplify the functional genes particularly because of a conserved aberrant light chain gene from the myeloma fusion partner. We propose a method using specific endonucleases to destroy the aberrant transcripts that will help to identify and characterize the immunoglobulin (Ig) functional genes from hybridomas. Total RNA was extracted from hybridomas, and cDNA was synthesized using gene specific primers. Primer pools were created that span from either the variable heavy (VH) or variable light (VL) chain regions of the framework region (FR1) to their corresponding constant region. These primer sets were used to amplify out the functional genes through polymerase chain reaction (PCR). Amplified aberrant products were then cut with restriction enzymes specific to different complementary determining regions (CDR) on the aberrant sequences. Any remaining un-cut amplified products will then be sequenced to identify the functional gene segments. This approach of using different restriction enzymes to cut each CDR could prove to be an efficient way of isolating Ig functional genes and potentially allow for better use in downstream applications such as recombinant antibodies.

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(Brent Shigano, Matthew Tuthill, Ph.D, John Berestecky, Ph.D)

Poster #126
Objectives: Malaria is a febrile illness caused by Plasmodium falciparum. WHO estimates over 200 million cases of malaria occur in 91 countries with 445,000 deaths annually. In this study, the magnetic nanoparticle PCR enzyme linked gene assay (MELGA), was optimized to detect submicroscopic infections of P. falciparum (Pf). The assay is user-friendly, has high sensitivity, is able to detect small concentrations of Pf DNA in blood, and is semi-quantitative. Three primer sets were used with the MELGA assay to enhance sensitivity. The primer sets that were compared included 18s rRNA, mitochondrial cytochrome c oxidase III (mito cox3), and the acidic terminal sequence varATS.

Methods: P. falciparum DNA was first isolated from 150 decoded, archival human blood samples from the Simbok village located in Cameroon, West Africa. In the MELGA assay, modified forward primers covalently coupled to magnetic nanoparticles and biotinylated reverse primers were used to amplify the isolated DNA. Then, the resulting PCR products with covalently bound magnetic nanoparticle were incubated with Streptavidin-Horseradish Peroxidase. TMB ELISA solution was added and color change was observed after 8 minutes. H2SO4 stop solution was added and optical density was measured.

Conclusions: The results from the MELGA optimization assay revealed that mito cox3 and varATS primer sets were the most sensitive, detecting as little as 5.2x10-3ng/ul of P. falciparum DNA. The 18s rRNA primer followed by detecting 1.3x10-1ng/ul of Pf DNA. The results from the Simbok blood samples revealed that the mito cox3 primer set was able to detect 9.3% and 21.6% more submicroscopic infections compared to 18s rRNA and varATS, respectively. The MELGA assay is simple and user-friendly and has high sensitivity and will be useful in countries with little resources.

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Coauthors: Jovikka Antallan, Bradley Thomas, Yukie M. Lloyd, Rose G.F. Leke, Diane W. Taylor

Poster #127
TILAPIA PROBIOTICS

With nearly eighty percent of fish consumed by the general public being farm raised, the challenge is to produce healthy robust fish without the use of antibiotics and other harmful chemicals. The daunting task of yielding a high percentage of healthy hatchlings in overpopulated man-made conditions has proven to be quite exigent.

OBJECTIVE: The purpose of this research was to determine if any potential probiotic microbes could be isolated from the normal GI tract of adult farm raised tilapia. This would allow for further research as to whether these microbes could be used as markers of healthy fish, and to potentially use probiotics to supplement tilapia feed.

METHODS: Contents from the GI tract of five (5) farm raised adult tilapia which had not been fed for forty-eight hours were obtained via sterile methods. Contents procured from specimens were separated to observe microbial growth in distal versus proximal areas of the intestine. Serial dilutions were implemented, followed by the inoculation of dilutions onto various media to allow for microbial growth. The 16S rRNA gene was amplified and sequenced with the intention of identifying isolated microbes. Results were recorded and charted to visualize the varying ratios of bacteria identified.

CONCLUSION: Successful identification of microbes was accomplished. Aeromonas genus was prevalent in both the distal and proximal intestine as well as Staph and Plesiomonas Shigelloides. Remarkably, Micrococcus was only present in the proximal intestine. Ongoing research is being conducted to establish the probable application of these microbes as indicators of vigorous fish and to be used as enhancements to tilapia feed.

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Co-authors: Alyssa McDonald, Dr. Helmut Kae, Dr. Kabi Neupane, Akira “Zoe” Byerly

Poster #128
IDENTIFYING DIFFERENTIAL GENE EXPRESSION IN THE LIVER OF MALE SCLY KNOCKOUT MICE

Introduction: Selenium is a nonmetal element in with characteristics similar to sulfur and particularly useful for redox reactions. Selenide is utilized to produce the amino acid selenocysteine, which can be incorporated into selenoproteins. Selenocysteine lyase (Scly) is a protein which recycles the selenium from selenocysteine by decomposing it into selenide and alanine. When the Scly gene is disrupted, mice were observed to suffer from hyperlipidemia, hypercholesteremia, hyperinsulinemia, and other metabolic dysregulation than mice with the Scly gene. The liver is a major contributor to the homeostasis of the body’s glucose, lipid, cholesterol, and selenium levels. It is currently unclear what the affected hepatic molecular pathways are after Scly gene disruption. Purpose: Our objective is to compare the transcriptome of Scly-/- mice with control mice (C57BL6) under two different diets, containing low and adequate selenium levels. Method: Scly-/- and wildtype mice were given normal and low selenium diet. RNA sequencing (RNA-Seq) was done to determine which genes are being affected by disruption of Scly. Results: RNA-Seq data analysis is currently ongoing in collaboration with the JABSOM Bioinformatics Core. Our results will provide insights into pathways influenced by the expression of Scly. For future experiments, we plan on confirming our RNA-Seq findings with real-time PCR and Western Blots or ELISA.

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Co-authors: Ann Hashimoto, Mark Menor, Vedbar Khadka, Youping Deng, Maarit Tiirikainen, Karolina Peplowska

Poster #129
Selenium (Se) is an essential element for organisms, and Se deficiency causes neurological problems. Selenoproteins, which incorporate Se, carry out a variety of functions that include antioxidant function and metal homeostasis. We are investigating the potential role of Se and selenoenzymes glutathione peroxidases 1 (GPx1) and 4 (GPx4) in Alzheimer’s disease (AD) and other neurodegenerative disorders. We examined the relation of Se levels to intracellular zinc (Zn), as elevated Zn can increase risk of AD by increasing phosphorylated Tau (pTau) to promote neurofibrillary tangles.

To investigate Se and pTau levels, protein was extracted from neuronal SH-SY5Y cells cultured in media with different Se levels, as well as from hippocampi of mice with a genetic deletion in the Se transporter selenoprotein P (Sepp1) and control animals. The levels of pTau protein were measured by western blot with pTau-specific antibodies. To determine if Se can alter free intracellular Zn levels, SH-SY5Y cells were grown in different Se concentrations as well as other antioxidants and selenoprotein antagonists. Free intracellular Zn was measured by fluorometry using the cell-permeant indicator FluoZin-3.

SH-SY5Y cells grown in media with 0 Se levels increased phosphorylation of tau at Ser 214. Additionally, the GPx1 antagonist mercaptosuccinate (MCS) and the GPx4 antagonist RSL-3 also increased pTau S214. In Sepp1 KO mice hippocampi, which are deficient in Se levels, levels of pTau at Thr 231 and Ser 396 were significantly increased compared to the wild-type controls. Additionally, Sepp1 KO mice raised on Se-supplemented diets did not have increased pTau levels.

We have previously shown that Sepp1 KO mice have increased intracellular brain Zn levels. In SH-SY5Y cells, 0 Se conditions as well as treatment of Se-sufficient conditions with RSL-3 resulted in elevated levels of free intracellular Zn. The Zn increase from RSL-3 was reversed by treatment with vitamin E and with the GPx mimetic ebselen.

Our results indicate that Se can regulate intracellular Zn levels that impact phosphorylation of Tau, likely by controlling the levels of GPx4.

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Poster #130
Angiostrongylus cantonensis is a parasitic nematode that infects and matures in rats and is carried by intermediate hosts the most common of which in Hawai‘i is the semi slug, Parmarion martensi. An emerging disease, angiostrongyliasis, is caused when humans ingest A. cantonensis in its L3 larval stage leading to the development of eosinophilic meningitis. One source of potential transmission is through rainwater catchment systems when an intermediate host enters the system, drowns, and releases infective larvae. One study in 2010 estimated that up to 60,000 people use rainwater catchment systems in the state of Hawai‘i, most of which exist on the island of Hawai‘i. With no federal, state, or county agencies providing oversight or regulations for rainwater catchment systems, the most widely endorsed method for water treatment in the US is a series of sediment filters followed by a UV disinfection system. A survey conducted in 2015 of rainwater catchment users found that only 49% of responders had a UV disinfection system installed in their home. Results from a recent pilot study found live A. cantonensis larvae penetrate some sediment filters leaving UV disinfection systems as the last defense. However, infective L3 larvae of three species of mammalian parasitic nematodes showed mortality two to six days after prolonged UV exposure. This study is testing the effectiveness of UV disinfection systems against infectious stages of A. cantonensis larvae. Viability of the larvae after UV exposure is determined through propidium iodide (PI) assay. Briefly, uptake of PI into the larvae and its fluorescence detection is quantified and interpreted as a value for viability. Preliminary data shows elevated fluorescence in larvae six days after UV exposure. Continued monitoring of these larvae plus additional tests will strive to determine larval mortality rate. Prolonged mortality of A. cantonensis following brief radiation with a UV disinfection system may negatively impact human health. These results may also help explain why 30% of 186 individuals living in the Puna district of the Island of Hawai‘i tested positive for antibodies against an A. cantonensis antigen, yet most of these individuals were seemingly asymptomatic.

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(Lisa M. Kaluna, John Jacob, Ingo Koomoa-Lange, and Susan I. Jarvi)
SEARCHING FOR BIOAVAILABLE PHOSPHATE IN HAWAIIAN SOIL AND WATER

Phosphorus (P) is an essential element to life on Earth. The industrial use of P to sustain food production through manipulating soil fertility begins a cycle of human consumption dependent on a finite amount of P resources. This cycle also produces P waste that can be quantified in soil and water runoff. There are complications with these measurements as P exists in many forms in all parts of the cycle, from production to consumption and then to waste product. To recover the P in treated soils and prevent the eutrophication of natural waters that receive runoff, a process is needed to manage the P. Exposing the bioaccessibility of P in soils and water runoff is the first step in developing this management process. Andisols in Hawai'i are comprised of high concentrations of free short-range order iron (Fe) and aluminum (Al) due to intense soil weathering. The intensity of soil weathering (implying different magnitudes of Fe and Al) seems to have an impact on how strongly the soil adsorbs oxyanions such as phosphate. This can change the residual value of fertilizers over time. The objective of this project is to identify the bioaccessibility of phosphate in soils that received large-scale treatments of fertilizer.

Soil samples will be taken from agricultural lands of native forest along the Hamakua coast of Hawai'i Island and processed to a filtrate by the Modified Truog method. Water samples will be collected from different water sources on Hawai'i Island. The phosphate in the filtrate and water samples will be quantified by the molybdenum-blue procedure and read by UV-Vis. Eutrophication typically occurs when phosphate levels in water exceed 0.050 ppm, but studies recommend no more than 0.02 ppm of phosphate in the water as a preventative measure. It is expected that the water samples will have some phosphate from runoff, but not enough to promote eutrophication (> 0.02 ppm). It is also expected that there will be less bioavailable phosphate in the water samples when compared to the soil samples. By analyzing the bioaccessibility of phosphate in the soils and water samples, future studies can explore a relationship between the large-scale fertilizer treatments and the presence of phosphate.

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Poster #132
**THE ROLE OF TET1 IN CRANIAL NEURAL CREST CELLS AND CRANIOFACIAL DEVELOPMENT**

Insufficient closing of the neural tube in the head can lead to malformations of the brain and face. Cranial neural crest cells (CNCC) give rise to head structures during development and therefore, anything that affects the normal function of CNCC will affect the formation of craniofacial structures. There are many factors that can affect cranial neural crest cell migration and the formation of head structures. By understanding the key components, we could potentially provide a possible solution to prevent such defects in humans.

TET1 is an epigenomic modifier that plays an important part in DNA demethylation and the expression of genes. We found TET1 expressed in the neuroectoderm during neural tube closure of the head in mouse embryos through whole mount in situ hybridization and immunohistochemistry. Mice homozygous for a nonsense mutation in TET1(TET1tuft) resulted in craniofacial malformations and neural tube defects (NTDs) with a significant reduction of TET enzyme activity in their heads. Previous RNA-Seq analysis indicated certain genes associated with CNCCs were found to be downregulated in the frontonasal region during neural tube closure. Our hypothesis is that TET1 plays a role in establishing how genes are expressed in CNCCs. Our investigation attempted to demonstrate the co-localization of both TET1 and WNT1, a CNCC marker, by immunohistochemistry. We further confirmed genes associated with CNCCs, such as PDGFR and PTPN11, were downregulated in embryos homozygous for the TET1tuft allele through the use of RT-PCR.

We then sought to identify potential biomarkers for craniofacial malformations resulting from the loss of TET activity in TET1tuft virgin female mice. Through analyzing levels of serum metabolites using mass spectrometry, we found elevated levels of 3-oxoalanine (2-formylmethionine) in the serum of virgin TET1tuft mice. This metabolite is a unique post-translational modification of sulfatases that was not prevalent in age-matched wildtype background strain mice. Excess 3-oxoalanine may indicate an excess in the sulfation of proteoglycans that regulate cell signaling and migration during neural tube closure and cranial morphogenesis. Thus, 3-oxoalanine is a potential biomarker for the detection or prediction of such defects indirectly through the loss of TET1 enzyme activity.

