Undergraduate Research Symposium May 20, 2016 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

MGH 241, Easel 151

11:00 AM to 1:00 PM

Preferential Sites of Initial Distant Metastatic Disease in Merkel Cell Carcinoma

Jamiluddin John (Jamil) Qazi, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Hannah Josline (Hannah) Thomas, Senior, Neurobiology
Mentor: Paul Nghiem, Dermatology

Merkel cell carcinoma (MCC) is an aggressive skin cancer with a propensity for early dissemination. As little is known about this process, we sought to identify the preferential sites of initial distant metastatic MCC in order to guide imaging evaluation and clinical follow up. In this retrospective study, we analyzed the clinical and imaging records of 442 initial distant metastases from 305 MCC patients enrolled in our data and tissue repository. Initial distant metastases were defined as the first lesions detected beyond the regional lymph nodes of the primary tumor. Among 305 patients, 69% had 1 initial metastasis, 19% had 2, 9% had 3, and 3% had 4 or more. Of the 305 patients, 50% were evaluated only by radiologic and/or clinical criteria, while 50% also underwent biopsy-confirmation of MCC metastases. Of the 442 initial distant metastases, the distribution of sites was: 26% to distant lymph nodes (typically supraclavicular, retroperitoneal, or iliac), 15% to liver, 13% to skin, 13% to bone, 13% to other visceral sites, 6% to lung, 5% to pancreas, 3% to brain, 2% to kidney/adrenal gland, 2% to gonads, 1% to chest wall, and 1% to stomach. Although there were similarities in initial metastatic sites between MCC and melanoma, the brain (3%) and lung (6%) were markedly less common in MCC than in melanoma (20% and 36%, respectively), while the pancreas was more common in MCC (5%) than melanoma (<1%). Given the aggressive nature of MCC, with ˜40% of patients eventually developing distant metastatic disease, these findings may facilitate improved detection and management.

POSTER SESSION 1

MGH 241, Easel 133

11:00 AM to 1:00 PM

Rhodopsin Expression can be Down Regulated in Mice through the use of Small Molecules CA88, CA95 and CA97

Andy Andreyevich (Andy) Shimchuk, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Thomas Reh, Biological Structure
Mentor: Paul Nakamura, Biological Structure

Retinitis Pigmentosa, a progressive neurodegenerative disease, is a significant contributor to loss of vision due to retinal degeneration. The retina is made up of light sensing cells called rods (for night vision) and cones (for day vision). In patients afflicted with this disease, there is a degeneration of rod photoreceptors over time, which leads to a decreased visual field and night blindness. At a certain point, the loss of rod cells leads to the breakdown and loss of cone cells, which are responsible for color vision. Mutations in the rod gene Rhodopsin have been linked to the development of this disease. By down regulating this defective gene, the degeneration of rods would likely be prevented. Subsequently, cone degeneration would also be prevented, limiting the loss of vision in afflicted individuals. The expression of Rhodopsin is controlled by a nuclear hormone-like receptor transcription factor called Nr2e3. In collaboration with another lab (Dr. Sheng Ding, UCSF), we have identified small drug-like molecules that can block Nr2e3, and we have called them CA88, CA95, and CA97. We predict these antagonists will lower the expression of rod genes. Wildtype mouse retinas were treated in culture with CA88, CA95, or CA97. The use of these small molecules resulted in the significant down regulation of a number of rod genes. These genes include Rhodopsin, NRL, NR2E3, and GNAT1. Stained tissue sections further confirmed a decrease in Rhodopsin with treated retinas. When retinal ex plants were done using mice with a P23H mutation in the Rhodopsin gene, treated retinas were found to have a significantly thicker photoreceptor layer than non-treated, indicating the ability for these compounds to prevent retinal degeneration. With a way to reliably down regulate problematic genes such as Rhodopsin, a potential avenue for treatment of humans affected by Retinitis Pigmentosa is opened.

