Study Title

ENLIGHTEN:

Establishing Novel Antiretroviral Imaging for Hair To End Nonadherence

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by:

Division of AIDS, US National Institute of Allergy and Infectious Diseases

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Time vs. Concentration Curve
CBC	Complete Blood Count
C_{max}	Maximum Concentration
CPT	Cell Preparation Tube
DAIDS	Division of AIDS
DBS	Dried Blood Spots
DOT	Daily Observed therapy
DTG	Dolutegravir
EAE	Expedited Adverse Event (reporting)
EC	Ethics Committee
FDA	(United States) Food and Drug Administration
FTC	emtricitabine
FTC-TP	emtricitabine triphosphate
HepBsAg	Hepatitis B Surface Antigen
HIV	Human Immunodeficiency Virus
IDS	Investigational Drug Service
IRB	Institutional Review Board
LLN	Lower Limit of Normal
MALDESI	Infra-red Matrix Assisted Laser desorption electrospray ionization
MRV	maraviroc
MSI	Mass Spectrometry Imaging
NIAID	(United States) National Institute of Allergy and Infectious Diseases

NIH	(United States) National Institutes of Health
PD	Pharmacodynamics
PK	Pharmacokinetics
PoR	Pharmacist of Record
PrEP	Pre-Exposure Prophylaxis
PSRT	Protocol Safety Review Team
PT	Prothrombin Test
QA	Quality Assurance
QC	Quality Control
RPR	Rapid Plasma Reagin Test for Syphilis
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
SSP	Study Specific Procedures (manual)
STI	Sexually Transmitted Infection
TDF	tenofovir disoproxil fumarate
TDF/FTC	Truvada® (tenofovir disoproxil fumarate and emtricitabine in fixed dose combination)
TFV	tenofovir
TFV-DP	tenofovir diphosphate
ULN	Upper Limit of Normal
ULPC	Upper Layer Packed Cells

TERMINOLOGY FOR TENOFOVIR, EMTRICTABINE, MARAVIROC, DOLUTEGRAVIR, AND THEIR DERIVATIVES

Abbreviation	Compound name	Comments
TDF	Tenofovir disoproxil fumarate	This is the inactive, oral formulation of tenofovir (trade name: Viread). The ester form enhances oral absorption and bioavailability. TDF is rapidly metabolized after dosing to the de-esterified pro-drug, tenofovir (TFV), which is also inactive.
TFV	Tenofovir	This is the inactive, de-esterified form of TDF. This is the form of the drug that is measured in serum, blood, other body fluids, and tissue samples.
TFV-DP	Tenofovir diphosphate	This is the active, phosphorylated form of tenofovir that is generated in cells. This is the form of the drug that is measured in cells (e.g., PBMCs). It is rapidly dephosphorylated to the inactive form outside of cells, and has a very short half-life outside of cells in tissue.
FTC	Emtricitabine	This antiretroviral drug is co-formulated with TDF in Truvada® (TDF/FTC). FTC is an inactive pro-drug that is activated in cells by phosphorylation. This is the form of emtricitabine that is measured in serum, blood, other body fluids, and tissue samples.
FTC-TP	Emtricitabine triphosphate	This is the active, phosphoylated form of FTC that is generated in cells. This is the form of the drug that is measured in cells (e.g., PBMCs).
Truvada®	Tenofovir plus Emtricitabine	This is the co-formulated drug produced by Gilead Sciences, Inc. Each pill contains 300 mg of TDF and 200 mg of FTC.
MRV	Maraviroc	This is the active oral formulation of maraviroc (Selzentry®).
DTG	Dolutegravir	This is the active oral formulation of Dolutegravir (Tivicay®)

IRB 17-0546 PROTOCOL TEAM ROSTER

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PROTOCOL SIGNATURE PAGE

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m24 August 2017

I, The Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I have read and understand the information in this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____

Name of Investigator of Record

_____ _____

Signature of Investigator of Record **Date**

PROTOCOL SUMMARY

Protocol Chair: Angela DM, Kashuba, BScPhm, PharmD, DABCP

Sample Size: 36 healthy adults divided into 3 different drug groups (N=12 per arm)

Study Population: Healthy volunteers, between 18-70 years of age with an intact gastrointestinal tract and greater than 1cm caput hair

Study Site(s): The University of North Carolina Clinical Translational Research Center
Chapel Hill, NC USA

Study Design: Single-center, open-label, 3-arm, triple stage study

Study Duration: Participants will be enrolled in the study for approximately 90 days, depending on the timing of their scheduled visits in comparison to the screening visit

Study Products: Emtricitabine, tenofovir, dolutegravir, maraviroc

Study Regimen: Participants will be sequentially assigned to enroll in dosing arm, beginning with Maraviroc, then Tenofovir/Emtricitabine and ending with Dolutegravir

- All participants will take a single observed dose of their assigned study product in Phase 1 on Day 0 (Maraviroc 300mg, Tenofovir/Emtricitabine 300mg/200mg, and Dolutegravir 50mg)
- All participants will take a single daily observed dose of their same assigned study product in Phase 2 beginning on Day 0 (Maraviroc 300mg, Tenofovir/Emtricitabine 300mg/200mg, and Dolutegravir 50mg)
- All participants will be randomized to a dosing schema in Phase 3 (N=4 per group) for their assigned study product (Maraviroc 300mg, Tenofovir/Emtricitabine 300mg/200mg, and Dolutegravir 50mg) beginning on Day 0
    - No further doses

- Dose once per week
- Dose three times per week

Primary Objectives:

1. Assess dose-proportionality of Maraviroc, Tenofovir/emtricitabine, Dolutegravir and intracellular TFV-DP and FTC-TP
2. Describe intra- and inter-individual variability of hair and blood antiretroviral concentrations at steady-state

Secondary Objectives:

1. Describe the relationship between single dose (Ct) and decaying concentrations of MRV, TFV/FTC, DTG and their phosphorylated derivatives (TFV-DP and FTC-TP) in blood and hair at steady state and end of study
2. Correlate blood and hair concentrations of antiretrovirals after single and multiple doses
3. Assess Grade 2 or higher study-related adverse events that lead to a temporary or permanent hold in study product

OVERVIEW OF STUDY DESIGN AND ALLOCATION SCHEME

FIGURE 1: Allocation Scheme

Figure 7: Schema of Clinical Activities to Determine Hair MSI Dose Proportionality in Healthy Volunteers. After screening, subjects will be randomly enrolled into one of three arms: Tenofovir+Emtricitabine, Maraviroc, or Dolutegravir. 10 hair strands will be collected 5 times in each 28 day period.

Description of Study Phases

Screening: Participants will be recruited from a variety of advertisements, and pre-screened using a telephone IRB-approved questionnaire. If participants are interested and pass the initial screening, a screening study visit in the research center will be scheduled. This visit should take approximately 90 minutes, during which full physical examination and medical history will be obtained, as well as physical diagnostics to assess for eligibility. This visit must be completed within the 28 days prior to enrollment.

Phase 1: Consists of a 28-day study period, with a single dose of study product on Day 0 (maraviroc, tenofovir/emtricitabine, or dolutegravir). Day 0 can be scheduled on either Mondays or Tuesdays, and once eligibility is confirmed on the day of enrollment, a witnessed dose of study product will be administered. Participants will return to the clinic for hair and blood sampling on Days 3/7/14/21/28 days post-dose. Adverse events will be assessed at every visit. Safety labs will be drawn at the midpoint and at the end of the study phase, and at any time indicated due to suspected adverse events. These visits should last less than 30 minutes.

Phase 2: Consists of a 28-day study period, with each subject receiving a single daily observed dose of study product beginning on Day 0. Day 0 can be scheduled on either Mondays or Tuesdays, once continued eligibility is confirmed. Target scheduling will have Phase 2 begin within 2 weeks of completing Phase 1, but could be extended up to 28 days as clinic availability dictates. Participants will return to the clinic daily for dosing, and for hair and blood sampling on Days 3/7/14/21/28 days post-dose. Adverse events will be assessed at every visit. Safety labs will be drawn at the midpoint, and at the end of the study phase, and at any time indicated due to adverse events. These visits should last less than 30 minutes. Dosing only visits should last less than 5 minutes.

Phase 3: Consists of a 28-day study period, with three randomized drug-dosing schemas. Day 0 can be scheduled on Mondays, Tuesdays or Fridays, once continued eligibility is confirmed. Target scheduling will have Phase 3 begin as soon as possible after completing Phase 2, on a Monday/Tuesday/Or Friday within the week. On Day 0, participants will be randomized to one of 3 potential dosing schemes:

a—No further doses

b—One dose weekly (Day 0, 7, 14, 21) 4 doses

c—Three Doses weekly (Mondays, Wednesdays, Fridays)

(Days 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25) 12 doses

Participants will return to clinic for observed dosing as scheduled, and for hair and blood sampling on days 3/7/14/21/28). Safety labs will be drawn at the midpoint, at the end, and at any time indicated due to adverse events. These visits should last less than 30 minutes. Dosing only visits should last less than five minutes.

Follow-Up: Within 2 weeks of the final sampling visit, all participants will return to the clinic for a final safety visit. Updated exam and medical history will be obtained as indicated, as well as vital signs and adverse events assessments. Safety labs and EKG will be collected.

