UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

SESSION 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.

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.

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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.