UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

1H

HIV: Diagnostics, Drug Resistance, and Antibodies

Session Moderator: Dara Lehman, Global Health, Human Biology

MGH 251

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Point-of-Care Nucleic Acid Amplification Diagnostic for HIV-1 Using Isotachophoresis

Amanda Moon Levenson, Senior, Chemical Engineering
CoMotion Mary Gates Innovation Scholar, Mary Gates Scholar
Mentor: Jonathan Posner, Mechanical Engineering
Mentor: Andrew Bender, Mechanical Engineering

As of 2015, approximately 36.7 million people worldwide are living with HIV/AIDS, of which almost 15 million receive antiretroviral therapy (ART). These patients require regular HIV-1 viral load (VL) tests to monitor ART effectiveness and compliance. However, the majority of affected people live in low-resource settings, where accurate diagnosis and disease monitoring through lab-based instrumentation systems, such as nucleic acid amplification tests (NAATs), are inaccessible. Thus, there is an increasing need for accurate, affordable HIV-1 VL tests at the point-of-care (POC). We have leveraged an electrokinetic separation and preconcentration technique, isotachophoresis, and an isothermal nucleic acid amplification method to develop a fully integrated POC NAAT for extraction, amplification, and detection of HIV-1 nucleic acids in whole blood. We have demonstrated the ability to extract these nucleic acids from human serum and quantify the nucleic acid amplification with fluorescent detection. The fluorescent read-out is correlated to the initial sample concentration, providing semi-quantitative VL information. Our device operates in a single step with limited equipment in 15 minutes, consequently cutting the time, cost, and complexity of NAATs for infectious disease diagnosis, especially in low-resource settings. Our POC NAAT has the potential to improve patient care, and we are currently investigating applying this technology to diagnose other infectious diseases.

The Origin of Low-Frequency Resistance Present during Acute HIV Infection in East Africa

Dana Renae (Dana) Arenz, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Dara Lehman, Global Health, Human Biology

Mutations that confer resistance to antiretrovirals (ARVs) for HIV can arise from drug selection pressure, HIV error-prone replication, or transmission of resistant variants. Understanding the source of resistant HIV strains present during early acute infection, including those present at low-frequency of the viral population, is important for developing interventions to reduce the spread of HIV drug resistance. Phylogenetically linked cases of HIV transmission were identified in a prospective study of HIV serodiscordant couples in Kenya and Uganda. Deep sequencing by 454 technology identified 29 seroconverters that had HIV variants with resistance mutations present during acute infection. Here we sought to determine whether similar resistance mutations were present in their linked index partners. Plasma samples collected within 6 months prior to and after the estimated date of HIV transmission were available from 19 index partners, resulting in 36 plasma samples tested for resistance by 454 deep sequencing. Mutations with a resistance score of $\geq$30 per the Stanford HIV Drug Resistance Database were assessed. Bioinformatic analysis of the deep sequencing data is complete for 15 of the 19 linked partners. Of the 15 linked partners, 6 (40%) had resistance mutations. Four (27%) had the same mutations detected in the corresponding seroconverter. The resistant variant was present at $<$10% of the viral population in the index partner in 1 of these 4 cases of transmitted resistance. Data to date suggests that transmission is not the primary source of resistance during acute infection in this cohort. In addition to resistance testing of the remaining 4 index partners, we also plan to deep sequence and perform phylogenetic analysis of HIV variants from genital samples from the linked index partners to determine whether the transmitted virus is more closely related to virus in the blood or genital tract.

Development of a Simplified Enzymatic Reaction System for a Paper-Based HIV Drug Resistance Test

Nikki Toshie Higa, Senior, Bioengineering
Mary Gates Scholar
Mentor: Barry Lutz, Bioengineering
Mentor: Nuttada Panpradist, Global Health

Human immunodeficiency virus (HIV) is a serious global health issue with 36.7 million people infected worldwide and over 70% of these people living in low- to middle-income countries. Despite increased access to antiretroviral therapy (ART) to manage HIV and recent declines in HIV-related deaths throughout these countries, the emergence of drug-resistant (DR) strains of HIV threatens the continued efficacy of available ART. Drug resistance testing can be used to inform proper ART regimens, improve patient outcomes, and prevent further transmission. However, the gold-standard method (Sanger sequencing) is costly, requires sophisticated laboratory resources, and is unable to detect low amounts of DR HIV that can lead to treatment failure. To address this technology gap, the Frenkel Lab at Seattle Children's Research Institute developed a sensitive, inexpensive, laboratory-based Oligonucleotide Ligation Assay (OLA). The OLA identifies single nucleotide polymorphisms (SNPs) known to confer resistance to ART via highly specific ligation of DNA probes targeting a SNP of interest. In order to expand application of the OLA to lesser-equipped settings, the Frenkel Lab is collaborating with the Lai and Lutz Labs (UW Bioengineering) to re-engineer the OLA into an integrated, paper-based device. As part of this effort, the present project aims to simplify the critical ligation process into a solid-phase enzymatic reaction system that can be integrated with a previously developed paper-based detection device. Compared to the laboratory assay, the solid-phase format provides a less intensive user protocol, protection from contaminants, and seamless transfer of analyte to downstream detection. Several affinity-based and bioconjugation-based enzyme immobilization methods will be investigated to create the solid-phase system. Quantitative analysis of enzymatic activity and immobilization efficiency will then be used to identify the best-performing method(s). Ultimately, simplification of the ligation process is expected to move the OLA closer to implementation in resource-limited areas impacted by HIV.