Clinical Laboratories

McLendon Labs

The following samples/tests will be processed at UNC Hospitals McLendon Core Lab:

- Urinalysis
- Pregnancy test
- Syphilis (RPR) titer
- HIV diagnostic testing
- Hepatitis testing
- Safety labs
    - CBC
    - Liver function tests
    - Serum Chemistries
    - Lipid Panel

UNC School of Pharmacy Clinical Pharmacology and Analytical Chemistry (CPAC) Lab

The following analyses will be performed at the CPAC lab:

- Plasma antiretroviral concentrations
- PBMC antiretroviral concentrations
- Hair imaging analysis
- Dried blood spot analysis

This laboratory, directed by Angela Kashuba, was created to provide clinical pharmacology expertise and laboratory support to facilitate clinical and preclinical HIV/AIDS research. The Core has been CLIA certified since December 2004 and successfully participates in four proficiency testing activities per year. The Core has > 90% accuracy for all antiretrovirals tested, and has been awarded certificates of excellence and is approved to perform therapeutic drug monitoring. Sample storage, processing, and HPLC methods are performed in the lab located in the Genetic Medicine Building in the Eshelman School of Pharmacy. This laboratory has validated analytical methods for all currently marketed antiretrovirals.

Study Operations

The Clinical Research Unit of the Clinical Pharmacology and Analytical Chemistry laboratory operates within the guidelines and regulations as described in ICH/GCP, The Code of Federal Regulations, Office

for Human Research Protections (OHRP) and FDA regulations and guidances, as well as any additional requirements of the National Institutes of Health, Division of AIDS and the sponsoring networks.

The Principal Investigator (PI) accepts ultimate responsibility for the quality of the data, subject safety and protocol adherence for all research conducted through the UNC clinical research unit. She also accepts responsibility for the overall functioning of the site.

The PI is responsible for oversight of activities and responsibilities delegated to other staff. All staff are to be appropriately educated in their obligations, as described above, and trained in the areas for which they have delegated responsibility.

1. INTRODUCTION

1.1 The Need for Quantify Antiretroviral Adherence

Adherence to antiretroviral (ARV) therapy is critical for achieving HIV RNA suppression in HIV-infected patients and for preventing HIV acquisition in uninfected individuals using pre-exposure prophylaxis (PrEP). Yet a high level of adherence is challenging for HIV-infected individuals on life-long ARVs, and for HIV-negative individuals using daily PrEP who are not at daily risk for HIV acquisition. Poor adherence was primarily responsible for a lack of drug effectiveness in multiple recent double-blind, placebo-controlled PrEP studies. These studies found that counting product returns and using patient self-report significantly over predicted adherence as measured by ARV concentrations in blood plasma, dried blood spots, or cells.

Since the consequences of poor or intermittent adherence are significant, valid measures of adherence are critical for optimizing the effectiveness of both HIV treatment and prevention, in both the clinical and research settings. Measuring drug concentrations is a more accurate measure of adherence than is self-report. Blood plasma or intracellular concentration monitoring have been considered the “gold standard” for determining if an ARV has been ingested, and is a common marker for therapeutic drug monitoring or clinical trial adherence monitoring. However, this approach has its own set of limitations, including being invasive, requiring advanced processing or storage (e.g. intracellular measures), being a short-term measure of drug taking behavior (depending on the half-life of the analyte), and requiring long turn-around times or substantial sample processing prior to analysis. Upper layer packed cells (ULPC) and Dried blood spot (DBS) analysis have been used as alternatives to plasma. DBS was generated as a less invasive, simple, and validated alternative to traditional venous sampling. The advantages are that it can involve more minimally invasive sampling, even with a small finger prick. Most analytes are more stable in DBS than in frozen sample, which also translates to convenient storage and transport. Since blood plasma, ULPC, and dried blood spot analysis are valid industry standards to assess patient adherence, we will use them both in this research as comparators to hair concentrations.

We propose the use of infra-red (IR) matrix-assisted laser desorption electrospray ionization (MALDESI) technology for mass spectrometry imaging (MSI) to visualize and quantify ARV concentrations in hair. Our hypothesis is that IR-MALDESI MSI can rapidly quantify ARV concentrations, provide evidence of drug ingestion non-invasively and longitudinally, and allow for clinician/researcher and patient/study participant feedback on adherence performance. We were awarded a grant from the NIH to begin to evaluate the feasibility and acceptability of this technology. Three specific aims of our grant are proposed: 1) Develop IR-MALDESI MSI hair protocols for high sensitivity and accuracy to quantify 11 ARVs in 5 therapeutic

drug classes, 2) Conduct 3 structured dose proportionality studies to develop mathematical benchmarks for real-time IR-MALDESI hair adherence monitoring in both PrEP and HIV treatment applications, and validate the benchmarks with a Phase 2 PrEP study, and 3) In the setting of REAL TIME clinical monitoring, investigate the acceptability, appropriateness, and feasibility of using hair IR-MALDESI MSI to provide HIV+ patients with feedback regarding longitudinal patterns of medication adherence. The goal of this work is to develop a simple, noninvasive, longitudinal depiction of ARV adherence that will provide high clarity feedback for both clinicians and patients.

In AIM 1, over the last year we have completed collection of numerous hair samples from participants of different genders, races, and evaluated the effect of hair treatments (i.e. coloring, chemicals, etc.) to facilitate formulating this protocol in the most informed way. In our AIM 1 work, we assessed the ability of the technology to see drug in hair of all genders, races and ethnicities. We also evaluated if any treatments to hair, such as bleach, dye or other chemicals, affected the quality of the data obtained from hair. Both of these evaluations helped us delineate our inclusion and exclusion criteria for our AIM 2 work. This protocol is to cover the work necessary in AIM 2, to further the investigation into the use of hair as measure of drug adherence.

1.2 Rationale

It is important to identify if someone is taking a medication regularly and as prescribed, to optimize their health and well-being. Sometimes, patients have trouble remembering if, and when, they miss doses; other times, even though patients are taking their medication, it is not getting into the body in the correct amount. Quickly monitoring medications in 5-10 hair strands using our novel imaging technology called IR-MALDESI will allow patients and their doctors to see how much medication they are exposed to over one or more months, and help identify challenges to taking medication in both research and clinical settings. This proposal will optimize and explore the accuracy and feasibility of using IR-MALDESI for monitoring medications in hair in comparison to the current industry standards.

1.3 Dose Proportionality

Dose proportionality occurs when an *r* fold increase in dose results in an *r* fold increase in exposure, which is typically measured by C_{max} or AUC (Hummel et al. 2009). Dose proportionality is important when considering how to predict the change in drug exposure and its corresponding biologic response with a change in dose. We are concerned with the proportionality between dose and the corresponding concentration in the hair. In order to reliably predict hair exposure, confidence in dose proportionality is necessary. Our goal is to prove this dose proportionality relationship in order to develop mathematical benchmarks for real-time IR-MADLESI hair adherence monitoring in both PrEP and HIV treatment applications.

1.4 Background (See Appendix III)

Tenofovir Exhibits Modest Dose-Proportionality in Hair with LC-MS/MS Analysis Data from Liu et al (88; **Figure 5**) determined that a log-linear relationship can be seen between TFV dose and hair concentrations, with an estimated 76% (95% CI 60-93%) increase in hair level per 2-fold dose increase. However, the between-patient variability is large. Assuming this relationship holds for other ARVs, this precludes the use of LC-MS/MS methods in determining, with precision, <3 fold differences in weekly drug dosing. Our preliminary data with IR MALDESI MSI suggest that we may be able to reduce variability and increase the power to detect differences between dose frequencies.

Tenofovir, Emtricitabine, Maraviroc and Dolutegravir Can Be Visualized by IR-MALDESI MSI Our preliminary data in Aim 1 demonstrate our ability to detect ARVs in hair with a linear response. We propose to conduct three dose-proportionality investigations utilizing these ARVs representing distinct physicochemical properties across 3 drug classes. These investigations are described in detail below, and will be based on directly observed dosing.

Experience with Healthy Volunteer Studies and Observed Dosing We have extensive experience in conducting ARV pharmacokinetic investigations in healthy volunteers, and have over 5 years of experience investigating the pharmacokinetics of these ARVs in particular (3,25,37,63,113). We also have experience with enrolling and retaining healthy volunteers in observed dosing studies: our participation in HPTN066 successfully enrolled and followed 19 (60% of total enrolled) patients with the following dosing strategies for the fixed dose combination of tenofovir disoproxil fumarate (TDF) + FTC: 1 tablet weekly, 1 tablet twice weekly, 2 tablets twice weekly, and 1 tablet daily for 35 days (68).