POSTER SESSION 1

MGH 241, Easel 126

11:00 AM to 1:00 PM

Conserved *Hox* Gene Expression during Larval Stages in the Bat Star, *Patiria miniata*

Alia Shifa (Alia) Hidayat, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: Paul Minor

Hox complex genes constitute an essential and ubiquitous component in the construction of the animal body. Despite their wide range of body plans, every member of the bilaterian clade utilizes these key genes to pattern the anteroposterior (AP) axis, with one potential exception - radial echinoderms. In addition to the possession of an adult radial body plan, Echinodermata are also mostly characterized by indirect development, beginning their lives as bilateral larvae, which undergo radical metamorphosis into a radial adult. The echinoderms, outside the sea urchin, *Strongylocentrotus purpuratus,* remain poorly studied, and it is unknown how classically bilateral planning mechanisms like *hox* are involved in patterning these very different life stages. To help begin to resolve this, we present *in situ* hybridization data of four *hox* genes present in the indirect developing bat star, *Patiria miniata* in its larval stages. *Hox* expression was observed in the posterior during the development of larval forms, similar to the expression patterns seen in other bilaterian animals. These results suggest that, despite a greatly divergent radial body plan of the adult, echinoderms use many of the same developmental mechanisms found in bilateral organisms to pattern their larval stages prior to metamorphosis.

SESSION 1J

IMPROVING HEALTH CARE THROUGH NEW DIAGNOSTIC TESTS AND BACTERIAL MONITORING

Session Moderator: Paul Yager, Bioengineering
MGH 284
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Development of a Competitive Inhibition Assay for Implementation in Fluorescence-Based Point-of-Care Diagnostics

Louise Lyth (Louise) Hansen, Junior, Bioengineering
Mary Gates Scholar
Mentor: Paul Yager, Bioengineering
Mentor: Josh Bishop, Bioengineering

Due to the rise of pathogen genetic knowledge and implementation methods of diagnoses pathways for patients, an increase in access to speedy and efficacious therapies is needed. The development of inexpensive, high-performance point-of-care (POC) tests does not only improve healthcare in low resource settings, but also moves the diagnoses out of hospitals and into homes and primary care offices. Paper-based microfluidic devices help both identify and screen for pathogens by allowing for more rapid care and treatment. The multiplexed, autonomous, disposable nucleic acid amplification test devices fabricated in the Yager Laboratory performs sequential functions - sample preparation, nucleic acid amplification, and lateral flow detection – for target identification. My project aims to bridge the last two segments into a single unit by introducing a method of inhibitory competition that permits for measurable, fluorescent-based analysis of isothermal DNA amplification from dry reagents in porous media. To prove this concept, a simplified experimental model of a multi-region, paper-based nucleic acid amplification test was designed, and new interference amplification mixtures developed. The isothermal strand displacement amplification (iSDA) technique utilized in these devices incorporates a fluorescent hybridization probe that allows for the detection of the increase in amplicons using an optical detection method. By incorporating a dilution of comparable, secondary strand of DNA as an internal control, a competition for reagents is established which creates different chemical sensitivities in the amplification zones. As a result, target DNA from the pathogen is amplified against a competitive threshold in a range. In the long run, fluorescence imaging of all regions can yield a quantitative measure of the concentration of pathogens in a sample. Overall, this project tests the fundamental groundwork for a new family of optical-based, quantitative devices by testing the use of fluorescent detection for POC tests, and investigating the principle of inhibitory competition.

POSTER SESSION 2

Commons West, Easel 19
1:00 PM to 2:30 PM

Exposure to Polycyclic Aromatic Hydrocarbons in Wildland Firefighters

Niloufar Ghodsian, Junior, Environmental Health
Mentor: Michael Paulsen, Environmental and Occupational Health Sciences

