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Project Title: Quantifying Drug Adherence and Drug Exposure to Antiretroviral Medicine

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I. Hypotheses and Specific Aims:

Sustained antiretroviral exposure is required to achieve virologic suppression in HIV-infected patients. The dominant factor impacting long-term drug exposure is adherence. Sub-optimal adherence is problematic and clinically significant for both HIV treatment and prevention, and over-exposure to antiretrovirals may result in biochemical and clinical toxicities. Few informative measures of drug exposure and adherence have been developed, and no gold standard measure is available in clinical practice.

Tenofovir (TFV), one of the main antiretrovirals for the treatment of HIV infection, has distinctive advantageous characteristics to monitor adherence and exposure. The activity of TFV depends on the intracellular concentration of tenofovir diphosphate (TFV-DP), which accumulates in higher concentrations (1.3 fold) with a longer half-life (~17 days) in red blood cells (RBC) vs. peripheral blood mononuclear cells (PBMC). These unique pharmacological features make RBC TFV-DP a promising and accurate indicator of cumulative drug exposure, particularly to quantify cumulative dosing. RBC are easily obtained in large numbers and can be stored for prolonged periods as dried blood spots (DBS). In this application we propose to evaluate DBS for the quantification of cumulative RBC TFV-DP drug exposure in HIV-negative and HIV-infected patients.

Our main hypotheses are that: 1) RBC levels of TFV-DP are an accurate and precise measure of long term drug exposure in HIV-infected individuals, and; 2) Exposure thresholds will be predictive of poor adherence and virologic failure in HIV infected patients. To test these hypotheses we propose the following specific aim:

Aim 1: To characterize the association of TFV-DP levels in DBS with viral suppression and toxicity in HIV-infected patients.

II. Background and Significance:

Sustained and durable ARV exposure is critical to achieve viral suppression in HIV-infected patients and to prevent transmission to HIV-negative individuals. Drug exposure is directly related to host factors; however, the dominant factor impacting long-term drug exposure is adherence. Modern regimens are more forgiving to lower levels of adherence, likely due to their higher potency and more favorable pharmacokinetics. However, adherence remains the major predictor of HIV outcomes. A major challenge to the adherence field has been the lack of an accurate and objective assessment tool that can be utilized in everyday clinical practice. TFV is a nucleotide that is phosphorylated to TFV-DP in RBC. Our group has discovered that TFV-DP persists with a 17-day half-life in these cells. Tenofovir is the most commonly prescribed antiretroviral in the US with a very favorable safety profile and availability in co-formulations. Therefore, adherence monitoring for TFV would apply to the majority of HIV-infected patients. TFV disposition is impacted by various host-related factors such as gender, age, body weight and pharmacogenomics, but insufficient data are available to consider a dose modification based on these factors.

The presence of TFV-DP in RBC suggests that DBS are an ideal matrix for TFV-DP testing. DBS have been historically used for neonatal screening of inborn errors of metabolism and are widely used for HIV diagnosis and viral load monitoring. The efficacy of DBS for the measurement of drug concentrations has become a topic of great interest given the many potential advantages it has over PBMC, RBC, whole blood and plasma sampling (i.e. less cumbersome storage and transportation, substantial cost reduction and less variability). DBS samples can be stored for prolonged periods of time and can be shipped without special biohazard precautions for analysis in a centralized laboratory. To date, few published studies have shown a direct correlation in ARV plasma drug and DBS. Our group has successfully quantified TFV and TFV-DP in DBS and we have also demonstrated that TFV-DP has a long half-life in DBS, reflecting RBC. However, no studies have evaluated whether TFV and TFV-DP levels in DBS can be used to measure drug exposure and drug adherence in the clinical setting, and thus this approach will be assessed in this proposal.

III. Preliminary Studies/Progress Report:

Our laboratory recently evaluated the single-dose pharmacokinetics of TFV and TFV-DP in plasma, PBMC and RBC and identified that TFV-DP has a long half-life and exhibits different PK characteristics in RBC vs. PBMC. A follow-up study determined that the TFV-DP half life in RBC was 17 (15-19) days vs. 4 (3-5) days in PBMC and the predicted steady state levels in RBC were 130 fmol/10^6 cells, which was ~1.3-fold above that in PBMC. This study also showed that TFV-DP in RBC accumulates approximately 25-fold and remains detectable for at least 30 days after drug discontinuation. Furthermore, we confirmed that there is very strong correlation between TFV and TFV-DP RBC and DBS levels and we found a tendency towards lower concentrations in HIV-negative vs. HIV-infected subjects, which suggests that different DBS thresholds may exist among these individuals. Lastly, we evaluated the feasibility and relevance of measuring TFV-DP in DBS in HIV-infected women on long-term HAART. In this analysis, we compared TFV-DP DBS levels with pharmacy refills and found that it was inversely correlated with increasing number of days between pharmacy refills and that HIV viremia was associated with the lowest TFV-DP in DBS.