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**Co-authors:** Guoxiang Xie, Vedbar Khadka, Wei Xia, Keith S. K. Fong

**Poster #133**
C. ELEGANS CLAUDIN PROTEINS DIFFERENTIALLY REGULATE OSMOTIC TOLERANCE ACROSS DEVELOPMENTAL STAGES

Inflammatory bowel disease (IBD) affects about 3 million adults in the US and involves dysfunction of the intestinal epithelium and its ability to maintain homeostasis within the gastrointestinal (GI) tract. Tight junctions are responsible for creating a selectively permeable barrier within the paracellular pathway. Tight junctions require claudin proteins to maintain ion balance, while blocking toxins and pathogens. Dysregulation of tight junctions has been linked to the unfavorable symptoms of IBD such as diarrhea, abdominal pain, or rectal bleeding. Fully understanding claudin proteins and their role in IBD pathogenesis may lead to therapeutic treatments for those who suffer from IBD.

Objective: The purpose of this study is to better understand the ion selectivity of claudin proteins throughout development in the roundworm C. elegans. Genomic sequencing and homology alignment show that claudin proteins are well conserved between humans and C. elegans. In comparison to mammals, C. elegans expressing a limited complement of claudin protein, provides a relatively simple platform for testing the function and ion selectivity of claudin proteins in barrier function. We therefore tested the hypothesis that earlier developmental stages of C. elegans are more osmotolerant due to changes in CLC expression.

Methodology: To examine the role of CLC-1 in osmotic stress tolerance, we measured salt toxicity between wild type and CLC-1 deficient worms. Worms were exposed to increasing sodium and potassium concentrations over a 24-hour time course and survival rates were determined. Additionally, osmotic tolerance was assessed at different development stages by subjecting worms to various concentrations of KCl and NaCl.

Results: Our findings show that CLC-1 deficient worms are more resistant to higher NaCl and KCl salt concentrations than wild-type worms that possess functional CLC-1 proteins. CLC-1 deficient worms show a higher rate of recovery from toxicity than wild-type animals when returned to a low osmotic stress environment.

Conclusion: The increased survivability and recovery shown in CLC-1 deficient worms supports the hypothesis that the CLC protein plays a role in in the paracellular pore pathway and the facilitation of ion transport. Further studies will need to be performed to support the hypothesis that CLC-1 is responsible for developmental changes in osmotic tolerance.

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Co-authors: Genardine Arizala, Brandon E. Johnson

Poster #134
Zika virus (ZIKV) is an arbovirus belonging to the Flavivirus genus of the Flaviviridae family. During the 2015-16 epidemic ZIKV emerged as sexually transmitted virus, an unexpected finding not reported for other mosquito-borne flaviviruses. The detection of ZIKV in semen and sperm for months after symptoms onset indicates the virus establishes persistent infection in the testes. Our recent work shows that human Sertoli cells (hSeC) support robust ZIKV replication for up to 9 days post-infection without compromising cell survival. In contrast, we found that human Leydig cells (hLC), another important cell type of the testes responsible for producing testosterone, does not support productive ZIKV infection. Collectively, this suggests that ZIKV has a distinct tropism for different testes cell types. Numerous reports have identified Axl, a TAM (Tyro3, Axl, Mer) receptor tyrosine kinase, as a potential entry factor for ZIKV. Published data indicates that the Axl receptor is highly expressed in hSeC whereas hLC is shown to exhibit low Axl expression. Our objective here was to test the effect of blocking Axl on ZIKV infection of hSeC. Pre-incubation of hSeC with an anti-Axl antibody that blocks ligand binding of Axl or with the Axl-specific kinase inhibitor R428 prior to ZIKV infection significantly reduces ZIKV titers in hSeC by 24 hours post-infection. Furthermore, we observed a dramatic decrease in type I interferon response in R428 treated hSeC infected with ZIKV at 24 and 72 hours post-infection. Taken together, this indicates that Axl promotes ZIKV infection in hSeC and that Axl expression may be a determining factor for testes cell tropism.

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Poster #135
TRANSFORMATION OF CAMPYLOBACTER JEJUNI BY METHYLATION OF THE PLASMID PWM1007

Objectives: Campylobacter jejuni (C. jejuni) is the leading cause of bacterial gastroenteritis. Despite being naturally competent, C. jejuni has been difficult to transform. Recently, it was discovered that this organism is able to readily take-up foreign plasmid DNA if it contains methylated RAATTY motifs. 1 To date, an Escherichia coli (E. coli) derived plasmid containing green fluorescent protein (GFP) successfully transformed two strains of C. jejuni: 81-176 and 11168. This study aimed to replicate this transformation protocol to identify potential for transformation across multiple selected strains of Campylobacter.

Methods: After the plasmid, pWM1007, containing genes encoding for GFP and kanamycin resistance was grown in E. coli, it was extracted and then methylated with EcoRI methylase. A single, isolated colony of each C. jejuni or C. coli strain was grown in a Brucella slant biphasic for 24 hours. 2µL of the methylated GFP plasmid was added to each tube and placed in a CO2 incubator for 24 hours. The following day the broth culture was spread on brucella agar plates containing 50µL of kanamycin and monitored for growth. Successful transformation was assessed using the fluorescent microscope.

Conclusion: Initially, successful transformation indicated by fluorescent expression was achieved with 2 of the strains while the 6 other strains appeared to be non-fluorescent but kanamycin resistant. However, the fluorescent strains eventually loss their fluorescence and were not able to gain back this property even after re-streaking. Loss of fluorescence could be attributed to recombination leading to loss of the GFP gene, however, PCR results showed the presence of the GFP gene in the cells. Our results indicate that the methylation of RAATTY sites does appear to facilitate the uptake of foreign DNA amongst Campylobacter, but that expression of the introduced protein is unstable and maybe unique to the various Campylobacter strains. The reasons for this are unclear and will be investigated further. Further studies and applications of Campylobacter, particularly where the fluorescent plasmid can be used as a marker, such as adhesion studies, the transformation of other fluorescent plasmids, and growth curves comparing transformed to non-transformed in particular, could provide insight into the growth kinetics of Campylobacter and other mechanisms that influence pathogenicity of the bacteria. The evaluation of success in relation to Campylobacter phenotypes could also be studied and the results could give clues as to why the fluorescence was lost in strains used for this study while it remained in the originally transformed 81-176 and 11168.

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(Co-authors: Nataliya Panova, Melissa Takaaze, Dr. John Berestecky, Robert Oda, Draven Aquino, and Colleen Allen)

Poster #136
PRODUCTION OF RECOMBINANT ZIKA VIRUS FUSION PROTEINS IN BACTERIAL AND MAMMALIAN EXPRESSION SYSTEMS

Zika virus (ZIKV) is a member of the Flavividae family, genus Flavivirus, which also contains the more familiar dengue, West Nile, and Yellow Fever viruses. Flaviviruses are enveloped, positive strand RNA viruses and the genomic RNA encodes a single polyprotein. The polyprotein is processed into 3 structural proteins, the capsid (C), pre-membrane (prM), and the envelope (E) proteins, and 7 non-structural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 within the cytoplasm of the infected cell. Although the Zika virus (ZIKV) has been known since 1947, it did not gain much public attention until recently when reports of an association between ZIKV infection in pregnant women and microcephaly in newborn infants and Guillain-Barre syndrome were reported.

Objective: Our overall objective is to understand the role of ZIKV NS proteins in the biogenesis of ZIKV infection. We therefore plan to - (i) Create recombinant pET-15b plasmid carrying ZIKV NS1 and Env genes for expression in bacteria; and (ii) Develop a fusion construct of ZIKV NS1 and Env genes with GFP and V5-His tags for expression in mammalian cells. These plasmids will assist in understanding in vitro ZIKV pathogenesis.

Methods: ZIKV Env and NS1 genes were amplified by PCR, and cloned in pET-15b (His-tag bacterial expression vector) plasmid at the NdeI and BamH1 insertion sites using a Gibson Assembly kit. The recombinant plasmid was propagated in E. coli, and the purified plasmid was sequenced to ensure proper DNA sequence, length, and reading frame before being transformed into BL21 DE3 cells for protein expression. Both genes were subsequently inserted into pcDNA3.1/V5-His-TOPO and pcDNA3.1/CT-GFP-TOPO via TA Cloning for transfection and expression in human HEK293T cells.

Results and Conclusions: ZIKV NS1 and Env genes were successfully cloned into pET-15b. Alignment of NS1 sequence data to the NS1 wild-type sequence revealed nucleotide similarity of 90.3% and a protein similarity of 99.4%. NS1-V5-His and -GFP fusions showed a nucleotide similarity of 90.5% and 90.7%, and a protein similarity of 98.6% and 98.9%, respectively. The NS1-GFP fusion construct was successfully expressed in HEK293T cells. ZIKV Env gene exhibited a nucleotide similarity of 96% and a protein similarity of 99% on BLAST search. Ongoing research is focused on using these fusion plasmids in understanding the biogenesis of ZIKV infection in mammalian cells.

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Poster #137
CONSTRUCTION AND EXPRESSION OF RECOMBINANT PLASMIDS CONTAINING WEST NILE VIRUS NS1 AND NS4B GENES FUSED WITH BACTERIAL CYTOLYSIN (CLYA)

Background: Since its introduction to North America in 1999, human infection with West Nile virus (WNV) has resulted in considerable acute morbidity and mortality. So far, no human vaccine is available to prevent new WNV outbreaks and to avoid worldwide spread.

Objective: Create fusion constructs that will allow WNV NS1 and NS4B proteins to load in the bacterial microvesicles (MV).

Methods: WNV NS1 and NS4B genes were PCR amplified with the appropriate primer sets containing the restriction sites, and about 23-26 bp overlaps on both ends of the two genes to facilitate Gibson Assembly. The PCR products were gel-purified and digested with the BamH1 and Ndel enzymes. The digested fragments were ligated with the linearized pET-15b plasmid, and propagated in E. coli. To fuse the WNV genes with the bacterial clyA gene, a gene product that enriched in the MV, two Gibson Assemblies were performed using an NEbuilder HiFi DNA Assembly Cloning Kit according to the manufactures instructions. The resulting fusion plasmids (pClyA-NS4B and pClyA-NS1) were transformed into E. coli DH5α cells and the purified plasmids were sequenced to verify the DNA sequence, the size, and open reading. Verified plasmids were then transformed into E. coli BL21 (DES) cells, and the protein expression was verified using western blotting (WB) assay.

Results and Conclusions: pClyA-NS4B and pClyA-NS1 were successfully inserted into pET-15B vector, and the DNA sequence data revealed the two genes to be in frame. WB assay on the total proteins isolated from the E. coli BL21 (DES) is in progress to verify the protein expression. Immunostaining of the expressed proteins with specific antibodies to ClyA and WNV proteins in the cell lysates is expected to visualize the bands of the expected sizes. Subsequent biochemical and immunoblotting assays will be conducted on the supernatant collected from various expression systems to confirm the presence of the WNV proteins in the MV. The loaded MV will be tested in in-vitro immune cell activation assay, and the potential candidates will be evaluated in animal model for protection.

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Poster #138
PRODUCTION OF VARIOUS FRAGMENT PROTEINS OF ARC

Arc (activity-regulated cytoskeleton-associated protein) is a 396 amino acid protein encoded by the immediate early gene, and its expression has been directly linked to information processing in brain. Studies have shown that Arc-knockout mice exhibit deficits in long-term memory consolidation although their short-term memory is unaffected. Arc is believed to interact with dynamin and endophilin proteins to enhance the endocytosis of AMPA receptors and reduce their surface expression. These interactions are considered important for late-phase synaptic plasticity and memory consolidation. The overall goal of our research group is to study the structure of the full-length Arc protein. Arc is difficult to be crystalized and it forms oligomers in solution; therefore, traditional structural methods, such as X-ray and NMR, cannot be used to study its structure directly. Our group approaches the problem by solving the structures of Arc fragment proteins and using them to construct the structure of the full-length Arc protein. The goal of this SRE project is to produce various fragment proteins of Arc. These proteins will be utilized as tools for characterizing the structure of the full-length Arc protein and analyzing the interactions between Arc and its binding partners. To produce these biomolecular tools, mutagenesis experiments were performed on the full-length Arc DNA generating a series of Arc fragment proteins. Their expression in E. coli and solubility in physiological buffer were examined. The fast protein liquid chromatography (FPLC) was employed to purify these fragment proteins using affinity, size exclusion, and ion-exchange columns.

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Poster #139
SELENOPROTEIN K IS CRUCIAL FOR MELANOMA STEMNESS, CELL GROWTH, MIGRATION

The importance of effective calcium flux from the endoplasmic reticulum (ER) has been recently demonstrated for the growth and migration of human melanoma cells. Because Selenoprotein K (SELENOK) has been implicated in calcium flux in immune cells during activation and migration, we hypothesized that it also plays a critical role in the proliferation and migration of melanoma cells. To test this hypothesis, we developed an in vitro human melanoma cell model using the NCI-60 validated human melanoma cell line, SK-Mel28. CRISPR/Cas9 techniques were used to generate a SELENOK-null clone and effective mutation of SELENOK was confirmed by DNA sequencing as well as western blot analyses showing truncated protein. The SELENOK-null SK-Mel28 cells were compared to w.t. control SK-Mel28 cells for proliferation and invasion was analyzed using a soft agar colony formation assay. All three functions were impaired in the SELENOK-null cells compared to controls. These assays were repeated in the SELENOK-null SK-Mel28 cells rescued with transient transfection of GFP-SELENOK or as a control just GFP. Results showed that transfection of the SELENOK-null SK-Mel28 cells with GFP-SELENOK but not GFP alone restored capacity to proliferate and migrate. Calcium flux was evaluated using fluorescence based assays and showed reduced levels in the SELENOK-null SK-Mel28 cells compared to the controls. The regulation of calcium dependent signaling pathways were evaluated via next-generation transcriptome profiling tools, and calcium dependent enzyme nuclear factor of activated T-cells (NFAT) showed decreased functionality in SELENOK-null SK-Mel28 cells. Altogether, these data suggest that SELENOK is important for the stemness, growth, and migration of melanoma cells and suggest that SELENOK may serve as a therapeutic target for treating melanoma in humans.