Multi-compartment Pharmacokinetics and Dose Proportionality Investigations In this aim, we will conduct population PK modeling of IR MALDESI MSI data in hair with multiple dosing strategies using a multi-compartmental approach (plasma, PBMCs [for intracellular TFVdp and FTCtp], and hair). We have significant experience modeling complex systems. Recently, we have developed models to predict ARV active metabolite exposure, based on dose, in mucosal tissues (174,35; **Figure 6**). This model was based on dose proportionality investigations we recently completed for TFV, TFVdp, FTC, FTCtp, and MRV in plasma, PBMCs, and tissues (35-35c,174). Finally, the Kashuba laboratory is participating in HPTN 069 (NEXT-PrEP, [Novel Exploration of Therapeutics (NEXT) for Pre-Exposure Prophylaxis (PrEP)]), performed to evaluate the safety and acceptability of 4 different drug combinations of TFV, FTC, and MRV when used as PrEP by men who have sex with men and women. 600 participants > 18years are being enrolled to one of 4 arms and will be required to take 3 pills (active + placebo) for 1 year. A number of substudies are planned, including an analysis of ARVs in hair, provided by the UCSF Hair Analytical Laboratory and led by Dr. Monica Gandhi. To date, 145 men and 126 women have had hair samples (~200 strands/collection) collected at weeks 24 and 48 of the study (*personal communication, Dr. Roy Gulick, Protocol Chair, March 18 2015*). We have been approved by the HPTN leadership and Dr. Gandhi (*see attached memorandum from Drs. El-Sadr and Cohen, and letter of support from Dr. Gandhi*) to analyze hair strands from these Participants. The Hair Analysis Laboratory has recently developed a method to measure TFV+FTC+ MRV in hair resulting in more than adequate numbers of hair strands available for IR-MALDESI MSI.

2. STUDY OBJECTIVES AND DESIGN

2.1 Identification of Study Design

This will be a single center open-label 3-arm pharmacokinetic study of the hair and blood concentrations of four antiretrovirals, measured after a single dose and multiple doses. Each phase is divided into three dosing groups, with twelve participants per dosing group. In Phase 1, a single dose of study product will be administered on Day 0, and then samples will be collected over a 28-day post-dose period. In Phase 2, participants will take the same study product once daily for 28 days straight, with samples of plasma and hair collected at set intervals over that subsequent month. Participants will immediately follow into Phase 3, where they will be randomized to one of 3 dosing schemas including: no further doses, a single dose once weekly, and three doses per week.

Participants will return to the clinical research center during all 3 phases, on days 3/7/14/21/28 post-dose to provide blood and hair samples. Vital signs and safety laboratory assessments will be obtained as

noted in the schedule of events. Adverse events will be assessed at every visit, with the participants encouraged to contact study staff at any time as needed.

The participants will be sequentially assigned to one of three dosing groups: Maraviroc, Tenofovir/Emtricitabine, and then Dolutegravir. After participant education, informed consent, and screening for study eligibility, participants will be evaluated at baseline (before the dose).

Within 2 weeks of the final study sampling, all participants will return to the clinic for a final safety visit and assessment.

2.2 Study Sampling

The following samples will be collected during all three phases, in all study arms, on Days 3, 7, 14, 21, and 28 days post dose:

- Blood (Plasma, ULPC, and Dried Blood Spots)
- Hair
- PBMCs (In the Tenofovir arm ONLY)

2.3 Study Experience

The UNC Chapel Hill Center for AIDS Research has considerable prior experience with the conduct of intensive blood and hair sampling studies. Most recently, in the study generated during AIM 1 of this grant, the study team obtained 84 hair samples from clinic patients within 9 months. These were performed successfully and there were no adverse events or unanticipated problems. As part of the screening and informed consent process, every participant will receive a detailed description of the procedures involved and the intensity of the protocol.

2.4 Summary of Major Endpoints

2.4.1 Primary Endpoints:

- Plasma, ULPC, and dried blood spot concentrations of FTC (and FTC-TP), TFV (and TFV-DP), MRV, and DTG to generate preliminary PK parameters
- Hair imaging of FTC (and FTC-TP), TFV (and TFV-DP), MRV, and DTG to generate preliminary parameters

2.4.2 Secondary Endpoints:

- Blood plasma concentrations of FTC, TFV, MRV, and DTG
- PBMC concentrations of FTC-TP, TFV-DP
- Whole blood concentrations of FTC, TFV, MRV, and DTG via dried blood spots and upper layer packed cells
- Adverse Experiences, including safety laboratory studies (hematological, renal, hepatic function)

2.5 Description of Study Population

This study will consist of approximately 36 healthy volunteers, between 18-70 years of age, able to maintain a minimum of one centimeter of caput hair during the entire study period, with an intact gastrointestinal system.

2.6 Time to Complete Accrual

Based on our prior experience with other similar pharmacokinetic studies, we anticipate recruiting for 18 months.

2.7 Study Groups

The study includes 3 dosing groups to include twelve Participants each on maraviroc, tenofovir/emtricitabine, and dolutegravir

2.8 Expected Duration of Participation

Once enrolled in the study, patients will participate in the study for 3 months on treatment, and then have a follow up visit within 14 days after the last sampling. No study data will be collected after the follow-up visit unless the participant has an adverse event (AE), which will be followed until resolution.

2.9 Clinical Study Site

The University of North Carolina Clinical Translational Research Center, Chapel Hill, NC USA

3. STUDY POPULATION

This study will consist of 36 healthy volunteers of any race or gender between the ages of 18-70. Participants will be selected for the study according to the criteria described in this protocol.

3.1 Selection of the Study Population

3.1.1 Recruitment

Our study Participants will be recruited from the Chapel Hill-Raleigh-Durham area (an area with a population of approximately 3 million). We will use the following recruitment strategies:

- Placing IRB approved advertisements in the university-based (Daily Tar Heel) and two local newspapers (Chapel Hill News, and Daily News and Observer)
- Posting IRB approved flyers around the UNC-CH campus
- Sending IRB approved emails to the UNC-CH campus listserv
- Posting IRB-approved text to ResearchMatch.org
- Contact a list of Participants who have expressed interest in any of our previous healthy volunteer studies

Potential Participants are given an email address or a phone number to call if they are interested, and the study coordinator conducts a brief initial IRB-approved screening interview over the phone in a private area to determine further eligibility. If the subject is potentially eligible, a screening visit is scheduled; if one or more of the basic inclusion criteria are not met, the subject is informed that they are not eligible.

3.1.2 Subject Withdrawal

There is a low chance of participant withdraw from the study. However, if a subject does withdraw before completion of at least four of the five sampling visits per phase, it will be necessary to replace the subject

in order to have a complete set of data.

3.1.3 Inclusion Criteria

- Healthy Participants between the ages of 18 and 70 years, inclusive on the date of screening, with an intact gastrointestinal tract. (Healthy is defined as no clinically relevant abnormalities identified by a detailed medical history, full physical examination, including blood pressure and pulse rate measurement, and clinical laboratory tests.)
- Participants must have, and be able to maintain caput hair greater than one centimeter in length for the duration of the study period.
- All Participants must have an estimated calculated creatinine clearance (eCcr) of at least 80 mL/min by the Cockcroft-Gault formula where:
  eCcr (female) in mL/min = [(140 - age in years) x (weight in kg) x 0.85] / (72 x serum creatinine in mg/dL).
  eCcr (male) in mL/min = [(140 - age in years) x (weight in kg)] / (72 x serum creatinine in mg/dL).
- All participants of childbearing potential must have a negative serum pregnancy test at screening and negative urine pregnancy tests on other designated days. Participants of childbearing potential must be willing to maintain at least one form of effective concentration during the entire study period. The definition of childbearing potential on this study is cisgender premenopausal females engaging in heterosexual intercourse who still have a uterus. Examples of acceptable forms of birth control, but not limited to, are:
  - Systemic hormonal contraceptive (oral, depot, transdermal or implant)
  - IUD placed at least 1 month prior to study enrollment
  - Bilateral tubal ligation (Sterilization)
  - Vasectomized male partners
  - Condom + Spermicide
  - Same sex partners
  - Abstinence of at least 3 months, with no plan to become sexually active during the study period
- Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the trial.
- Willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other trial procedures.
- Subject must be HIV-1 and Hepatitis B surface antigen negative as documented on screening labs.
- Subject must not be actively involved in the conception process.
- Subject must be able to swallow pills and have no allergies to any component of the study products.

3.1.4 Exclusion Criteria

- Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease (including

documented drug allergies, but excluding untreated, asymptomatic, seasonal allergies at time of dosing).

- Participants who are pregnant, possibly pregnant or lactating
- History of febrile illness within five days prior to first dose.
- Any condition possibly affecting drug absorption (eg, gastrectomy).
- A positive result for HIV.
- Active Hepatitis B infection as determined by positive Hepatitis B surface antigen (HBsAg) or Hepatitis B core antibody (HBcAb) tests (in the absence of HBsAb).
- Active Hepatitis C infection as defined by positive HCV Ab (determined by multi-antigen EIA) and detectable Hepatitis C RNA.
- A positive test for syphilis at screening.
- Any clinically significant laboratory result Grade 2 or greater according to the DAIDS Laboratory Grading Tables
- Treatment with an investigational drug within 4 months preceding the first dose of trial medication.
- History of regular alcohol consumption exceeding 14 drinks (1 drink = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of spirits) per week.
- Participation in a clinical trial involving hair samples within 3 months preceding the first dose of trial medication.
- Blood donation of approximately 1 pint (500 mL) within 56 days prior to dosing.
- Any condition, which, in the opinion of the investigator, is likely to interfere with follow-up or ability to take the study medication appropriately.
- Unwilling or unable to comply with the following dietary and concomitant drug restrictions about study drug administration as outlined in the study procedures and prohibited medications sections.

