HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

DAIDS ID: 10851

A Study of the HIV Prevention Trials Network

Sponsored by:

Division of AIDS, U.S. National Institute of Allergy and Infectious Diseases
U.S. National Institutes of Health

Pharmaceutical Support Provided by:

Gilead Sciences

Protocol Chair(s):
Craig W. Hendrix
Johns Hopkins University
Baltimore, MD, U.S.A

Protocol Co-Chair:
Ken Mayer
Brown University
Boston, MA, U.S.A

Kristine Patterson
University of North Carolina, Chapel Hill
Chapel Hill, NC, U.S.A

Version 2.0
29 November 2010

Non-IND study

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

TABLE OF CONTENTS

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome
aPTT	activated partial thromboplastin time
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BV	bacterial vaginosis
Ca	calcium
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CORE	(HPTN) Coordinating and Operations Center
Cl	chloride
CRPMC	(NIAID) Clinical Research Products Management Center
CT	Chlamydia (Chlamydia trachomatis)
DAIDS	Division of AIDS
DOT	Directly observed therapy
EAE	expedited adverse event (reporting)
EC	ethics committee
FDA	(United States) Food and Drug Administration
FTC	emtricitabine
FTC-TP	emtricitabine triphosphate
GC	gonorrhea (Neisseria Gonorrhoeae)
GCLP	good clinical laboratory practice
GCP	good clinical practice
HBsAg	hepatitis B surface antigen
HC03	bicarbonate
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
IDS	investigational drug service
IRB	institutional review board
K	potassium
LDMS	Laboratory Data Management System
LL	local laboratory
LLN	lower limit of normal
Na	sodium
NAAT	nucleic acid amplification test
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health

NL	(HPTN) Network Laboratory
NSAID	Non-steroidal anti-inflammatory drug
OHRP	Office for Human Research Protections
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
RTI	reproductive tract infection
RSC	Regulatory Support Center (formerly RCC)
RUQ	right upper quadrant
SAE	serious adverse event
SDMC	(HPTN) Statistical and Data Management Center
SMC	Study Monitoring Committee
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
Trich	trichomoniasis (Trichomonas vaginalis)
ULN	upper limit of normal
UTI	urinary tract infection

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

LIST OF TABLES AND FIGURES

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

TERMINOLOGY FOR TENOFOVIR, EMTRICITABINE, 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 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 (trade name: Emtriva®) 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 form of FTC that is generated in cells. This is the form 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.

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

PROTOCOL TEAM ROSTER

David Borasky
Office of Research Protection
RTI International
3040 Cornwallis Rd
RTP, NC 27709 , U.S.A.
Phone: 919-316-3903
Fax: 919-316-3897
E-mail: dborasky@rti.org

Ying Chen
SCHARP/FHCRC
1100 Fairview Avenue N
M2-C200
PO Box 19024
Seattle, WA 98109, U.S.A
Email: yqchen@scharp.org
Phone: 206-667-7051
Fax: 206-667-4812

Myron Cohen
University of North Carolina
School of Medicine,
Department of Medicine,
Division of Infectious Diseases
130 Mason Farm Road
Bioinformatics Bldg.
Chapel Hill, NC 27599,
U.S.A
Email:
myron_cohen@med.unc.edu
Phone: 919-966-2536
Fax: 919-966-6714

Vanessa Elharrar
Medical Officer
Prevention Science
Program/DAIDS/NIAID/NIH
6700-B Rockledge Drive, 5th
Fl. Rm. 5125
Bethesda, MD 20892, U.S.A.
Email:
elharrarva@niaid.nih.gov
Phone: 301-827-0845

Lynda Emel
Senior Project Manager -
Perinatal and Prevention
SCHARP/FHCRC
1100 Fairview Avenue North
LE-400
PO Box 19024
Seattle, WA 09109 U.S.A
Email: lemel@scharp.org
Phone: 206-667-5803
Fax: 206-667-4812

Susan Eshleman
Johns Hopkins University
720 Rutland Avenue
Ross Bldg., Room 646
Baltimore, MD 21205, U.S.A
Email: seshlem@jhmi.edu
Phone: 410-614-4734
Fax: 410-502-9244

Alexia Exarchos
Pharmaceutical
Representative
Gilead Sciences
333 Lakeside Drive
Foster City, California 94404
U.S.A
Email:
Alexia.Exarchos@gilead.com
Phone: 650-522-1201
Fax: 650-522-5418

Fang Gai
Statistical Research Associate
SCHARP/FHCRC
1100 Fairview Ave. N.
LE-400
P.O. Box 19024
Seattle, WA 98109-1024
U.S.A
Email: fang@scharp.org
Phone: 206-667-7524
Fax: 206-667-4812

Craig Hendrix
Protocol Chair
Johns Hopkins University
600 North Wolfe Street
Harvey 502
Baltimore, MD 21287, U.S.A
Email: cwhendrix@jhmi.edu
Phone: 410-955-9707
Fax: 410-955-9708

Angela Kashuba
3318 Kerr Hall,
School of Pharmacy
UNC-Chapel Hill
Chapel Hill NC, 27599,
U.S.A
Email: akashuba@unc.edu
Phone: 919-966-9998
Fax: 919-962-0644

Cheryl Cokley
FHI
2224 E. NC Hwy 54
Durham, NC 27713, U.S.A
Email: ccokley@fhi.org
Phone: 919-544-7040 ext. 11359
Fax: 919-544-0207

Ken Mayer
Protocol Co-Chair
Fenway Health
1340 Boylston Street
Boston, Ma, U.S.A
Email:
kenneth_mayer@brown.edu
Phone: 401-793-4711
Fax: 401-793 4709
Fax: 617-536-8602

Laura McKinstry
Project Manager
SCHARP/FHCRC
1100 Fairview Ave. N.
LE-400
P.O. Box 19024
Seattle, WA 98109-1024
U.S.A
Email:
lmckinst@scharp.org
Phone: 206-667-7322
Fax: 206-667-4812

Ayana Moore
FHI
2224 E. NC Hwy 54
Durham, NC 27713, U.S.A
Email: amoore@fhi.org
Phone: 919-544-7040 ext. 11244
Fax: 919-544-0207

Kristine Patterson
Protocol Co-Chair
Division of Infectious Diseases
CB# 7030, Bioinformatics Building
130 Mason Farm Road, 2nd Floor
Chapel Hill, NC 27599,
U.S.A
Email:
kristine_patterson@med.unc.edu
Phone: 919-966-2536
Fax: 919-966-6714

Paul Richardson
HPTN NL QC Representative
Johns Hopkins Univ. Dept. of Pathology
Pathology Building 306
600 N. Wolfe St.
Baltimore, MD 21287,
U.S.A.
Email: pricha18@jhmi.edu
Phone: 410-502-0435
Fax: 410-614-0430

James F. Rooney
Pharmaceutical Representative
Gilead Sciences
Vice President Medical Affairs
333 Lakeside Drive
Foster City, California 94404
U.S.A
Email:
jim.rooney@gilead.com
Phone: (650) 522-5708
Fax: (650) 522-5854

Katherine Shin
Pharmacist
Pharmaceutical Affairs Branch
Division of AIDS
6700-B Rockledge Drive,
Room 4227
Bethesda, MD 20892
Phone: 301-594-1517
Fax: 301-402-1506
Email:
KaShin@niaid.nih.gov

Nirupama Sista
FHI
2224 E. NC Hwy 54
Durham, NC 27713, U.S.A
Email: nsista@fhi.org
Phone: 919-544-7040 ext. 11590
Fax: 919-544-0207

Lei Wang
SCHARP/FHCRC
1100 Fairview Avenue N
M2-C200
PO Box 19024
Seattle, WA 98109, U.S.A
Email: lwang@scharp.org
Phone: 206-667-7049
Fax: 206-667-4812

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

PROTOCOL SIGNATURE PAGE

Version 2.0
29 November 2010

A Study of the HIV Prevention Trials Network (HPTN)

Sponsored by:
Division of AIDS, U.S. National Institute of Allergy and Infectious Diseases
U.S. National Institutes of Health

I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I agree to maintain all study documentation for a minimum of three years after submission of the site's final Financial Status Report to the Division of AIDS (DAIDS), unless otherwise specified by DAIDS or the HIV Prevention Trials Network (HPTN) Coordinating and Operations Center. Publication of the results of this study will be governed by HPTN policies. Any presentation, abstract, or manuscript will be made available by the investigators to the HPTN Manuscript Review Committee and DAIDS for review prior to submission.

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

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

Version 2.0
29 November 2010

SCHEMA

Purpose: Describe the dose-proportionality and intra-individual variability of tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) at steady-state in healthy human participants taking Truvada® (FTC 200mg/TDF 300 mg) under direct observation.

Design: Phase 1, multi-site, open label, randomized, 4-arm, dose-ranging, pharmacokinetic (PK) study.

Study Population: HIV-uninfected, healthy, sexually-active volunteers, including both men and women recruited from two sites in the United States (U.S.).

Study Size: **32** participants distributed into four 8-person cohorts with 4 men and 4 women per cohort. **16** of the original 32 participants (2 men, 2 women per dosing cohort), will undergo intensive tissue sampling.

Treatment Regimen: Research participants will receive doses under the direct observation of study personnel for 5 weeks. Research participants will be enrolled randomly into one of four study arms:

1. FTC 200 mg/TDF 300 mg, one tablet orally once weekly for 5 weeks
2. FTC 200 mg/TDF 300 mg, one tablet orally twice weekly for 5 weeks
3. FTC 200 mg/TDF 300 mg, two tablets orally twice weekly for 5 weeks
4. FTC 200 mg/TDF 300 mg, one tablet once daily for 5 weeks

Study Duration: Approximately 12 months total. Accrual will require approximately 6 months. Each participant will be followed for approximately 2 months (screening, treatment, and follow-up).

Primary Objectives:

1. Assess dose-proportionality of intracellular TFV-DP and FTC-TP from weekly to daily dosing.
2. Describe intra-individual variability in intracellular TFV-DP and FTC-TP concentrations at steady-state (comparison of Day 28 and Day 35)

Secondary Objectives:

1. Describe the relationship between pre-dose (C_τ) and decaying concentrations of TFV, FTC, and their phosphorylated derivatives (TFV-DP and FTC-TP) in blood serum, peripheral blood mononuclear cells (PBMCs), CD4+ blood cells, total tissue cells, CD4+ tissue cells, tissue homogenate, semen, and luminal fluid at steady-state (Day 35 [pre-dose] and Day 49 [decaying, greater than one half-life for TFV-DP]).
2. Describe differences in intracellular TFV-DP and FTC-TP steady-state C_τ between men and women.
3. Characterize the safety profiles of four different TDF/FTC regimens for pre-exposure chemoprophylaxis (PrEP).

Study Sites:

- Johns Hopkins University, Baltimore MD; USA
- University of North Carolina at Chapel Hill, Chapel Hill NC; USA

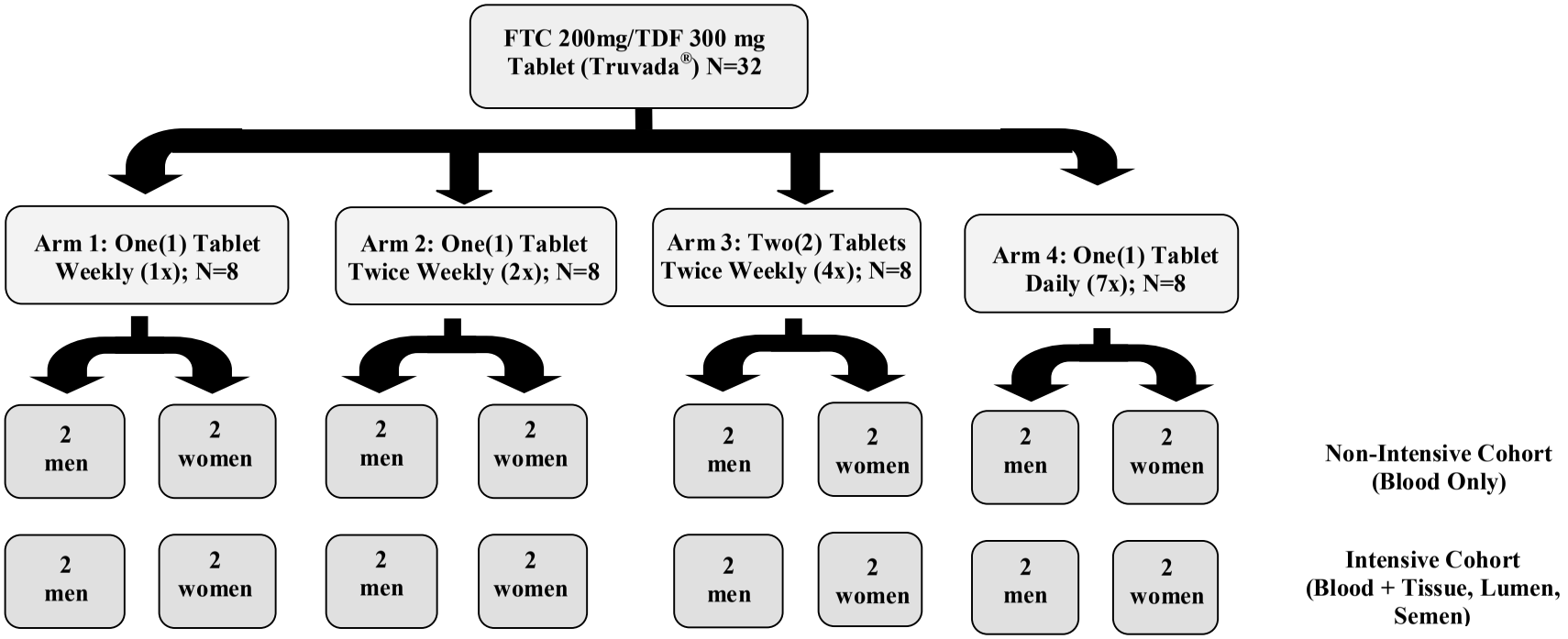
HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

