HIV AND STIs: FROM THE BENCH TO THE BEDSIDE AND BEYOND

Session Moderator: Geoffrey Gottlieb, School of Medicine
JHN 175
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Genetic Mechanisms to Second-Generation Integrase Inhibitor Resistance in HIV-2
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Mary Gates Scholar
Mentor: Geoffrey Gottlieb, School of Medicine
Mentor: Robert Smith, Pathology

Human immunodeficiency virus (HIV) infection is an important health issue worldwide and consists of two main types, HIV-1 and HIV-2. In the fight against HIV, integrase strand transfer inhibitors (INSTIs) are a relatively newer class of antiretroviral drugs aimed at blocking viral replication. FDA-approved first generation INSTIs include raltegravir (RAL) and elvitegravir while the second generation includes dolutegravir and cabotegravir (CTG). The high mutation rate of HIV often results in resistance to antiretrovirals and leads to treatment failure. However, the mechanisms and mutations that confer INSTI resistance in HIV-2 are not completely understood, despite the growing importance of INSTIs for HIV-2 treatment. The purpose of my research is to identify and characterize genetic changes responsible for INSTI resistance in HIV-2. Specifically, I engineered mutations that have been reported in sequences from patients receiving INSTI treatment into a laboratory strain of HIV-2, and tested the mutants for resistance to RAL and CTG in culture. Initial results indicate that a mutation at integrase position 263 (R263K) does not confer resistance to either RAL or CTG. However, a second variant encoding the mutations G140S+Q148H+N155H is highly resistant to both drugs, with 50% effective concentrations (EC_{50} values) that were at least 1000-times greater than the wild-type strain. Experiments with additional HIV-2 integrase mutants are in progress. In the future, the mutant HIV-2 clones produced in this study will serve as a reference library for evaluating the resistance profiles of newer integrase inhibitors, thus helping to identify therapeutic strategies for HIV-2 patients that harbor drug-resistant virus.

Investigating Neutrophil Related Cytokines in Acute Simian Immunodeficiency Virus Infection
Lydia Alice Sweet, Senior, Microbiology
Arina Wu, Junior, Environmental Health
Mentor: Nichole Klatt, Pharmaceutics

Chronic inflammation in human immunodeficiency virus (HIV) infected individuals is associated with increased mortality, even in patients who are virally suppressed by antiretroviral therapy. The goal of this research is to understand how the gastrointestinal (GI) tract is damaged during early HIV/SIV infection. In many infections, neutrophils play an important part during early response to infection because of their microbial killing properties. The role of neutrophils in the establishment of HIV infection and subsequent chronic inflammation has not been examined. Our purpose was to study neutrophil supporting cytokines before and during acute simian immunodeficiency virus (SIV) infection and their potential role in the kinetics of the neutrophil response. We used SIV in the Rhesus macaque model instead of HIV so that we could obtain longitudinal samples very early after infection to observe the neutrophil kinetics. The neutrophil supporting cytokines we looked at were IL-8, IL-17, and G-CSF. We hypothesized neutrophils would decrease during acute SIV infection in peripheral blood in response to the decreasing neutrophil-supporting cytokines. We used Luminex to measure cytokine levels in the blood at each time point, and used Complete Blood Count to get blood neutrophil concentrations. After infection, neutrophils initially increased, but then 14 days post-infection there was a decrease in most of the animals. Prior to the post-infection decrease of neutrophils, we saw a decline of IL-8 levels, which may contribute to neutrophil decline. In contrast to other types of infection, we saw no increase in G-CSF or IL-17. However, we did observe increases in pro-inflammatory cytokines 14 days post-infection, and the pro-inflammatory cytokine IL-15 correlated with neutrophil decrease. These data suggest that reduced neutrophil supporting cytokines and increased pro-
inflammatory cytokines may be contributing to the neutrophil decline observed in acute HIV and SIV infection.