*Screening evaluations may be repeated once during the screening window to verify eligibility as needed.

3.1.5 Co-enrollment Guidelines

Participants cannot have participated in a clinical trial in which they received treatment with an investigational drug within 3 months preceding the first dose of trial medication or have participated in a clinical trial involving hair samples within 3 months preceding the first dose of trial medication. No participants may co-enroll in other studies that collect hair samples while enrolled here, nor may they enroll in any other study that would exceed the NIH recommended 8 week blood draw limit of 550mL.

4. STUDY TREATMENT/PRODUCT/INTERVENTION

4.1 Study Product (s) Description

Maraviroc 300mg tablets (commercially available)/ “Medication A”
Dolutegravir 50mg tablets (commercially available) / “Medication B”
Tenofovir /Emtricitabine 300mg/200mg (commercially available) “Medication C”

4.2 Administration

Participants will remain on the same assigned study product throughout all phases of the study. Drugs will be coded as Medication A, B or C, to increase patient confidentiality, as per our standard practices with Investigational Drug Services. All participants will know the medication they are receiving, but we will

label the bottle with Medication A, B, or C to protect their confidentiality. They will take their first dose in the clinical research center on Day 0 of Phase 1, observed and monitored by study staff. All participants will be closely monitored post-dose, and will be followed for sampling over the subsequent month. Phase 2 will require daily observed dosing at approximately the same time window during each 24 hour period (ideally within the same 6 hour window each day). The exact time of the doses will be recorded on the study records and will include the signatures of the witnesses. Participants will be randomized to their dose frequency for Phase 3. All doses will still be observed by study staff with close observation of study participants.

4.3 Supply and Accountability

The medications will be provided by the Investigational Drug Service at UNC Hospitals. Prescriptions for study product will be ordered electronically via the UNC Chapel Hill electronical medical records system, with paper copies of prescriptions as back up. All prescriptions will be filled for one week increments at a time. All participants will be given a 2-dose take-home supply of their assigned study product for emergency purposes during Phases 2 and 3. Situations in which the emergency supply may be utilized would be in the case of severe weather or other emergencies that would prevent a participant from traveling to the clinic. Attempts will be made to witness dosing in these extreme circumstances via telephone or internet video chat. Only a max of 3 doses during Phase 2, and two doses in Phase 3 can be unwitnessed and have the patient still be evaluable. At the end of each Phase, the emergency supply will be collected and returned to IDS. Once all study dosing has been completed, remaining drug will be disposed of per standard institutional procedures.

4.4 Directly Observed Therapy

Drug adherence is an essential component of this study design. DOT is defined as directly observed therapy. A person identified by the research site must observe and document the doses of study products taken by the study participant. Participants randomized to all arms will have all doses (as per randomized study arm) observed and documented. The method by which DOT is to occur should be discussed at length with the participant at study screening. Participants and coordinators will formulate a well-described plan for DOT prior to entry.

Study product can be picked up on behalf of the study subject from the Investigational Drug Pharmacy from any coordinator, study physician or other designated study staff at the enrollment visit and as indicated throughout the three phases. The prescription will be cross-checked with the dosing scheme prior to dispensing by at least two people, such as study physicians, mid-level providers, coordinator or research center nurses and staff.

- For Phase 1 Dosing, participants will be sequentially assigned to receive one of the study products. On the morning of enrollment, study staff will obtain a single dose dispensed from the UNC Investigational Drug Services (IDS). Study product will be dispensed in clinic, and witnessed in person by study staff.
- For Phase 2 and 3 dosing, all participants will receive 28 straight days of dosing, which will include dosing on some days on which study samples will not be collected. Study product will remain the same as in Phase 1, and will be dispensed by IDS. Doses can be observed in many preselected locations, by any approved study staff member. Study-related staff including: physicians, mid-level providers, research coordinators, research assistants, nurses, residents or fellows, social workers, community health promoters, mobile health unit staff, pharmacists, interns, and volunteers. All study staff will have completed HSP, GCP and HIPAA training per

unit SOPs. Campus locations may include the hospital pharmacy, medical clinics, research centers, or other pre-selected hospital locations. Off campus locations may include the participant's place of residence or business, community research or health centers, health departments, public libraries, mobile health units, internet based video chat, or pre-selected other public locations.

- Participant identification will be a top priority for all visits. Upon presentation to the research clinic, in a HIPAA controlled area, the participant will verify personal health information to verify their identity, such as full name and date of birth. For any out of clinic dosing visits, the patient will give their HIPAA code word, instead of PHI, to the study staff member prior to dosing. The dosing HIPAA code word will be selected at the screening visit.

4.5 Toxicity Management

Participants will be dosing in the UNC Clinical and Translational Research Center where study staff will be able to monitor for and immediately address allergic reactions or anaphylaxis. Laboratory tests will be taken before study drug administration to look for baseline abnormalities and these will be compared to the laboratory tests to be taken at the follow-up visit after study drug exposure. Any side effects will be managed symptomatically on a case-by-case basis. Participants will be asked to contact study staff if any adverse effects occur and to seek medical attention as they see appropriate. Additional study visits or safety assessments may be needed to evaluate toxicity, as deemed appropriate by the study medical team.

4.6 Possible Adverse Events of a Single Dose

4.6.1 Emtricitabine

Single dose emtricitabine safety data has not been published. For multiple doses, greater than 10% of patients have shown the following adverse effects: headache, dizziness, tiredness, inability to sleep, unusual dreams, loose or watery stools, upset stomach (nausea) or vomiting, abdominal pain, rash, itching, skin darkening of the palms and/or soles, increased cough, runny nose. Less than 10 % of patients experienced abnormal liver function tests, increases in pancreatic enzyme, increased triglycerides, and increased creatinine phosphokinase.

4.6.2 Tenofovir

A phase I single-dose study with tenofovir 300 mg showed it was generally well tolerated. The most frequently reported adverse events were headache (19%) and dizziness (13%). Flatulence (7%), hot flush (3%), and an increase in alanine aminotransferase (3%) were also reported.

4.6.3 Maraviroc

Phase I single-dose studies for doses of maraviroc up to 1200 mg have shown to be generally well tolerated. AEs occurring in greater than 10% of patients include nausea, fatigue, dizziness, headache, pharyngolaryngeal pain, and hypotension. AEs occurring less commonly (< 10%) include dry eye, blurry vision, abdominal discomfort, upper abdominal pain, diarrhea, dry mouth, dyspepsia, flatulence, toothache, vomiting, infection, back pain, myalgia, somnolence, rash, hematoma, and hot flush.

4.6.4 Dolutegravir

The most common (≥10% of Participants) treatment related AEs reported by study volunteers following single and multiple dosing with dolutegravir have been mild to moderate headache and mild to moderate nausea.

4.7 Adverse Effects Associated with the Use as Treatment of HIV Infection

4.7.1 Emtricitabine

Black Box Warnings associated with emtricitabine include a warning of acute, severe exacerbations of HBV with discontinuation of antiretrovirals in HIV/HBV co-infected patients and a warning of lactic acidosis and severe hepatomegaly with steatosis with nucleoside reverse transcriptase analogues that can be fatal. Additional warnings and precautions exist for fat redistribution and immune reconstitution syndrome, as well as warnings for use in patients with renal impairment.

4.7.2 Tenofovir

With multiple dosing, greater than 10% of patients experienced insomnia and depression that was more common in patients with decompensated liver disease, rash, increased triglycerides, nausea, diarrhea, creatinine kinase elevations and weakness. Less than 10% of patients have experienced fatigue, headache, anxiety, hyperglycemia, dyspepsia, flatulence, hematuria, increased transaminases, peripheral neuropathy, myalgia, serum creatinine increases, and renal failure and upper respiratory infection. Black Box Warnings associated with tenofovir include severe acute exacerbation of hepatitis B with medication discontinuation of tenofovir in HIV/HBV co-infected patients and a warning of lactic acidosis with severe hepatomegaly with steatosis with nucleoside reverse transcriptase analogues that can be fatal. Additional warnings and precautions exist for decreased bone mineral density, fat redistribution, immune reconstitution syndrome, and renal toxicity with warnings of use in patients with hepatic and renal impairment.

4.7.3 Maraviroc

With multiple dosing, greater than 10% of patients have experienced fever, rash, upper respiratory infection and cough. Less than 10% of patients have experienced dizziness, insomnia, anxiety, consciousness disturbances, depression, folliculitis, appetite disorders, constipation, lipodystrophy, increased transaminases and bilirubin, neutropenia, urinary symptoms, joint disorders, peripheral neuropathy, respiratory infections, A Black Box Warning exists for possible drug induced hepatotoxicity with allergic features. Additional warnings and precautions exist for immune reconstitution syndrome, infections and postural hypotension.

4.7.4 Dolutegravir

The most common (≥1% of Participants) clinical and laboratory events seen in previous studies of dolutegravir include headache, nauseas, diarrhea, nasopharyngitis, cough, influenza, insomnia, pyrexia, bronchitis, respiratory tract infection, abdominal pain; increased serum cholesterol, glucose, lipase, phosphorous, LDL cholesterol, aspartate aminotransferase, magnesium, creatine phosphokinase, alanine transaminase, and total bilirubin; low serum sodium, calcium and glucose; and increased urine protein (by dipstick).