Wildland firefighters are trained to combat forest fires by prescribed burning or wildfire suppression. Prescribed burning is a planned fire lit intentionally by firefighters to reduce fire hazard and to provide greater safety for firefighters and the public. Wildland firefighters often work on controlled burns using one of two work tasks: lighting, which consists of laying a strip of fire on the forest floor; and holding, keeping fire inside specified boundaries. It is the exposure inherent to these work tasks that our research is primarily concerned with. During these operations, firefighters are exposed to various combustion products, including fine particulate matter (PM2.5), carbon monoxide, and polycyclic aromatic hydro-

carbons (PAHs). Many PAHs are carcinogens that undergo metabolism and are excreted in urine. In order to measure the PAH exposure severity, urine samples from wildland firefighters were collected before and after prescribed burn operations as well as on non-burn days. Urine samples were hydrolyzed by treatment with sulfatase and glucuronidase enzymes, and cleaned up with solid phase extraction. High performance liquid chromatography with fluorescence detection was used to analyze the samples for PAH metabolites. Our data analysis compares PAH exposure by work task, by season, and between burn and non-burn days. We anticipate that urinary concentrations of PAHs in firefighters will be higher after the prescribed fire operation compared to pre-burn levels. Findings of this research can be used to measure PAH exposure not only in wildland firefighters, but also in other occupational or residential settings where people are exposed to high concentrations of PAHs to reduce risk factors of developing cancer.

POSTER SESSION 2

Balcony, Easel 86

1:00 PM to 2:30 PM

Efficient Selection of Genetically Modified T Cells for Human Immunotherapy using Methotrexate

Teresa Einhaus, Sophomore, Molecular Sciences, Bellevue College
Mentor: Gita Bangera, RISE Learning Institute, Bellevue College
Mentor: Bish Paul

Genetically modified T cells have the potential for a number of therapeutic uses in anti-cancer immunotherapy, but a current limitation is the low yield of modified cells. Methotrexate is an FDA-approved drug used to destroy rapidly dividing cells by blocking their metabolism of folic acid. With the addition of a construct containing a mutant DHFR (dihydrofolate reductase) for methotrexate resistance, modified cell populations can be selectively expanded. Our mutant DHFR gene, delivered by lentivirus, has a Tyr-22 mutation which confers methotrexate resistance by changing its binding site. The purpose of this study is to determine the optimal concentrations and timing for the addition of methotrexate for selection of our target cell population. First, we grew cultures of Jurkat cells, an immortal T cell line, to find their normal growth and viability curves. Next, we transduced the cells by the addition of lentivirus at 1 or 2 X 10^4 viruses per 10^6 cells. We measured gene expression of a green fluorescent protein reporter gene using flow cytometry, which showed the lower viral concentration produces a peak protein expression of 14% on day two. Finally, we used various concentrations of methotrexate and found the optimal dosage for chemoselection to be 50 nM, with around 90% of the population showing expression on day five. We demonstrate a six-fold increase in gene modified cells in the presence of methotrexate and that the growth rate of modified resistant cells is comparable to non-modified cells. Currently, we are testing this selection protocol in human CD4 T cells. Overall, this study has major implications for the use of gene therapy requiring T cell products in clinical trials.

SESSION 2O

ASTRONOMY

Session Moderator: Suzanne Hawley, Astronomy

JHN 026

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Cataclysmic Pulses: Investigating Temperature Instability of Accreting White Dwarf Pulsators

Donald Francisco (Donald) Serna Grey, Senior, Astronomy, Physics: Comprehensive Physics
NASA Space Grant Scholar
Mentor: Paula Szkody, Astronomy

The compact core that remains after a star like our Sun dies is called a white dwarf. Some of these stars have close orbiting companion stars that they will accrete matter from constantly. This accretion eventually causes outburst events called dwarf novae. These binary star systems are known as cataclysmic variables. The six telescope observing targets for this project also undergo variable brightness pulsations due to temperature instabilities in the near pure hydrogen atmpspheres of these white dwarf stars. These variable brightness pulsations make these stars ZZ Ceti pulsators in addition to being cataclysmic variables. We present time series photometry in the optical range from cataclysmic variable white dwarf ZZ Ceti pulsators as part of an ongoing long term investigation of accretion dynamics and white dwarf internal structure. These light curves are used in comparison with UV light curves gathered from the Hubble Space Telescope to probe the temperature and pulsation properties of these targets. Previous observations suggest a temperature instability strip that is broader than current models predict. This investigation will empirically measure the instability strip as our pulsator telescope targets cool and restart regular pulsations after dwarf nova outburst events that were observed near the start of this project. Measurements of the time it takes the pulsations to reappear allows for an estimation of accreted mass and heating that occurred during the dwarf nova outburst. These measurements have confirmed the target SDSS0755+14 as a ZZ Ceti pulsator in the target pool and allow for tighter constraints and refined models of binary and accretion interactions of white dwarf systems, especially Type Ia supernova progenitor models.