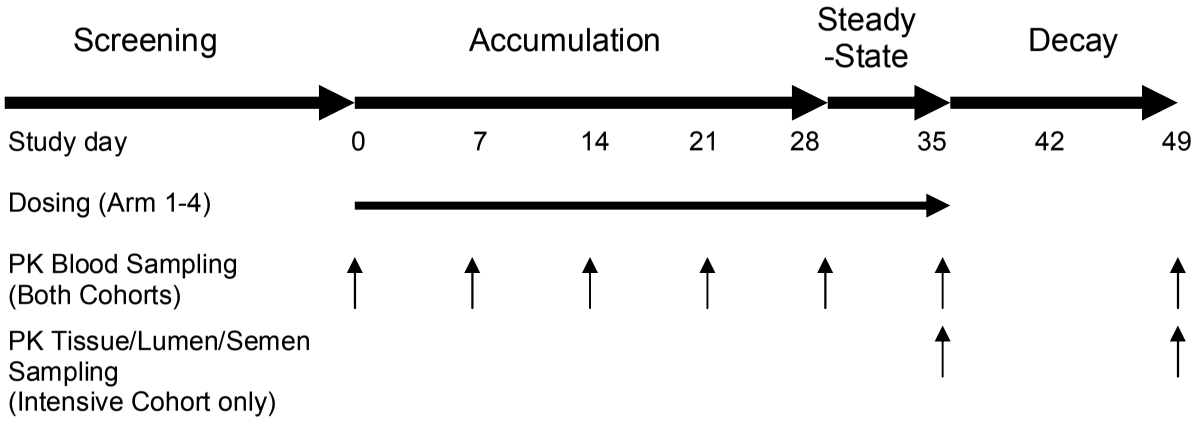
Version 2.0
29 November 2010

OVERVIEW OF PARTICIPANT DISTRIBUTION AND STUDY DESIGN

A) Distribution Scheme



B) Study Design



1.0 INTRODUCTION

1.1 Background and Prior Research

1.1.1 Using PrEP for HIV Prevention

With 2.5 million new HIV infections expected in the coming year, there is an urgent need to find effective ways to reduce HIV transmission (UNAIDS, 2008). Pre-exposure antiretroviral chemoprophylaxis (PrEP) has been shown to decrease viral transmission in animal models (Garcia-Lerma et al., 2008; Subbarao et al., 2007; Veazey et al., 2005), and post-exposure prophylaxis has decreased HIV transmission in health care workers (Cardo et al., 1997) and men who have sex with men (MSM) (Schechter et al., 2004). The first human efficacy trials of daily PrEP are likely to be completed by late 2010. These trials are only evaluating daily dosing. For the majority of the "at-risk" populations, HIV risk-taking is unlikely to occur on a daily basis. Therefore, less frequent drug dosing (i.e., intermittent PrEP) will result in decreased drug costs and lower risk of toxicity. However, any benefit from intermittent PrEP as compared to daily PrEP could be mitigated by an increased risk of HIV infection if intermittent PrEP were to provide insufficient drug concentrations in relevant tissues. The most encouraging data suggesting that intermittent PrEP may be a plausible prevention strategy comes from a study by Garcia-Lerma and colleagues (Garcia-Lerma et al., 2009), in which monkeys were well-protected from SIV infection after receiving one dose of tenofovir disoproxil fumarate (TDF) plus emtricitabine (FTC) at either 7 days, 3 days, or 2 hours pre-SIV exposure followed by a second dose 2 hours post-exposure. While encouraging, these data cannot be directly extrapolated to humans due to the differences in simian anatomy and pharmacokinetics as well as differences between HIV and the SIV challenge strain. Moreover, an efficacy trial of intermittent PrEP can be initiated only after the following pharmacological issues are understood. First, the overall drug concentrations achieved in humans receiving various intermittent PrEP regimens must be understood. Secondly, the intra-individual variability exhibited between proposed intermittent PrEP regimens must be carefully considered. Lastly, drug concentrations must then be evaluated in light of *in vitro* and animal model data to estimate the range of concentrations that may be necessary to provide preventive concentrations of the relevant antiretroviral drugs.

1.1.2 Significance of Determining Pharmacology of Intermittent PrEP

The data generated by the proposed study will be used to develop a comprehensive multi-compartment pharmacokinetic (PK) model. Assuming dose proportionality is found, this model will be used to estimate extracellular and intracellular drug exposure in multiple compartments for any dose and dosing interval combination. This model will be critical to the following three areas: 1) developing an optimal pharmacology-based adherence intervention for future PrEP studies, 2) assessing adherence in ongoing studies with currently stored samples, 3) developing a

pharmacokinetic-pharmacodynamic (PK/PD) model to determine target intracellular drug exposures that prevent HIV infection.

1.1.3 Need for Accurate Adherence Data

All large-scale PrEP trials will need to determine medication adherence accurately in order to interpret the success or failure of any pharmacologic intervention. Acquisition of HIV infection during such trials may result from failure to achieve protective drug concentrations at the site of action. This failure may be from either (1) full adherence that did not achieve adequate drug concentrations from the prescribed regimen, or (2) poor adherence to the prescribed regimen, resulting in concentrations too low to provide protection from HIV infection. Differentiating between these two etiologies of PrEP failure are impossible using traditional adherence measures [pill counts, medication history and computer chips in the cap of pill containers (e.g., MEMS caps)] as none of these actually document pill-taking behavior. Using a pharmacologic measure in combination with a robust PK model will allow interpretation of adherence using stored samples from completed studies, and will provide real-time information that could be used to guide behavioral interventions.

1.1.4 Drug Concentration as an Adherence Measure

In clinical trials, measurement of drug concentrations has been proposed as a potential adherence measure to assess medication adherence more accurately. In this method, blood and other clinical samples of interest are sampled at specified times and the resultant "observed" drug concentrations are compared to the "expected" drug concentrations. The proportional difference between the expected and observed drug concentrations is taken as a rough estimate of the proportion of prescribed doses that were actually taken. Any differences between the observed and expected values are attributed primarily to adherence, since the expected value estimates (described below) control for adherence, and since other significant sources of variation (e.g., assay variability and environmental variables) are reasonably assumed to be stable over time.

1.1.5 Drug Concentration Simulation Models

In computer simulation models, the "expected" drug concentration is based on the PK characteristics of the drug and the prescribed dosing regimen of the study. To facilitate these simulations, PK data are available from several reports in which TDF (Viread®) has been administered to human research participants. From these studies, one can make some estimates of the effective intracellular half-life for tenofovir diphosphate (TFV-DP), the active moiety of the drug, which appears to be approximately ~150 hours (Hawkins et al., 2005; Pruvost et al., 2005; Pruvost et al., 2009). From these studies, however, the inter-individual variability for half-life and peak concentration (C_{max}) for TFV-DP is relatively large (coefficient of variation around 50% to 65%). Accordingly, the best parameter estimates for such a simulation come from

individual study participants. To avoid intensive, frequent PK sampling, which would be cumbersome in a large clinical trial, one can employ a combination of (1) population-based estimates from the studies like those cited above, and (2) data from a few samples from each individual in a prospective study. To remove variability in adherence in estimation of individual PK parameter estimates, samples collected from the individuals tested should follow an observed dose, thus insuring 100% adherence without variability. With an observed dose, the possibility of variable adherence affecting subsequent drug concentrations is removed. This leaves the following variables to consider: inter-individual PK variability, assay variability, and unknown sources of noise that may vary with time in the environment. Examples of simulations of proposed dosing strategies of TFV-DP in PBMCs (Figure 1-1) and TFV in blood serum (Figure 1-2) are shown below.

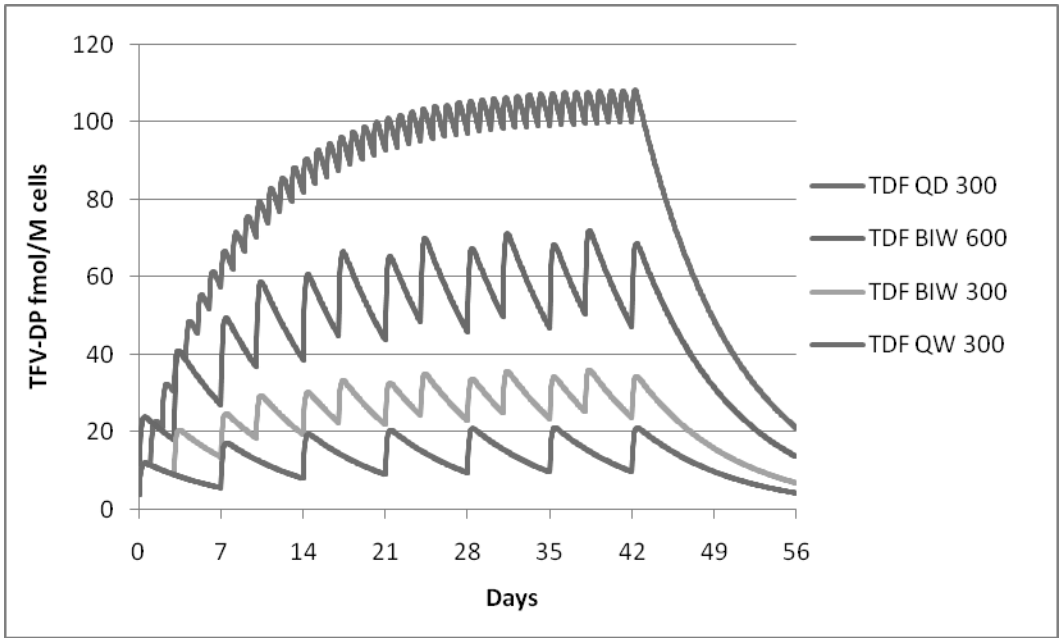


Figure 1-1. Simulation of tenofovir diphosphate (TFV-DP) concentration in PBMCs (y-axis) **versus time** (x-axis) **anticipated with the four different regimens planned for this study**: TDF 300 mg daily (blue, QD 300), TDF 600 mg twice weekly (Monday & Thursday, red, BIW 600), TDF 300 mg twice weekly (Monday & Thursday, green, BIW 300), TDF 300 mg weekly (Monday, purple, QW 300); note all regimens are administered as Truvada® (TDF/FTC). Note the minor increase in C_{tau} and C_{max} between Day 28 and Day 35 where intra-individual variability will be estimated. Simulation parameters are based on data from previous studies (Hawkins et al., 2005; Pruvost et al., 2005; Pruvost et al., 2009). Simulations assume dose-proportionality and do not include intra-individual variation.

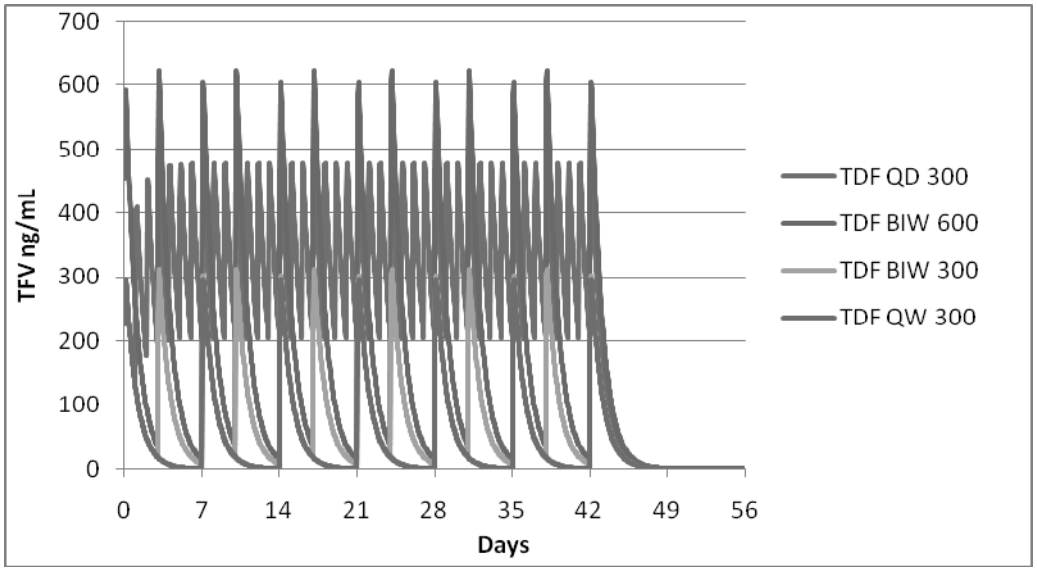


Figure 1-2. Simulation of tenofovir concentration in blood serum (y-axis) **versus time** (x-axis) **anticipated with the four different regimens planned for this proposal**. The regimens are described in Figure 1-1. Note the near complete overlap in concentration profiles between all regimens on days when the dose is taken. This is due to a very low accumulation index (see text below).

To use the computer modeling methods described above, *two key facts must be determined with regard to the interaction of drug, participant, and regimen: intra-individual variability and dose-proportionality.* Knowledge of these variables is essential for planning future studies that use estimated PK parameters based on individual drug concentrations to evaluate adherence.

1.1.6 Importance of Intra-individual Variability

Knowledge of intra-individual variability in drug concentrations is necessary to determine accurate sample size estimates for studies that plan to use drug concentration monitoring as an adherence measure. Without this parameter, estimates can be made from inter-individual variability from existing unpaired studies. However, the inter-individual approach typically results in overestimates of variability and hence a larger than necessary sample size. Alternatively, some conservative assumptions can be made for intra-participant correlation, though this introduces an additional variable that may cause estimates to be either too high or too low.

1.1.7 Importance of Dose-proportionality

In order for drug concentration to be used as an adherence measure, the dose-proportionality (i.e., drug concentration at specified times changes proportionally with change in the frequency and amount of drug taken) must be stable at concentrations throughout the adherence range of interest: from concentrations associated with 100% adherence down to concentrations associated with lower levels of adherence. While dose-proportionality is closely related to first order PK,

there are other conditions that will cause disproportionate kinetics, even for a first order drug. Very few drugs exhibit mixed or zero order PK in the clinically relevant concentration range (e.g., aspirin, phenytoin, and fluoxetine). For these drugs, a proportional increase in dose results in a disproportionately large increase in concentration. For other drugs, a proportional increase in dose may result in a disproportionately small increase in concentration. This can be seen in drugs with saturable absorption, and with drugs that are anabolized to phosphorylated forms within cells, such as TFV, FTC, and zidovudine (AZT). For example, because phosphorylation of AZT-monophosphate within cells is the rate-limiting step in AZT-triphosphate anabolism, increasing the AZT dose can result in disproportionately small or no increase in AZT-triphosphate. For either cause of disproportionate PK, the assumption of proportionate change in adherence (as with expected and observed drug concentrations) becomes more complex.

While the dose-proportionality for both TDF and FTC are well-established (Barditch-Crovo et al., 2001; Gish et al., 2002), this proportionality has not been determined for the phosphorylated moieties of these drugs and must be established for one to use TFV-DP or FTC-TP as measures of adherence to a Truvada® (TDF/FTC) regimen.

Other proportionality factors are dose frequency and the accumulation of drug concentration with time – characteristics that are unique to each drug and dosing regimen. Drugs that are dosed at intervals more frequent than their half-life accumulate over time and achieve concentrations that are higher at steady-state than following a single dose. This accumulation – the so-called "accumulation index" or R_{ac} – varies with the ratio of the drug half-life to dose frequency as follows:

$R_{ac} = 1/(1 - e^{-k\tau})$ where k is the elimination rate constant [Ln(2)/half-life] and τ is the dosing interval.