Poster #140
ROLE OF FRACTONES IN THE EXTRACELLULAR MATRIX OF A MAMMALIAN DISEASE MODEL

Objectives (Statement of the study’s purpose): Fractones are part of the extracellular matrix (ECM) in the stem cell niche of the brain that controls the growth and proliferation of stem cells to suit specialized functions. Fractones directly contact stem cells to promote the effect of the growth factors they bind and encourage differentiation to form and renew brain tissues. Neurogenesis, once thought to occur only in embryonic development, has been shown to occur in adulthood in specific regions like the subventricular zone. We will characterize the role of fractones in a cancer disease model.

We have optimized a model of the neurogenic explant, a 1mm x 1mm piece of the adult neurogenic zone that retains the interaction between stem cells, fractones and growth factors in a near-physiological context. We have used this brain explant model and microinjection of neuroblastoma cells to examine the specific growth factor/fractone molecular interactions that lead to the control of neurogenesis and disease proliferation. Neuroblastoma cells transfected with GFP were injected into the explants to visualize stem cell fate via on-live fluorescence microscopy after the addition of various fluorescently labeled growth factors and antibodies.

Fluorescence microscopy imaging and immunohistochemistry on explants will allow us to develop the use of explants as a model to study pathology. Use of explants in the context of a neuroblastoma model will allow us to examine the development of diseases and elucidate the interactions between ECM structures such as fractones in the tissue and the state of disease progression.

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(Dr. Frederic Mercier)

Poster #141
EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF BOCCONIA FRUTESCENS

Bocconia frutescens was introduced to Hawai‘i as an ornamental plant. However, it is considered a noxious weed at the moment. Nevertheless, B. frutescens is used in Mexican traditional medicine to treat respiratory and skin problems. Phytochemical screening indicated B. frutescens containing alkaloids.

Objectives: This project has two aims: (1) To evaluate B. frutescens crude extract for antioxidant and anti-inflammatory activities; (2) To isolate and identify compounds from B. frutescens

Methods: Extraction and purification of crude extract: Fresh plant materials will be cut into pieces and air dried. The plant materials were extracted thrice with methanol at room temperature, and the solid material is then removed. The filtered extraction then evaporated under vacuum using rotary evaporator. The crude extract was subjected to silica gel by column chromatography (CC), eluting with a CHCl3−MeOH gradient solvent to afford several fractions. Two of the subfractions were subjected to silica gel and Sephadex LH-20 for further purification.

FRAP Assay: All samples and the positive control were prepared at 4 mg/mL, 1mg/mL, 0.25 mg/mL, 0.1mg/mL, 0.01mg/mL, and 0.001mg/mL concentrations by serial dilution. Samples were dispensed in triplicate into 96-well plates and incubated for 8 minutes at room temperature after dispensing the working FRAP reagent. The working FRAP reagent was made by making an acetate buffer from NaOAc·6H2O at pH 3.6, 10mM TPTZ, and 20mM FeCl3·6H2O into a 10:1:1 ratio, respectively. The FRAP assay was monitored at 593 nm.

Nuclear factor kappa B Assay: Human embryonic kidney cells 293 Panomic are used to observe changes along the NF-κB pathway. Stable constructed cells are applied to a 96-well plate at 20 10^3 cells/well. The cells are kept in Dulbecco’s modified Eagle’s supplement with 10% fetal bovine serum and antibiotics. The medium is then replaced after 48 hr of incubation and treated with different concentrations of the experimental extract and compounds. TNF-α is used as an activator (2 ng/mL). The plate is incubated fo 6 hr. The cells are lysed with 50 μL of Promega’s Reporter Lysis Buffer. The luciferase assay is done with the Luc assay system from Promega. The gene product, luciferase enzyme works by reacting with luciferase substrate. This reaction emits light that is detected with a luminometer and the data is expressed in IC50 values which is the concentration required to inhibit TNF-activated NF-κB activity by 50%.

Conclusion: This study support the hypothesis that Bocconia frutescens containing natural products with anti-inflammatory and antioxidant activities. B. frutescens crude extract demonstrated inhibitory effects against TNF-α-induced NF-κB activity using stably-transfected human embryonic kidney cells. Pure isolate structure identification was determined via 1H, 13C NMR spectra in comparison with known compound.

Co Authors & Acknowledgments:  
(Mengke Zhang, Sasha Kovacs, Tamara Kondratyuk, Leng Chee Chang, Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720) (This project was supported by grants from the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS), IDEA Networks of Biomedical Research Excellence (INBRE), Award number: P20GM103466. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.)
GENETICALLY ENGINEERING HUMAN CENTROSOME PROTEINS WITH FLUORESCENT PROTEINS TO OBSERVE CENTROSOME BEHAVIOR IN MITOTIC CELLS EXPOSED TO A WEAKLY ESTROGENIC COMPOUND BISPHENOL-A (BPA)

Since 2008, studies with Bisphenol-A (BPA) have shown effects of the formation of multipolar spindles leading to defects in chromosome segregation in early developing sea urchin embryos and in mitotic human cancer cell lines. Additionally, in vitro studies with BPA and mitotic extracts showed that it bound specifically to centrosome components like gamma tubulin. Since these studies, minimal research has been done to understand how BPA directly disrupts the function of the centrosome. For our project, we constructed and engineered constructs of human gamma-tubulin tagged with Green Fluorescent Protein (GFP) and human centrin tagged with Yellow Fluorescent Protein (YFP). These constructs were then expressed in a bacterial expression system to ensure that the proteins are still active and functional. Eventually, we would like to express our constructions in human tissue cultured cells exposed to BPA. Gamma-tubulin and Centrin proteins are involved in centrosome mechanisms but with different roles, one is involved in nucleation of microtubules and the other is involved in position and segregation of the centrosome, respectively. By observing the behavior of these two proteins in the presence of BPA, we can determine if the interactions of BPA and gamma tubulin is fragmenting centrosomes creating these multipolar spindles. Further, BPA only affects cells that are in mitosis (dividing cells), but it does not discriminate against normal and cancer cells. Because cancer cells include uncontrolled division, we can take what we learn about BPA effects on mitotic cells to see how that can help with therapeutics of cancer cells.

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Poster #143
Objective: In the 2016 Hawai‘i Behavioral Health Risk Factor Surveillance System data, over one out of ten Native Hawaiians adults have been diagnosed with diabetes and over a third have been diagnosed with hypertension, which is higher compared to other ethnic groups in Hawai‘i. Preventable hospitalization are hospital admission for chronic conditions that could have likely been prevented with better access to care. The purpose of this study was to obtain patient perspectives of the factors and barriers that led to preventable hospitalizations among Native Hawaiian patients with heart disease and diabetes.

Methods: Mixed-method study of 36 Native Hawaiians admitted to the Queens Medical Center (QMC) with heart disease and diabetes-related complications from June 2013 to February 2016. Participants were 21 years and older, self-reported Native Hawaiian, resident of Hawai‘i, and admitted to QMC for heart disease and/or diabetes complications. Patients unwilling to participate, non-English speaking, admitted to the Intensive Care unit, clinically unstable, pregnant, suffering from with memory loss, non- Hawai‘i resident and a resident of nursing home, hospice, prison, or other similar institution were excluded. Participants completed a face-to-face interview with a research nurse focused on reasons for their hospitalization from their perspective. Using the framework approach, two coders reviewed the audio files for each patient independently to identify themes in patients’ stories about factors leading to their potentially preventable hospitalization. Coders then met to discuss the themes for each patient and come to a consensus on the patient’s pathway to hospitalization. Adapted the pathway model developed by “Pathways to potentially preventable hospitalizations for diabetes and heart failure: a qualitative analysis of patient perspective” paper to understand an individual’s pathways to hospitalizations through grouping of factors. These factors were immediate (urgent clinical reasons for the hospitalization), precipitating (practical explanation for that urgent clinical reason), and underlying factors (causes for that practical challenge).

Conclusions: The Native Hawaiian patient stories provided patient perspectives regarding the primary factors and barriers that led to their preventable hospitalization for chronic conditions. Approximately half (48.4%) of participants’ hospitalizations were for heart disease complications and almost half were for diabetes complications (immediate factor). 6 out 10 of Native Hawaiians identified medication-related issues, such as not taking medication due to cost, as a primary precipitating factor to their hospitalization. Underlying factors that were commonly reported by Native Hawaiian patients were social, avoidance or denial, and healthcare system issues. 4 out of 10 Native Hawaiian participants identified social factors as the most common factor that led to their hospitalization. These included financial challenges, limited social support, and housing challenges. Avoidance/denial, where patients said they don’t want to deal with their chronic conditions or were resigned to fate, were also common reasons reported. Healthcare system issues, such as no health insurance or poor communication between patients and provider, were important underlying factors for Native Hawaiians. These findings add data about the importance of considering patients’ perspectives of the barriers they face inside and outside the healthcare facilities towards managing chronic conditions. Understanding patient perspectives is critical to reduce hospitalizations for diabetes and heart disease.

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Co-authors: Todd Seto, MD, Kathryn L. Braun, DrPH, and Tetine Sentell, PHD

Poster #144
INVESTIGATION OF THE EFFECTS OF FOOD PRESERVATIVE ADDITIVES ON THE GROWTH OF BENEFICIAL BACTERIAL SPECIES

Objectives: Previous experiments showed that the food preservatives sodium bisulfite and sodium sulfite have inhibitory effects against four species of beneficial human gut bacteria at levels “Generally Regarded as Safe” (GRAS) for human consumption. Bacteria were subsequently challenged with four different food preservatives, calcium propionate, methylparaben, sodium nitrite, and sodium benzoate, at concentrations including and exceeding levels determined GRAS by the Food and Drug Administration (FDA) in otherwise optimal conditions. The GRAS limits for calcium propionate, methyl paraben, sodium nitrite, and sodium benzoate are 4,000 parts per million (ppm), 1,000 ppm, 200 ppm, and 1,000 ppm respectively.

Methods: Bacteria were grown in appropriate broth media at 37°C in 5% CO2. Starting in early log phase, bacteria were challenged with all four preservatives at various concentrations ranging above and below GRAS levels. OD600 readings were taken using a plate reader at one hour intervals for a period of up to eight hours and in some cases plate counts were done to confirm viability of cells.

Conclusion: Preliminary results have shown that methylparaben has a varying (species and concentration dependent) inhibitory effect on all four species of bacteria. Calcium propionate had no significant impact on the growth of the Lactobacillus species but did have a slight inhibitory effect on S. thermophilus. Sodium nitrite and sodium benzoate had little to no effect on the growth of all four species. Further analysis is being conducted to determine if the observed preservative effects occurred before or after exceeding the GRAS limit. Additional tests, such as BacTiter-Glo which is based on ATP production of living cells, are being developed to determine cell viability of bacteria in human saliva before and after exposure to all food preservatives tested.

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Poster #145
WILL THE “RAINFORESTS OF THE SEA” SURVIVE CLIMATE CHANGE?

Objective: To determine any changes in the thermal tolerance of reef corals. Ocean temperatures have been accelerating at an alarming rate mainly due to anthropogenic fossil fuel emissions. This has led to an increase in the severity and duration of coral bleaching events. Predicted projections for the state of reefs do not take into account the rates of adaptation or acclimatization of corals as these have not as yet been fully documented. To determine any possible changes in thermal tolerances, manipulative experiments were conducted to precisely replicate the initial, pivotal research defining threshold temperatures of corals nearly five decades ago.

Methods: Six 1m x 1m mesocosms (n=3 per temperature treatment) and a water delivery system and flow rates identical to the ones designed by the original authors were used in this experiment. Twenty colonies of each of three coral species were collected from Kāne‘ohe Bay, randomly placed in each of the six aerated 660-liter mesocosms, for a total of 360 colonies, and weighed. Three replicate mesocosms remained under ambient conditions while three mesocosms were heated to 31°C, 2.7°C above ambient conditions for the 31-day experimental period, followed by a 28-day recovery period. Coral colonies were assessed in terms of calcification, partial mortality and survivorship. Calcification rates were determined using buoyant weights expressed as mean solid radius to compensate for differences in corallum size and morphology. Partial mortality is defined as percentage of dead skeletal area. Survivorship was characterized by the number of individuals alive within a treatment during the experimental and following recovery period. Results were assessed in terms of the long-term potential for upward regulation of thermal thresholds for coral bleaching and species interspecific capacity for change. Statistically higher calcification rates (using a GLM), survivorship, and lower mortality (using ANOVA model comparison) were observed in Montipora capitata, Pocillopora damicornis, and Lobactis scutaria in the present study at 31°C compared to the original 1970 findings. Bleaching was reported sooner in the original experiment as compared to the current one. Although ambient temperatures increased by 2.2°C from the original experimental conditions, no significant difference in growth or mortality was found between the heated (31.4°C) and the ambient treatments (28.7°C) for L. scutaria in the current study.