5. STUDY PROCEDURES

5.1 Pre-screening

Persons interested in participating will be pre-screened by telephone interview using a standardized IRB-approved questionnaire to assess general eligibility prior to scheduling a screening visit. Persons who pass pre-screening will be eligible for a screening visit.

5.2 Screening Visit

After providing written informed consent, potential participants may be screened for eligibility.

The screening visit will consist of the following activities:

- Review study overview and obtain written informed consent
- Assign Participant ID
- Assign study product
- Collect demographic information
- Collect medical/surgical history
- Perform physical exam
- Vitals (blood pressure, heart rate, respiratory rate, height, weight, orthostatic BPs and HRs)
- Obtain safety labs:
    - CBC with differential (includes hemoglobin, hematocrit, WBC and differential count, platelets)
    - Basic Metabolic Panel (Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, phosphate, , aspartate aminotransferase, alanine aminotransferase, Magnesium, alkaline phosphatase, total bilirubin)
    - Urinalysis (includes protein, glucose, color, clarity, pH, specific gravity, ketone, leukocyte esterase, nitrite, urobilinogen, bilirubin, blood, cells, casts)
    - Lipid Panel (HDL, LDL, Total cholesterol, triglycerides)
- Serum Pregnancy Test (for participants of child-bearing potential)
- Virology tests (HIV Ag/Ab, HBsAg, Hep C Ab)
- Syphilis test
- 12-lead ECG

5.3 Phase 1 Enrollment Visit (Day 0)

Eligible Patients will return to the outpatient ward of the Center for Translational and Clinical Research (CTRC) at the University of North Carolina Chapel Hill no sooner than 24 hours and no later than 28 days from their screening visit. The participant will be sequentially assigned to take ONE of the THREE anti-retrovirals for all 3 of their phases: the first 12 participants will receive Maraviroc, the next 12 will receivetenofovir/emtricitabine, and then the final group of 12 participants will receive dolutegravir. (*this sequence may go out of order if participants do not complete all study evaluations and thus a replacement subject is enrolled)

This visit will consist of the following activities:

- Brief physical examination, if indicated
- Vitals (BP, HR, RR, weight, orthostatic BPs and HR)
- Urine Pregnancy Test (for Participants of childbearing potential)
- Hair and Blood Sampling
    - Two 3mL EDTA (purple top tube)
    - One 8mL CPT Tube (blue black tiger top) (tenofovir arm ONLY)
    - One hair collection (10 strands)

- Witnessed dose of study medication
- Adverse event assessment

Since it is not possible to collect samples simultaneously, we will prioritize collecting the blood sample first, followed by the hair sample, if possible. During visits in which both sample collection and dosing will occur, samples will be collected before the subsequent dose is given.

5.4 Phase 1 Sampling Visits (Post-Dose Days 3, 7, 14, 21, 28)

All participants will return to the outpatient CTRC for study sampling on Days 3, 7, 14, 21 and 28 post-dose. Hair samples and blood samples will be obtained on all participants at all visits during all three phases.

Phase 1 sampling visits will consist of the following activities:

- Vital Signs (Days 14 and 28 only)
- Urine Pregnancy Test (for Participants of childbearing potential) (Days 14 and 28, or if indicated)
- Safety Labs (Days 14 and 28 only)
    - CBC
    - Liver Function tests (AST, ALT, ALKP, Tbili)
    - Chemistry Panel (Mg, Phos, Na, K, Cl, CO2, BUN, Cr, Glu
    - HIV Diagnostic testing (Day 28 only)
    - Urinalysis (Day 28 only)
- Hair and Blood Sampling
    - Two 3mL EDTA (purple top tube)
    - One 8mL CPT (blue black tiger top) (Tenofovir arm only)
    - One hair collection (10 strands)
- Witnessed dose of study medication
- Adverse event assessment

5.5 Phase 2 Enrollment (Day 0)

After continued eligibility is confirmed, participants will return to the research clinic as soon as possible, but no later than 28 days, after the completion of Phase 1. Participants will remain on the same assigned antiretroviral as in Phase 1, and begin 28 days of observed daily dosing.

The Phase 2 enrollment visit will consist of the following activities:

- Verification of Continued Eligibility
- Confirmation of Daily Dosing plan
- Adverse Event Assessment
- Witnessed Dose of Study Product

5.6 Phase 2 Dosing visits

The dosing visits will consist of the following activities:

- Witnessed Dose of Study Product
- Adverse Event Assessment

5.7 Phase 2 Sampling Visits

The Phase 2 sampling visits on Days 3, 7, 14, 21 and 28 will consist of the following activities:

- Vital signs (Day 14 and 28 only)
- Urine Pregnancy Testing (Day 14 and 28 only) (for participants of childbearing potential)
- Safety labs (Days 14 and 28 only):
  - CBC
  - Serum Chemistry Panel (Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, phosphate, magnesium, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin)
- Urinalysis (Day 28 only)
- Witnessed Dose of Study Product
- Adverse Events Assessment
- Hair and Blood Sampling
  - Hair Sampling (10 Strands), (Additional Hair sample Day 28 ONLY in the Dolutegravir arm)
  - One 8mL CPT (Blue black tiger top) (Tenofovir arm ONLY)
  - Blood Sampling (two 3mL EDTA purple top tube)

5.8 Phase 3 Enrollment (Day 0)

Continued eligibility will be verified at the end of Phase 2, and participants will come back to the research clinic to enroll into Phase 3 as soon as possible. Participants will be randomized in a 1:1:1 block randomization scheme into one of three dosing schemas with the same study product: no further doses, doses three times weekly, or one dose weekly.

The Phase 3 enrollment visit will consist of the following activities:

- Verification of Continued Eligibility
- Witnessed Dose of Study Product (Cohort Specific)
- Adverse Event Assessment

5.9 Phase 3 dosing visits

The dosing visits will consist of the following activities:

- Witnessed Dose of Study Product
- Adverse Event Assessment

5.10 Phase 3 Sampling visits:

The Phase 3 sampling visits on Days 3, 7, 14, 21 and 28 will consist of the following activities:

- Vital signs (Day 14 and 28 only)
- Urine Pregnancy Testing (Day 14 and 28 only) (for participants of childbearing potential)
- Safety labs (Days 14 and 28 only):

  - CBC
  - Serum Chemistry Panel (Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, phosphate, magnesium, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin)
- Urinalysis (Day 28 only)
- Witnessed Dose of Study Product (cohort specific)
- Adverse Events Assessment
- Hair and Blood Sampling
  - Hair Sampling (10 Strands), (Additional hair sample Day 28 in Dolutegravir arm ONLY)
  - Blood Sampling (two 3mL EDTA purple top tube)
  - Blood Sampling (one 8mL CPT blue black tube) (Tenofovir arm ONLY)

5.11 Follow-up Visit

The follow up visit will consist of the following activities:

- Targeted physical examination, as indicated
- Vitals (BP, HR, RR, weight, orthostatic BP and HR)
- Safety labs:
  - CBC with differential
  - Serum Chemistry Panel (Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, phosphate, aspartate aminotransferase, alanine aminotransferase, magnesium, alkaline phosphatase, total bilirubin)
  - Lipid Panel
- Urinalysis
- HIV testing
- Urine Pregnancy test (for participants of childbearing potential)
- 12-lead ECG

6. SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1 Clinical Data and Safety Review

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are the first layer of this tiered system and are responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the Protocol Safety Review Team (PSRT) if unexpected concerns arise. The PSRT will consist of the following study site investigators: Angela Kashuba, PharmD (Principle Investigator), Cindy Gay, MD (Study Physician), Heather Prince, PA-C (Study Physician's Assistant), Amanda Poliseno (Research Coordinator) along with the Biostatistician.

During the trial, the PSRT will review safety reports (all AEs included, independent of determination of relatedness to study products) and conduct calls to review the data as appropriate. The content, format and frequency of the safety reports will be agreed upon by the PSRT in advance of study implementation. In addition to these routine safety data reviews, the PSRT will convene on an ad hoc basis to make decisions regarding the handling of any significant safety concerns. If necessary, experts external to the protocol team, representing expertise in the fields of microbicides, biostatistics, and medical ethics may be invited to join the PSRT safety review.

6.2 Adverse Events Definitions and Reporting Requirements

An adverse event is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. This definition is applied to all the study groups, and is applied to all groups beginning from the time of sequential assignment. The term "investigational product" for this study refers to all study products listed in this protocol. Study participants will be instructed to contact the study site staff to report any AEs they may experience at any time between screening and follow-up. In the case of a life-threatening event, they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. Sites will obtain written permission from the participant to obtain and use records from non-study medical providers to complete any missing data element on a CRF related to an adverse event. All participants reporting an untoward medical occurrence will be followed clinically, until the occurrence resolves (returns to baseline) or stabilizes.

The site physicians will determine AE resolution or stabilization in their best clinical judgment, but may seek PSRT medical consultation regarding follow-up or additional evaluations of an AE. Study site staff will document in source documents all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. Study staff also will record all AEs on case report forms.