The greater the drug half-life, the greater the accumulation index. The higher the accumulation index, the greater the magnitude by which drug accumulation will exceed sources of noise in drug concentration assessment (e.g., dosing time variation, assay variability, assay sensitivity, and inter- and intra-individual variability). When measuring adherence with drug concentration, one is assessing the magnitude of difference between the expected concentrations of drug at (1) prescribed dosing intervals assuming 100% adherence, and (2) actual drug concentrations from an unobserved dosing period. The difference is attributed to less than 100% adherence. A reduction in adherence has the equivalent effect on concentration as a reduction in the frequency of dosing (i.e., the concentration falls). Drugs with a large accumulation index provide a greater degree of detectable difference between 100% adherence and lesser adherence. For drugs with little accumulation (dose frequency less than their half-life), it may be very difficult to discriminate the concentration associated with a single dose (without any prior doses) from perfect adherence. Therefore, a participant with poor adherence can appear to a have high level

of adherence with a low accumulation index drug and drug regimen combination. In contrast, large accumulation index drug and drug regimen combination is very resistant to "white-coat adherence", in which a participant takes one or several doses within the day prior to a scheduled research clinic visit.

Table 1-1. **Accumulation index for parent and phosphorylated moieties of TDF and FTC dosed either weekly (168 hours) or daily (24 hours).** Drug moieties and regimens are ranked by accumulation index. An index of 2 indicates a doubling of concentration at steady-state compared to a single dose. An index of 1 indicates no accumulation at steady-state. For example, the concentration observed with 100% adherence can look the same as no adherence if the non-adherent participant takes a single dose prior to the observed blood collection when TFV, FTC or TDF/FTC are used with prescribed weekly dosing regimens. By contrast, the concentration expected with 100% adherence to Truvada® will be 9.6 (TFV-DP) or 2.7 (FTC-TP) times the concentration observed if a participant has taken only a single dose prior to the scheduled observation. (Hawkins et al., 2005; Pruvost et al., 2005; Pruvost et al., 2009; Wang et al., 2004)

Table 1-1. Accumulation index for parent and phosphorylated moieties of TDF and FTC dosed either weekly (168 hours) or daily (24 hours).

Drug moiety	Half-life (hours)	Dosing interval (hours, tau)	Dosing interval / half-life (tau/HL)	Accumulation index (R_{ac})
TFV-DP	150	24	0.2	9.5
FTC-TP	36	24	0.7	2.7
TFV-DP	150	168	1.1	1.9
TFV	17	24	1.4	1.6
FTC	10	24	2.4	1.2
FTC-TP	36	168	4.7	1.0
TFV	17	168	9.9	1.0
FTC	10	168	16.8	1.0

For this reason, TFV-DP (the intracellular form of TFV) is an excellent candidate for drug adherence monitoring, especially with daily dosing (Table 1-1). With an intracellular half-life of 150 hours, daily to weekly dosing of TDF has a TFV-DP accumulation index ranging from of 9.5 to 1.9, offering a great range of sensitivity for determining reduced adherence over a large range of dose frequencies. In contrast, TFV concentration (measured in blood serum), when TDF is dosed daily, would be expected to perform much less well, with a small difference in drug accumulation at steady-state during daily dosing when compared to a single dose (e.g., taken on the day of observation). When using TDF on a weekly dosing regimen, there is no anticipated difference between drug concentrations of serum TFV compared to taking a single dose of TDF without any prior doses. Contrasting Figure 1-1 with Figure 1-2 illustrates this point graphically. The different dose regimens are easily distinguishable in Figure 1 where intracellular TFV-DP is measured. In contrast, the TFV serum levels are largely overlapping with daily and weekly dosing at least one day per week.

1.2 Rationale

1.2.1 Overall Study Rationale

This PK study is designed to establish the dose-proportionality of TFV and FTC (serum and intracellular forms) with daily to weekly dosing. This information is essential to (1) employ drug concentration as an adherence measure in future PrEP studies, and (2) to estimate the anticipated concentration of parent and active moieties of TFV and FTC in intermittent PrEP regimens associated with full adherence to a prescribed regimen. In addition, intra-individual variability will be assessed to improve sample size estimates in future PrEP studies that use drug concentration as an adherence measure or where PK-PD correlations are planned. The dose-proportionality of intra-cellular phosphates of the two components of the study product has not previously been established. Given the complexity of movement of these drugs between body compartments (central compartment, vaginal mucosal tissue, vaginal lumen, gastrointestinal mucosal tissue, gastrointestinal lumen), into cells within these compartments, and intra-cellular phosphorylation, it is possible that non-dose proportional kinetics, such as mixed or zero-order saturable processes may be operating.

1.2.2 Dosing Regimen Rationale

We propose to study four different dosing regimens of Truvada® (tablets include 300 mg TDF and 200 mg FTC): one tablet daily, two tablets twice weekly, one tablet twice weekly, and one tablet weekly. To provide adequate data on dose-proportionality, these regimens are selected to cover the range of concentrations achieved between daily and weekly dosing, representing a 7-fold range of anticipated drug concentrations. These regimens will achieve drug concentrations anticipated in current PrEP studies that employ daily dosing, as well as drug concentrations likely to be achieved with proposed intermittent PrEP regimens with dosing as infrequent as weekly or twice weekly dosing. In addition to studying three different dosing frequencies (daily, twice weekly, and weekly), we will study two different doses given twice weekly: a twice-weekly regular dose (one Truvada® tablet), and a twice-weekly double-dose (two Truvada® tablets). This will provide data on dose-proportionality for regimens between the weekly and daily dosing. Using the weekly one Truvada® tablet as reference, these four dosing regimens represent anticipated drug exposures of 1x (weekly), 2x (twice weekly), 4x (double-dose twice weekly), and 7x (daily). These proportions will provide sufficiently rich data to assess dose-proportionality. If dose-proportionality is determined not to hold between weekly and daily dosing, the middle dose regimens provide intermediate points to increase the accuracy with which we can determine the point of deviation from dose-proportionality. Further, they will provide rich drug concentration data to understand these intermediate dosing regimens, which may prove useful in future PrEP efficacy studies should the concentrations of drug prove insufficiently low with weekly dosing.

1.2.3 Dosing Duration Rationale

Dose-proportionality can be assessed at any point of observation following initiation of dosing. Intra-individual variability, however, can best be assessed at two sampling times not anticipated to vary based on PK principles. Essentially, by definition, this condition is achieved when steady-state has been reached. This study requires direct observation of dosing to rule out adherence as a cause for variation in drug concentrations. Therefore, we plan to begin sampling for intra-individual variability assessments as early as possible after dosing, balancing logistical difficulties (up to daily dosing for study staff and research participants) and PK limitations. Intracellular TFV-DP concentrations are estimated to achieve between 93% and 97% of steady-state Cmin (trough) concentrations after 4 half-lives and 5 half-lives, respectively, have elapsed after initiation of Truvada® dosing. Based on an estimated 150-hour TFV-DP intracellular half-life (FTC-TP will achieve steady-state earlier, given its far shorter 36-hour half-life), the 4 and 5 half-life points coincide with 28 and 35 days of dosing. Laboratory assay variation for all of the assays planned for this study are more than twice as large as the very small 4% difference in concentrations that we expect to measure between Day 28 and Day 35. Accordingly, we plan to dose for 35 days, and to use samples from Day 28 and Day 35 for our intra-individual variability estimates. One could argue for longer periods of dosing to further reduce differences between doses in the asymptomatic approach to a true steady-state, but we believe that our proposed plan provides a reasonable balance between logistical complexity (daily dosing under direct observation) and reasonable experimental variation.

The double-dose (two Truvada® tablets) twice-weekly regimen is only $4/7^{th}$ (57%) of total weekly dosing for the single dose (one tablet) daily regimen. However, the double-dose twice-weekly regimen includes doses within each dosing day that are twice the dose recommended in the package insert for both FTC and TDF, namely 400 mg and 600 mg, respectively. We believe that there is ample evidence that this dose will be safe in these healthy volunteers based on previous clinical testing and based upon pharmacological principles. First, regarding prior clinical studies, as part of their drug development, both TDF and FTC were studied at these doses with daily dosing from 2 to 8 weeks without any observation of dose-dependent adverse effects (Barditch-Crovo et al., 2001; Rousseau et al., 2003; Rousseau et al., 2001).

Kearney, et al., demonstrated bioequivalence for TDF and FTC compared to the Truvada® combination (Kearney et al., 2004). Accordingly, the data based on dosing single products is provided as evidence of each individual component within the combination.

In the Barditch-Crovo 2001 paper, one cohort of HIV-infected patients received 600 mg TDF for 35 days – the highest dose administered (Barditch-Crovo et al., 2001). The drug demonstrated dose-proportionality for peak concentration and area under the concentration time curve in blood plasma over the range from 75 mg to 600 mg TDF daily. With regard to safety, 1 placebo and 3

TFV subjects discontinued the study early due to lab abnormalities. Six subjects had grade 3 or 4 adverse events (1 placebo, 5 on TDF). None of the 10 subjects receiving TDF 600 mg daily were discontinued for either lab abnormalities or experienced a grade 3 or 4 clinical event. Accordingly, there was no dose-response noted in adverse effects in this study over the dose range from 75 mg to 600 mg daily for 35 days. We will dose for the same 35 day duration, but the 600 mg dose will only be administered twice weekly rather than daily as in this study.

In the Rousseau 2001 paper, one cohort of 9 HIV-infected subjects received 400 mg daily FTC for 14 days. Dose-proportional increases in FTC and FTC-TP concentrations were seen over this period. Among all 38 subjects in the study, FTC was well tolerated at all dosing levels. No serious or severe adverse events were noted. Moderate events possibly related to FTC were seen and were not dose-related. While this study only dosed for 14 days, it is still highly relevant since the only dose that is anticipated to exceed the intracellular concentrations of the single daily dose cohort is the first dose of the double dose twice weekly regimen. Thereafter, all intracellular concentrations will fall below those of the single daily dose cohort.

In contrast, we plan to administer the double-dose only twice-weekly. Accordingly, we believe the double-dose twice-weekly regimen will be at least as safe as the daily dosing regimen.

Based on pharmacological principles, published estimates of PK parameters for both drugs as summarized in Table 1-1 above, only the very first dose of the double-dose regimen will give an intracellular concentration higher than the concentration following the first dose of the single dose daily regimen. At all other times, the intracellular concentration for the double dose twice weekly falls below the single dose daily regimen The accumulation index of the fully phosphorylated moieties of tenofovir and emtricitabine dosed daily is 9.5 and 2.7, respectively. Thus, at steady-state, the intracellular concentration of the fully phosphorylated moieties exceeds that of the peak concentration following a single dose by multiples of those values. Accordingly, even with a doubling of a single dose, the peak intracellular concentrations falls well below the steady-state concentrations with daily dosing. With twice weekly dosing of Truvada® at double the recommended dose, the peak intracellular concentrations are still below those of daily Truvada® dosing (since the total weekly dose is only $4/7^{th}$ the daily dose total. After the first dose, the double-dose regimen results in concentrations below the daily single dose regimen (see Figure 1-1 above).

The serum TFV concentrations will be higher with double-dosing twice weekly when compared to daily dosing of a single tablet (Figure1- 2). This is because the accumulation index for TFV and FTC are only 1.6 and 1.2, respectively, indicating very little accumulation. Accordingly, the double dose will exceed the concentration of the daily single dose TFV and FTC, but only on the 2 days of the week when the double dose is taken. In addition, the use of double doses with a twice-weekly frequency provides the daily dose equivalent most closely associated with coitally-dependent dosing, in which a dose is taken both before and following intercourse. We believe these blood plasma concentrations are less relevant for safety issues as the phosphorylated

moieties are the ones associated with the desired antiretroviral and undesired (non-viral) effects of the drugs.

2.0 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objectives

The primary objectives of this study are to:

- Assess dose-proportionality of intracellular TFV-DP and FTC-TP from weekly to daily dosing (Arms 1-4).
- Describe intra-individual variability in intracellular TFV-DP and FTC-TP concentrations at steady-state (comparison of Day 28 and Day 35).

2.2 Secondary Objectives

The secondary objectives of this study are to:

- Describe the relationship between pre-dose (C_τ) and decaying concentrations of TFV, FTC, and their phosphorylated derivatives (TFV-DP and FTC-TP) in blood serum, peripheral blood mononuclear cells (PBMCs), CD4+ blood cells, total tissue cells, CD4+ tissue cells, tissue homogenate, semen, and luminal fluid at steady-state (Day 35 [pre-dose] and Day 49 [decaying, greater than one half-life for TFV-DP]).
- Describe differences in intracellular TFV-DP and FTC-TP steady-state C_τ between men and women.
- Characterize the safety profiles of four different TDF/FTC PrEP regimens.

2.3 Study Design

2.3.1 Identification of Study Design

HPTN 066 will be a Phase 1, multi-site, randomized, four-arm, open label study of the concentrations of TFV, FTC, and their metabolites in various body compartments following oral Truvada® dosing under direct observation by the site staff (directly observed therapy [DOT]) to assure 100% adherence to prescribed dosing regimen. The prescribed dosing regimens range from weekly to daily FTC/TDF at one of two possible doses. The overall study schedule and sample collection summary is provided in Appendix I. After participant education, informed consent, and screening for study eligibility, participants will have baseline procedures performed (e.g. phlebotomy) before the first dose.

2.3.2 Study Sampling

Sampling will occur over 7 weeks: at enrollment (pre-dose), pre-dose #2 (Day 1 for treatment arm 4, Day 3 for treatment arms 2 and 3, and Day 7 for treatment arm 1) and Days 7, 14, 21, 28, 35 and 49 to support each of the several objectives as follows:

1. Sample blood serum and blood cells (total cells and CD4+ cells) weekly for 5 weeks (Enrollment and Days 7, 14, 21, 28, and 35) to assess rate of accumulation and dose-proportionality in concentrations of extracellular TFV and FTC and intracellular TFV-DP and FTC-TP.
2. Sample blood serum and blood cells at steady-state (Day 28 and Day 35) to assess intra-individual variability.
3. When participants consent to participate in HPTN 066, they will be offered the opportunity to participate in the intensive sampling cohort (to provide mucosal samples). The slots in the intensive sampling cohort will be balanced by gender, so that there will be two men and two women who will undergo mucosal sampling in each of the four arms of the study. Potential research participants who do not choose to participate in or are inevaluable for the intensive sampling cohort will be offered participation in the non-intensive cohort. The study team will sample rectosigmoid, seminal and vaginal fluid, cells, and tissue (in addition to blood) at Days 35 and 49 to compare extracellular concentrations of TFV and FTC and intracellular concentrations of TFV-DP and FTC-TP, and to estimate the elimination rate of drug from these compartments.

The sampling described must occur on the days prescribed given the need to synchronize post-dose time (7 days) across the four regimens. The blood sampling time should precede the established dosing time by no more than one hour. If the participant cannot have tissue sampled as prescribed, sampling should take place within the visit window, described in the SSP. In all cases, there must be accurate recording of sample collection times.