Novel Strategies to Measure HIV-2 Resistance in Real-Time

Sara Masoum, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Geoffrey Gottlieb, School of Medicine
Mentor: Dana Raugi, Medicine/Allergy & Infectious Diseases

Two human retroviruses cause AIDS: HIV-1 and HIV-2. HIV-2 is less pathogenic than HIV-1, with lower plasma viral loads, decreased transmission and mortality rates, and a slower decline in CD4+ T cells. Despite this, many patients progress to clinical AIDS and may benefit from antiretroviral therapy (ART). HIV-2–infected patients failing first-line ART often harbor drug-resistant strains of the virus that limit second-line treatment. Consequently, there is a need for rapid, inexpensive HIV-2 drug resistance testing that can guide second-line therapy. In this study, we optimized and validated a novel genotypic drug resistance test for HIV-2 using dried blood spots (DBS). Specimens for this project were collected as a part of an ongoing cohort study of ART for HIV-2 infection in Senegal, West Africa. Blood samples from HIV-2–infected patients were spotted onto filter paper and shipped via courier "overnight" to Seattle at ambient temperature. We then extracted viral RNA and DNA from the DBS, amplified genes of interest using nested PCR, and identified mutations at major drug resistance sites after population sequencing. After obtaining genotypes from only three of 42 (7%) of DBS from a previous protocol, we designed a new, optimized protocol and were able to genotype 10 of 22 (45%) DBS, including five of seven (71%) with HIV-2 plasma viral loads over 50 copies/mL. Drug resistance mutations V47A (protease) and M184V (reverse transcriptase; RT) were observed in 63% and 75% of genotypes, respectively – 75% had evidence of multi-class resistance to both protease and RT inhibitors. These results show that DBS genotyping can identify major drug resistance mutations in HIV-2 patient samples, thereby guiding second-line ART for HIV-2 infection. Because DBS-based resistance testing is a low-cost testing approach which provides results within a clinically-actionable timeframe, we expect that this innovation will improve HIV-2 patient care in resource-limited settings.

Antiviral Activity of EFdA against NRTI-Sensitive and NRTI-Resistant Strains of HIV-2

Vincent Huang Wu, Senior, Biology (Molecular, Cellular & Developmental), Informatics
Mary Gates Scholar
Mentor: Geoffrey Gottlieb, School of Medicine
Mentor: Robert Smith, Pathology

Human immunodeficiency virus (HIV) infection is a global health issue and consists of two main types, HIV-1 and HIV-2. As the backbone of all first-line treatments of HIV, nucleoside reverse transcriptase inhibitors (NRTI) have a critical role in antiretroviral therapy. EFdA (4'-ethynyl-2-fluoro-2'-deoxyadenosine; MK-8591; Merck & Co.) is an investigational NRTI that blocks HIV-1 replication in culture with 50% effective concentrations (EC_50) in the low nanomolar to picomolar range. However, studies evaluating the activity of EFdA against HIV-2 and against NRTI-resistant mutants of HIV-2 are lacking. As a result, HIV-1 and HIV-2 isolates from antiretroviral-naïve individuals were tested

against EFdA in single-cycle infections of a human cell line (MAGIC-5A cells). Site-directed mutants of HIV-2 reverse transcriptase were generated in a full-length plasmid clone and were evaluated for EFdA resistance in the single-cycle assay. EFdA inhibited HIV-2 infection of MAGIC-5A cells with mean EC_50 values ($\pm$ SD) of 0.55 $\pm$ 0.17 nM for 8 HIV-2 group A isolates and 0.50 $\pm$ 0.12 nM for 6 HIV-2 group B isolates (range = 0.25–0.76 nM for all 14 HIV-2 strains tested). In contrast, the mean EC_50 for 10 HIV-1 isolates, including group M (subtype A, B, C, D and F) and group O isolates, was 2.56 $\pm$ 0.56 nM (range = 1.96–3.78 nM; $p<.0001$ for HIV-1 versus HIV-2, Welch's t-test). EFdA is the most potent inhibitor of HIV-2 replication described to date and is more active against HIV-2 than against HIV-1 in culture. EFdA also inhibits multi-NRTI–resistant HIV-2 mutants with single-cycle EC_50 values $<$12 nM. Our data indicate that EFdA should be evaluated in clinical studies involving HIV-2-infected individuals.

Studying the Longitudinal Development of HIV-specific Antibodies that Mediate Antibody-dependent Cellular Cytotoxicity (ADCC)

Megan Maurine (Megan) Stumpf, Senior, Microbiology
Mary Gates Scholar, NASA Space Grant Scholar
Mentor: Laura Noges, Human Biology

A more complete understanding of protective HIV-specific immune responses is critical to designing effective vaccine strategies. A recently concluded Phase III HIV vaccine trial identified antibodies that mediate antibody-dependent cellular cytotoxicity (ADCC) as a correlate of protection. While researchers have characterized hundreds of neutralizing antibodies (Abs), much less is known about Abs that mediate ADCC. ADCC can be measured through a distinct assay known as the rapid and fluorometric ADCC assay (RF-ADCC). This assay quantifies HIV-coated cell lysis mediated by antibodies and primary effector cells. Here we describe seven ADCC-mediating Abs isolated from an individual who developed a potent HIV-specific response. This project aims to examine the biological development of these seven ADCC Abs. To accomplish this, we deep sequenced longitudinal blood samples to reconstruct phylogenetic trees that illustrate the mutative developmental pathway of this patient's Abs. We selected and cloned Ab sequences that represent intermediate states between germline and mature ADCC-capable Abs. These intermediaries are currently undergoing HIV binding studies. Next, we will test these Abs in the RF-ADCC assay to assess when the Ab lineages acquired ADCC activity. Finally, epitope mapping studies will be conducted on Ab intermediates of interest. This project will offer understanding on the developmental pathways of ADCC-mediating Abs and inform us on how we can exploit these antibodies in vaccine development.