6.3 Serious Adverse Events

Serious adverse events (SAEs) will be defined by the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.1, dated March 2017) as AEs occurring at any dose that:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization

The following are examples of hospitalization that will not be considered to be AEs:

- Protocol-specified admission (e.g. for procedure required by study protocol)
- Admission for treatment of target disease of the study, or for pre-existing condition (unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator)
- Diagnostic admission (e.g. for a work-up of an existing condition such as persistent pretreatment lab abnormality)
- Administrative admission (e.g. for annual physical)

- Social admission (e.g. placement for lack of place to sleep)
- Elective admission (e.g. for elective surgery)

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Study staff will report all AEs that meet serious adverse event (SAE) reporting requirements according to the DAIDS-defined "standard" reporting requirements. Information on all AEs will be included in reports to the US FDA, and other applicable government and regulatory authorities. Study staff will report information on all AEs and SAEs to the IRB in accordance with all applicable regulations and requirements.

6.4 Adverse Event Relationship to Study Product

The relationship of all AEs to study product will be assessed per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.1, dated March 2017). Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.1, dated March 2017), the relationship categories that will be used for this study are:

Related: There is a reasonable possibility that the AE may be related to the study agent(s)

Not related: There is not a reasonable possibility that the AE is related to the study agent(s)

6.5 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009), Addenda 1 and 3 (Female Genital and Rectal Grading Tables for Use in Microbicide Studies) will be the primary tools for grading adverse events for this protocol. Adverse events not included in those tables will be graded by the DAIDS AE Grading Table Version 1.0, December 2004 (Clarification dated August 2009). In cases where an AE is covered in all tables, the DAIDS AE Grading Table, Version 1.0, December 2004 (Clarification dated August 2009), Addendum 1 (Vaginal Grading Table for Use in Microbicide Studies),and Addendum 3 (Rectal Grading Table for Use in Microbicide Studies) will be the grading scale utilized.

6.6 Pregnancy and Pregnancy Outcomes

Pregnancy-related data will be collected using the pregnancy CRFs for all pregnancies detected during the study. Pregnancy outcomes will not be expeditiously reported to DAIDS, unless there is an associated adverse event in the pregnant participant that meets expedited reporting criteria or the pregnancy results in a congenital anomaly meeting the Manual for Expedited Reported of Adverse Events to DAIDS (Version 2.1, March 2017) guidelines for expedited reporting. Fetal losses without congenital anomalies or maternal complications that require expedited reporting will not be expeditiously reported but data will be captured via the pregnancy CRFs.

After the participant's follow-up study visit, the study team will make every attempt to follow the participant

until an outcome of the pregnancy can be ascertained to allow reporting of these outcomes meeting EAE criteria. Pregnancy outcomes that meet criteria for EAE reporting as described above (e.g., maternal complications, congenital anomalies) occurring among participants known to be pregnant at the Final Study Visit will continue to be expeditiously reported.

6.7 Social Harms Reporting

Although study sites make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result. For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities. Social harms that are judged by the Investigator of Record to be serious or unexpected will be reported to the responsible site IRB at least annually, or according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed. While maintaining participant confidentiality, study sites may engage their CABs in exploring the social context surrounding instances of social harm.

7. CLINICAL MANAGEMENT

In general, the site investigator has the discretion to discontinue study product at any time if she feels that continued product use would be harmful to the participant or interfere with treatment deemed clinically necessary. Unless otherwise specified below, the investigator should immediately consult the PSRT for further guidance regarding permanent discontinuation.

The site investigator or designee will document all discontinuations on applicable case report forms.

7.1 Dose Modification Instructions

Study doses will not be modified on this protocol.

7.2 Discontinuation of Study Product(s) in the Presence of Toxicity

Stopping rules have been created to protect the health and safety of study participants. If a single subject experiences a Serious Adverse Event, enrollment will be held while the protocol undergoes review. If 2 or more participants experience Grade 3 or Grade 4 adverse events, enrollment will be held while the protocol undergoes review. If 5 or more participants experience Serious Adverse Events or Grade 3/Grade 4 adverse events deemed by the study physician to be related to the drug under study, the study will be stopped while the safety concerns of using these variable doses of study medication are reviewed.

Any Grade 3 or 4 or serious adverse event related to a study intervention (blood draw, biopsy) or the study drugs (tenofovir, maraviroc, emtricitabine, Dolutegravir) will result in subject discontinuation. Participants who are withdrawn for safety reasons will complete a follow-up visit within 14 days of discontinuation.

7.3 Pregnancy

Pregnant and lactating women are excluded from this study. Routine serum pregnancy testing is being performed at the screening visit and routine urine testing is being performed at every study visit and prior to the administration of study medication. If a participant becomes pregnant at any time during the course

of the study, the participant will remain in the study and will continue with follow-up visits and safety lab assessments.

Pregnancy-related data will be collected using the pregnancy CRFs for all pregnancies detected during the study. The study team will make a very strong attempt to maintain contact with the pregnant participant until the outcome of the pregnancy is determined or it becomes clear that it will be impossible to obtain outcome information. The protocol team will prepare and provide to DAIDS a quarterly report on all pregnancies and their outcomes.

7.4 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. The site investigator also may withdraw participants from the study to protect their safety and/or if they are unwilling or unable to comply with required study procedures, after consultation with the PSRT. Participants also may be withdrawn if the study sponsors, government or regulatory authorities (including the Office of Human Research Protections), or site IRBs terminate the study prior to its planned end date. Every reasonable effort is made to complete a final evaluation of participants who withdraw or are withdrawn from the study prior to completing follow-up. Study staff members will record the reason(s) for all withdrawals in participants' study records. In the event that participants who voluntarily withdraw from the study wish to re-join the study, they may resume product use (if applicable) and follow-up through their originally scheduled study exit date.

8. DATA MANAGEMENT AND RECORDKEEPING

8.1 Data Management Responsibilities

The study site will maintain source data/documents in accordance with Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (http://www3.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/PDF/Source DocPolicy.pdf).

The investigator will maintain, and store securely, complete, accurate and current study records throughout the study. The investigator will retain all study records for at least three years following the completion of the study, unless directed otherwise by the National Institutes of Health (NIH). Study records must be maintained on site for the entire period of study implementation.

8.2 Quality Control and Quality Assurance

The study site will conduct quality control and quality assurance procedures in accordance with Requirements for Clinical Quality Management Plans at DAIDS Funded and/or Supported Clinical Research Sites available at: (http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/QMPPolicy.pdf).

8.3 Study Coordination

All procedures, documentation, subject contact, data management, clinical monitoring, personnel training and regulatory requirements will be performed in accordance to policies governed by the University of North Carolina Clinical Pharmacology and Analytical Chemistry Laboratory. These policies are located in department SOPs.

9. STATISTICAL CONSIDERATIONS

The Center for AIDS Research (CFAR) Biostatistics Core is collaborating with us for our statistical plan on this grant.

9.1 Statistical Analysis Plan

Linear mixed models with random intercepts will be used for multivariable analyses of predictors of hair MSI output. Inter- and intra-subject variability will be determined from these models. Dose proportionality will be assessed by employing a power model of the form $E\{\log(y)\} = \beta_0 + \beta_1 \log(dose)$, where y denotes drug (ARV) concentration and $\beta_1 = 1$ corresponds to perfect dose proportionality. The power model will be fit and the 90% confidence interval (CI) for β_1 will be compared to a pre-specified critical region. If the CI falls within this critical region, then the data support dose proportionality. This critical region is $\left(1 + \frac{\ln(\theta_L)}{\ln(r)}, 1 + \frac{\ln(\theta_U)}{\ln(r)}\right)$ where r is the ratio of the highest dose to the lowest dose, $\theta_L = 0.80$, and $\theta_U = 1.25$.

9.2 Power and Sample Size Calculation:

Through a series of simulation studies conducted using R-project, it was determined that 12 participants per ARV treatment group will be sufficient to achieve > 99% power to establish dose proportionality (i.e., assuming $\beta_1 = 1$). For these simulation studies, we assumed dose levels ranging from 1 dose per 28 days to 1 dose per day over the three phases of study, yielding $r = 28$. Concentrations where no drug is given (0 doses per day) cannot be used when fitting the power model described above. To account for the same individual contributing repeated observations (corresponding to the three 28 day phases of observation), a mixed effects power model was used in the empirical power simulations. Values for the between- and within-individual variability were assumed based on a natural-log transformation of pilot IR-MALDESI response per voxel data from participants taking efavirenz. The estimated within-individual standard deviation [SD] was 0.10 and estimated between-individual SD was 0.52.