Biopsies will not be performed during menstruation. As such, the timing of the first dose will need to take into consideration menstrual patterns and will be adjusted accordingly.

2.3.3 Summary of Major Endpoints

2.3.3.1 Primary Endpoints:

- Concentrations of TFV and FTC in blood serum
- Concentrations of TFV-DP and FTC-TP in PBMCs and CD4+ blood cells

2.3.3.2 Secondary Endpoints:

- Concentrations of TFV, FTC, TFV-DP and FTC-TP in blood
- Concentrations of TFV and FTC in vaginal and rectal secretions
- Concentrations of TFV and FTC in vaginal/cervical and rectal tissue homogenates
- Concentrations of TFV-DP and FTC-TP in unselected and CD4+ vaginal/cervical and rectal tissue cells and tissue homogenates
- Concentrations of TFV, FTC, TFV-DP and FTC-TP in fluid (vaginal, rectal, semen), cells, and tissue homogenate
- Adverse experiences, including safety laboratory studies (hematological, renal, hepatic function)

2.3.4 Description of Study Population

The study population will include approximately 32 evaluable (defined Section 7.3), generally healthy 18-44 year-old men and women, who are HIV-uninfected and not pregnant or breastfeeding. The upper age limit was selected to provide comparability in age for male participants with our pre-menopausal female participants and reduce the variability that might be introduced by a wider age range that could have a significant impact in this small study.

2.3.5 Trial Duration

The total duration of participation from the Enrollment Visit to the Termination Visit is 7 weeks. Visits may be completed within specified windows around target dates. The approximate time to complete study enrollment is expected to be six months. The time of total study duration is expected to be approximately twelve months, including the study follow-up period. Detailed information regarding visit windows will be thoroughly described in the HPTN 066 Study Specific Procedures (SSP) Manual.

2.3.6 Expected Duration of Participation

The expected duration of participation for enrolled participants approximately two months. No study data will be collected after the Day 49 final study visit.

3.0 STUDY POPULATION

Thirty-two HIV-uninfected participants will be included in this study. Participants will be selected for the study according to the criteria in Section 3.1 and 3.2. They will be recruited as described in Section 3.3 and assigned to a study arm as described in Section 7.4. Issues related to

participant retention and withdrawal from the study, are described in Sections 3.4 and 3.5, respectively.

3.1 Inclusion Criteria

Participants who meet **all** of the following criteria are eligible for inclusion in this study:

- 18 to 44 years of age, inclusive on the date of screening.
- Provides informed consent for the study.
- Non-reactive HIV rapid test results at the screening and enrollment visits.
- An estimated calculated creatinine clearance (eCcr) at least 70 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).
- Participants are sexually active, defined as at least one sex (vaginal or anal intercourse) act in the 30 days prior to screening.
- Participants must agree to use condoms for all coital events during study participation.
- Intensive sampling cohort only:
    - Not using spermicide as a means of birth control (in conjunction with a condom or diaphragm)
- Women must:
    - Be pre-menopausal
    - Have regular menstrual cycles with at least 21 days between menses (unless on contraception that causes amenorrhea or irregular menses)
    - Have a negative urine pregnancy test at screening and enrollment
    - Be utilizing an alternative method of birth control in addition to condoms (hormonal contraceptive, diaphragm or have undergone surgical sterilization) or have a vasectomized exclusive male partner.
    - Intensive sampling cohort only:
        - Have a cervix
        - Have documentation of a normal Pap smear within 12 months

3.2 Exclusion Criteria

- At screening:
    - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than 1.5 X the site laboratory ULN (upper limit of normal)
    - Hemoglobin less than 10.0 g/dL
    - Platelet count less than 100,000/mm^3
    - Serum phosphate level below site laboratory LLN (lower limit of normal)
    - INR or aPTT greater than site laboratory ULN
    - Other safety tests (bicarbonate (HCO3), potassium (K), chloride (Cl), sodium (Na), calcium (Ca), fasting glucose) with results outside of the laboratories reference range
    - 1+ or greater protein on urine dipstick testing
    - 1+ or greater glucose on urine dipstick testing
- Culture-confirmed urinary tract infection
- Co-enrollment in any other HIV interventional research study (excluding behavioral only interventions) or prior enrollment in the active arm of a HIV vaccine trial.
- Clinically apparent or patient report of active skin disorders including: rash, pruritus, maculopapular rash, urticaria, vesiculobullous rash, and pustular rash.
- Women who are pregnant or breastfeeding.
- One or more reactive HIV rapid test results at screening or enrollment, even if HIV infection is not confirmed.
- Positive hepatitis B surface antigen (HBsAg) test.
- Excessive use of alcohol (more than 4 drinks a day on a regular basis).
- Interleukin therapy; medications with significant nephrotoxic potential, including but not limited to amphotericin B, aminoglycosides, cidofovir, foscarnet and systemic chemotherapy; and medications that may inhibit or compete for elimination via active renal tubular secretion (including but not limited to probenecid).
- Participants with a history of having a gastrectomy, colostomy, ileostomy, or any other procedure altering the gastrointestinal tract or drug absorption.
- Intensive sampling cohort only:
    - A positive test for syphilis, gonorrhea, or Chlamydia
    - A positive test for HSV-2 (individuals with active lesions only)
    - Findings consistent with bacterial vaginosis, vaginal candidiasis, or trichomonas (women only)
    - History of STI within 3 months prior to enrollment
    - Medications that prolong clotting time (e.g., warfarin, heparin, clopidogrel classes.)
    - Abnormalities of the colorectal mucosa, or significant colorectal symptom(s), which in the opinion of the clinician represents a contraindication to biopsy

(including but not limited to presence of any unresolved injury, infectious or inflammatory condition of the local mucosa, and presence of symptomatic external hemorrhoids).
    - Clinically apparent pelvic exam finding (observed by study staff) of genital lesions, erythema, edema or any other abnormal physical or pelvic exam finding that, in the opinion of the investigator or designee, would contraindicate study participation.
    - Women who have had cervical procedures (conization, LEEP procedure, cryosurgery) within the previous 6 months.
    - Spermicide as a method for contraception within last 30 days
- Any other reason or condition that in the judgment of the investigator, would make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

3.3 Recruitment Process

Participants will be recruited from a variety of venues. Examples of such venues include local health clinics, local health departments, and family planning clinics. In order to reach a broader audience, college and local newspaper advertisements will be utilized. Fliers will be posted on frequently visited local venues. Electronic advertisement will include secure list-serves (university) and craigslist.com. All advertising materials must undergo approval by local IRB's.

3.4 Participant Retention

Once a participant enrolls in this study, the study site will make every effort to retain him/her for 7 weeks of the study. Retention goal for the study is 95%. For each participant who is not fully evaluable (as defined in Section 7.3), an additional participant will be enrolled. Study site staff are responsible for developing and implementing local standard operating procedures (SOPs) to target this goal. Components of such procedures include:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit.

- Thorough explanation of the importance of all study treatment groups to the overall success of the study.

- Collection of detailed locator information at the study Screening Visit, and active review and updating of this information at each subsequent visit.

- Use of appropriate and timely visit reminder mechanisms.

- Immediate and multifaceted follow-up on missed visits.

- Mobilization of trained outreach workers or "tracers" to complete in-person contact with participants at their homes and/or other community locations.

3.5 Participant Withdrawal

Regardless of the participant retention methods just described, participants may voluntarily withdraw from the study for any reason at any time. The Investigator also may withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures after consultation with the Protocol Chair, DAIDS Medical Officer, SDMC Protocol Statistician, and CORE Protocol Specialist. Study visit and dosing requirements for research participant eligibility for safety and PK analyses is described in Section 7.2.

Participants also may be withdrawn if the study sponsor, government or regulatory authorities (Including the Office for Human Research Protections (OHRP) and FDA), or site IRBs/ECs terminate the study prior to its planned end date.

Every reasonable effort will be made to complete a final evaluation (as described in Section 5.5) of participants who terminate from the study prior to the final study visit, and study staff will record the reason(s) for all withdrawals from the study in participants' study records.

4.0 STUDY TREATMENT/PRODUCT/INTERVENTION

4.1 Study Product Formulation/Content/Storage

The study product to be dosed in all treatment arms is Truvada® (FTC/TDF) tablet. Truvada® tablets are fixed dose combination tablets containing emtricitabine (FTC) 200 mg and tenofovir disoproxil fumarate (TDF) 300 mg in each tablet. Each bottle contains a silica gel desiccant canister that should remain in the original container to protect the study product from humidity.

Study Product Storage

FTC/TDF (Truvada®) study products should be stored at 25°C with excursions permitted to 15°C - 30°C (59°F-86°F).

4.2 Study Product Regimen(s)

Study participants who meet the inclusion/exclusion criteria (see Sections 3.1 and 3.2) will be randomized to one of four open-label treatment arms with emtricitabine (FTC) 200 mg/tenofovir disoproxil fumarate (TDF) 300 mg (Truvada®).

Table 4-1. Study Product Dosing Arms

Treatment Arm	Study Product	Number of Tablets	Route	Frequency	Duration
1	FTC 200 mg/TDF 300mg	One(1)	Orally	Once Weekly	5 Weeks
2	FTC 200 mg/TDF 300mg	One(1)	Orally	Twice Weekly	5 Weeks
3	FTC 200 mg/TDF 300mg	Two(2)	Orally	Twice Weekly	5 Weeks
4	FTC 200 mg/TDF 300mg	One(1)	Orally	Once Daily	5 Weeks

4.3 Study Product Administration

FTC/TDF (Truvada®) tablets are administered orally with or without food.

4.3.1 Directly Observed Therapy (DOT) Guidelines

DOT is defined as directly observed therapy. A person identified by the Clinical Research Site (CRS) 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 should formulate a well-described plan for DOT prior to entry. It is possible that participants may start coming to the clinic for DOT for the first few weeks and then switch to an alternate location as CRS study staff and alternative facilities permit.

Each CRS will be responsible for determining who can perform DOT for that site. The individual(s) could administer the study product or observe the study products being taken, depending upon his/her certification and legal requirements of the local jurisdiction. Someone who is not medically trained cannot offer advice about the study product.

The DOT observer must be an objective third party arranged by the CRS. Friends or family members are not permitted as observers. Individuals who might perform DOT include physicians, registered nurses, licensed practical nurses, physician's assistants, nursing assistants, certified home health aides, pharmacists, social workers, outreach workers, or other responsible individuals designated by the CRS.

DOT may be delivered in any location deemed appropriate by the CRS (i.e., in the home, clinic, pharmacy, or outreach center). The study staff are responsible for ensuring that confidentiality is maintained at the selected locations. Examples of clinic-based DOT used at sites include:

- Participant presenting daily to the site pharmacy for observed dosing by the site pharmacist who then documents on DOT participant log.

- Participant presenting daily to the entrance of the hospital/clinic and study coordinator bring participant's prepared study product to participant and study coordinator then documents on DOT participant log.

- Participant and study coordinator meet at a designated off-campus location (coffee shop) each morning for DOT and study coordinator documents on DOT participant log.

CRSs that plan to use off-site locations for DOT are advised to give careful consideration to the following issues:

- Confidentiality: Sites should consider where study paper work will be stored, how participants will be identified, how participant's prepared study product will be transported, and where participants will be observed. Confidentiality should be discussed with participants as plans for their DOT are developed. The participant should be comfortable with the person who will be observing DOT. Sites must also discuss confidentiality with the DOT observer, noting situations where breaches are most likely to occur. DOT observers may be required to sign a confidentiality agreement.

The place of DOT administration should be captured on the study treatment record. The primary location of DOT administration and any alternate or secondary locations of DOT administration should be documented in the participants' study files as well. A DOT log will be used as a primary source document to record date, time, place, study product administered or taken, observer signature, participant signature, and any intervention for missed DOT visit (i.e., telephone call).

Flexibility and creativity are needed as sites explore options for DOT delivery. It is recognized that sites will have some variability in the way they choose to administer DOT, but do need to report the delivery mechanisms they choose in the participants' study files. Sites will be asked to inform the study team of the methods they are considering before they are used. Sites that have questions should contact the HPTN 066 protocol team for guidance.

4.3.2 Follow-up for Missed Appointments

Participants who miss a DOT visit must be contacted by the clinic. Sites can be flexible (e.g., some sites may choose to do a home visit if this is an available option while others may choose telephone contact). A tracking form has been developed for missed DOT visits as part of the study treatment record. Missed visits and reasons for missed visits must be recorded on the appropriate CRF.

4.3.3 Take-home Supply (for emergencies only)

Take-home dosing may be provided to participants for emergency purposes. Additional details on the use of the emergency dose will be provided in the SSP manual.

4.4 Product Supply and Accountability

Study Product Supply, Distribution and Accountability

Emtricitabine (FTC) 200 mg/tenofovir disoproxil fumarate (TDF) 300 mg study product tablets are manufactured and provided by Gilead Sciences, Inc. under the trade name Truvada®.

Study Product Acquisition

Emtricitabine (FTC) 200 mg/tenofovir disoproxil fumarate (TDF) 300 mg study product will be supplied by the NIAID Clinical Research Products Management Center (CRPMC). The study site pharmacist can obtain FTC/TDF study products through the CRPMC by following the instructions in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trial Networks*, and instructions in the SSP Manual.

Study Product Accountability

The site pharmacist is required to maintain records of all FTC/TDF study products received from the CRPMC and subsequently dispensed to study participants. All unused study products are to be held until the study is completed, terminated, or otherwise instructed by the sponsor. Specific instructions will be provided by the sponsor for return or destruction of the study products.

4.5 Toxicity Management

Guidelines for clinical management and product hold/discontinuation are outlined in this section.

In general, the site investigator has the discretion to hold study product at any time if s/he feels that continued product use would be harmful to the participant, or interfere with treatment

deemed clinically necessary according to the judgment of the investigator. Unless otherwise specified below, the investigator should immediately consult the PSRT for further guidance in restarting study drug or progressing to permanent discontinuation.

4.5.1 Grading System

The grading system is located in the Division of AIDS Table for Grading Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004; Clarification August 2009, which can be found on the Regulatory Support Center (RSC) Web site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

4.5.2 Dose Modification

No dose modifications are permitted.

If absolutely necessary, dosing may be delayed for 2 days in Arms 1-3 during the first 3 weeks on study. Thereafter, all doses in week 4 and 5 must be taken as scheduled. All doses for research participants in Arm 4 must be taken as scheduled. Failure to comply with these schedule requirements will result in termination of the participant from the protocol after a final safety evaluation.

4.5.3 Discontinuation of Study Product in the Presence of Toxicity

Grade 1 or 2
In general, participants who develop a Grade 1 or 2 AE regardless of relatedness to study product, and that is not specifically addressed below may continue use of the study product per protocol.