Conclusion: These findings provide the first strong evidence of coral acclimatization or adaptation to increasing ocean temperatures. However, this increased temperature tolerance may not be occurring rapidly enough to escape the projected increased intensity of bleaching events, as evidenced by the recent 2014 and 2015 high coral mortality in Hawai‘i (34%) and in the tropics worldwide.

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PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST ZIKA VIRUS NON-STRUCTURAL PROTEIN 1 – A TOOL FOR THE DEVELOPMENT OF VIRAL DIAGNOSTICS

Background and Objective: The purpose for the experiment is to test the immunogenicity of recombinant Zika virus (ZIKV) nonstructural protein 1 (NS1) protein in BALB/c mice and to attempt to generate monoclonal antibodies (mAbs) against the antigen using hybridoma technology. ZIKV is a member of the family Flaviviridae and genus Flavivirus and was first isolated 1947 in the Zika forest in Uganda. It has been shown to be transmitted by infected mosquitos of various Aedes species. Since 2015 ZIKV has been implicated in causing birth defects such as microcephaly in babies born to mothers infected during the early stages of pregnancy, which presents an urgent need for sensitive and specific serodiagnostic tests in endemic areas where other flaviviral infections occur. Generating hybridoma cell lines secreting mAbs specific to ZIKV NS1 protein can aid in development of viral diagnostics and a better understanding of flavivirus epidemiology. Detailed study of the generated mAbs can also be used to help further the understanding of the importance for cross-reactivity with other flaviviruses such as West Nile virus or dengue viruses (DENVs). ZIKV NS1 has a >50% conserved sequence with other flaviviruses and has potential to serve as a diagnostic marker for early detection of ZIKV infection. Also, determination of ZIKV NS1 functionality can help distinguish its role. Other flavivirus NS1 proteins are shown to play a role in RNA replication, associate with infected cell membranes and are being secreted from infected cell as a hexamer. Since NS1 is not completely conserved, there is the possibility that it may assume other roles for ZIKV, as it may further aid the virus in assembly of the structural proteins to produce infectious particles. Creating antibodies specific to (whole) ZIKV NS1 protein is a logical choice for diagnostic purposes for ZIKV since the NS1 protein is most abundant early on in the infection. Since the ZIKV NS1 protein is about 40 kDa in size, we can expect there to be multiple epitopes that produce antibodies with different specificities.

Methods: Recombinant ZIKV NS1 protein adsorbed to Alum was administered twice to five BALB/c mice to induce potent IgG responses. Two weeks after the second dose, IgG titers against ZIKV NS1 protein were determined using a flavivirus multiplex immunoassay (Luminex). Followed by another booster immunization, splenocytes prepared from the spleens of the two animals showing the highest antibody responses were then fused with P3 myeloma cells to create hybridoma cells and grown in HAT (Hypoxanthine-Aminopterin-Thymidine) select media. The hybridoma cells were cultured in five 96-well plates for one week and screened for the highest production of mAbs using the same immunoassay as described previously. Those hybridomas with highest IgG titers detected in the supernatant were subcloned to establish monoclonality. The obtained mAbs were subsequently tested for binding to ZIKV NS1, as well as to DENV NS1 using Western blot analysis. Subsequent characterization included epitope mapping to determine the point of binding on the antigen as well as determination of the antibody isotype/IgG subtype using Enzyme-linked immunosorbent assays (ELISAs).

Results and Conclusion: Successful vaccinations of two mice led to the production of eight different subcloned mAbs. All eight subcloned mAbs were determined to be IgG1 and were not cross reactive to DENV NS1. The results of Western blotting and Dot Blot showed that these identified mAbs reacted to ZIKV NS1 and none were anti-His antibodies. Each mAb was purified from cell culture supernatants with a Protein G Column on FPLC. Some insight has been gained on characterizing each of the mAbs, and ongoing experiments are to determine the epitopes of these mAbs. Overall, we have successfully generated high quantities of anti-ZIKV NS1 mAbs using hybridoma technology and they can be utilized to develop a serodiagnostic assay for ZIKV infection.

(Co-authors: Teri-Ann S. Wong, Alan Garcia, Draven Aquino, Liana Medina, Brien Haun, Madhuri Namekar, Axel T. Lehrer, John M Berestecky; This project was supported by grants from the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS), IDeA Networks of Biomedical Research Excellence (INBRE), Award number: P20GM103466. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.)

Poster #147
Hedychium coronarium is one of about 1,300 species belonging to the ginger family Zingiberaceae known widely for their use as perfumes and spices and is the national plant of Cuba. The aromatic herb is commonly referred to as the white ginger lilly, native to the Himalayas can be characterized by their aromatic scent, tall green leaves and white flowers with white stamens. It is widely found in tropical environments in Southeast Asia and prefers shaded wet soil making the Hawaiian Islands an ideal environment for the plant to grow. In Hawaii, as well as other countries like South Africa and Australia, the plant is considered to be an invasive species. The rhizomes of H. coronarium are edible and are used in some cultures as a medicinal plant, while in others it is considered a weed. The goal of this research project is to identify and isolate a pure compound from H. coronarium.

A compound was isolated by reflux of the extract in acetone from the rhizomes of H. coronarium. The extracted compound was isolated using flash column chromatography. It was then identified by spectroscopic HNMR and CNMR data, UV-VIS and FTIR to determine the chemical structure of the isolated chemical.

The isolated chemical from H. coronarium in this research project was identified as being the ent-isomer, (E)-Labda-8(17),12-diene-15,16-dial. Labdadienedials such as the chemical isolated in this research project are known for their cytotoxic, antimicrobial and hepatoprotective activity (González, 2010). This isolated compound will be used in future studies to determine its activity against inflammatory diseases.

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Poster #148
RELATIONAL SIZE DISCRIMINATION OF LANDMARKS IN HONEYBEES

The honeybee is the only invertebrate species that has been systematically studied in learning experiments. The results show extensive similarity to the learning of vertebrates. The invertebrate and vertebrate brains are very different, so such similarities raise questions about the evolutionary development of learning capabilities and their underlying mechanisms. Recently, studies of honeybees have shifted to more difficult cognitive phenomena, such as relational/concept learning, even though a prevailing view is that such phenomena are exclusive to vertebrates. Again, the results for honeybees are similar to those for vertebrates. One example is that honeybees can learn an oddity concept; they learn to choose the odd shape or color when presented a series of novel sets of three stimuli. The experiments reported here were designed to study size discrimination of three-dimensional landmarks. The hypothesis was that honeybees can learn a relative size concept.

The methodology employed a free-flying procedure with foraging honeybees. Individual bees were pre-trained to visit a laboratory window, modified into an open-access box, to obtain a drop of sucrose reward placed on a wooden block. The bee drank the sucrose on the block, flew to the hive to unload the sucrose, and then returned to the window for another trial. After pre-training, the bees in both experiments were trained with wooden blocks arranged horizontally on the floor of the window. The wooden blocks (landmarks) used in the experiments represent a continuum of different lengths from small to large (2.2 cm, 4.5 cm, 9 cm, 14.5 cm). In Experiment 1, four bees were trained with the two smallest block sizes, and four bees were trained with the two largest block sizes. For half of the bees, choice of the smaller block was rewarded with sucrose, and choice of the larger block was punished with stevia solution. The bees learned to choose the correct block size. In Experiment 2, eight bees were trained with all possible pairs of block sizes. Four bees were rewarded for choosing the larger block in each pair, and four bees were rewarded for choosing the smaller block. The bees gradually learned to choose correctly, although the discrimination was relatively difficult.

The results demonstrate that honeybees can discriminate block size. The bees had to compare the blocks in the pair to make a choice, and therefore the choices must be based on the size relationship between blocks. These experiments extend the study of concept learning in honeybees to relative size, and the findings contribute to understanding the formation of concepts in honeybees. In a planned experiment, bees will be trained in an oddity problem to choose the odd size in a set of three blocks.

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Poster #149
ANTIMICROBIAL POTENTIAL OF HAWAIIAN ALGAE

Potentially lethal infections from the bacteria Staphylococcus aureus is a cause of concern due to its increasing resistance to antibiotics. Therefore, it is extremely imperative to study alternative compounds to combat S. aureus while preventing multidrug resistance. Marine natural products, given the extensive taxonomic diversity of the ocean, should be highly considered as potential antibiotic agents. Algae produce secondary metabolites in response to ecological pressures including competition for space, maintenance of an un fouled surface, deterrence of predation, and the ability to successfully reproduce. Environmental stresses from sunlight, temperature, and salinity as well as the life stage, reproductive stage and age of algae have been shown to influence these metabolites. This research strives to determine the benefits of various algae as a natural antibiotic against human related bacteria. Samples of Asparagopsis taxiformis, Sargassum echinocarpum, and Ulva fasciata were collected, cleaned of epiphytes, and homogenized using mortar and pestle. 0.4 gram samples were saturated for 24 hours in 1mL of 60% methanol 40% water, acetone, or chloroform. 20 μL of algae extract was pipetted and dried onto 1 cm Ø discs of filter paper. Discs were plated on TSA media, that had been spread with Bacillus subtilis or Staphylococcus epidermidis. Water was used as a negative control and tea tree oil as a positive control. After 24 hours of incubation at 37 °C, visible zones of inhibition were measured. In a previous study, we found Asparagopsis taxiformis to demonstrate antimicrobial properties when extracted with ethyl acetate, while other species of algae did not inhibit any bacterial growth. According to other studies, the effectiveness of the algal extract to dispose bacteria is influenced by the polarity of the extraction solvent. I expect Asparagopsis taxiformis, Sargassum echinocarpum, and Ulva fasciata to display antimicrobial activity when tested with methanol, acetone, or chloroform. If adequately researched, antimicrobial as well as antioxidant properties in various algae may hold distinctive medicinal value.

(This project was supported by grants from the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS), IDeA Networks of Biomedical Research Excellence (INBRE), Award number: P20GM103466. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.)
A multitude of health complications arise from obesity. In pregnant women, maternal obesity predisposes both the mother and fetus to additional health problems. One health complication is macrosomia, a condition in which the fetus is large for gestational age. This may lead to complications during delivery as well as a higher likelihood of developing early onset diabetes, hypertension, and metabolic syndrome later in life. The placenta, the organ responsible for transporting nutrients to the fetus, represents a promising target for interventional strategies. Limiting nutrient transfer from the placenta to the fetus presents an innovative approach in preventing fetal overgrowth. Since glucose is one of the main molecules transported through the placenta, it has been hypothesized that excess glucose is one of the contributing factors to this condition. This project is focused on providing a way to study the role Glut1, the most abundant glucose transporter in the placenta, has on fetal growth. Consequently, we’ve constructed a plasmid containing a copy of Glut1 under the control of Cyp 19I.1, a promoter that restricts expression to the placenta. This vector was also designed to include a transposon system, PiggyBac, that will be used to create a line of transgenic Glut1 knock-in mice. We tested this vector in vitro and compared transgene expression and genomic integration in human placental choriocarcinoma (BeWo) and human embryonic kidney (Hek293) cells.

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**ISOLATION AND PURIFICATION OF 15-METHOXYLABDA-8(17),13-TRIEN-15,16-OLIDE FROM HEDYCHIUM CORONARIUM**

The purpose of this study is to isolate and identify chemical constituents from the rhizomes of Hedychium coronarium. Commonly known as white ginger, it is cultivated in China, Taiwan, Myanmar and the Indian subcontinent. This is an invasive species in Hawaii. This plant’s young flowers are edible and it has a strong fragrance. Rhizomes of H. coronarium were known to treat intense discomfort due to rheumatism in addition to headaches and inflammation.

The extraction of dried H. coronarium rhizomes was done using several different solvents, however, hexane provided the best results for this particular isolate. The hexane fraction was separated into 60 fractions using flash column chromatography system with 5% ethyl acetate: 95% hexane as the mobile phase. Fraction 27 was further purified using HPLC with an analytical silica-based column. The resulting pure compound was characterized through its H-NMR, C-NMR, HMBC, HSQC and COSY data. Comparison with literature data identified the pure isolate as 15-methoxylabda-8(17),13-trien-15,16-olide.