IV. Research Methods

A. Outcome Measure(s):

Our primary objectives through this study are to evaluate TFV and TFV-DP in RBC/DBS as a potential measure of drug adherence and exposure in the clinical setting and the association of DBS TFV-DP with viral suppression, adherence and toxicity in HIV-infected individuals.

B. Description of Population to be Enrolled: Study Design and Research Methods

Subjects

Aim 3 is an observational, 48-week prospective cohort of HIV-infected individuals treated with TFV in which we will compare DBS TFV-DP levels in virologically suppressed vs. non-suppressed individuals and evaluate the utility of this measure as a predictor of virologic failure. Our sample analysis has calculated a sample of 1,200 patients to achieve the desired power for our analysis. Viral suppression will be defined as an HIV VL <200 copies/ml. To accomplish our study, we will approach all patients actively taking TFV who present to the Infectious Diseases Group Practice (IDGP) at University of Colorado Hospital for their regular follow-up appointment and will have HIV-related blood drawn (i.e. CBC, chemistry, CD4 count and HIV VL) as ordered by their healthcare provider. Once a patient has consented and is enrolled in the study, we will draw blood at each subsequent visit to our clinic for a period of 48 weeks (we anticipate an average of 3 visits per patient in a 48-week period). Recruitment will continue until we have reached our targeted enrollment. Potential study subjects will be identified via the daily IDGP schedule and the healthcare provider for those subjects will be asked for permission to approach the patient while at the clinic. At the time of enrollment all individuals will also undergo pertinent questions on demographics and medication history and provide consent that will allow the PI to contact their

pharmacy to obtain history on refills to antiretrovirals. At every visit (including initial visit), individuals will also be evaluated for self-reported adherence using a visual analog scale.

Inclusion criteria:

1. HIV-infected males and females, ages 18 years and older, who are taking any TFV-based regimen and who will have blood drawn as part of their clinic visit.
2. Able and willing to give informed consent.

Exclusion criteria:

1. Individuals not taking a TFV-based regimen.
2. Inability to give informed consent.
3. Pregnancy.
4. Refusal to participate.

Process of Informed Consent

Consent will be obtained in a quiet, private setting (e.g., outpatient clinic room) by trained study personnel. Potential subjects will be asked if they feel comfortable. They will be given time to read the consent, answer questions, and consider whether or not to be involved in the study. Comprehension and autonomy will be assessed by asking questions about the study and assessing their responses. Potential subjects will be told that their participation is voluntary, and that participation in this study (or election not to participate) will not affect their care in any way. A signed copy of the consent and will be provided to the subjects. Consent will be obtained prior to any research procedures being performed.

Sample Collection

We will collect up to 30 mL of blood through venipuncture from individuals who are already having blood drawn as ordered by their healthcare provider while at the IDGP. In addition, a subset of 30 individuals will have 5 drops of blood obtained through finger stick using a lancet. Blood will be collected in EDTA tubes and will be immediately transported to the Colorado Antiviral Pharmacology Laboratory (Directed by co-investigator Dr. Peter Anderson), where samples for DBS will be processed and stored at -20^{o}C until analysis using liquid chromatography-tandem mass spectrometry (LC/MS/MS).

We are also asking subjects to sign a separate addendum consent that would allow us to obtain blood to isolate human genomic DNA. This would allow us to investigate relationships between genetics and drug levels, should there be a rationale such as abnormally high or low drug levels or unusual or exaggerated medication responses are encountered. We are only interested in genes which encode enzymes or transporters responsible for the metabolism or transport of anti-infective drugs. As examples, we have interest in genes which encode for drug efflux transporters, cytochrome P_{450} enzymes, or glucuronosyl transferase enzymes. We are not interested in genes that determine parentage or susceptibility to a disease.

Participants will be compensated $10.00 every time they agree to a blood draw.