POSTER SESSION 3

Commons West, Easel 27

2:30 PM to 4:00 PM

Incidence of Dementia Subtypes in a Community-Based Prospective Cohort Study: The Adult Changes in Thought (ACT) Study

Julia Bauman, Sophomore, Pre-Sciences
Mack Paller Moore, Junior, Public Health-Global Health
Mentor: Paul Crane, Medicine

Recent proposed guidelines for Alzheimer's disease (AD) stress the importance of AD subtypes (DuBois et al., Lancet Neurology 2014). There are limited data available regarding incidence rates of AD- and non-AD dementia subtypes. Better understanding of these subtypes is an important objective for improving clinical diagnoses and planning future research targets. Using data from the Adult Changes in Thought (ACT) Study we identified groups of individuals with pronounced deficits in memory, executive functioning, language, visuospatial ability, and attention, defined as $>$1SD below their average score on the battery of cognitive tests administered at the dementia evaluation. We performed two sets of analyses on these groups. The first set of analyses was on the subset of the ACT cohort who died and came to autopsy (636 individuals). In this group, we analyzed Braak stage and CERAD level, which are measures of disease progression, using data from 274 cases and 344 controls, as well as the presence and location of microinfarcts, macroscopic infarcts, Lewy bodies, and amyloid angiopathy. The second set of analyses was on the entire ACT cohort regardless of autopsy status. In this group we analyzed dementia onset, years of education, self-reported race, and medical comorbidity as estimated from pharmacy data. We also analyzed APOE genotype in this group; one variant of this gene is a well-know risk factor for AD. In ACT, of 867 cases of dementia with probable or possible AD, 745 (86%) had complete data with non-missing scores for all cognitive domains. Of these, 517 (69%) had a typical cognitive profile while 228 (31%) had an atypical cognitive profile. We concluded that clinical AD with a typical pattern of cognitive deficits is the most common form of incident dementia in the community, and that clinical AD with an atypical pattern of cognitive deficits is more common than non-AD dementia. Further work may reveal associated risk factors for these groups.

POSTER SESSION 3

Commons East, Easel 60

2:30 PM to 4:00 PM

Role of Post-Operative Radiation Therapy in Stage IA Merkel Cell Carcinoma of the Limbs and Trunk

Ilsa Daniela Victoria (Ilsa) Juhlin, Junior, Mechanical Engineering
NASA Space Grant Scholar
Maclean M (Maclean) Cook, Junior, Neurobiology
Mentor: Upendra Parvathaneni, Radiation Oncology
Mentor: Paul Nghiem, Dermatology

Merkel cell carcinoma (MCC) is a rare, and often aggressive skin cancer with a disease-associated mortality of approximately 40%. About 1,500 cases of MCC are diagnosed annually, and the incidence is increasing. Following surgery, post-operative radiation therapy (PORT) is usually recommended to minimize local recurrences. However, it is unclear whether patients with low-risk Stage IA MCC arising on the limbs and trunk should also receive PORT. We retrospectively identified 80 low-risk patients from our database who met all of the following criteria: 1) primary tumor $\leq$2cm in diameter, 2) microscopically negative margins on surgical excision, 3) no microscopic evidence of nodal metastasis in the sentinel lymph node biopsy, and 4) absence of immunosuppression. Of these 80 patients, 20 patients were treated with surgery alone and 60 received surgery and PORT. There were no significant differences between the two groups in terms of demographics (age, sex, race), location of primary tumor, margin status, depth, or lympho-vascular spread. However, patients treated with PORT tended to have larger primary tumors (p=0.009). The median long-term follow up time was similar for both groups, and was $>$ 4 years. We defined a local recurrence (LR) as a recurrence within 2 cm of the primary tumor operative bed, and Fisher's exact (2 tailed) was used. There was no statistically significant difference in LR between patients treated with surgery alone versus surgery + PORT (p=0.25). One patient (5%), who had surgery alone, had a LR. There were no LRs among those who had PORT. There was also no statistical difference in regional nodal metastases (p=1), distant metastases (p=0.33), MCC specific survival (p=0.06), and overall survival (p=0.06) between the two groups of patients. In conclusion, PORT was not associated with a reduced risk of local recurrence in low-risk, Stage IA, MCC arising on the limbs and trunk.