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Poster #152
NOVEL STUDY OF HUMAN MEMBRANES USING A 3D CO-CULTURE OF AMNIOTIC EPITHELIAL AND MESENCHYMAL CELLS

Human parturition, or the act of giving birth, normally occurs after 38-42 weeks of gestation. This process involves a series of physiological events including uterine activation, ripening and dilation of the cervix, myometrium contractility, and fetal membrane rupture. These events must occur in a synchronous and precise manner for a normal and healthy birth to occur. In particular, rupture of membranes (ROM) is a pivotal event for the onset and development of labor. However, an occurrence of ROM prior to the completion of gestation may lead to premature delivery of the fetus. Approximately one third of all preterm deliveries (birth prior to 37 gestational weeks) are caused by premature preterm rupture of fetal membranes (PROM). Inflammation plays a key role in human parturition, including the rupture of fetal membranes. Recent studies show that transcription factor Nrf2 may play a role in the regulation of inflammation. The amnion mesenchymal layer is of chief importance in normal membrane rupture due to its high tensile strength provided by its composition of the protein collagen. The amniotic mesenchymal cells (AMCs) of the amniochorionic membrane have been extensively studied in several fields including stem cell therapy and tissue regeneration due to their pluripotent abilities. Consequently, this stem cell characteristic predisposes AMCs to differentiate into multiple lineages when studied under in vitro conditions. Given this, it is important that a careful study of the role of Nrf2 in fetal membranes is done in physiologically relevant models and this necessitates the establishment of a model that closely mimics the three-dimensional and multicellular environment of the fetal membrane. Therefore the aim of this work is to establish a co-culture 3D model to study the role of Nrf-2 in the human fetal membrane amnion. The wider hypothesis that this work contributes to testing is that Nrf2 regulation has a key role in the mechanisms of the initiation of parturition. Human amniotic tissue was collected at term repeat caesarian section from Kapiolani Medical Center for Women and Children (with IRB approval). Tissues were isolated for amniotic mesenchymal and epithelial cells as routinely performed in the lab. Preceding the establishment of a co-culture, amniotic mesenchymal and epithelial cells were individually seeded onto Alvetex® polystyrene scaffolds. A thin layer of basement membrane was layered on top of the Alvetex® scaffold to facilitate cell adhesion of a single epithelial monolayer. Mesenchymal cells were seeded onto a scaffold primed with Poly-L-lysine solution. Scaffolds were then fixed and dehydrated in EtoH prior to H&E staining. Immunohistochemistry was performed on fetal membrane tissue sections embedded in paraffin. The expression and localization of NRF2 (1:100, ThermoFischer) were determined using the Vectastain ABC kit (Vector Laboratories, Inc., CA) and diaminobenzidine. Sections were counterstained with hematoxylin and mounted with Permount (Fischer Chemicals, MA). Primary AECs and WISH cells (1.25 x 105 cells/well) were seeded into 4-well chamber slides pre-coated with poly-L-lysine, grown to 60% confluency before fixation with 4% para-formaldehyde in 1X PBS and used for immunocytochemistry. Expressions of Nrf2 (1:100, ThermoFischer), was determined by fluorescence microscopy (Nikon C1 Plus Ti Eclipse epi-fluorescence). DAPI (1:5000, Calbiochem, MA) was used as a nuclear indicator. On morphological examination it could be seen that the human AEC grew and proliferated over time in the 3D model, the majority of the (>85) cells adhered to the apical surface however, it was difficult to visualize the basement membrane collagen IV. Subsequent experiments have focused on optimizing the numbers of cells and amount of collagen in the model. Nrf2 expression has been described in the human fetal membranes before the onset of labor, we are currently confirming the level of expression in our Hawai’i patient population. To further characterize the role of Nrf2 in vitro, the establishment of a 3D co-culture model will help demonstrate a more physiologically relevant way to manifest an in vivo-like function. This model will serve as a tool to accurately study Nrf2 expression in fetal membranes and its role in human labor.
DIFFERENCES BETWEEN CAMPYLOBACTER JEJUNI STRAINS AFTER TRANSFORMATION WITH PLASMID CONTAINING GFP MARKER

Objectives: Campylobacter jejuni is a foodborne pathogen recognized as a worldwide leading cause of human gastrointestinal enteritis. C. jejuni is known to be naturally competent but only with DNA of other Campylobacter. Recently, C. jejuni was discovered to readily uptake foreign DNA when a methylated EcoR1 site is present, thus, the ability of these organisms to be transformed with foreign DNA opens the door to analyze the species for potential therapeutic intervention. In this study, various strains of C. jejuni were transformed with plasmids containing GFP as a reporter gene.

Methods: Eight strains of C. jejuni were grown using Brucella agar plates and isolated colonies were grown for 24 hours in biphasic slants. Transformation was achieved via methylation of the EcoR1 site of the pWM1007 plasmid by using an EcoR1 methyltransferase. Strains of bacteria were incubated with methylated plasmid at 37°C for 24 hours with kanamycin as a selecting agent. Transformed strains were then visualized using immunofluorescence, and a combination of PCR and gel electrophoresis was used to verify the presence of the GFP gene inside the bacteria.

Conclusion: C. jejuni showed growth on kanamycin supplemented agar plates which suggested successful transformation. However, the expression of GFP appeared to have different results across strains. Under immunofluorescent imaging, some strains presented red fluorescence instead of green. PCR results showed the presence of GFP which confirms that plasmid DNA was successfully inserted into the strains of bacteria. Red fluorescence of GFP can be potentially explained by the photoactivation of GFP due to low oxygen concentration, while the loss of fluorescence over time by possible recombination of the T1 promoter site present on the plasmid. Further investigation of this transformation process could lead to a deeper understanding of the mechanisms of protein synthesis in the C. jejuni and could potentially open new avenues toward knockout strategies for therapeutic intervention in human gastrointestinal enteritis.

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Co-authors: Robert Oda, Draven Aquino, Rebecca Kagami, Melissa Takaaze and Colleen Allen

Poster #154
IN VITRO FERMENTATION EFFECTS OF FIBROUS FEEDSTUFFS, WITH AND WITHOUT Xylanase Enzyme Supplementation, ON Cecal Microbiota OF BOILER CHICKENS ANALYZED USING 16S Ribosomal DNA Typing

This study evaluated the effects of adding wheat mill run (WMR) and xylanase enzyme to the diet of broiler chickens, and the resulting impact on their cecal microbiota profile. WMR, a byproduct of wheat flour mill that is rich in fiber, is a more economical feed ingredient that may be fermented by the gut microbiota in boiler chickens. The experimental design was completely randomized with 8 treatments ran in two batches with 12 replications per treatment. The treatments were i) blank, ii) inulin while others were a corn/soybean-based diet supplemented with iii) 0% WMR + 0% xylanase, iv) 5% WMR + 0% xylanase, v) 10% WMR + 0% xylanase, vi) 0% WMR + 0.01% xylanase, vii) 5% WMR + 0.01% xylanase, and viii) 10% WMR + 0.01% xylanase. The remaining residue from processes that mimicked the digestion in the stomach and small intestine of a chicken, was used to perform In vitro fermentation. The digested residue was dried and mixed with inulin and 100 mg of each treatment feed. 10 ml of inoculum containing chicken cecal contents in an anaerobic dilution solution (1:100) were added to it. This was transferred to serum vials under a constant stream of CO2. These sealed serum vials were inoculated in a shaker water bath at 39 ± 0.5 ºC and 50 rpm for 48 hours. The pellets extracted after fermentation were then used for microbial diversity determination by polymerase chain reaction-based denaturing gradient gel electrophoresis (PCR-DGGE) of the V3 region of the 16S rDNA amplicons. No significant polymorphism was observed in DDGE gels between the 8 treatments. The addition of xylanase had little to no impact on bacterial diversity. A dendrogram revealed that all of the feed samples were closely related in their cecal microbiota diversity. In summary, while the DGGE analysis shows a lack of bacterial diversity amongst all the experimental feed samples, further sequencing must be done in order to identify if these bacteria are beneficial or pathogenic.

Poster #155
AN ANALYSIS OF GENE EXPRESSION OF THE ANTERIOR CRUCIATE
LIGAMENT VERSUS THE SEMITENDINOSUS TENDON

Introduction: Tearing of the ACL is one of the most common and serious sports injuries, often requiring surgery. There are many options when repairing the ACL, and a frequently used graft is the semitendinosus tendon. The purpose of this project is to analyze the differences in gene expression of the anterior cruciate ligament (ACL) versus the semitendinosus tendon (ST).

Specifically, it will look at the differences between expression of collagen I alpha-1 chain gene (COL1A1) and collagen III alpha-1 gene (COL3A1) as a function of the forkhead Box O3 gene (FOXO3A). COL1A1 and COL3A1 are genes that code for the alpha chains of type I and type III collagen respectively. Differences in levels of these proteins affect the physical properties of the tissue including density, stringency, and tensile strength. The FOXO3A gene codes for transcription factors that regulate a variety of cellular processes such as apoptosis, stress resistance, and metabolism among others.

Objective: The hypothesis is that the ACL and ST will show a significant difference between expression of COL1A1 and COL3A1. It is also hypothesized that levels of expression of FOXO3A will have a relationship with levels of expression of COL1A1 and COL3A1. By analyzing the differences in COL1A1 and COL3A1 expression in these two tissues, this experiment can provide insight into the molecular differences between the ACL and ST. Differences in gene expression could also help explain the differences in functionality after an ACL graft. Correlation between FOXO3A and COL1A1 and COL3A1 could reveal a relationship between the FOXO3A gene and the different physical properties between these genes.

Methods: ACL and ST samples were extracted from two cadavers (CAD A and CAD B) with no previous knee injuries. The mRNA was extracted and subsequently converted to cDNA. The cDNA was then used to perform q-RTPCR. Hypoxanthine guanine phosphoribosyltransferase (HPRT) was used as a reference gene. FOXO3A, COL1A1, and COL3A1 were analyzed in the experiment. The samples were run in triplicate and “no reverse transcriptase” (NoRT) and “no template controls” (NTC) were used to ensure validity of the results. The values were analyzed against the reference gene.

Results: Experimentation has been completed and data analysis is ongoing.

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Poster #156
H. CORONARIUM: ISOLATION AND CHARACTERIZATION OF ETHOXY CORONARIN D

Background: Hedychium coronarium, commonly known as white ginger, belongs to the Zingiberaceae family. It has been naturalized in places such as the U.S., Australia, South Africa, and Central America. The rhizomes of H. coronarium have been used to treat inflammation and aid in digestion; it is anti-inflammatory, stomachic, and carminative.

Objective: The purpose of this study was to extract, isolate, and identify a pure chemical from the rhizomes of H. coronarium.

Methods: Rhizomes of H. coronarium were ground into a fine powder and subjected to heated reflux distillation using methanol. The mixture was extracted with chloroform and purified using silica-based flash column chromatography with hexane/ethyl acetate. The purified extract was characterized using H NMR, C NMR, UV-VIS, FTIR, and mass spectroscopy.

Results: The amber colored oil was identified as Ethoxy Coronarin D, a labdane diterpene with [M+H]+ at m/z 347.2588 and chemical formula C22H34O3. Labdane diterpenes are potential pharmacological agents against diseases such as cancer and heart disorders.

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Hedychium coronarium, also known as the White Ginger Lily plant, is an invasive plant found throughout the islands of Hawai‘i. The rhizomes of the plant are used for a variety of purposes, such as an ingredient in cooking and baking, stewed in water for tea, or made into a sweet candy. Medicinally, ginger has been used for the treatment of nausea, rheumatism, reduction in contusion inflammation, and alleviation of headaches. Antifungal and antimicrobial properties of the ginger have been also reported, however, the mechanism by which it acts on bacterial cells has not been documented. To further study the plant’s potential, coronarin D, a bioactive compound in H. coronarium, was purified using high performance liquid chromatography. Analysis of antibacterial properties was performed by the disk-diffusion method on selected Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Pseudomonas aeruginosa. Results indicate that coronarin D has an antibacterial activity against gram-positive bacteria, but is not effective in inhibiting Gram-negative bacterial growth. To test the susceptibility of S. aureus to coronarin D, the minimum inhibition concentration (MIC) was determined by microplate serial dilution, which is 27.5 µg/ml. In a time-kill assay using S. aureus, coronarin D showed a fast kill time (< 1 hours) at the 2-fold of MIC, suggesting a relatively high antibacterial efficacy. To understand the antibacterial mechanism involved, cell wall and cell membrane integrity of S. aureus was analyzed in presence of coronarin D. Triton X-100 lysis assay indicated that coronarin D does not affect the cell wall of S. aureus. However, analysis of SYNTOX Green uptake showed disruption of the cell membrane by coronarin D. These results demonstrated the antibacterial properties of coronarin D, suggesting that it could be a good potential therapeutic agent against Gram-positive bacterial infections.

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THE POTENTIAL ROLE OF ABCC6 IN PURINERGIC SIGNALING AND IN REGULATION OF CARDIAC FUNCTIONS

Introduction: Ischemic cardiac diseases, including acute myocardial infarction (MI), represent a significant health problem that can lead to heart failure. Understanding innate mechanisms of cardioprotection is essential to develop novel therapy for patients with acute MI. In the heart, purinergic signaling, including adenosine and ATP, has a well-established role in mitigating myocardial damage from ischemia. ABCC6, an ATP-binding cassette membrane transporter, is primarily expressed in the liver and kidney and contributes to calcification inhibition by facilitating the efflux of cellular ATP into the extracellular space. The released ATP is rapidly converted into pyrophosphate (PPi) and adenosine by ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) and ecto-5'-nucleotidase (NT5E). PPi is a major inhibitor of calcification and adenosine indirectly prevents calcification by inhibiting the expression of the tissue nonspecific alkaline phosphatase (TNAP). Mutations in ABCC6 result in two currently incurable human disorders, pseudoxanthoma elasticum (PXE) and some cases of generalized arterial calcification of infancy (GACI). Both disorders lead to extensive ectopic calcification, notably affecting cardiovascular tissue. ABCC6 also influences genes involved in nucleotide and purinergic signaling. Our preliminary data shows that mice with a deficiency in ABCC6 is associated with improved cardiac functions after MI than in wild type mice. Therefore, it seems that ABCC6 is also involved in the regulation of cardiac functions.

Objective: We hypothesize that ABCC6 acts as an upstream modulator of extracellular purinergic signaling in distal tissues, and that it contributes to the regulation of cardiac functions. This study aims to understand and evaluate the cardioprotective effects of ABCC6 deficiency during ischemia in in vivo and ex vivo models.

Methods: We used wild type (WT) and Abcc6-/- knockout (KO) mice to study the cardioprotective effects of ABCC6 deficiency. As an in vivo model, WT and KO mice were given permanent coronary ligation to imitate MI. As an ex vivo model, WT and KO mice hearts were harvested and placed into a Lagendorff system. Cardiac function were measured in both models and a transcriptome analysis was performed.