Grade 3
Participants who develop a Grade 3 AE or toxicity that is not specifically addressed below and is judged to be related to study product should have that study product permanently discontinued. In general, and unless otherwise decided in consultation with the PSRT, the investigator should re-evaluate the participant at least weekly up to 2 weeks to document resolution of toxicity to less than Grade 2.

Grade 4
Participants who develop a Grade 4 AE or toxicity that is not specifically addressed below regardless of relationship to study product should have the study product permanently discontinued. The participant should be re-evaluated at least weekly up to 2 weeks to show that the adverse event resolves to less than Grade 2.

4.5.4 General Criteria for Discontinuation of Study Product

Participants may voluntarily discontinue the study products for any reason at any time. Site IoRs will permanently discontinue participants from study product per protocol for any of the specific criteria below, which may be further clarified in the SSP. Site IoRs also may permanently discontinue participants from study product for use of prohibited medication (per Section 4.9), for reasons not shown here or in the SSP (e.g., to protect participants' safety and/or if participants are unable or unwilling to comply with study product use procedures). In such cases, the Site IoRs should first query the PSRT for review. The PSRT will provide a written response to the site indicating whether the PSRT has recommended permanent discontinuation of study product based on careful review of all relevant data.

The criteria for permanent discontinuation of study product use for an individual participant are:

- Study product-related toxicity requiring permanent discontinuation of study product per the guidelines above and below
- Completion of regimen as defined in the protocol
- Request by participant to terminate study product
- Clinical reasons determined by the physician
- Acquires HIV infection, hepatitis B infection
- Becomes pregnant

Any research participant who discontinues participation prematurely for any reason should, prior to discontinuation and dismissal from the study, undergo the safety evaluations regularly scheduled for the final study visit on Day 49, at the time of discontinuation.

4.6 Management of Specific Toxicities

Specific guidance related to permanent discontinuation is also noted here as it pertains to the clinical management of toxicities.

4.6.1 Nausea and Vomiting

Participants with Grade 1 or 2 nausea or vomiting may be treated symptomatically with hydration, oral antiemetic therapies or antiemetic suppositories at the discretion of the site investigator. Participants should be instructed to take oral study product with food.

Participants with Grade ≥ 3 nausea and vomiting for which an alternative etiology is not established must permanently discontinue the study product and be treated symptomatically.

4.6.2 Diarrhea

Participants with diarrhea of any toxicity grade may be treated symptomatically with permitted antimotility agents and rehydration at the discretion of the site investigator.

Participants with new onset Grade ≥ 3 diarrhea that is unresponsive to antimotility agents and for which an alternative etiology (e.g., infectious diarrhea) is not established must permanently discontinue study product and be treated symptomatically.

4.6.3 AST/ALT Elevations

Careful assessments should be done to rule out the use of alcohol, non-study medication-related drug toxicity, or viral hepatitis as the cause of elevation in AST or ALT of any grade. The participant must be carefully assessed for any symptoms or signs of hepatotoxicity, including fatigue, malaise, anorexia and nausea, jaundice, acholic stools, right upper quadrant pain or hepatomegaly. If the AST/ALT elevation is considered most likely to be due to concomitant illness or medication, standard management, including discontinuation of the likely causative agent, should be undertaken. If symptoms or signs of clinical hepatitis are present, study treatment must be discontinued (see below).

Grade 1

For study participants with less than Grade 1 ALT and AST at study entry, an increase to Grade 1 ALT or AST even in an asymptomatic participant may be of concern.

ALT or AST must be repeated as soon as possible (at most within 1 week of the receipt of the results) of a new Grade 1 ALT or AST. Study treatment may be continued while repeating ALT and AST at the discretion of the investigator provided the participant is asymptomatic.

Grade 2

Participants should have ALT or AST re-checked as soon as possible (at most within 1 week) and then be followed weekly until levels are Grade ≤ 1. The frequency of follow up may be altered at the discretion of the site investigator following consultation with the PSRT. Study treatment may continue at the discretion of the investigator provided the participant is asymptomatic.

Grade 3/4

Study product should be permanently discontinued for any ALT or AST of Grade 3 or 4. Participants should have ALT or AST re-checked as soon as possible (at most within 1 week). Participants should then be followed weekly until levels are Grade ≤ 1.

4.6.4 Creatinine Clearance

If the creatinine clearance is <50mL/min, it should be confirmed within 1 week of the receipt of the results in consultation with the PSRT. If the creatinine clearance is confirmed to be <50mL/min, the study product must be permanently discontinued. Participants who fail to have a confirmed test will permanently discontinue the study product.

4.6.5 Hypophosphatemia

Grades 1 and 2.
The phosphate should be repeated within 1 week of the receipt of the results. Supplemental phosphate should be given with phosphate-rich food or fluid with or without neutral phosphate solution. Other causes of phosphate loss should be thoroughly evaluated.

Grade 3/4
The phosphate should be repeated within 48 hours of the receipt of the results. Supplemental phosphate should be given with phosphate-rich food or fluid with or without neutral phosphate solution, and other causes of low phosphate should be thoroughly investigated. During the time that supplemental phosphate is provided to the participant and the time that testing is repeated, sites should follow product hold guidelines described in Section 4.5. If alternative etiologies for Hypophosphatemia cannot be identified, the study team will give consideration to discontinuation of study drug. Study medication should be discontinued for resistance to supplemental phosphate intervention after 2 days.

4.6.6 Genital Sexually Transmitted Infection/Reproductive Tract Infection

Testing for STIs will be performed at screening for all participants. Persons identified as having an STI will be excluded from the intensive cohort and will be referred for management according to the local standard of care. Testing will be performed at other study visits only if the participant develops signs/symptoms of an STI or expresses a concern about having acquired a STI infection after enrollment. If any participant acquires an STI during the study, clinical management will be provided in accordance with current CDC guidelines. Probenecid may not be given with study medications. If probenecid is necessary for STI treatment, the study dosing will be discontinued. Study product need not be held in the event of genital STI/ reproductive tract infection (RTI) requiring treatment, unless other product hold guidelines apply. An STI will result in removal from the study only if treatment is not complete within one week prior to the tissue sampling days in the protocol (intensive sampling cohort only).

4.6.7 HIV and Hepatitis B

HIV Infection

HIV testing will be performed at screening and enrollment. Individuals with one or more reactive HIV rapid test results (regardless of subsequent test results) will be excluded from the study and will be referred for management according to the local standard of care. HIV diagnostic testing will also be performed at the end of the study, and at any other study visit at which a participant has signs/symptoms of acute HIV infection or expresses a concern about having acquired HIV infection after enrollment.

Hepatitis B

Hepatitis B testing (HBsAg) will be performed at screening. Persons with a positive HBsAg test will be excluded from the study and will be referred for management according to the local standard of care. Hepatitis B testing will be performed at the end of the study and at other study visits if the participant expresses a concern about having acquired hepatitis B infection after enrollment or as clinically indicated for AST/ALT elevations.

4.6.8 Management of sampling complications

Tissue and fluids will be obtained in the intensive sampling cohort on Days 35 and 49. All subjects will be required to abstain from sexually activity for 72 hours following sample collection. Subjects will be contacted by phone 24 hours after sample collection to assess for any adverse events.

Cervicovaginal Fluid

Collection of CVF requires women to be recumbent for 5 minutes to allow fluid to pool in the posterior fornix. The vaginal sample aspirator is then gently inserted into the vagina without the placement of a speculum. This procedure may cause very slight discomfort, pressure, or irritation that should occur only during the procedure (<5 minutes). Any uncomfortable sensations should resolve shortly after the procedure.

Subjects will not undergo CVF sampling if evidence of vaginitis is present on initial examination.

Cervical Cells

Cervical cells will be collected following the insertion of a speculum into the vagina by inserting a cytobrush (two full 360 degree rotations) into the endocervical canal. Minimal discomfort may occur with placement of the speculum. Slight bleeding or spotting may occur after the cytobrush is removed but no more than that which occurs during a normal pap smear.

Seminal Fluid

Semen will be self-collected in a private room in the research unit. Subjects may experience psychological distress and local irritation or discomfort.

Vaginal Biopsies

During this procedure subjects may experience some mild pain when the sample is taken. Subjects will receive topical anesthetic prior to the procedure. Bleeding may also occur at the specimen collection site. Bleeding may be stopped at the biopsy site with application of a local anti-coagulant. In very rare cases, a stitch might need to be placed in order to stop bleeding. Under such circumstance, female participants may feel slight soreness or discomfort in the vagina for one to two days after the vaginal biopsy. Participants may experience some vaginal spotting or bleeding and slight discharge for up to one week after the biopsy. In rare instances, a vaginal biopsy may cause infection or prolonged bleeding.

Subjects will not undergo tissue sampling if evidence of vaginitis is present on initial examination.

Colon Biopsies

Subjects will be requested to follow a low fiber diet for 3 days prior to the procedure and a clear liquid diet for 12 hours prior to the procedure. With the procedure, subjects may experience abdominal pain, rectal irritation, bloody bowel movements, dehydration, and changes in heart rate. There is rare risk of bleeding, perforation and infection with this procedure. In the event that other pathology is observed during flexible sigmoidoscopy (anorectal lesions, polyps, ulcerations, etc.), the subject will be informed of the findings and a referral for treatment will be provided.

Study procedures may be terminated in the event that any subject experiences any of the following: bleeding from the site that requires aggressive intervention to stop bleeding following the sample collection, experiences severe abdominal pain, any increased tenderness or distention in the abdominal region, or a change in vital signs from baseline deemed clinically significant by the endoscopist/study physician.

Any subject who experiences excessive bleeding (in the opinion of the local study physician) following any biopsy procedure will not have further tissue sampling.

Rectal Luminal Fluid

To collect the rectal luminal fluid, the clinician will gently insert a clear, plastic, lubricated tube into the rectum (no more than 4 inches) through which the luminal fluid will be collected with a swab or sponge.

While this is a common medical procedure, it is possible that the participant could experience mild discomfort, embarrassment or, rarely, pain (should they have another condition that is already causing pain in the area). If there is any pain experienced during the procedures, the study clinician will adapt or stop the procedure.

4.7 Clinical Management of Pregnancy

All study participants are required to use condoms for all coital events during study participation. Women should also be utilizing a second form of contraception as indicated in Section 3.1. Study staff will provide contraceptive counseling to enrolled participants as needed throughout the duration of study participation and will facilitate access to contraceptive services through direct service delivery and/or active referrals to local service providers. Study staff also will provide participants with male condoms and counseling on use of condoms during every sex act during study participation.

Pregnancy testing will be performed at screening, enrollment, Day 28, Day 35 (intensive sampling cohort only), and Day 49 (pregnancy testing will be done prior to any vaginal procedures). Participants will be encouraged to report all signs or symptoms of pregnancy to study staff. The site IoR or designee will counsel any participants who become pregnant regarding possible risks to the fetus according to site-specific SOPs. The IoR or designee also will refer the participant to all applicable services; however, sites will not be responsible for paying for pregnancy-related care.

Participants who are pregnant at the termination visit will continue to be followed until the pregnancy outcome is ascertained (or, in consultation with the PSRT, it is determined that the pregnancy outcome cannot be ascertained). Pregnancy outcomes will be reported on relevant CRFs. Outcomes meeting criteria for expedited adverse event (EAE) reporting also will be reported on EAE forms (see Section 6.3.4 below). Follow-up of pregnant participants beyond study duration is only intended to capture pregnancy outcomes. On-going or regular study visits will not take place.

NOTE: Participants who become pregnant during the course of the study will permanently discontinue study product.

4.8 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. Site IoRs may, with the approval of the PSRT, withdraw participants before their scheduled termination visit to protect their safety, and/or if participants are unable or unwilling to comply with study procedures. Participants also may be withdrawn if the study sponsors, government or regulatory

authorities (including the OHRP and FDA), or site IRBs/ECs terminate the study prior to its planned end date. Site investigators are required to consult the Protocol Chair and Protocol Biostatistician prior to the termination of any study participant. Study staff will record the reason(s) for all withdrawals in participants' study records. Study visit and dosing requirements for research participant eligibility for safety and PK analyses is described in Section 7.2.

4.9 Concomitant Medications

With the exception of medications listed as prohibited (see below, this section), enrolled study participants may use concomitant medications during study participation. All concomitant medications, over-the-counter preparations, vitamins and nutritional supplements, recreational drugs, and herbal preparations reported throughout the course of the study will be recorded on CRF designated for that purpose.

All concomitant medications taken or received by participants within the 4 weeks prior to study enrollment will be reported on applicable study CRFs. Medications used for the treatment of AEs that occur during study participation also will be recorded on applicable study case report forms.

Should participants report use of any of the following medications, they will be required to discontinue use of study products and have termination visit evaluations: interleukin therapy; medications with significant nephrotoxic potential, including but not limited to amphotericin B, aminoglycosides, cidofovir, foscarnet and systemic chemotherapy; and medications that may inhibit or compete for elimination via active renal tubular secretion (including but not limited to probenecid).

Medications that prolong clotting time (e.g., warfarin, heparin, clopidogrel classes) are exclusionary as are the conditions they treat. Occasional use of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) for analgesia is allowed with restriction. Aspirin cannot be taken within 1 week of scheduled biopsies. NSAID cannot be taken within 24 hours of scheduled biopsy. Detailed instructions for preparation for biopsies will be included in the SSP.

Spermicide should not be used by participants who undergo mucosal sampling during the study. Non-study vaginal and rectal products (other than tampons during menstruation, diaphragms and female condoms) are also prohibited for individuals undergoing mucosal sampling. Participants who report current use of these products will be counseled regarding the use of alternative methods. Participants are not expected to require gynecologic surgical procedures during enrollment period; however, should such a procedure be required, the site IoR or designee will consult the PSRT regarding ongoing product use by the participant.

5.0 STUDY PROCEDURES

An overview of the study visit and procedures schedule is presented in Appendix I. Presented below is additional information on visit-specific study procedures. Detailed instructions to guide and standardize all study procedures across sites will be provided in the SSP manual.

In addition to any Interim Visits that may occur in accordance with guidance outlined in Section 5.6, the following visits should take place for study participants:

- Screening
- Enrollment (first dosing day; Day 0)
- Pre-dose #2, Sample visit for single blood draw (accumulation index)
- Day 7, Non-Intensive Sampling visit
- Day 14, Non-Intensive Sampling visit
- Day 21, Non-Intensive Sampling visit
- Day 28, Non-Intensive Sampling visit
- Day 35, Non-Intensive plus Intensive[*†] Sampling visit
- Day 49, Non-Intensive plus Intensive[*†] Sampling visit, Termination Visit

[*] *Note*: Sixteen of the total 32 participants will participate in the intensive sampling. The intensive sampling cohort will be divided evenly between men and women from each dosing cohort.
[†] Site staff will follow-up with participants via telephone, 24 hours after biopsy to inquire about any complications possibly resulting from the biopsy.