POSTER SESSION 3

Commons East, Easel 49

2:30 PM to 4:00 PM

Sample Preparation Concerning RNase Activity in Point-of-Care Settings

Anne Marie Elizabeth (AnneMarie) Welch, Senior, Microbiology
Mentor: Paul Yager, Bioengineering
Mentor: Paula Ladd, Bioengineering

In 2012, the World Health Organization (WHO) estimated that in low resource countries 4 in 10 deaths among children under the age of 15 were associated with infectious disease. Point-of-care diagnostic devices that can quickly and effectively identify the source of the infection will drastically improve patient treatment times increasing rates of survival. We therefore investigated whether traditional RNA handling techniques used in a laboratory setting are necessary for these point-of-care devices, by assessing the stability of RNA under various conditions. Using respiratory syncytial virus (RSV) as an example, we discovered that RSV can be thermally lysed without significantly degrading the integrity of the RNA. In addition, the use of RNase inhibitors or tRNA does not have a statistically significant effect on sample integrity. A comparison between RSV RNA derived directly from patient nasal swabs, with swabs containing purified RSV RNA, shows that RSV virions protect their RNA prior to lysis. Amplification by qRT-PCR, indicates that RSV RNA is directly amplifiable suggesting that RNases are inactivated at 95C, and do not regain activity prior to amplification. Our data provides the necessary framework for performing point-of-care testing without the need for traditional RNA handling procedures. The results have important implications since we expect that the research concerning RNA handling and integrity will translate to other RNA based viruses which include Ebola haemorrhagic fever, influenza, hepatitis C, West Nile fever, polio, and measles. With recognition of these capabilities, we can implement minimal RNA sample processing into point-of-care devices.

POSTER SESSION 3

Commons East, Easel 48

2:30 PM to 4:00 PM

Point-of-Care Diagnostics, MAD NAAT

Matthew Henry (Matt) Turner, Sophomore, Pre-Sciences
NASA Space Grant Scholar
Mentor: Paul Yager, Bioengineering
Mentor: Josh Bishop, Bioengineering

According to the World Health Organization, more than 100 million people annually drop into poverty because of health care related costs. Point-of-care (POC) diagnostic testing works to alleviate this issue. Point-of-care diagnostics is testing that delivers cheap, accurate, and timely diagnosis in the hospital, the field, the office, or the home. In this way, POC testing can provide vital health information in a way that reduces the burden of health care globally. The lab of Dr. Paul Yager has developed a POC diagnostic device known as the multiplexed autonomous disposable nucleic acid amplification test, or MAD NAAT, which returns results in less than one hour. This device uses a two-dimensional paper network (2DPN) to automate the fluidic steps of a nucleic acid-based diagnostic assay, which detects the presence of one or more pathogens (e.g. MRSA, RSV) in a patient's test sample. The downstream region of the 2DPN uses nitrocellulose strips to support a visual, colorimetric detection system. The detection system produces a series of visible red lines on the nitrocellulose strip that indicate the result of the test and associated controls. My research is focused on the optimization of the detection system, specifically: increasing the visible intensity of these red lines without increasing device cost. I have investigated the effects of varying the geometry of the 2DPN through the detection region. The goal of this research is to obtain the most intense test line signal with the least amount of expensive reagents, at the lowest possible pathogen count, in the least amount of time. I have also used MATLAB to implement patient sample analyzations for the MAD NAAT device. These efforts support further development of an affordable POC device, which will improve patient health outcomes in communities that lack proper healthcare.