**Multifunctional Nanoparticles for Dendritic Cell-Based Intravaginal Vaccine against Sexually Transmitted Infections**

*Natacha Lou (Natasha) Comandante, Senior, Bioen: Nanoscience & Molecular Engr*

Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee

*Mentor: Kim A. Woodrow, Bioengineering*

Recent disease models have shown that majority of sexually active individuals will acquire sexually transmitted infections (STIs) sometime in their lives. Compartmentalization of vaginal mucosal immunity, as well as the immunosuppressive immunity exhibited by the Langerhans cells in vaginal mucosal tissue, pose challenges in developing efficacious STI vaccines. To address both of these challenges, we are developing multifunctional nanoparticles for programming ex vivo dendritic cells (DCs), which subsequently can be delivered intravaginally to stimulate Langerhans cells (LCs) in the vaginal epithelial tissue. We designed our DC transfection nanoparticles using a polycationic protein called protamine to complex antigen-encoded DNA plasmids for antigen presentation, and a double stranded RNA adjuvant, poly(I:C), for provoking DC maturation. In determining the optimal formulation, nanoparticles were formulated at four different protamine to mock DNA mass ratios (P:D ratios), 8:1, 2:1, 1:1 and 0.5:1. The nanoparticles were analyzed for their size, stability and DNA loading efficiency. Integrating results from these studies, we determined that nanoparticles with P:D ratio of 2:1 is the best candidate since it is the smallest P:D ratio that yields nanoparticles with the optimal size for DC uptake, as well as high particle stability and DNA loading efficiency. Additionally, DC transfection efficiency of the nanoparticle formulated at this ratio is also discussed. Successful development of the DC programming nanoparticles will demonstrate the feasibility of developing novel DC-based intravaginal vaccine, which will enhance vaginal mucosal immunity and can potentially be translated to efficacious vaccines against various types of STIs.

**Optimization of a "Fiber-in-Fiber" System for HIV Prevention in Females**

*Christina Nhan, Senior, Bioengineering*

Mary Gates Scholar, NASA Space Grant Scholar

*Mentor: Kim A. Woodrow, Bioengineering*

*Mentor: Anna Blakney, Bioengineering*

Many biological, socioeconomic, and cultural factors increase women’s risk of acquiring HIV, especially in low-resource settings. Currently, there aren’t any options that allow women to discreetly protect themselves from HIV acquisition. To address this, we developed a topical nanofiber mesh to be inserted into the vagina to deliver antiretrovirals for HIV prevention. Benefits of this dosage form include high drug loading, tunable drug release, and imperceptibility to users while protecting them from HIV over the course of a week. We designed a “fiber-in-fiber” system: the “burst” release nanofiber delivers an initial bolus of drug, while the “sustained” release nanofiber (polyester) prolongs drug release over a week. Nanofibers are synthesized via electrospinning, which involves exposing a polymer and drug solution to an electric field to create a nanofiber mesh. After polyester fibers are electrospun, they are micronized using a food processor and electrospun directly into the burst release fibers. The final product is a burst release nanofiber mesh that contains small polyester fiber particles. Initially, the polyester fiber particle sizes were large and variable, ranging from 8 to 2000 micrometers. After optimizing the micronization process, the median particle size was reduced to 179 micrometers. We compared this to the median particle size of post-electrospinning, which was 26 micrometers. The data indicate that smaller nanofibers are more likely to be electrospun into the fiber-in-fiber mesh. Thus, minimizing particle size is essential to achieve the desired drug loading. Furthermore, loading the micronized fibers with a fluorescent dye revealed that they are evenly distributed throughout the burst release fiber mesh. Recent experiments assessed the drug release profiles of this fiber-in-fiber system, which showed that it is able to sustain drug release. Evaluating the drug release benefits of this fiber-in-fiber system will allow this system to be applied to other topical drug delivery systems.

**Fabrication and Characterization of Electrospun Agarose and Polyvinyl Alcohol Blend Nanofibers as an Antiretroviral Drug Delivery Platform**

*Namratha Potharaj, Senior, Bioengineering*

Mary Gates Scholar

*Mentor: Kim A. Woodrow, Bioengineering*

*Mentor: Shih-Feng Chou, Bioengineering*

The Joint United Nations Program on HIV/AIDS (UNAIDS) reported in 2014 that approximately 36.9 million people in the world are currently living with HIV/AIDS. Many of these individuals reside in low and middle income countries where access to STD prevention methods is limited and risk of infection is high. Electrospin nanofibers are a promising drug delivery platform in development, with the potential to provide either pericoital or sustained protection at an affordable cost. Here, we fabricate and characterize nanofibers composed of polyvinyl alcohol (PVA) and agarose (AGR), a naturally occurring polysaccharide that is both mechanically strong and biocompatible. Previous studies in our group have reported challenges in electrospinning polymer blends from AGR and PVA, likely due to the high gelling properties of agarose. Subsequently, a non-uniform distribution of fiber diameter...
resulted in variable mechanical properties with an undesirable burst release of hydrophilic small molecule drugs (tenofovir, TFV). In this study, the electrospinning process was improved to achieve higher AGR loading in the nanofibers. We used a melt-electrospinning technique where the polymer solution was heated at a constant temperature of approximately 60°C, which is below the glass transition temperature of PVA. Constant heating of the polymer solution reduced AGR gelation and improved fiber throughput. The melt-electrospinning process enabled fabrication of nanofibers with up to 30% AGR content. Mechanical testing on the nanofibers revealed that the Young’s Modulus was approximately 100 – 200 MPa. Drug release studies revealed that AGR loading in the nanofibers did not significantly affect the burst release of TFV from the fibers. Fiber properties indicate that the PVA-AGR system must be optimized to further validate experimental results. In general, the melt-electrospinning process was key to enable the fabrication of PVA-AGR nanofibers with increased AGR content, which allows for enhanced characterization of these fibers as an antiretroviral drug delivery platform.