All sampling will occur no more than one hour prior to the scheduled dosing time.

As dosing will be observed by a study team member, participants will have up to daily contact with the study team. These will be termed “dosing visits” to distinguish them from study visits listed above.

Reimbursement for study visits will either take place at the end of each study visit, at the end of the study, or in a pro-rated manner, depending on site specific scheduling and IRB approvals.

5.1 Screening Visit

After providing written informed consent, potential participants may be screened for eligibility over two or more visits if necessary, and eligibility must be confirmed at the enrollment visit (i.e. values from screening need to be checked against the eligibility requirements). For participants who do not meet the eligibility criteria, screening will be discontinued when ineligibility is determined.

For participants who are found to be presumptively eligible based on the evaluations listed below at these visits, final eligibility will be confirmed at the Enrollment Visit (Day 0), scheduled to take place within 30 days of the Screening visit.

Table 5-1. Screening Visit (up to 30 days prior to Enrollment Visit)	
Component	**Procedure/Analysis**
Administrative	• Obtain written informed consent for screening/enrollment • Verify age • Assign Participant ID (PTID) • Collect demographic information • Collect locator information • Assess behavioral eligibility • Schedule next visit
Clinical	• Collect medical/menstrual history • Collect concomitant medications • Perform physical exam • Perform pelvic exam (intensive sampling cohort) • Provide counseling o Contraceptive o HIV pre- and post-test o HIV/STI risk reduction and condom • Refer to care for UTI/RTIs/STIs • Provide male condoms
Blood	• Collect blood samples o HIV diagnostic testing (see SSP) o Safety testing (see SSP) o Coagulation testing (intensive sampling cohort, see SSP) o HBsAg (confirmatory testing if positive) o Syphilis testing (confirmatory tests if positive) o Store plasma
Urine	• Collect urine sample o Pregnancy testing (women only) o Dipstick urinalysis (if positive for leukocyte esterase or nitrates, perform urine culture) o GC/CT testing (intensive cohort only)
Pelvic	• Collect pelvic samples (intensive sampling cohort women only) o Vaginal fluid for wet mount (BV, candida, trichomonas), vaginal fluid pH o Pap smear*
Genital	• HSV-2 testing (if active lesions) (intensive cohort only)
Rectal	• GC/CT testing (intensive cohort only)

*Not required if a normal PAP smear result was documented within 12 months of screening.

5.2 Enrollment Visit

Table 5-2. Enrollment Visit (Day 0)	
Component	**Procedure/Analysis**
Administrative	• Review/update locator information • Confirm behavioral eligibility, required for eligibility assessment • Randomization • Schedule next study visit • Follow procedures for study arm assignment
Clinical	• Update medical/menstrual history, required for eligibility assessment • Update concomitant medications, required for eligibility assessment • Review pre-existing conditions, required for eligibility assessment • Perform physical exam (See Appendix IB), required for eligibility assessment • *Refer for UTI/RTIs/STIs, treat or refer for other findings • Provide counseling o Contraceptive o HIV pre- and post-test o HIV/STI risk reduction and condom o Protocol adherence, product use/adherence • Provide male condoms
Blood	• HIV diagnostic testing (see SSP) • Store plasma • Collect PK blood specimens o Serum o PBMCs o CD4+ blood cells
Urine	• Pregnancy testing (women only)
Study Product Supply	• Administer first dose of study product • Provide an emergency dose(s) of participant's prepared study product

*If indicated (see SSP)

5.3 Non-Intensive Sampling Visits

Table 5-3. Non-Intensive Sampling Visits (Days 1‡, 3 ‡, 7, 14, 21, 28, 35, and 49)	
Component	**Procedure/Analysis**
Administrative	• Review/update locator information • Schedule next study visit

Clinical	• Collect interval medical/menstrual history
	• Review/update concomitant medications
	• Record/update adverse events
	• Coagulation tests (Day 28 only, see SSP, intensive sampling cohort only)
	• Perform directed physical exam
	• Reinforce counseling
	  o Contraception
	  o HIV/STI risk reduction and male condom
	  o Protocol adherence, product use/adherence
	• Provide male condoms

Blood	• Pre-dose #2: single blood draw for accumulation index (Day 1 for Treatment Arm 4, Day 3 for Treatment Arms 2 and 3, Day 7 for Treatment Arm 1)†
	• Safety testing (Days 14, 28, 35 and 49 only, see SSP)
	• Hepatitis B testing (Day 49 only, see SSP)
	• Store plasma (Days 28 and 49 only)
	• HIV testing (Day 49 only)
	• Collect blood specimens for PK analysis
	  o Serum
	  o PBMCs
	  o CD4+ blood cells

Urine	• Collect urine sample (women only, Day 28 and 49 only) o Pregnancy testing
Study Product Supply	• Administer study product (if applicable) • Collect unused emergency dose of participant's prepared study product on Day 28 Visit for all arms. Provide a new emergency dose of participants' prepared study product on Day 28 Visit in Treatment Arms 2, 3, and 4. • Collect unused emergency dose of participants' prepared study product on Day 31 Visit for treatment Arms 2 and 3, and on Day 34 Visit for Treatment Arm 4.

***** If indicated (see SSP)

† Sample collection for each cohort should be done no more than 1 hour prior to the second dose for each cohort. These will occur on day 7 (Cohort 1), Day 3 (Cohort 2 and 3), and Day 1 (Cohort 4), as indicated.

‡ Day 1 and Day 3 visits are only for the pre-dose #2 blood draw. Only administrative, blood collection, and study product supply procedures will take place one these days.

5.4 Intensive Sampling Visits

Table 5-4. Intensive Sampling Visit (Days 35 and 49)	
Component	**Procedure/Analysis**
Administrative	• Review/update locator information • Schedule next study visit

Clinical	• Collect interval medical/menstrual history
	• Review/update concomitant medications
	• Record/update adverse events
	• Perform directed physical exam
	• Perform pelvic exam
	• Reinforce counseling
	  o Contraceptive
	  o HIV/STI risk reduction and male condom
	  o Protocol adherence
	• Male condoms

Blood	• HIV diagnostic testing (Day 49 only, see SSP)
	• Hepatitis B testing (Day 49 only, see SSP)
	• Safety testing (see SSP)
	• Store plasma (Day 49 only)
	• Collect blood specimens for PK testing
	  o Serum
	  o PBMCs
	  o CD4+ blood cells

Urine	• Collect urine sample (women only) o Pregnancy testing
Vaginal sampling†	• Biopsy, cervical cytobrush, and luminal fluid (see SSP)
Rectosigmoid Specimens†	• Flexible sigmoidoscopy with biopsies (up to 30) and collection of luminal fluid (see SSP)
Semen†	• Collect semen sample (Men only, see SSP)

*If indicated (see SSP)

† Sixteen of the total 32 participants will participate in the intensive sampling which includes all procedures listed above. The intensive sampling cohort will be divided evenly between men and women from each dosing cohort. For the remaining subjects, that is, the non-intensive cohort, there will be no rectosigmoid, vaginal, or semen sampling on Day 35 and 49.

5.5 Follow up Procedures for Participants who Discontinue Study Product and for Participants who Receive a Biopsy on the Last Study Visit.

Participants who permanently discontinue study product for any reason will be encouraged to return to the clinic to have exit safety labs drawn (see SSP). This will be their final scheduled visit for the study.

Participants who are enrolled in the intensive sampling cohort will have biopsies collected on Day 49. As an additional safety check, these participants will receive a follow-up phone call the day following the biopsy to evaluate for biopsy related adverse events.

5.6 Interim Contacts and Visits

Interim contacts and visits (those between regularly scheduled follow up visits) may be performed at participant request or as deemed necessary by the investigator or designee at any time during the study. All interim contacts and visits will be documented in participants' study records and on applicable CRFs.

Some Interim visits may occur for administrative reasons. For example, the participant may have questions for study staff. Interim visits at which no data is collected are not documented on case report forms. Other interim contacts and visits may occur in response to AEs experienced by study participants. When interim contacts or visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically, record the event on the case report form, and provide or refer the participant to appropriate medical care.

5.7 Clinical Evaluations and Procedures

See Appendix I for an outline of physical exam and pelvic exam components. For directed physical exams (occurring during visits on Day 0 through 49), the extent of the examination is determined by the clinical judgment of the clinician based on interim medical history.

5.8 HIV and STI Counseling

At any time during the study when HIV or STI testing is done on study participants, they will be informed of their test results as soon as they become available. Sites should follow their local standard of care for providing HIV and STI counseling and should document these procedure in site specific SOPs before study initiation.

5.9 Participant Preparation for Sampling

Preparation and safety evaluations for biopsies

As stated in sections 4.6.8, participants should start a low fiber diet 3 days before each rectal biopsy procedure and have a clear liquid diet 12 hours prior to the procedure. They should also refrain from meals (or coffee) the day of the procedure. Enemas will be administered before the biopsy procedures take place. Additional details on the dietary preparation and enemas will be included in the SSP.

Following enrollment, coagulation testing is done on participants in the intensive sampling cohort at the Day 28 visit. If test results are a Grade 2 or higher, the intensive tissue sampling will be cancelled. If possible, these participants may be transferred into non-intensive sampling cohort if there is an available slot. If a slot is not available, they will be terminated from the study.

Semen sampling preparation

Men should refrain from sexual activity 24-48 hours prior to providing semen samples to allow for collection of an adequate number of seminal cells.

6.0 SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1 Safety Monitoring

Close cooperation between the Protocol Chair(s), study site Investigator(s), NIAID Medical/Program Officer, CORE Protocol Coordinator, SDMC Biostatistician, and other study team members will be necessary in order to monitor participant safety and respond to occurrences of toxicity in a timely manner. The team will have regularly scheduled conference calls during the period of study implementation, and additional ad hoc calls will be convened if required.

The study site Investigators are responsible for continuous close monitoring of all AEs that occur among study participants, and for alerting the PSRT if unexpected concerns arise. Accrual (but not dosing) will be suspended if two or more study participants experience the same grade 3 or higher AE (as defined by the DAIDS Standard Toxicity Tables) judged to be related to product use. The protocol team then will review all pertinent safety data and determine whether to continue accrual and product use. A decision to stop the trial may be made by the protocol team at this time or at any such time that the team agrees that an unacceptable type and/or frequency of AEs has been observed.

A sub-group of the Protocol Team, including the Protocol Chair, DAIDS Medical Officer, one or more site clinicians, and the SDMC Clinical Affairs safety Associate, will serve as the Protocol Safety Review Team (PSRT) to be chaired by the Protocol Chair. The HPTN SDMC will prepare routine safety data reports for review by the PSRT, which will meet via conference call approximately once per month or as needed throughout the period of study implementation to review safety data, discuss product use management and address any potential safety concerns. The content, format and frequency of safety data reports will be agreed upon by the PSRT and the SDMC in advance of study implementation.

Termination Visit. If the participant has an adverse event (AE) at the termination visit, the AE, for study purposes, is closed out as "continuing at the end of study participation" but will be followed clinically until resolution or stabilization with updates on status provided to the PSRT.

Participants who have AEs at the Termination Visit that have not resolved or stabilized will be followed beyond the Termination Visit until a clinically acceptable resolution of the AE(s) is confirmed and documented. Clinical acceptability of resolution will be determined by the site investigator of record (IoR) in consultation with the Protocol Safety Review Team (PSRT). For participants who are pregnant at the Termination Visit, study site staff will make every effort to follow the participant until such time that her pregnancy outcome can be ascertained and documented.

6.2 Clinical Data Safety Review

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the PSRT if unexpected concerns arise. Participant safety is also monitored at the Network level through a series of routine reviews conducted by the SDMC Clinical Affairs staff, the PSRT and study sponsors. Additional reviews may be conducted at each of these levels as dictated by the occurrence of certain events.

HPTN SDMC Clinical Affairs staff will review incoming safety data on an ongoing basis. Events identified as questionable, inconsistent, or unexplained will be queried for verification. Adverse event reports submitted in an expedited manner to the DAIDS Safety Office will be forwarded to the DAIDS Medical Officer and SDMC Clinical Affairs staff for review.

The PSRT will meet regularly via conference call to review clinical data reports generated by the HPTN SDMC. The content, format and frequency of the clinical data reports will be agreed upon by the PSRT and the SDMC in advance of study implementation. In addition to the 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 HPTN representing expertise in the fields of antiretroviral therapy, biostatistics, HIV transmission and medical ethics may be invited to join the PSRT safety review. A recommendation to stop the trial may be made by the PSRT at this time or at any such time that the team agrees that an unacceptable type and/or frequency of AEs has been observed.

In the unlikely event that the protocol team or PSRT has serious safety concerns that lead to a decision to permanently discontinue the study product for all participants and stop accrual into the study, the protocol team or PRST will request a review of the data by the HPTN Study

Monitoring Committee (SMC) before recommending that the study be stopped. If at any time, a decision is made to discontinue the study product in all participants, the site investigators of record will notify the responsible IRBs/ECs expeditiously.

6.3 Adverse Event Definition and Reporting Requirements

6.3.1 Adverse Events

An AE 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. The term "investigational product" for this study refers to the oral medication Truvada® (tenofovir disoproxil fumarate/emtricitabine).

Study participants will be provided instructions for contacting the study site to report any untoward medical occurrences they may experience, except for possible life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where a study clinician is based, and to request that the clinician be contacted upon their arrival. With appropriate permission of the participant, whenever possible, records from all non-study medical providers related to untoward medical occurrences will be obtained and required data elements will be recorded on study CRFs. All participants reporting an untoward medical occurrence will be followed clinically until the occurrence resolves (returns to baseline) or stabilizes.

Study site staff will document in source documents and the appropriate AE Log CRF all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. AE severity will be graded per the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009).

6.3.2 Serious Adverse Event

Serious adverse event (SAE) will be defined per U.S. Code of Federal Regulations (CFR) 312.32 and International Conference on Harmonization (ICH), "Good Clinical Practice: Consolidated Guidance" (E6) and "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" (E2A), as AE 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

This includes important medical events that may not be immediately life-threatening or result in death, or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed above.

Per ICH SAE definition, hospitalization itself is not an adverse event, but is an outcome of the event. The following types of hospitalization do not require expedited reporting to DAIDS:

- Any admission unrelated to an AE (e.g. for labor/delivery, cosmetic surgery, administrative, or social admission for temporary placement for lack of place to sleep)
- Protocol-specified admission (e.g. for procedure required by protocol)
- Admission for diagnosis or therapy of a condition that existed before receipt of study agent(s) and has not increased in severity or frequency as judged by the clinical investigator

6.3.3 Adverse Event Relationship to Study Product

Relatedness is an assessment made by a study clinician of whether or not the event is related to the study agent. Degrees of relatedness will be categorized according to current DAIDS-approved guidelines.