POSTER SESSION 4

MGH 241, Easel 137

4:00 PM to 6:00 PM

Independent Chemical Verification of Electrically-Evoked Dopamine Release

Dorothy Anne (Dorothy) Cabantan, Junior, Neurobiology
Mentor: Paul Phillips, Psychiatry & Behavioral Sciences
Mentor: Stefan Sandberg, Psychiatry and Behavioral Sciences

Fast-scan cyclic voltammetry (FSCV) is an electrochemical method that measures and identifies *in vivo* brain analytes during behavioral tasks in rats. FSCV identifies analytes through a chemical fingerprint called a cyclic voltammogram (CV). When an unknown sample is collected, it is important to verify that the analyte being measured is the analyte of interest. To this end, five criteria have been proposed to identify an unknown analyte being measured in the brain, namely: electrochemical, anatomical, physiological, pharmacological, and independent chemical analysis. The brain analyte dopamine has been established under these five criteria, except for independent chemical analysis. We hypothesize that the reason for failing to establish independent chemical analysis is that tissue impedance leads to a discrepancy between *in vitro* calibrations (independent chemical analysis) and *in vivo* FSCV measurements. Indeed, recent work in our lab has demonstrated a 50 kOhm difference between the reference and working electrode, *in vivo* vs *in vitro*. This difference may cause a shift in the CV for dopamine, making it difficult to use for analyte verification. In order to test whether this 50 kOhm differential can explain the discrepancy between *in vivo* and *in vitro* dopamine, we added a 50 kOhm resistor to the circuitry between the reference and working electrode. We then measured a range of *in vitro* dopamine concentrations in the absence and presence of the

50 kOhm resistor. The CVs generated from these measurements are then statistically compared to *in vivo* dopamine CVs in terms of qualitative and quantitative agreement. Subsequently, dopamine along with pH measurements at zero and/or 50 kOhm resistance will be used to test the performance of a statistical calibration method known as chemometrics.

POSTER SESSION 4

MGH 241, Easel 131

4:00 PM to 6:00 PM

The Influence of Conserved Elements Responses during the Acute Infection of HIV on the Response to ART and DNA Therapeutic Vaccination

Nika Hajari, Senior, Microbiology
Mary Gates Scholar, UW Honors Program
Mentor: Deborah Fuller, Microbiology
Mentor: Paul Munson, Microbiology

While HIV treatment is improving, there are still problems associated with it. Antiretroviral therapy (ART) has unfavorable side effects, is expensive, and requires a lifelong daily pill intake. However, it has been proposed that a therapeutic vaccine could provide durable control of viremia in the absence of ART. This is a difficult approach due to the high mutation rate of the virus. Research has shown that there are conserved elements (CE) within the virus that do not change because they are important for the viral fitness. Accordingly a therapeutic DNA vaccine is being investigated that targets the CE epitopes. To address this, rhesus macaques were infected with SIV (Simian Immunodeficiency Virus), put on ART, vaccinated with four doses of DNA vaccine expressing CE, removed from ART, and their viral loads monitored. A successful vaccine is expected to reduce the viral loads to negligible amounts post ART cessation. My research project investigates the role of CE responses developed during acute infection. *I hypothesize that macaques that naturally develop higher CD4+ and CD8+ T cells (types of white blood cells) with CE specificity in response to the acute SIV infection will respond better to ART and CE vaccinations*. To investigate this I collected plasma and lymphocytes from the blood during acute infection and analyzed the CD4+ and CD8+ T cells using enzyme linked immunospot (ELISpot) and intracellular cytokine stain (ICS) assays. The viral loads were monitored by measuring the concentration of viral RNA in the plasma. T cell responses measured at each time point was then correlated to viral loads post ART-pre vaccinations or to CE responses measured after the final DNA vaccination by Spearmann Rank correlative test. My findings will help us better understand the significance of conserved elements in controlling the HIV and thus design better human therapeutic vaccines.