A Study of Potential HIV Transmission Hotspots among Men who Have Sex with Men (MSM) and Transgender Women (TGW) in Lima, Peru
Audrey V.M. (Audrey) Brezak, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Ann Duerr, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center

Innovative prevention strategies for MSM/TGW that effectively reduce incidence are critically needed; building these strategies will require identifying transmission networks and associated drivers of ongoing HIV transmission. The objective of this study was to identify drivers and sources of transmission among MSM/TGW in Lima, Peru through two aims: 1) To recognize geographic transmission hotspots through mapping locations of venues at which participants reported having sexual encounters and their residences; 2) To identify clusters of related incident infections through phylogenetic analysis and link these clusters to real-time data on sexual encounters, high-risk behaviors, and attendance at social venues (bars, clubs, saunas, etc.). Between 9/2013 and 10/2015, MSM/TGW (n=3,191) were screened for participation in a 24-month follow-up study (HIV prevalence=20.5%). HIV-uninfected individuals (n=2,078) agreed to monthly HIV testing and completing surveys covering drug/alcohol use, sexual activity, and attendance at venues. Cohort HIV incidence was 8.6 per 1000 person-years (n=303). To identify HIV hotspots, locations of HIV-infected participants’ residences and venues where participants reported a sexual encounter were mapped and analyzed using the Getis-Ord-Gi* method. Putative transmission networks were identified by phylogenetic analysis of partial pol sequences from incident HIV infections. In the geographic analysis, 7 of the 20 social venues were identified as transmission hotspots (99% confidence); no neighborhoods were identified as hotspots. Phylogenetic analysis indicated 13 clusters of highly-related infections (bootstrap values >90%). Within clusters with sufficient behavioral data covering the time of infection (n=7), all or most members reported sexual encounters in the 60 days prior to HIV diagnosis with partners they met at specific venues that had been identified as transmission hotspots. Phylogenetic and geographic cluster analyses identified related incident HIV cases associated with specific venues indicating increased risk of HIV acquisition. These results support offering HIV prevention services, testing, and linkage-to-care efforts at venues rather than current services centered around neighborhoods.

Impact of Medical Plurality on Care Engagement and Treatment Outcomes among People Living with HIV in Limpopo, South Africa
Taylor Lee (Tay) Boyd, Senior, Neurobiology
UW Honors Program
Mentor: Maya Wright
Mentor: Rebecca Dillingham, Infectious Disease and International Health, University of Virginia

Medical plurality, the concurrent existence of multiple medical systems, is particularly important in the South African context. Our research during the summer of 2015 utilized an in-depth survey to document the engagement of individuals living with HIV, with alternative health sectors: traditional medicine, herbal medicine, and faith healers. The participants in the study were both male and female patients living with HIV between the ages of 18 and 65, who had been on antiretroviral therapy (ARTs) for at least one year. Every participant was a patient at either the Fhulufelo HIV Clinic or the Thohoyandou Health Center in Limpopo, South Africa. After listening to a brief explanation of the study and its purpose, patients had the option of volunteering to take a survey, which inquired about their adherence to ARTs, engagement with traditional health sectors and their understanding of HIV. Verbal and written consent were obtained from every participant and the survey data from every participant was included in the statistical and thematic analysis. We found that 34% of the participants had an unsuppressed viral load (n=35). Of the patients that were unsuppressed, 46.3% of them were among the participants who had ever engaged in any of the alternative sectors, while 22.2% of those unsuppressed had never engaged in these alternative sectors. This research suggests engagement with alternative health care sectors is associated with unsuppressed viral loads. This study is important as it may encourage the development of interventions to support better individual and population health outcomes and collaboration between biomedical providers and traditional healers. One limitation was the relatively small sample size (n=102),
in comparison to the total population of people living with HIV in the Vhembe District of South Africa.