C. Description, Risks and Justification of Procedures and Data Collection Tools:

Risks

There are no medication-associated risks for the participants who will be enrolled in this study because we plan to enroll HIV-infected individuals who are already receiving antiretroviral therapy (under the care of their healthcare provider) and are taking TFV as part of their regular antiretroviral regimen.

There are risks associated with venipuncture and lancet finger stick, but are limited to pain, bruising and, rarely, superficial phlebitis. However, these risks are not inherent only to the study as these individuals will be already undergoing blood draw as recommended by their healthcare provider. The study personnel will take all necessary measures and adequate care to prevent and minimize these risks or correct them should any of them arise. Universal precautions will be used as recommended by the Center for Disease Control (CDC), the National Institutes of Health and the University of Colorado-Anschutz Medical Campus, including the appropriate disposal of needles and human wastes. These precautions will be carefully exercised by the study personnel to prevent the accidental transmission of HIV (or other viruses). Stored DBS cards will be properly labeled, handled and disposed according to the universal standard blood and body fluid precautions as recommended by the CDC.

Additional risks include the potential for personal participant information to become public through failure of confidentiality. Every effort will be made to maintain the confidentiality of patient study records and all individuals working on the project will be informed of the need to maintain strict confidentiality at all times. To ensure this, we will collect patient's information on a case report form that includes only a subject's unique study identification number (PID) and not their name. The "link" between a subject's name and PID will be kept in a separate case report form. All research records will be kept in a locked office or laboratory in our research group. Randomly assigned PIDs will not indicate whether or not a subject has an infectious disease.

If a subject agrees to provide blood for future genetic testing, there are risks associated with this including possible discrimination or compromised insurability. We will take steps to minimize these risks. As noted, we are not looking at genes that determine parentage or susceptibility to a disease and the analyzed samples will not be coded with any identifiable information, only a subject's unique PID. These samples would be kept in restricted-access at the Colorado Antiviral Pharmacology Laboratory. As part of our study, genetic analysis in a subset of these samples will be performed at The DNA Resources Core (DNARC) of the Vanderbilt University Center for Human Genetics Research (CHGR) under the supervision of Dr. David Haas.

Data Collection Tools

Data will be collected on case report forms (CRFs). With the exception of the demographic CRF, CRFs will contain PID numbers instead of patient identifiers.

D. Potential Scientific Problems:

We do not anticipate problems with investigating the pharmacokinetics of the antiretrovirals analyzed. However, as this is a hypothesis driven study, we may face problems identifying correlates of drug levels and clinical outcomes.

E. Data Analysis Plan:

Data collected during the course of this study may be assembled electronically for analysis. Some examples of the analysis include:

- Comparison of TFV-DP levels in DBS in HIV-infected patients with viral suppression vs. virologic failure.
- Correlation of TFV-DP in DBS with adherence and drug toxicity.
- Efficacy of TFV-DP in DBS as a predictor of virologic failure.

Appropriate parametric statistical tests, with log transformation of the data as necessary, will be used for analyses.

Data Management and Security

Patient identifiers will not be stored electronically for this study. Datasets assembled for this study will include only the unique PID and relevant demographic information (race, age, sex, etc.), relevant clinical data and antiviral drug concentration results. Electronic data will be stored on our laboratory server which has limited access (members of our CAVP research team only and SOP IT specialists) and is backed-up nightly. Paper records for this study (consent, HIPAA, CRFs) will be

stored in a locked office or our entrance-restricted laboratory. Data will be destroyed per the timelines specified in HIPAA regulations.

Data Safety and Monitoring

Dr. Jose R. Castillo-Mancilla will assess adherence to the protocol procedures. All adverse events related to subject's participation in this protocol will be reported to Dr. Jose R. Castillo-Mancilla, who will keep track of their frequency and report any serious adverse events or unexpected problems to COMIRB according to standard procedures**.** Dr. Jose R. Castillo-Mancilla will also provide medical oversight for the study. He will be notified of all study-related adverse events and will ensure appropriate medical management (i.e., follow-up visits, additional laboratory tests, referral to primary care provider, etc.).

F. Summarize Knowledge to be Gained:

Drug exposure to antiretrovirals is mainly driven by drug adherence, which is a strong predictor of HIV treatment efficacy. Measuring adherence has proven to be difficult and there is no gold standard measure of drug exposure and drug adherence in clinical practice. The proposed research will develop a new pharmacologic approach to quantify drug exposure and adherence, which could lead to more efficacious HIV treatment and prevention strategies.

G. References:

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