Conclusion: Abcc6 deficient mice were shown to have a better heart recovery when compared to WT mice following myocardial infarction in both in vivo and ex vivo experiments. Notably, the onset and time to peak contracture were markedly delayed in Abcc6-/- mice. As the rapidity of contracture onset is more relevant to injury than the actual extent of contracture, our data suggested that the absence of ABCC6 is beneficial during the ischemic phase.

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(Chris Brampton, Briana Shimada, Viola Pomozi, Janna Zoll, Bianca Calio, Olivier Le Saux)

Poster #159
Neuroblastoma (NB) is a rare pediatric cancer that develops in the sympathetic nervous system. The number of treatment options can diminish during the course of treatment as the neuroblastoma cells often develop drug resistant mechanisms. As much as 50% of NB patients diagnosed with high-risk disease will develop resistance to treatment. Therefore, new effective treatment options for high risk and refractory NB need to be developed. A common source for possible new drugs is plant extracts. Junipers have been widely used in traditional medicines from cultures all around the globe throughout history. Previous studies have shown that the juniper extracts produced from the stem, leaves, and roots have potent anti-cancer effects and significantly reduced NB cell proliferation. The purpose of this experiment was to test different fractionations and compounds isolated from the juniper plant extract used in the previous study on non-drug resistant and drug-resistant neuroblastoma.

The juniper fractions and compounds were tested on two cell lines from the same patient. The SK-N-Be1 cells were isolated from a patient pre-treatment and were responsive to chemotherapeutic drugs. The SK-N-Be2c cells were isolated from the same patient post treatment after relapse and were resistant to chemotherapeutic drugs. The cells were seeded into 96-well plates and the juniper fractions and compounds were administered. Doxorubicin was used as control. To test for cell proliferation, a Sulforhodamine B assay was used. The plates were read in a microplate reader and the absorbance at 510 nm was recorded. The absorbance for the treated cells were compared to the control cells to determine the amount of cell growth. Student T-tests were used to determine whether the plants extract’s effect was statistically significant.

The results show that four different fractions and compounds worked on both cell lines. Further tests will need to be conducted in order to determine the active compound in the extracts, to determine the potency of said compounds and to determine the mechanism of action of the active compounds.

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Poster #160
**PRODUCTION OF RECOMBINANT PLASMIDS FOR THE EXPRESSION OF ZIKA VIRUS NS5 GENE IN BACTERIAL SYSTEM**

**Background:** The recent outbreak of Zika virus (ZIKV) has infected over 1 million people in over 30 countries. Unique features of Zika virus infection are sexual and transplacental transmission and associated neurological Guillain–Barré syndrome and fetal microcephaly. Nonstructural protein 5 (NS5) is essential for the replication of the ZIKV RNA genome.

**Objective:** Create recombinant plasmid of ZIKV NS5 in pET-15b for expression in E. coli bacteria. This recombinant plasmid will assist research efforts in dissecting the dynamics of ZIKV pathogenesis.

**Methods:** ZIKV NS5 gene was PCR amplified using the appropriate primers, and cloned in frame in pET-15b (His-tag bacterial expression vector) at the NdeI and BamH1 insertion sites using a Gibson Assembly. The plasmid was propagated in E. coli and the isolated plasmid was fully sequenced to ensure correct sequence, proper length, and reading frame.

**Results and Conclusion:** NS5 gene was successfully cloned into pET-15b. Subsequent assays include using this NS5 fusion construct for bacterial expression, and also serve as template to clone the NS5 gene into a mammalian expression system to examine the NS5 role in ZIKV pathogenesis.

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EFFECTS OF REPLACING CORN WITH SUN-DRIED CASSAVA CHIPS IN DIETS ON THE INTESTINAL MICROBIOTA OF HYBRID TILAPIA (OREOCROMIS NILOTICUS X O. MOSSAMBICUS)

The dietary fibers fermented from different substrates affects the composition of the intestinal microbiota, which in turn, affects the digestion, health, and well-being of the host animals. Cassava (Manihot esculenta) is rich in fiber and can be a cost-effective energy source in fish diet. The objective of this study was to investigate the effects of replacing the corn in tilapia feed with sun-dried cassava chips at different concentrations (0%, 12.5%, 25%, 50% and 75%) on the intestinal microbiota of hybrid tilapia, with the purpose of increasing healthy fish production and reducing production costs.

Four hundred tilapia fingerlings (~10g initial body weight) were randomly and equally placed in 20 tanks and fed with one of the 5 diets for 12 weeks. Then intestinal digesta samples were collected monthly and DNA was extracted. Polymerase chain reaction-based denaturing gradient gel electrophoresis (PCR-DGGE) analysis of 16S rRNA genes was used to determine the microbial diversity present in the intestines of the tilapia.

Based on the comparison from the cluster analysis of the DGGE banding patterns of each of the different feeds containing varying concentrations of the cassava, cassava inclusion in the tilapia diet does affect the diversity of intestinal microbiota of the tilapia.

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(Co-author: Jordan Yoshioka)

Poster #162
THE EFFECTS OF SODIUM SULFITE AND OTHER FOOD PRESERVATIVES ON ENZYME ACTIVITY IN HUMAN SALIVA

Previous research has indicated that sodium sulfite food preservatives have bacteriostatic and bactericidal effects on some beneficial members of the gut microbiome. This suggests that these compounds may be altering the gut and/or the mouth microbiome. Other experiments with rats have shown that sulfite compounds affect the activity rate of some gut enzymes. This brings into question the possible effects of sodium sulfite on the function of enzymes in the human digestive system, particularly in the mouth. Amylase, which converts starch and glycogen to simple sugars; and lysozyme, an enzyme known to lyse cell walls of bacteria, are both abundant in human saliva and have an influence on the mouth flora as well as initial steps in food digestion.

Experiments were developed to determine the effects of sulfites on the activity of lysozyme in human saliva and chicken egg white lysozyme (EWL) by observing lysozyme activity utilizing a fluorescence-based assay. Lysozyme was exposed to sulfites in concentrations ranging between 156-5000 parts per million. Enzyme activity was observed as fluorescence when labeled bacterial cell lysis occurred. Egg white lysozyme and saliva with labeled cells were used as a control of uninhibited lysozyme activity to establish a standard curve. Fluorescence at 2-10 minute intervals of samples exposed to sodium sulfite when compared to the control showed a significant decrease in the overall reaction rate and peak activity of lysozyme in human saliva. To date, the data collected from experiments conducted on EWL with sodium sulfite in the tested concentrations did not provide conclusive evidence to suggest inhibition in its reaction rate or overall activity. A lack of inhibition may be linked to structural differences between chicken and human lysozyme or due to competition with a cofactor found in saliva. Amylase activity was not diminished by sulfite exposure as observed in a simple iodine test.

Current research will continue to better define the relationship between sodium sulfite and lysozyme and the resulting effect on its activity. Further research is in the initial stages of experimentation to determine if other food preservatives, including sodium benzoate, calcium propionate, and sodium nitrite; have a similar effect on lysozyme from chicken egg white and human saliva.

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(Co-Authors: Emily Graham, Peter Fisher, Richard Allen, Sally Irwin)

Poster #163
EXPRESSION AND PURIFICATION OF RECOMBINANT DYNAMIN 2 PLEXTRIN HOMOLOGY (516-625)

The objective of this SRE project is to produce the plextrin homology (PH) domain (residues 516-625) of the recombinant dynamin 2 protein and use the NMR technique to examine the interaction between this domain and its binding partner, the Arc protein, at the residue-specific level. Arc is a regulator of synaptic plasticity and a key player in the consolidation of long-term memory. Arc has also been observed at increased concentrations in the medial frontal cortex of Alzheimer’s patients making it a major point of interest in the study of neurodegenerative disease. However, the working mechanism of Arc at the molecular level remains unclear. It is known that Arc interacts with the dynamin 2 and endophilin 3 proteins to facilitate endocytosis of AMPA receptors at neuronal synapses. The C-terminus of Arc (Arc 155-396) was found to interact with the C-terminus of dynamin 2. In this SRE project, we produced a soluble PH domain protein of dynamin 2 using an E. coli expression system followed by fast protein liquid chromatography (FPLC). Our preliminary NMR data shows possible binding of this domain with the Arc protein, but further data must be collected to confirm this interaction.

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(Co-Authors: Melissa Boldridge and Lei Wang)
IDENTIFICATION OF NOVEL COMPOUNDS FOR THE TREATMENT OF DRUG RESISTANT NEUROBLASTOMA

Background: Neuroblastoma (NB) is a solid extra-cranial pediatric cancer originating from neural crest cells. It is the second most common pediatric cancer resulting in 12% of deaths in cancer patients under the age of 15. High-risk NB poses an even poorer prognosis, with a survival rate of about 40%, often due to the development of multi-drug resistance (MDR) and high rate of disease relapse. Despite a plethora of treatment options including monotherapies, combined therapies and multi-modal treatments, there are currently no effective treatments for refractory and relapsed NB. To address this limitation, this study is focused on identifying novel compounds for the treatment of drug resistant NB.

Objective: To identify novel compounds with potent anti-cancer effects in NB cells, and to elucidate the mechanism of action of these compounds.

Methods: Model system: MYCN-amplified drug sensitive (DS) neuroblastoma cells (SKN-Be1) and MYCN-amplified drug resistant (DR) neuroblastoma cells (SKN-Be2(c)) may be used to elucidate mechanisms that promote NB drug resistance. DS cells were cultured from tumors removed from a 24 month old patient prior to chemotherapy treatment upon diagnosis. DR cells were cultured from tumors removed from the same patient post chemotherapy treatment after relapse. Using these cells from the same patient provides a model for an accurate indicator into the effectiveness and impact that newly developed cancer fighting compounds might have on drug resistant neuroblastoma cells. Cell proliferation: The sulfo-rhodamine B (SRB) assay was used to screen a library of novel compounds for anti-cancer effects in NB cells. Western Blot: Whole cell lysates were prepared from DS and DR NB cells treated with compound A, compound B, doxorubicin (positive control) and untreated NB cells. The lysates were analyzed by western blot to compare the presence of cleaved PARP (apoptosis marker), cyclin D1 (cell cycle marker) and LC3 (autophagy marker). Imaging: Plasmid based fluorescent indicators were used to measure ER and mitochondrial calcium levels. Using laser-scanning confocal microscopy, the impact of the known cancer drug doxorubicin and the compounds A and B on calcium levels in the mitochondria and ER were observed in real time in the DS and DR cell lines.

Conclusion: The results indicate that compounds A and B inhibit cell proliferation in DR and DS neuroblastoma cells. Western blot analysis showed that compound A is more potent than B, based on the changes observed in cleaved PARP, cyclin D1 and LC3. Compound A and B also induced ER calcium signaling, mitochondrial calcium signaling, and loss of mitochondrial membrane potential, an indication of mitochondrial damage and early apoptosis. Further testing is required to identify the exact mechanisms of action of these compounds. However, the results suggest that the anti-cancer effects may involve mitochondrial calcium overload, loss of mitochondrial membrane potential, opening of the mitochondrial permeability transition pore and induction of apoptosis.

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(Co-authors: Italo Fuenzalida Espinoza, Nathan Sunada, Ingo Lange, Dana-Lynn Koomoa)

Poster #165
L-TYPE CALCIUM CHANNELS MEDIATE RESPONSES TO METH

The purpose of this project is to study how methamphetamine (METH) affects calcium signaling, and how METH interacts with calcium channels to promote changes in dopaminergic neuron function. METH, a powerful and addictive drug that targets the central nervous system (CNS), belongs to a class of stimulants called amphetamines. The incidence of METH use in the state of Hawaii is one of the worst in the nation. METH abuse has been shown to be associated with neurotoxicity and neural injury. Previous studies demonstrated that METH enhances calcium levels in dopaminergic neurons, and calcium overload was shown to play a role in mediating neurotoxicity. Data from our laboratory have shown that METH acutely inhibits calcium currents; however, the long-term effect of METH is an upregulation of L-type calcium channel expression. Determining which calcium channel subtypes promote calcium overload, and subsequently, neural injury, would help us determine the best way to reduce, if not prevent, the adverse effects of METH in the brain.

To study the effects of METH on calcium signaling, we used differentiated SH-SY5Y cells as a dopaminergic cell model. SH-SY5Y cells were plated and differentiated in poly-l-lysine coated glass-bottom Petri-dishes at 37°C and 5% CO2 conditions. Cells were loaded with 1µM FURA-2AM fluorescent dye for 10-15 minutes at room temperature in the dark. Cells were washed with saline solution and then imaged. FURA-loaded cells were illuminated with 340nm and 380nm wavelength of light while perfusing with normal saline solutions for 3-4 minutes, then with 50µM METH (with or without calcium channel blocker) for 20 minutes and followed by a 3-minutes washout. Emitted light from cells was captured at above 490nm wavelength using an intensified CCD camera. Intracellular calcium levels are expressed as ratios of fluorescent signals at 340nm to 380nm.

Our preliminary data show that METH-induced calcium influx during the 20-minute treatment. These responses were suppressed by L-type calcium channel blocker, nicardipine (2µM). Lopinavir (50ng/mL), a protease inhibitor, also suppressed calcium influx. Our findings suggest that reducing calcium overload by blocking L-type channels may useful in inhibiting neural injury as a result of chronic METH exposure.