6.3.4 Expedited Adverse Event Reporting

6.3.4.1 Adverse Event Reporting to DAIDS

Requirements, definitions and methods for expedited reporting of Adverse Events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact DAIDS-ES at DAIDS-ESSupport@niaid.nih.gov or from within the DAERS application itself.

Sites where DAERS has not been implemented will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/. For questions about EAE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

6.3.4.2 Reporting Requirements for this Study

The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.

The study agent for which expedited reporting is required is: Truvada® (tenofovir disoproxil fumarate / emtricitabine (TDF/FTC))

6.3.4.3 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) is should be used to grade adverse events and is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.

6.3.4.4 Expedited AE Reporting Period

- The expedited AE reporting period for this study is as per the EAE manual.
- After the protocol-defined AE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

6.3.5 Pregnancy Outcomes

Pregnancy outcomes will be collected for all study participants by contacting these women soon after anticipated parturition. After the participant's final study contact, any pregnancy outcomes that meet criteria for SAE reporting as described above (e.g., congenital anomalies) occurring among participants will continue to be expeditiously reported.

6.3.6 Regulatory Requirements

Information on all reported AEs will be included in reports to applicable regulatory authorities. The site IoR/designee will submit AE information in accordance with local regulatory agencies' or other local authorities' requirements. The site IoR/designee also will submit AE information and any other relevant safety information to the IRB in accordance with IRB requirements.

6.4 Social Harms

In order to prevent adverse social events related to study participation, social harms will be monitored throughout the study. Social harms are any untoward social occurrences that happen

to a participant as a result of their participation in the study. Examples include loss of employment, harassment by neighbors, shunned by family, rejection by partner, etc. Although social harms due to this study are expected to be negligible, they will be monitored closely throughout the study. Information on social harms will be actively solicited from participants at follow-up visits and recorded on case report forms and captured in the study database. Participants will also be encouraged to report any social harm on an ad hoc basis when it occurs. 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. Social harms that are judged by the Investigator of Record to be serious or unexpected will be reported to responsible site's IRB/EC at least annually, or according to their individual requirements. The nature and frequency of these social impact reports will be monitored by the protocol team on a regular basis. In addition, these data will be reviewed by the HPTN (SMC).

7.0 STATISTICAL CONSIDERATIONS

7.1 Review of Study Design

HPTN 066 is a Phase 1, multi-site, randomized, four-arm, open label study of TFV and FTC concentrations in various body compartments following oral Truvada® dosing under direct observation by the study team (directly observed therapy [DOT]) to assure 100% adherence to prescribed dosing regimen. The prescribed dosing regimens range from weekly to daily TDF/FTC (Truvada®) at one of two possible doses for a total duration of 5 weeks. Thirty two participants are sampled weekly for blood (serum and cells). At the end of the 5th week and 7th week, 16 of the 32 participants also undergo seminal fluid, rectosigmoid (tissue and luminal fluid) and vaginal sampling (tissue and luminal fluid). Samples are assayed for TFV, FTC and phosphorylated moieties of these drugs.

7.2 Endpoints

7.2.1 Primary Endpoints

Consistent with the primary study objective, the following endpoint will be assessed on Day 28 and Day 35 across all 4 dosing arms:

- Concentrations of TFV and FTC in blood serum
- Concentrations of TFV-DP and FTC-TP in PBMCs and CD4+ blood cells

7.2.2 Secondary Endpoints

Consistent with the secondary study objective, the following endpoints will be assessed on Day 35 and Day 49 across all 4 dosing arms:

- Concentrations of TFV, FTC, TFV-DP and FTC-TP in blood
- Concentrations of TFV and FTC in vaginal and rectal secretions
- Concentrations of TFV and FTC in vaginal and rectal tissue homogenates
- Concentrations of TFV-DP and FTC-TP in unselected and CD4+ vaginal and rectal tissue cells and tissue homogenates
- Concentrations of TFV, FTC, TFV-DP and FTC-TP in fluid (rectal, vaginal, semen), cells, and tissue homogenate.
- Adverse experiences, including safety laboratory studies (hematological, renal, hepatic function)

7.3 Accrual, Follow-up, Retention, and Sample Size

A total of 32 evaluable men and women will be enrolled over a period of approximately 6 months at multiple sites. An equal number of participants (8 per arm) will be randomized into each of the four dosing arms. Each enrolled participant will be followed for 7 weeks.

Research participants may miss one of the following pre-steady-state study visits when on drug (Day 7, Day 14, Day 21) and remain in the study and remain eligible for evaluation in the PK outcome analysis of the study. Day 0, Day 28, Day 35, and Day 49 are required for a participant to be considered evaluable. Optimally, all study product dosing will occur on the date prescribed. However, some forgiveness of strict adherence to the schedule will be tolerated as long as the total dose within each week is consistent with the prescribed total. Therefore, for arms 1, 2, and 3, study drug dosing can be delayed by 2 days, during the first 3 weeks of the study. Thereafter, all doses in week 4 and 5 must be taken as scheduled. In Arm 4, all doses for research participants must be taken as scheduled. Failure to comply with these scheduled requirements will result in discontinuation from the study after a final safety evaluation.

Evaluable participants are expected to represent 95% of participants enrolled into the study. No adjustments are made for retention losses since all discontinued or inevaluable participants will be replaced. Each study site will establish participant retention procedures to target lost-to-follow-up rates of <5% in order to minimize additional enrollments to compensate for inevaluable participants.

For the intensive cohort, a subject must have all blood on Days 0, 28, 35 and 49, and vaginal, seminal, and rectal samples on Days 35, and 49 to be evaluable. If the subject is inevaluable for

the intensive cohort, but has met evaluability criteria for the non-intensive cohort AND there is an opening in the non-intensive arm-gender cohort matching the subject, then they may be switched in assignment to the non-intensive cohort. If the intensive subject fails to complete all required samples OR there is not an opening consistent with their gender/arm, then the participant is discontinued after the final safety evaluation. In either case, the subject needs to be replaced in the intensive sampling group.

Typical sample sizes for phase I PK studies generally range between 6 and 12 subjects per arm. A number of different approaches have been used to assess dose proportionality in the literature (Gough et al., 1995; Hummel et al., 2009; Smith et al., 2000). We consider the sample size using the following two approaches:
1) fitting a simple linear regression model between the PK parameter (y) and dose $y = \alpha + \beta dose$, where the hypothesis that $\alpha = 0$ is tested and the lack of fit of the model is generally tested by adding a quadratic term; and
2) using the power model approach to fit $\log(y) = \alpha + \beta \log(dose)$, where 90% CI around β is compared to the limits $(1 + \frac{\ln(\theta_L)}{\ln(r)}, 1 + \frac{\ln(\theta_U)}{\ln(r)})$, where (θ_L, θ_U) are the pre-defined limits and r is the ratio of the highest to the lowest dose. The power model and the CI approach has become more preferred choice in recent years (Gough et al., 1995; Smith et al., 2000), however, Hummel et al. (Hummel et al., 2009) suggested that the choice of bioequivalence default values of $\theta_L = 0.8$ and $\theta_U = 1.25$ seems to be impractically strict when applied over a dose range that is more than doubling apart and proposed a more lenient criterion $\theta_L = 0.5$ and $\theta_U = 2$.
The empirical power of both approaches are computed using a simulation study with the following steps:

1. Generate random samples of size n from a normal distribution $N(\mu = 14 \times dose, sd = CV \times \mu)$, where 14 is the weekly dosing Day 35 C_τ from Figure 1-1, and dose = 1, 2, 4 and 7 for Arm 1 to 4 respectively.
2. Fit linear regression model $y = \beta_0 + \beta_1 \times dose + \beta_2 \times dose^2$ (Smith et al., 2000) and test for nonsignificant intercept and quadratic terms (Zhou et al., 2006).
3. Fit linear model $\log(y) = \alpha + \beta \log(dose)$ and compare 90% CI of β with the pre-specified critical interval $(1 + \frac{\ln(0.5)}{\ln(7)} = 0.64, 1 + \frac{\ln(2)}{\ln(7)} = 1.36)$
4. Repeat steps 1-3 for 1000 times. The empirical power is calculated as the proportion of the replications in which nonsignificant intercept and quadratic term holds for approach 1 or the CI falls entirely within the critical interval for approach 2.

There are at least 4 available groups of participants for estimating inter-individual variability to be used here for the purposes of estimating sample size. These studies, however, present a broad range of CV. In one HIV prevention study in which research participants took a daily oral dose of TDF, the inter-individual coefficient of variation in TFV-DP concentrations in PBMCs was 46%. Hawkins et al. (Hawkins et al., 2005) report range of median concentrations of 85-110 fmol/M cells (Table 7-1) for 7 subjects evaluated. Imputing values from the extremes of this range would yield a CV of approximately 14%. Pruvost et al. (Pruvost et al., 2009) report (Table 7-3) range of TFV-DP CV% as 28, 36, 35, 54 in a drug interaction study with LPV/r and NVP. Pruvost et al. (Pruvost et al., 2005) report (Table 7-1 groups 1 and 3) inter-individual CV% of 42% (group 1) and 83% (group 3). Note none of the above studies were done in healthy volunteers with one single drug dosed under direct observation. Given that the DOT used in this study is likely to reduce the variability, a CV estimate of 25-35% seems to be realistic. In the simulation study, we explored a range of CV values from 25% to 45%.

Table 7-1. Power for dose proportionality assessment, $\alpha = 0.05$

Sample size (per arm)	CV	Simple linear model and hypothesis testing approach	Power model and CI approach
N=6	25%	93.3%	98.4%
	35%	93.6%	74.9%
	45%	93.2%	33.3%
N=8	25%	95.0%	99.7%
	35%	95.1%	81.1%
	45%	93.8%	41.8%
N=10	25%	95.5%	99.8%
	35%	95.0%	85.0%
	45%	94.4%	43.5%
N=12	25%	96.1%	99.7%
	35%	93.9%	85.2%
	45%	94.6%	43.6%

Simulation results suggested that either 6 or 12 participants per arm would yield sufficient power for a range of coefficient of variation parameters (25% - 45%) when dose proportionality is assessed using hypothesis testing approach based on simple linear regression model. However, the empirical power associated with the power model and the CI approach is more heavily depend on the value of CV in the relevant range. With CV ≤ 35%, a reasonable degree of variation given the argument above, we would have ≥ 81% power to claim dose proportionality with 8 participants per arm. For this reason, in addition to cost and operational considerations, 8 subjects per arm was selected as the minimum size to provide adequate power for the dose-proportionality assessment, our primary objective.

For the secondary objective assessing difference in intracellular TFV-DP and FTC-TP steady state Day 35 C_τ (pre-dose concentration) between men and women, we assume the mean TFV-DP steady state concentration C_τ is 100 fmol/10^6 cells, and the coefficient of variation between people is 35%. Based on the results from Van Belle and Martin (Van Belle and Martin, 1993), we would have only 35% power to detect a 1.5 fold difference (i.e., 100 to 150) between men and women within each dosing arm if an arm has 4 men and 4 women (i.e., a total of 32 participants distributed evenly into four arms, 50% men and 50% women). If dose-proportionality holds, then we would be able to make the comparison between men and women across dosing arms. We then would have 89% power to detect a 1. 5 fold difference with a total sample size of 32 (16 men and 16 women) selected for this study. Table 7-2 provides power for a range of CV, sample size and minimal detectable difference. The power would reduce with smaller sample size, smaller minimal detectable difference, and larger CV.

Table 7-2. Power to detect the minimal detectable difference (in fold) for various sample sizes and coefficients of variance (CV)

		Minimal detectable X-fold difference		
CV	N per group	1.25	1.5	1.75
0.25	3	0.18	0.48	0.73
	4	0.24	0.60	0.85
	6	0.33	0.78	0.95
	12	0.58	0.97	0.99
	16	0.70	0.99	0.99
	24	0.86	0.99	0.99
0.35	3	0.12	0.28	0.45
	4	0.14	0.35	0.57
	6	0.19	0.49	0.74
	12	0.34	0.78	0.96
	16	0.43	0.89	0.99
	24	0.59	0.97	0.99
0.45	3	0.09	0.19	0.30
	4	0.10	0.23	0.38
	6	0.13	0.33	0.53
	12	0.22	0.57	0.82
	16	0.28	0.69	0.91
	24	0.40	0.86	0.98

7.4 Study Arm Assignment

At the time of consent, subjects will designate whether they will participate in the non-intensive or intensive sampling cohort. Assignment prioritization will go to the intensive sampling cohort. Within each of these 2 cohorts and each gender stratum, enrolled participants will be assigned at random to one of the four study arms in a 1:1:1:1 ratio. The permuted block randomization scheme will not be stratified by site and will be generated and maintained by the HPTN SDMC. For each randomized participant who is not fully evaluable (see section 7.3) an additional participant of the same gender will be enrolled into the same arm and the same cohort (intensive or non-intensive sampling). Details of the randomization system will be in the SSP Manual.

7.5 Blinding

There will be no blinding of the study. All of the outcome measures are objectively determined drug concentrations for which there should be minimal risk for bias.

7.6 Data Analysis

7.6.1 Primary Analyses

7.6.1.1 Dose Proportionality

Dose proportionality will be assessed by a confidence interval approach based on the power model, using Day 35 pre-dose concentration (C_τ) data from all evaluable participants in Arms 1-4. Dose-proportionality will be declared if the 90% confidence interval of the slope completely falls in the critical interval $(1+\frac{\ln(0.5)}{\ln(7)}=0.64, 1+\frac{\ln(2)}{\ln(7)}=1.36)$. Analyte concentrations assessed for dose-proportionality will include TFV and FTC in fluids and their phosphorylated moieties, TFV-DP and FTC-TP, in cells. TFV-DP and FTC-TP will also be assessed in tissue homogenates. Weight-based or creatinine clearance-adjusted intracellular PK parameters may also be used in evaluating dose-proportionality (as well as secondary outcomes). Biological matrices included in the analysis will include: serum, PBMC, CD4+ blood cells, rectosigmoid and vaginal luminal fluid, semen, and rectosigmoid and vaginal tissue specimens (total tissue cells, CD4+ tissue cells, tissue homogenate) (see Table 7-3).

Table 7-3. Biological matrices for analyses							
	Blood Samples			Vaginal, Rectosigmoid, and Semen Samples			
	Serum	PBMC	CD4+ blood cells	Total tissue cells	CD4+ tissue cells	Luminal fluid & semen	Homogenate
TFV-DP		X	X	X	X		X
FTC-TP		X	X	X	X		X
TFV	X					X	X
FTC	X					X	X

Descriptive statistics, such as means, medians, variance, and interquartile ranges, will be used to summarize Day 35 pre-dose concentration (C_τ) by gender and by dose arm. Simple comparison of median concentration level between dose arms will be done with standard non-parametric methods. Gender- or dosing-specific CVs of Day 35 pre-dose concentration (C_τ) will be estimated. These CV estimates would be informative to the future studies even if dose proportionality does not hold.