9.3 Pharmacokinetic Analysis Plan

Initial data exploration will be utilized to produce descriptive statistics and exploratory plots. Nonlinear mixed effects modeling with NONMEM (ICON plc, Ireland) will be used to construct population PK models describing the pharmacokinetics of TFV, FTC, MVC, and DTG in plasma, PBMC, and hair (101,102). The structural model will consist of a compartment for each matrix, with possible transit compartments from plasma to hair. Linearity will initially be assumed yet will be subsequently assessed. If the PK relationship

is found to be nonlinear, saturation terms can be added to the model parameters. Various structural models will be explored with Berkley Madonna software (University of California, Berkeley) to generate plausible candidate models (80). The best model will be chosen depending on the objective function and model performance diagnostics such as visual predictive checks. Bayesian analysis (83) using existing published PK models (12, 29,159,162) can also be added to model parameters if necessary for uncertain parameters such as drug uptake into hair (5, 38) and clearance. Once the base model is constructed, demographic covariates such as weight, BMI, creatinine clearance, age, gender, and race will also be tested for significance one at a time. Scientific input and previous research findings will be used to inform selection of a multivariable model. Monte Carlo simulations will be performed with the model to generate prediction intervals to both explore other possible dosing regimens and for adherence assessments in HPTN 069. (Aim 2b) In addition, localized drug concentrations within a hair strand will also be modeled using partial differential equations (e.g., via Matlab). This physiologic-based pharmacokinetic (PBPK) modeling approach has previously been done to characterize localized drug concentrations in tumor. (31, 42). This will enable a more granular estimation of dosing history from continuous drug concentration distribution within a hair strand. Initially, various models will be explored and one with the most physiologic relevance will be fit to the data. Variance will also be added to various parameters to describe the data on a population level. Monte-Carlo simulations can be performed to generate prediction intervals of drug concentration along the length of the hair under various dosing regimens.

10. HUMAN SUBJECTS PROTECTIONS

The investigators will make efforts to minimize risks to participants. Volunteers and study staff members will take part in a thorough informed consent process. Before beginning the study, the investigators will have obtained IRB approval. The investigators will permit audits by the NIH/DAIDS, the FDA or any of their appointed agents.

10.1 Institutional Review Board

This protocol, along with the informed consent, will be submitted for approval by the UNC IRB. Subsequent to initial review and approval, the IRB will review the protocol at least annually. The Investigator will make safety and progress reports to the IRB at least annually and within three months of the study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human participants or others.

10.2 Risks

- **Blood Draws:** Risks associated with blood draws include bleeding, discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection.

- **Hair Collection:** There are no known associated risks with collection of hair.

- **STI Screening/Reporting:** Disclosure of STI status may cause sadness or depression in volunteers. Partner notification of STI status may cause problems in their relationships with their sexual partners. Additionally, participants could misunderstand the current experimental status of the study medication and as a result increase their HIV risk behaviors while in the study. The following STIs are required by law to be reported to the NC State Department of Health if positive:

HIV, Syphilis, gonorrhea, Chlamydia, Hepatitis B and C.

- **Unknown Risks:** There are always a potential for unknown problems to arise. Participants will be encouraged to contact study staff any time they expect a problem.

- **Confidentiality:** Participation in clinical research includes the risks of loss of confidentiality and discomfort with personal nature of questions. Although the study site makes every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result.

- **Study Medications**
  The known toxicities for a single dose of these antiretrovirals in this protocol. Listed below are the pregnancy categories for the medications.

  - **Tenofovir:** TDF is a pregnancy category B medication. No controlled human studies of TDF among pregnant women have been conducted. Animal studies have shown decreased fetal growth and reduced fetal bone porosity. No increased risk of overall birth defects has been observed following first trimester exposure according to data collected by the antiretroviral pregnancy registry (Lexi-Comp).

  - **Emtricitabine:** FTC is a pregnancy category B medication. No controlled human studies of FTC among pregnant women have been conducted. Adverse events were not observed in animal studies and no increased risk of overall birth defects has been observed according to data collected by the antiretroviral pregnancy registry (Lexi-Comp).

  - **Dolutegravir:** Dolutegravir is a pregnancy category B. There have been no animal studies that have documented fetal risk, and no studies testing Dolutegravir in pregnant women.

  - **Maraviroc:** Maraviroc is a pregnancy category B. Adverse fetal effects were not seen in animal studies, however, there have been no studies testing maraviroc in pregnant women.

10.3 Benefits

Participants in this study will experience no direct benefit. Participants and others may benefit in the future from information learned from this study. Additionally, participants will be referred for treatment for any incidental findings detected during screening and other examinations.

10.4 Informed Consent Process

The investigators will obtain informed consent from each subject before starting any study procedures according to the standards set forth in the ICH Good Clinical Practice guidelines and per unit SOPs. The process will including reviewing consent forms with potential participants in a confidential setting and explaining all risks and benefits associated with participation of the study. This involves reading over the

IRB-approved consent form with the subject in a private space, soliciting questions from the subject, allowing the subject ample time alone to review the form, soliciting questions again, and then offering the subject the opportunity to sign the consent form. To ensure understanding, study staff may ask questions of the participants regarding study procedures. The consent forms will use language that is sufficiently simple for lay persons to comprehend. Participants will not be coerced into participating. Children under the age of 18 years, decisionally impaired adults and non-English speakers will not be enrolled in this study. Each subject will be provided with a photocopy of all documents that she signs. The informed consent process will cover all elements of informed consent required by research regulations. In addition, the process specifically will address the following topics of importance to this study:

- The unknown safety and unproven efficacy of the study products
- The need to practice safer sex behaviors regardless of study treatment group
- The importance of participants in all four study groups to the success of the study
- The importance of adherence to the study visit and procedures schedule
- The potential medical risks of study participation (and what to do if such risks are experienced)
- The potential social harms associated with study participation (and what to do if such harms are experienced)
- The real yet limited benefits of study participation
- The distinction between research and clinical care
- The right to withdraw from the study at any time

The informed consent process will include an assessment of each potential participant's understanding prior to enrollment and sequential assignment of concepts identified by the protocol team as essential to the informed consent decision. Participants who are not able to demonstrate adequate understanding of key concepts after exhaustive educational efforts will not be enrolled in the study.

10.5 Participant Confidentiality

Confidentiality will be maintained by storing all specimens for current and future use with a unique identifying number, which will be linked to the subject's name, social security number, address, telephone number, and hospital medical record (MR) number. The principal investigators and study staff will be the only people with access to the identifying information. Any information provided to other people working on this study will be given with the study ID number, not other identifying information. The records will be secured in a locked file cabinet in the principal investigator's office.

All electronic data for this study will be stored on a dedicated University server which contains extensive protections and securities.

10.6 Special Populations

Children under the age of 18 years, decisionally impaired adults and non-English speakers will not be enrolled in this study.

10.6.1 Pregnant Women

Women who test positive for pregnancy at the screening visit or prior to study drug administration, will not be eligible to participate in this study. A serum pregnancy test will be performed at the screening visit and urine pregnancy tests at all study visits, and additionally if clinically indicated. During the informed consent

process, women will be informed that none of the study products are methods of contraception and about the current knowledge of effects of these products on a developing human fetus.

10.6.2 Children

The NIH has mandated that children be included in research trials when appropriate. This study meets "Justifications for Exclusion" criteria for younger children as set forth by the NIH. Specifically, "insufficient data are available in adults to judge potential risk in children" and "children should not be the initial group to be involved in research studies." This study does not plan to enroll children under 18 years old.

10.7 Compensation

Pending IRB approval, participants will be compensated for their time and effort in this study. Stipends will be offered which are consistent with industry standards. UNC Hospitals parking deck tokens will be offered to all participants for their time on campus for study appointments.

10.8 Communicable Disease Reporting

Study staff will comply with all applicable local requirements to report communicable diseases including HIV identified among study participants to local health authorities. Participants will be made aware of all reporting requirements during the study informed consent process

10.9 Access to HIV-related Care

Participants found to be HIV-infected will be referred to available sources of medical and psychosocial care and support, and local research studies for HIV-infected adults.

10.10 HIV Counseling and Testing

HIV test-related counseling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study, and to all enrolled participants at each follow-up HIV testing time point. Counseling will be provided in accordance with standard HIV counseling policies and methods at the site and additionally will emphasize the unknown efficacy of the study products in preventing HIV infection. In accordance with the policies of the US NIH, participants must receive their HIV test results to take part in this study.

10.11 Care for Participants Identified as HIV-Infected

Participants will be provided with their HIV test results in the context of post-test counseling. Participants found to be HIV-infected will be referred to available sources of medical and psychosocial care and support, and local research studies for HIV-infected adults.

10.12 Study Discontinuation

This study may be discontinued at any time by NIAID/DAIDS, the US FDA, the OHRP, or the site IRB.

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12. APPENDICES

12.1 APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

Procedure	Screening	Phase 1						Phase 2						Phase 3						Follow Up
	(up to 28 days)	D 0	D 3	D 7	D 14	D 21	D 28	D 0	D 3	D 7	D 14	D 21	D 28	D 0	D 3	D 7	D 14	D 21	D 28	(within 14 days)
Informed Consent	X																			
Vital Signs[a]	X				X		X				X		X				X		X	X
PMH	X																			
Physical Exam[b]	X																			
Pregnancy Testing[c]	X	X			X		X	X			X		X				X		X	X
HIV Diagnostic Testing	X						X						X							X
Safety Testing[d]	X				X		X				X		X				X		X	X
Immunology Testing[e]	X																			
Lipid Panel	X																			X
Urinalysis	X						X						X						X	X
EKG	X																			X
Hair Sampling			X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	
Blood Sampling			X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	
Adverse Event Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a—Vital signs to be obtained at Screening include: Height, Weight, Temperature, Respiratory Rate, and orthostatic BP and pulse. Orthostatic vitals to be obtained at subsequent denoted visits t hroughout all 3 phases and follow up.

b—Full physical examination will be completed at screening, and targeted exams will be completed at all other visits as indicated.

c—Blood will be obtained for BetaHCG at screening and follow up. Urine POC testing will be conducted at other denoted visits in women of childbearing potential, or anytime pregnancy is suspected.

d—Screening and follow up safety labs to be obtained include: CBC with differential, BMP, Phosphorus, Mg, Liver function studies (AST, ALT, Tbili, AlkP), Urinalysis, and total lipid panel (HDL, LDL, TChol, Triglycerides). Intra-phase safety testing will include CBC, BMP with Mg, Phosphorus, Liver function studies (AST, ALT, Tbili, AlkP).

e—RPR, Hepatitis B and C testing will be obtained at screening.