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(Co-authors: Marianne Chen, Chena Bryan, Sadie Karratti-Abordo and Marilou A. Andres)

Poster #166
MEDICINAL PLANTS: EXAMINING THE DISTRIBUTION OF BIOACTIVE ALKALOIDS WITHIN PRICKLY POPPIES

Plants are commonly used medicinally. The family Papaveraceae (poppies) contains many well-known species with medicinal uses, such as the opium poppy, Papaver somniferum, which has been the subject of extensive chemical research. In contrast, the prickly poppies, genus Argemone, are relatively understudied despite their common use in ethnomedicine. We are addressing this gap in our knowledge by performing a survey of the secondary chemistry in 4 species of Argemone: the endemic Hawaiian A. glauca, and 3 species native to Mexico, including A. mexicana, A. ochroleuca, and A. platyceras. Previous work on A. mexicana has identified 2 alkaloids to be in high concentrations (sanguinarine and berberine). In medicinal use, the bioactivity is potentially being driven by these alkaloids. The other three species have not been previously investigated, and it is currently unknown whether they produce the same alkaloids and if so, which plant parts express the highest concentration. Because ethnomedicinal preparations with prickly poppies involve various plant organs, it is likely that intra-organismal variation in alkaloid distribution exists.

Alkaloids were extracted from seeds, roots, leaves, and flowers, as well as the latex exuded from leaves, of the four focal species. Samples were run on a high performance liquid chromatograph with a gradient solvent system of 2% Acetic Acid Aq. and 90/10 v/v Acetonitrile/0.1% v/v Triethylamine/10% H2O. Standards of berberine and sanguinarine were run under the same conditions. All chromatograms were extracted at a wavelength of 300 nm. Alkaloids were identified by retention time and standard comparison. Alkaloid concentrations were compared among plant parts within and across species to describe patterns of variation in secondary chemistry.

The results from this study provide the basis for future work analyzing alkaloid content in medicinal preparations of prickly poppies. Combining analytical chemistry with ethnomedicinal practice will provide novel insights into the role of plant secondary chemistry in medicine.

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Poster #167
The purpose of this study is to develop simple and accurate assays of phosphate ions in water samples as indicators of residual herbicides. Once a method is developed, it will be used to test water samples from around the island of Hawai‘i collected from areas such as beach parks and rivers. An area of focus is to investigate if the concentration of phosphate is higher in areas near the previous locations of sugar cane plantations.

Standard solutions of potassium phosphate were made in concentrations of \(10^{-2}\), \(10^{-3}\), \(10^{-4}\), \(10^{-5}\), and \(10^{-6}\) M. The molybdem blue solution was made by mixing 50mL of 4% ammonium molybdate with 25 mL 2.5 M sulfuric acid, and then slowly adding a sample 0.265 g of ascorbic acid dissolved in 50 mL deionized water. A volume of 4 mL of the molybdate reagent was then added to 20 mL of each standard solution and resulting stock solutions were then refrigerated for a minimum of 24 hours. The standards were then analyzed using a spectrometer to determine the light absorbance of each solution. The absorbance is found using the formula: \(A = \log_{10}(Io/I)\), where \(Io\) is the light that enters the spectrometer and \(I\) is the light that exits. The data gathered from the standard solutions was used to determine a narrower range of concentrations from \(0.5\times10^{-5}\) M to \(10^{-4}\)M. These solutions will also be mixed with the molybdate reagent and be analyzed for light absorbance in order to create a calibration curve. Once the calibration curve has been generated, water samples collected from local beaches and rivers will also be analyzed with the molybden reagent for absorbance and compared with the stock solutions to determine the phosphate ion content.

The information gathered will help draw conclusions about what areas are at higher risk of pollution and if these areas correlate with any pattern such as locations of previous sugar cane plantations.

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(Coauthors: Maggie Chen and Skyla Lee)

Poster #168
Parasitic nematodes that infect humans, animals, and plants can cause serious diseases that could be detrimental to the health of the host organism. Intermediate hosts such as slugs, snails, lizards, frogs, and crabs can carry, transmit, and spread infectious agents to accidental hosts. In Hawaii, the semi-slug Parmarion martensi has been found to carry high abundance of Angiostrongylus cantonensis larvae. Ingestion of infectious A. cantonensis larvae by humans and other accidental hosts can cause a form of eosinophilic meningitis, known as angiostrongyliasis. Transmission of infectious A. cantonensis larvae is believed to be primarily by ingesting an intermediate host, however other sources of transmission may include ingestion of or exposure to contaminated water or produce. Understanding the diversity of other nematodes associated with Hawaiian slugs and snails may be relevant to clinicians and veterinarians for diagnosis, treatment, or prevention of disease. The diversity of nematodes that are associated with Hawaiian slugs and snails also has implications for active research of A. cantonensis. Large quantities of the infectious L3 larvae are required for experimentation, yet there are no current in vitro protocols for establishing and maintaining pure A. cantonensis cultures. Live A. cantonensis larvae are isolated from drowned, wild-caught slugs and snails, however it is clear from our research that other species of nematodes are also present in the extract. This study is surveying the diversity of nematodes associated with Hawaiian slugs and snails. Polymerase chain reaction (PCR) with the universal nematode barcoding primer set, NC1F and NC2R, is being used on various slug and snail species and live nematodes isolated from slugs and snails. Amplified PCR products are separated using the TOPO TA Cloning Kit for Sequencing. Transformants are amplified by PCR using plasmid primers, M13F and M13R, the products are cleaned using ExoSAP-IT, and sequenced on an Applied Biosystems 3500 Genetic Analyzer. Results to date have shown the presence of A. cantonensis and the soil nematode, Caenorhabditis briggsae, which is closely related to Caenorhabditis elegans. While further tests are ongoing, the absence of additional parasitic nematodes is beneficial to clinicians, veterinarians, and researchers. Furthermore, limited nematode diversity within Hawaiian slugs and snails allows for easier development of an A. cantonensis culture in vitro.

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(Co-authors: Lisa M. Kaluna, Kirsten K. Cannoles, and Susan Jarvi)

Poster #169
INVESTIGATING THE TRANSMISSION OF ANTIBIOTIC RESISTANCE BETWEEN CAMPYLOBACTER JEJUNI AND ESCHERICHIA COLI, KAPI‘OLANI COMMUNITY COLLEGE

Background: Campylobacter jejuni (C. jejuni), is now the leading cause of bacterial gastroenteritis in humans. Another pathogen that is attributed to human digestive disease is Escherichia coli (E. coli). Identifying mechanisms by which these bacteria can lead to disease also open avenues to explore potential therapeutic options. A bacteria’s ability to resist antibiotic treatment is a major roadblock in therapeutic intervention. Antibiotic resistance is commonly spread via bacterial conjugation and allows for horizontal genetic modification through the transfer of DNA-containing plasmids. It was assumed that conjugation occurs most readily between closely related species, but recent data suggest gene transfer across very distant species. The purpose of this project is to first transform C. jejuni with a plasmid containing genes for green florescent protein (GFP) and kanamycin resistance, and then to determine the organism’s ability to transfer these foreign plasmid DNA to E. coli.

Methods: Eight strains of C. jejuni were grown on Brucella agar plates and isolated colonies were selected and grown in Brucella agar biphasic slants. Transformation was achieved via methylation of an EcoR1 restriction site motif on the pWM1007 plasmid with an EcoR1 methyltransferase. The bacteria strains were incubated with the methylated plasmid for 24 hours at 37°C. Strains of bacteria were visualized using a fluorescent microscope for verification of successful transformation.

Conclusions: Only the C. jejuni strain, 11168, glowed green under a blue laser; other strains either were not successfully transformed or glowed red under excitation by a green laser. Moreover, this red fluorescence appeared to subside after a few days, whereas the originally transformed C. jejuni retained its green fluorescence. This phenomenon will be investigated further to understand the mechanisms of protein synthesis of GFP in C. jejuni. Moving forward, C. jejuni will be co-cultured with E. coli to investigate the potential conjugal interaction between these distally related species. Successful transformation of E. coli strains could provide insight into mechanisms of C. jejuni and its pathogenicity. Further exploration could also lead to greater understanding of naturally competent bacterial species and their interactions with non-competent species.

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(Co-authors: Nataliya Panova and Rebecca Kagami; Draven Aquino; and Robert Oda)
Energy Metabolism in Breast Cancer Cells: Effect of Energy Substrate on Survival and Expression of Metabolic Enzymes

Objectives: Breast cancer is the most common cancer for women in the United States. One feature breast cancer has in common with almost all other cancer is altered energy metabolism, resulting in highly elevated uptake of glucose. Much of the glucose is ultimately fermented as glycolysis becomes uncoupled from the citric acid cycle, even in the presence of adequate oxygen concentration, a phenomenon named after its discoverer the Warburg Effect. This metabolic alteration is thought to promote the survival of cancer cells. Since cancer cells refrain from adapting their energy metabolism to changing oxygen concentrations, it becomes questionable if cancer cells retain the same metabolic plasticity as non-transformed epithelial cells. We therefore aimed to examine the effect of changing the energy substrate in breast cancer cell medium from glucose to ketone bodies to provide a rationale using glucose ablation interventions as potential cancer treatments. We chose ketones since it is well documented that carbohydrate restricted diets are ketogenic. We controlled our experiment by using a breast epithelial cell line besides two well characterized breast cancer cell lines.

Methods: Two breast cancer cell lines (MDA-MB-231 and MCF-7) were routinely maintained in DMEM medium with 10% FBS. The MCF-10A breast epithelial cell line was maintained in DMEM/F12 medium with 5% horse serum. Cell proliferation of these cells was measured after 48 h or 72 h of treatment using an MTT-Assay. Treatment conditions were various glucose concentrations (4.5 g/l to 0 g/l) and 25 mM beta-hydroxybuterate (BHB). Protein expression of enzymes involved in energy metabolism was asses by Western Blotting after 24h or 48h treatment in 0 g/l and 10 mM or 25 mM BHB.

Result: MCF-10A breast epithelial cells but neither of the breast cancer cell lines showed increased survival when supplemented with 25 mM BHB in no glucose conditions, indicating that breast cancer cells are not able to adequately adapt their metabolism to survive during low glucose conditions. MDA-MB-231 cells do not change expression of Hexokinase in no glucose/high ketone conditions, further indicating their inability to adapt their metabolism. Protein expression of metabolic enzymes (PDK-1, OXCT-1, and IDH-1) is ongoing.

Conclusion: These preliminary results indicate that human breast cancer cells are not able to use ketone bodies as energy substrates in the absence of glucose, while normal cells display sufficient metabolic plasticity to be survive glucose deprivation. We want to employ animal models to determine if radical glucose deprivation interventions are feasible for cancer treatment.

Poster #171
DOES PROPHYLAXIS USING SULFADOXINE-PYRIMETHAMINE PREVENT PREGNANT CAMEROONIAN WOMEN FROM BECOMING INFECTED WITH MALARIA?

Objectives: Plasmodium falciparum (Pf) infection is common in Africa, and is especially dangerous for pregnant women. Pf infection in pregnant women may result in maternal anemia, placental parasitemia, and neonatal mortality. The World Health Organization recommends pregnant women receive Intermittent Preventive Treatment during pregnancy using Sulfadoxine-Pyrimethamine (IPTp-SP) to reduce these negative birth outcomes. However, SP may not totally clear Pf parasites from all women. Recent research has shown that low-level Pf infection during pregnancy is associated with increased risk of malaria in the baby during the first year of life. Consequently, reducing Pf parasitemia without eliminating it may improve birth outcomes, but may paradoxically worsen the baby’s health after birth. The objective of this study was to determine if women who receive IPTp-SP are parasite-free or have low levels of Pf.

Methods: A total of 235 pregnant Cameroonian women treated with 2 or 3 doses of IPTp-SP were selected for inclusion. Women had peripheral blood drawn at delivery. Blood was stored as pelleted RBCs at -20°C until DNA extraction. Extracted DNA was used as template for PCR with Pf mitochondrial cytochrome c oxidase III (cox3) primers, a newly-described ultra-sensitive primer set. PCR products were visualized by gel electrophoresis with ethidium bromide.

Conclusion: Only 9/235(3.8%) of women were PCR-positive for Pf. IPTp-SP treatment did not result in a large percentage of low-level Pf infection in Cameroon, but ultra-sensitive PCR detection methods found infection in a small percentage missed by traditional detection methods. Further ultra-sensitive assays using samples taken from varying time points during pregnancy are necessary to confirm the effectiveness of IPTp-SP found in this experiment.

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(Co-authors: Michael Fernandez, Jovikka Antallan, Yukie Lloyd, Livo Esemu, Rose G. F. Leke, Diane W. Taylor)

Poster #172
Rainwater catchment systems are the primary potable water supply for many households on the island of Hawai‘i. There currently are no federal, state, or county agencies providing oversight or regulation of rainwater catchment systems for residential dwellings and the responsibility for adequate protection against waterborne pathogens is dependent on homeowners. It is estimated that only 66% of home designed systems are capable of producing safe potable water, according to ‘recognized best practices’. Poorly designed and/or maintained systems resulting in inadequate filtration or disinfection may result in human exposure to various infectious agents, including L3 larvae of Angiostrongylus cantonensis. In 2007, the semi-slug Parmarion martensi, was identified as a high-risk carrier of A. cantonensis larvae due to its abundance, behavior, and infection loads. Infected intermediate hosts such as P. martensi, may enter a water storage tank and release parasites upon drowning. A model water catchment system was used in a laboratory setting to test the effectiveness of various filtration types and sizes against A. cantonensis larvae. Sediment filters, for ‘universal fit’ housing, were donated from local retailers and ranged in size, material, presence/absence of end gaskets, and various ratings by the National Sanitation Foundation (NSF). Filters of 20 µm, 10 µm, 5 µm, and 1 µm pore size were tested, but other differences in structural elements existed among these filters. Two replicate filters were tested four times for the ability of nematodes to penetrate each filter. Nematodes were from drowned, wild caught, P. martensi slugs, photographed and length and diameter measured pre-filter. During the first, second, and third runs 250, 250, and 500 nematodes respectively were applied to the filter, whereas in the fourth run the system was turned on without adding additional nematodes to see if nematodes would penetrate the filter after a one-week incubation period. For each run, roughly ten liters of filtrate was collected, filtered across a 0.2 µm nylon filter, and nematodes were rinsed off of this filter and observed in a petri dish via microscopy. Post-filter nematodes were photographed, length and diameter measured, motility documented, and isolated for qPCR analysis to verify the presence of A. cantonensis larvae. Results to date have shown that live nematodes, including A. cantonensis larvae, were able to penetrate 20 µm and 10 µm wound polypropylene filters and a 5 µm spun polypropylene filter (4-7%, 14-39%, and 0-1.5% respectively). No nematodes were found in the filtrate of 5 µm carbon block and 1 µm spun polypropylene filters. However, control runs, where nematodes were added to the system with no sediment filters, estimate our methods can only detect 80-86% of nematodes post filtrate, giving our data a 14-20% margin of error. While the product information sheet of all tested filters claims only to reduce microorganisms, no filter provided size exclusion filtering as demonstrated by no statistically significant difference in the length or diameter of nematodes pre and post filter. Overall, the results of this pilot study highly emphasize the need for a further, comprehensive study of the structural design elements that influence effectiveness of these sediment filters. In this study, there were not enough replicates of various structural design elements to draw conclusions about type of filter material, filter size, end gaskets, and NSF ratings. Moreover, the data in this pilot study is only good for the specific brand and model of the tested filters, homeowners should be cautious about extrapolating these findings to other, non-test brands and models.

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(Co-authors: Lisa M. Kaluna, Yaeko Tagami, Kathleen Howe, and Susan Jarvi)
BIÓINFORMATICS ANALYSIS OF VIRULENCE GENES IN CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI STRAINS

Campylobacter jejuni is a common commensal bacterium found within poultry, and the leading cause of bacterial gastroenteritis in humans. The objective of this project is to use Unix command line tools, UH High Performance Computer, and Bioinformatic software to analyze Campylobacter virulence genes taken from the Campylobacter jejuni reference genome 11168. The genes of interest are those that are related to virulence mechanisms such as adherence, invasion, and toxin production. A custom Perl script was used to perform multiple sequence alignments corresponding to reference genes in the various whole Campylobacter jejuni and Campylobacter coli genomes. Campylobacter coli was selected due to its close relation to Campylobacter jejuni and the abundance of sequences on NCBI. All sequences were obtained from the NCBI database. The sequences were analyzed for SNPs, and phylogenetic trees were generated that examined the evolution of virulence genes within various strains. The resultant analytics identified that the Penner19 strain has the least percent identity to the reference genome. Moreover, within the Penner19 strain, as well as the other C. jejuni genomes, the flagellar genes flaA and flaB have the least homology to the reference genome in comparison to the other genes observed. According to literature, Penner19 is a strain of Campylobacter jejuni that is capable of causing Gullain-Barre syndrome, in which the host’s immune system attacks the peripheral nervous system which is seemingly unrelated to gastroenteritis. The difference in the sequences of the flagella genes compared to the representative genome, Campylobacter jejuni 11168, could provide insight to the adherence and invasion mechanisms of the Penner19 strain. Continuing work should be done using gene expression models of the flagella genes of strain 11168 and Penner19 to further study how bacteria that causes diarrhea can cause an auto-immune nerve damage syndrome.

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Co-authors: Dan Laspisa, Robert Oda, Dr. Amanda Lee, Dr. John Berestecky

Poster #174
Bacteriophages are ubiquitous in nature and play significant roles in ecology, alternative medicine, and scientific advancement. However, a relatively minute number of bacteriophages are completely sequenced and thoroughly studied, leaving gaps in knowledge. To fill these gaps, genomic analyses and biological characterization were conducted on two separate phages. Virion morphology revealed icosahedral head sizes of 60-62nm and 48-50nm, long rigid and flexible tails. Genome sequence analysis suggests linear dsDNA genomes consisting of 92,319 and 43,945 base pairs (bp), G+C contents of 49.4% and 54.4%, and 15 and 0 tRNA sequences. Coordinating respectively to a Pseudomonas aeruginosa infecting Myoviridae, PAKP1 like virus and an undocumented Escherichia coli infecting Siphoviridae species. Redundant terminal sequences of 127bp suggest a direct terminal repeat packaging mechanism in both phages. Genome annotation resulted in 174 and 57 ORFs. A few encoding essential genes for propagating infection and replication such as a large terminase subunit and peptidoglycan infection receptors. One-step growth curves estimate average burst sizes of 79.3 progeny for the E. coli phage and 48.5 progeny for the PAKP1 like phage per infected cell. UV exposure, chloroform sensitivity, temperature variability, and divalent cation restriction suggested different viability of infectious phage particles following treatment. The complete genome sequence and genome annotation will be uploaded to GenBank and provide public access to information about these phages that may be utilized in future studies.

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(Co-authors: Helmut Kae, Kabi Neupane, Alyssa Macdonald)

Poster #175
THE ACTIVATION OF TOLL-LIKE RECEPTOR 9 IN HUMAN AMNION EPITHELIAL CELLS

Background: Premature or preterm birth occurs when a baby is born before 37 weeks of development. In Hawaii, the preterm birth rate is 10.5% and has been steadily increasing since 2011 (March of Dimes). Prematurity can lead to many health challenges including, neurological, gastrointestinal and lung dysfunction, and depending on how early the baby was born, can also lead to death. Because of this, premature birth is the most frequent reason for infant mortality. Half of the cause for premature birth is due to infection, which is known to activate the innate immune response via Toll-Like Receptors (TLR). TLR9 is the second most highly expressed TLR in cells of the amnion in the human fetal membranes (Sato et al., 2016). TLR9 is activated by viral and bacterial DNA leading to NF-Kappa B activation, cytokine secretion and an inflammatory response. This inflammatory response may cause the release of Damage Activated Molecular Pathways (DAMPs) causing the activation of parturition. The purpose of this study was to understand the role of TLR9 in the human fetal membrane. We first tested if the TLR9 receptor was functional. Secondly, cells from the amnion were stressed with hydrogen peroxide to see if they produce possible TLR9 activating ligands. The hypothesis of this project is stressing the cells activates the cells, which then causes TLR9 activation and downstream inflammation that can contribute to the processes of parturition.

Methods: Fetal membranes were collected from repeat cesarean section at Kapi‘olani Hospital for Women and Children with IRB approval. Tissue collection was done with the McDonald and Casey Method for AEC isolation. AEC cells were isolated by a modified McDonald and Casey method, as routinely performed in the lab (Sato et al., 2016). The cells were put into 6-well plates with 10% FBS DMEM and allowed to adhere for 7-10 days. TLR9 stimulation experiments: The cells were serum starved for 15 hours in 0.5% FBS DMEM prior to treatment with TLR9 ligands (#CPG ODN; 2006, 2006C, 2395, 2395C, 2216 and 2243 at 5uM) for 6 hours. A Multi-Analyte ELISA was used to measure IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-14, IL-17A, IFN Gamma, TNF Alpha, and GM-CSF cytokines from the conditioned media according to the manufacturer’s instructions. The absorbance was read at 450nm. The production of cytokines was normalized to the amount of protein in each conditioned media sample by a standard BCA Assay. The average 562 nm absorbance of the blank standard replicates was subtracted from the media sample replicates. Cell stress experiments: AECs were treated with 6 different concentrations of H2O2: 0, 0.1, 1, 10, 100, and 1000 ng/ul of H2O2 over night (16-hrs). An LDH Assay was performed by adding 96.7 micro liters of super mix into each well in a 96-well plate. The super mix was composed of 32.4mL Tris HCl (0m2M, PH 7.3), 1.188mL NADH (6.6mM) and sodium pyruvate (30nM). The plate was incubated for 5 minutes at 25 degrees Celsius. 3.3mL of the H2O2 stressed media was then added to the cuvette in triplicates, and the absorbance at 340 nm was recorded every 10 seconds for 10 minutes for a total of 60 reads.

Results: All of the TLR9 ligands tested showed the up and down-regulation of several cytokines. However, different ligands affected different cytokines. 2006 caused the increase in the amount of IL-6 and GM-CSF secretion whereas, 2216 caused the upregulation of IL-6, IL-8 and IL-10, and 2395 only increased the levels of IL-17A. The cytokines that were down regulated were, IL-12 and IL-1β by 2006 and 2395 respectively. 2216 downregulated; IFNγ, TNFα and GM-CSF. The treatment of the cells by hydrogen peroxide lead to the increase in LDH activity with increasing dose. Measurement of DAMPs and PAMPs by this cell stress is ongoing.

Discussion: The results of these experiments show that the TLR9 receptors are able to be activated and induce the production of cytokines and that each ligand tested, produced a different response. Therefore, this shows that different synthetic ligands are able to produce different responses from the same receptor, and thus it is possible that perhaps different DAMPs and PAMPs may also produce different immune responses. The data from the hydrogen peroxide experiments allows us to now measure, which DAMPs are produced by this stress model.

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Poster #176
ANALYSIS AND CATALOGING OF HAWAIIAN SWEET POTATO THROUGH RAPD PCR

The purpose of this experiment was to identify sweet potato strains through RAPD PCR. Traditional cataloging techniques used for sweet potatoes contains numerous problems as there is immense difference among leaf shape, size and color of the tuber flesh as well as with different names of the strains. As DNA is a clear and concise form of cataloging, it well suited to apply to the different strains of sweet potato to determine if the different strains are closely related at the genetic level, or radically different. RAPD PCR differs from standard PCR in that it produces multiple bands of a DNA ladder, as opposed to the one to two bands produced by the standard PCR. RAPD PCR was selected because these multiple bands it produces are better suited for looking at complex and subtle differences in the Hawaiian sweet potato, more so than the standard PCR.

Samples of sweet potato were amplified using eight different primers that had been used successfully to catalog bacteria. After selecting three of the most promising primers, success with producing multiple banding patterns was achieved initially. However, the numerous subsequent experiments were yielded unsuccessful results. The final experiment was successful and showed that the banding pattern was highly similar between two strains with very different names. This confirmed that the two successful samples were either closely related or the same.

The potential for RAPD PCR as a cataloging method with auspicious for its ability to differentiate different strains and unify terminology for strains that have multiple names.

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Poster #177
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**ASSESSMENT OF GALECTIN-9 EXPRESSION IN THE CENTRAL NERVOUS SYSTEM OF HIV-INFECTED INDIVIDUALS**

Objectives: It is estimated that about 50% of HIV-infected individuals develop some degree of HIV-associated neurocognitive impairment (NCI) even after being virally suppressed on long-term antiretroviral therapy. This persistence has led to an effort to elucidate the underlying pathophysiology of HIV-associated NCI. Galectins, a family of proteins that bind β-galactosides, regulate inflammatory responses and are involved in peripheral and tissue homeostasis. Galectin-9 (Gal-9) is one of 10 human galectins, and contributes to HIV transcription and viral production, suggesting its role in HIV pathogenesis and persistence. Gal-9 is elevated in patients with compromised neurological function, such as multiple sclerosis and glioma, however the role of gal-9 in HIV-associated central nervous system (CNS) disease is unknown. The aim of this project was to determine the role of Gal-9 in the CNS with HIV-infection.

Methods: We examined RNA expression of galectins in the brain using NCBI GEO microarray datasets, while Gal-9 was analyzed at the protein level by immunohistochemistry staining of post-mortem brain tissue (n=17); tissue donors were HIV-, HIV+ with normal brain pathology, or HIV+ with encephalitis. Utilizing a novel human microglial latency cell with integrated HIV provirus, we assessed the effects of a recombinant Gal-9 stimulation on microglial response using flow cytometry and luminex multiplexing. Statistical analysis on results was conducted using either Prism Graphpad or SPSS software.

Conclusions: Our studies suggest a link between Gal-9 in the CNS and HIV infection and potential responses as a result of Gal-9 stimulation, which may provide additional information about the pathogenesis of CNS reservoirs and cognitive impairment development.

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IMPROVING A GOLD AND TITANIUM CONTAINING METALLODRUG’S EFFICACY AGAINST CANCER

The purpose for the experiment is to test a subset of metallodrug compounds on select cancer lines (kidney cancer) in order to determine how effective they are against kidney cancers. These compounds are composed of two metals (e.g. Titanium and Gold or Ruthenium and Gold) bound to a scaffold of methyl and carboxylate groups. Modifications will be made to the scaffold components of these components to improve efficacy. How these compounds kill kidney cancer will also be determined. The most effective metallodrugs may then be used for in vivo studies in mouse models of kidney cancer.

A basic cell number assay called XTT assay will be used to determine which compound variants are most effective at killing the target kidney cancer lines. The assays that will be performed to identify the mechanism of cancer cell death include apoptosis (cell death), proliferation, cell cycle, microtubule, DNA damage and binding, and cell motility. Previous results have shown that earlier versions of these compounds kill kidney cancer cells, with minimal toxicity. Furthermore, the scaffold for these metals determines the target and efficacy of the compound.

The major significance of this research is the potential to identify good quality metallodrug compounds to pursue in further pre-clinical models, which may someday prove valuable against kidney cancers in patients. Creating these metallodrug compounds is a logical choice to help discover diagnostic and therapeutic chemotherapies to be used in cancer patients. The results may also shed new light on developing better and more effective anticancer drug treatments. However, further characterization will be required to establish their diagnostic and therapeutic potentials.

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