12.2 Appendix II: HAIR STANDARD OPERATING PROCEDURES

G. SUPPLIES NEEDED FOR HAIR COLLECTION

A few basic supplies will be needed to collect and correctly store the hair sample: hair clips, scissors, aluminum foil, dessicant bags, and ziplock bags.

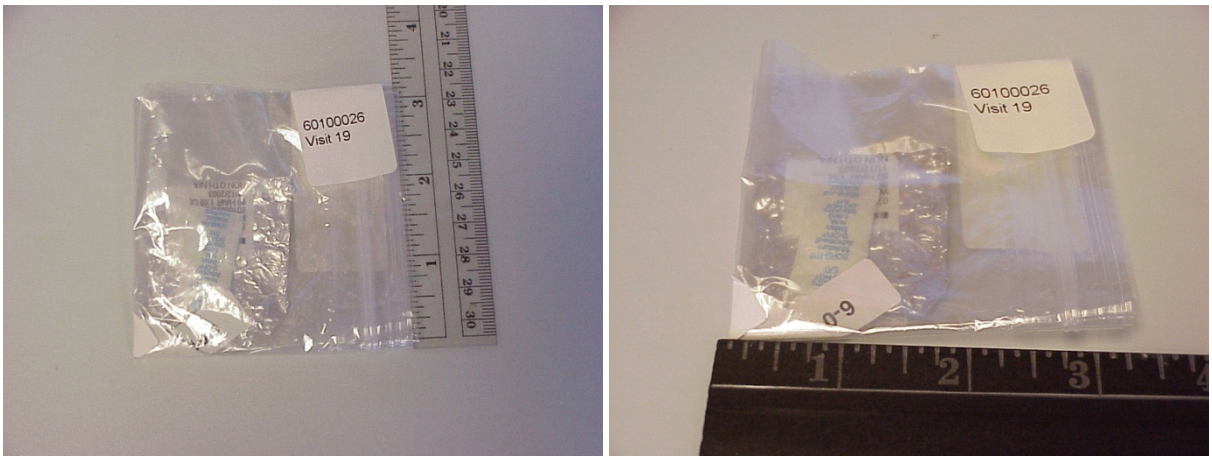
- Aluminum foil can be ordered from Quill Diagnostics. The product is called Handy Foil Standard Aluminum Foil, catalogue number 035-11205: 12 inches x 100 feet, $39.99. Alternatively, the foil can be purchased locally, if sites can find a better price. Aluminum foil should be cut into squares approximately 5cm x 5cm and folded into quarters.
- Dessicant bags should be ordered from U-Line. Phone: 1-800-295-5510; fax: 1-800-295-5571. The product is called 1/2 g Silica Gel Desiccants, catalogue number S-8032: 1 pail (6000 bags/container), $133.00.
- Scissors can be purchased locally.
- Hair clips can be purchased locally.
- Ziplock bags should be small and can be purchased locally.

H. HAIR COLLECTION PROCEDURE

1. Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry prior to use.
2. Unfold the piece of aluminum foil and have it ready, along with a small label for labeling the hair once cut
3. Lift up the top layer of hair from the occipital region of the scalp. A hair clip can be used to keep this top layer of hair out of the way. Isolate a small thatch of hair from *underneath* this top layer of hair from the occipital region.
4. 20-30 fibers of hair at least 1 centimeter long should be isolated and cut.
5. Cut the small hair sample off the participant's ahead *as close to the scalp as possible*.
6. Lay the small hair sample onto the piece of unfolded aluminum foil and place a small label with the participant's Study ID over the distal end of the hair thatch (affixing the hair sample to the tin foil in the process). The distal end is the portion furthest from the scalp. It is very important to place the label at the distal end as this will distinguish the scalp end from the distal end.
7. Refold the foil over to completely enclose the thatch of hair.
8. Place a StudyID label on outside of the folded piece of foil.
9. Place the folded piece of foil inside a Ziplock bag with a dessicant bag in it and seal the Ziplock bag.

10. The pair of scissors used to collect the hair samples should be cleaned prior to using on each participant. Reclean the blades of the scissors with an alcohol pad and allow blades to completely dry prior to reuse.
11. Hair samples should be kept at room temperature and in a dark place prior to pickup.

Each packet of hair is to be folded to approximately 3" x 3" and labeled with the StudyID and visit on the upper portion of the packet.



WIHS V- HAIR COLLECTION PICTURES

Material Required: Scissors, piece of tin foil, patient labels (2), ziplock bag, alcohol swabs, dessicant pellet (optional)

Suggest making these "hair kits" ahead of time

Step 1: Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry

Clean off blades of scissors between patients

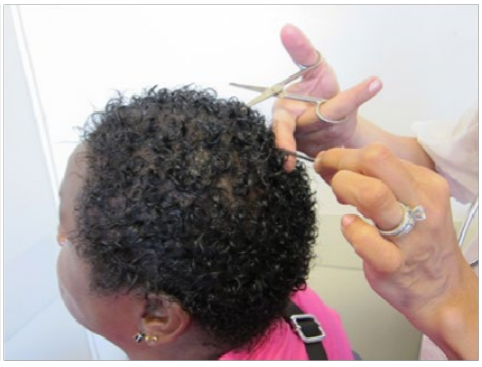
Step 2: Lift up the top layer of hair from the occipital region of the scalp. Isolate a small thatch of hair (20-30 strands) from underneath this top layer

Can use hair clip to keep top layer of hair away if easier

Step 3:: Cut the small hair sample as close to the scalp as possible

STRAIGHT HAIR


CURLY HAIR

BRAIDED HAIR

Cut hair thatch from in-between braids or dread locks

Step 4: Keep your fingers on the part of the hair that was FURTHEST away from the scalp and put the hair sample down on an unfolded piece of tin foil

Step 5: Put a thin label over the end of the hair sample that was FURTHEST away from the scalp

Step 6: Refold the foil over to completely enclose the hair and place a study ID label on the folded piece of foil

Step 7: Place the folded piece of foil inside the plastic (e.g., Ziplock®) bag (dessicant pellet in the bag is

optional) and seal the bag; Hair samples should be kept at room temperature and in a dark place at each site prior to pickup

12.3 Appendix III: LIST OF FIGURES

Figure 5: (See "Background")

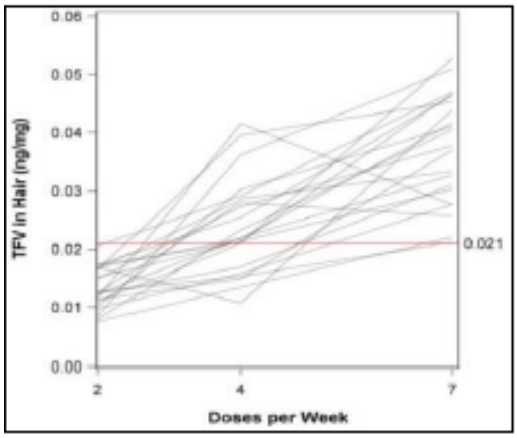


Figure 5: Tenofovir Concentrations in Hair Using LC-MS/MS Methods Only Uniquely Distinguish 2 and 7 Doses/Week (88)

Figure 6: (See "Background")

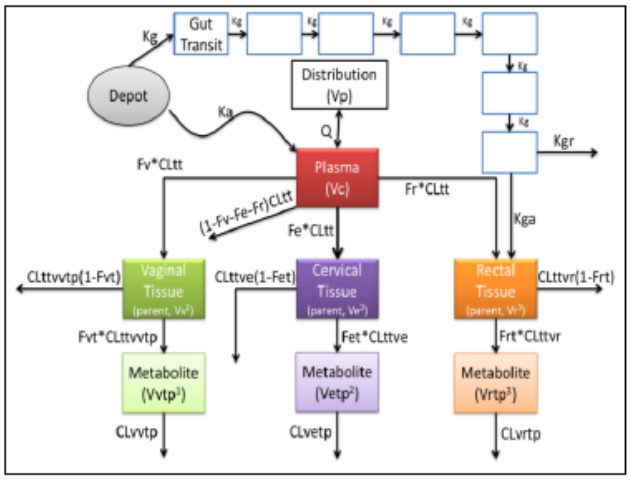


Figure 6 Multi-compartment Tissue Model of Extracellular and Intracellular TFV and TFVdp or FTC and FTCtp. Using goodness of fit plots, the model well predicted concentrations in all compartments.