7.6.1.2 Intra-Participant Variability

Intra-participant variability will be described by the coefficient of variation of concentrations assessed on Day 28 and Day 35, which are assumed to be at steady-state, using data from all evaluable participants across dosing arms. We will confirm that steady-state was achieved by evaluating drug concentration data from Day 7, 14, and 21. If the dose-proportionality is established as described earlier, then dose-adjusted concentrations will be used for a more robust assessment. These variability estimates will be calculated for all analytes in blood and blood cells. As supportive evidence, the accumulation data from Day 7, 14, and 21 will also be used to estimate the elimination rate constant. Tissue and luminal assessments cannot be calculated due to the absence of multiple steady-state samples.

7.6.2 Secondary Analyses

7.6.2.1 Intracellular Concentration Decay

The apparent elimination rate constant (k_e), for intracellular phosphates (TFV-DP and FTC-TP) will be determined by estimating the negative slope of the decline in the natural log of concentration between Day 35 and Day 49. Half-life will be estimated as the natural log of 2 divided by the elimination rate constant (k_e). These estimates will be performed for each research participant and summarized using descriptive statistics for each arm and the study as a whole. Biological matrices included in the analysis are the same as shown in Table 7-3.

7.6.2.2 Differences between Men and Women

Because the genital tracts of men and women differ greatly in terms of anatomy and physiology (e.g. pH differences) as well as drug metabolism, it is feasible that there may be gender-specific differences in the pharmacology of different doses of tenofovir and emtricitabine in different mucosal compartments. Differences in tissue concentrations of these drugs could be relevant in understanding if gender-related differences are detected in intermittent PrEP efficacy trials. Some gender-related differences may be primarily explained by differences in body mass index between men and women, so determining the specific reasons for potential gender-related differences may be challenging with relatively small sample sizes. Men and women will be recruited into the study in equal proportions for comparison. If dose-proportionality is established in the primary objectives, then dose-adjusted Day 35 C_τ concentration differences (each analyte, location, and matrix) between men and women will be tested pooling all research participants using ANOVA. If dose-proportionality is not established in the primary aim, intracellular PK parameters will be summarized by standard descriptive statistics (such as means, medians, and ranges) for each gender within each dose arm. Non-parametric methods may be used to compare C_τ between men and women. The lack of statistical power of these comparisons due to small sample size is acknowledged.

7.6.2.3 Safety Evaluation

Adverse events will be presented by individual listings and in frequency tables broken down by body system.

Laboratory test values will be presented in participant listings and in summaries. Graded laboratory values will be summarized. Boxplots of local laboratory values will be generated for baseline values and for values measured during the course of the study. Each boxplot will show the 1st quartile, the median, and the 3rd quartile. Outliers, or values outside the boxplot, will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

8.0 HUMAN SUBJECTS CONSIDERATIONS

8.1 Ethical Review

This protocol and the template informed consent form(s) contained in Appendices II and III — and any subsequent modifications — will be reviewed and approved by the HPTN Protocol Review Committee and DAIDS Prevention Science Review Committee with respect to scientific content and compliance with applicable research and human subjects regulations.

The protocol, site-specific informed consent form, participant education and recruitment materials, and other requested documents — and any subsequent modifications — also will be reviewed and approved by the ethical review bodies responsible for oversight of research conducted at the study site.

Subsequent to initial review and approval, the responsible IRBs/ECs will review the protocol at least annually. The Investigator will make safety and progress reports to the IRBs/ECs at least annually, and within three months of 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 subjects or others. Study sites will submit documentation of continuing review to the DAIDS Protocol Registration Office, via the HPTN CORE, in accordance with the current DAIDS Protocol Registration Policy and Procedure Manual.

8.2 Informed Consent

Written informed consent will be obtained from each study participant. Each study site is responsible for developing a study ICF for local use, based on the template in Appendices II and III, which describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations. The study site is also responsible for translating the template form into additional languages (if applicable), and verifying the accuracy of the translation by performing an independent back-translation. Literate participants will document their provision of informed consent by signing their informed consent forms. Participants will be provided with a copy of their informed consent forms.

8.3 Risks

Phlebotomy, STI Screening. Phlebotomy may lead to discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection. Pelvic examination may cause mild discomfort and/or vaginal bleeding or spotting. Disclosure of STI status may cause sadness or depression in volunteers. Participation in clinical research includes the risks of loss of confidentiality and discomfort with personal nature of questions.

Confidentiality. 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.

STI Reporting. Participants in sites requiring partner notification in response to diagnosed STI or HIV infection could have problems in their relationships with their sexual partners. Participants also could have problems in their partner relationships associated with use or

attempted use of study products. In addition, participants could misunderstand the current experimental status of the study medication and as a result increase their HIV risk behaviors while in the study.

Flexible Sigmoidoscopy with Biopsy. Flexible sigmoidoscopy is a commonly practiced medical procedure and the endoscopic procedures done in this trial will not involve any unusual risks or discomforts. The risks associated with these procedures include mild discomfort and the feeling of having a "bloated stomach". Endoscopic biopsies are painless and heal quickly within 3 days. On extremely rare occasions, the endoscopic procedure or biopsies may lead to pain, infection (sepsis), bleeding or perforation of the gastrointestinal tract. Perforation occurs approximately once out of every 100,000 procedures. If this extremely rare complication occurs, antibiotics and surgery to repair the tear may be necessary. Participants will be counseled to refrain from sexual intercourse for at least 72 hours following the biopsy.

Anoscopy and luminal fluid collection. It is possible that the participant may experience mild discomfort, embarrassment or, rarely, pain (should they have another condition that is already causing pain in the area) during this procedure. If there is any pain experienced during the procedures, the study clinician will adapt or stop the procedure.

Enema. The primary risk from having an enema is temporary discomfort. The procedure may cause a bloated or crampy feeling. Some air may be pumped into the rectum as well, causing flatulence. Very rarely, the enema tube could make a hole in the rectum causing a bowel perforation requiring surgery.

Vaginal Biopsy. Vaginal biopsy carries the risk of discomfort or pain during the procedure and for a few hours afterwards. Participants may have mild vaginal spotting (bleeding) for one or two days, and will be instructed to avoid sexual intercourse until bleeding stops. Some temporary discomfort with sexual intercourse may occur if the biopsy areas are still healing. There is a small risk of infection and heavier bleeding. Participants will be instructed to contact the clinic if symptoms are bothersome, if heavy bleeding is noted (soaking through a pad or tampon in an hour or less) or if the participant develops any abnormal odor or discharge from the vagina. Participants will be counseled to refrain from sexual intercourse for at least 72 hours following the biopsy.

Study Product

FTC/TDF Combination Tablet. Truvada® tablets are fixed dose combination tablets containing emtricitabine (FTC) 200 mg and tenofovir disoproxil fumarate (TDF) 300 mg in each tablet. There is very little experience in healthy volunteers taking Truvada®. No new or unexpected side effects are observed with the TDF 300 mg / FTC 200 mg combination tablet than those observed when each drug is given separately. For study Arm 4, the TDF/FTC dosing regimens are consistent with package insert recommendations. Participants in Arms 1 and 2 will be taking the typical TDF/FTC dose, but at reduced frequency. Participants in Arm 3 will be taking twice the recommended dose, but at reduced frequency (twice weekly rather than daily), representing 57% of the weekly recommended dose. Individual daily doses of this magnitude were studied during development of both TDF and FTC and these were well tolerated.

FTC. The following side effects have been associated with the use of FTC: 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, abnormal liver function tests, increases in pancreatic enzyme, increased triglycerides, increased creatine phosphokinase.

FTC is a pregnancy category B medication. No controlled human studies of FTC among pregnant women have been conducted.

TDF. The most common side effects associated with oral TDF in patients with HIV infection are nausea, headache, diarrhea, vomiting, asthenia, flatulence, abdominal distension/pain and anorexia. Less common side effects of TDF include kidney toxicities and low blood phosphate. Other side effects reported in the post-marketing period include weakness, pancreatitis, dizziness, shortness of breath, and rash. TDF has been associated with decreased bone mineral density in HIV-infected patients taking TDF tablets for 24-48 weeks.

TDF is a pregnancy category B medication. No extensive controlled human studies of TDF among pregnant women have been conducted.

Nucleotide Analogues. Lactic acidosis (elevated lactic acid levels in the blood) and severe hepatomegaly (enlarged liver) with steatosis (fatty liver) that may result in liver failure, other complications or death have been reported with the use of antiretroviral nucleoside analogues alone or in combination. The liver complications and death have been seen more often in women on these drug regimens. Some nonspecific symptoms that might indicate lactic acidosis include: unexplained weight loss, stomach discomfort, nausea, vomiting, fatigue, cramps, muscle

pain, weakness, dizziness and shortness of breath. These effects are not expected in this study involving a brief exposure to antiretroviral drug.

Antiretroviral Drugs. Redistribution/accumulation of body fat including central obesity, dorsocervical fat enlargement (buffalo hump), peripheral wasting, facial wasting, breast enlargement, and "cushingoid appearance" have been observed in persons receiving antiretroviral drugs. The mechanism and long-term consequences of these events are currently unknown. A causal relationship has not been established. These effects are not expected in this study involving a brief exposure to antiretroviral drug.

8.4 Benefits

There will be no direct benefits to participants in this study. However, participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of a safe and effective PrEP regimen that prevents HIV infection.

8.5 Incentives

Pending IRB/EC approval, participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits and time away from work. Site-specific reimbursement amounts will be specified in the study ICFs.

8.6 Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, process, and administrative forms will be identified by a coded number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Participant's study information will not be released without the written permission of the participant, except as necessary for monitoring by the NIAID and/or its contractors, Gilead Sciences; representatives of the HPTN CORE, SDMC, and/or NL, U.S. FDA, OHRP, and/or site IRBs/ECs.

The HPTN has obtained a Certificate of Confidentiality from the US Department of Health and Human Services that will be applicable for this study. Sites may register under the Certificate through the HPTN CORE once they have obtained local IRB approvals for the study. This Certificate protects study staff from being compelled to disclose study-related. information by any US Federal, State or local civil, criminal, administrative, legislative, or other body.

All protected health information (PHI) will be protected according to the provisions of the HIPAA and will only be used or disclosed as allowed by the privacy rule pursuant to relevant waivers or authorizations, or as required by federal law.

8.7 Communicable Disease Reporting Requirements

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

8.8 Study Discontinuation

The study may be discontinued at any time by NIAID, site IRBs/ECs, the HPTN, Gilead Sciences, OHRP or other governmental agencies as part of their duties to ensure that research subjects are protected.

9.0 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

9.1 Local Laboratory Specimens

As described in Section 5, the following types of specimens will be collected for testing at the local laboratory:

<u>Blood</u>

- HIV diagnostic testing (see SSP)
    - HIV rapid test
    - HIV Western blot (for HIV confirmatory testing)
    - APTIMA HIV-1 RNA Qualitiative Assay (GenProbe, Inc., GenAptima assay)
- Safety testing:
    - CBC
    - AST/ALT
    - Creatine and calculated creatinine clearance
    - phosphate
    - HCO3, Na, Cl, K, Ca, glucose
- Coagulation testing:

    - INR, PT and PTT
- Hepatitis testing (HBsAg)
- Syphilis testing
- Plasma storage
- Blood samples for PK analysis (drug levels)
    - Serum
    - PBMCs
    - CD4+ cells
- Quantitative HIV RNA assay (for any participant who acquires HIV infection during the study).
- CD4 cell count (for any participant who acquires HIV infection during the study)
- Genotyping and additional plasma storage (for any participant who acquires HIV infection during the study– see Appendix 1D)

Urine

- Pregnancy testing (women only)
- CT/GC testing
- Dipstick for urinalysis. If positive for leukocyte esterase or nitrates, perform urine culture

Pelvic Specimens (women only)

- Vaginal wet mount (for BV, candida, trichomonas)
- Vaginal pH (if Amstel criteria are used for diagnosis of BV)
- Pap smear (if a normal result is not documented within 12 months)

Responsibility of Study Sites

Study sites must adhere to standards of good clinical laboratory practice (GCLP), the HPTN Manual of Laboratory Operations, and local Standard Operating Procedures (SOPs) for proper collection, processing, labeling, and transport of specimens to the local laboratory.

Procedures Performed at Local Laboratories

General requirements

Local laboratories must adhere to standards of GCLP, the HPTN Manual of Laboratory Operations, and local SOPs for proper specimen receipt, testing, aliquoting, storage, and shipment of specimens (see below). Specimen testing and storage must be documented using the Laboratory Data Monitoring Systems (LDMS). Clinical sites in must document that their clinical laboratories are CLIA-certified and must meet DAIDS requirements for external quality assurance (QA) testing. External Quality Control (QC) of CLIA-waived tests (e.g. HIV rapid tests) may be required. HPTN NL staff will conduct period visits to each site to assess the

implementation of on-site QC procedures, including proper maintenance of laboratory testing equipment and use of appropriate reagents. HPTN NL staff will follow-up directly with site staff to resolve any QA or QC problems identified through proficiency testing and/or on-site assessments.

HIV diagnostic testing

HIV infection status will be assessed both at screening, enrollment, at the end of the study, and at any study visit at which a participant expresses a concern about having acquired HIV infection. Diagnostic testing algorithms are provided in the SSP. HIV rapid testing will be used to screen participants for HIV infection. HIV rapid test kits must be FDA-cleared. Preference is given to FDA-cleared OraSure Oraquick HIV-1/2, Clearview HIV-1/2 Stat Pak, or Clearview Complete HIV-1/2. Participants with one or more reactive HIV rapid test results will not be eligible for enrollment, regardless of subsequent test results. In those cases, HIV infection status will be confirmed using local HIV testing guidelines. After enrollment, if HIV infection is suspected or documented, the HPTN NL should be contacted. HIV infection must be confirmed for any enrolled participant using two independent samples.

Processing of samples for PK analysis (measurement of drug levels)

Blood samples will be processed locally to isolate serum and cells of interest. Detailed procedures for processing, freezing, storing, and shipping tissue samples are provided in the SSP Manual.

Shipping

Sites must have the capability for shipping samples to the HPTN NL for Pharmacology, Virology, QA, and other testing. All specimens must be shipped in accordance with International Air Transportation Association (IATA) specimen shipping regulations and must be documented in the LDMS.

Sample storage

Specimens will be stored at study sites and shipped to the HPTN NL upon request.

9.2 Network Laboratory Specimens

Testing Performed at the HPTN NL

Virology

The HPTN NL will perform testing to determine HIV infection status in selected cases and will perform HIV diagnostic testing for QC. The HPTN NL may also perform specialized assays to characterize HIV viruses and the immune response to HIV infection in any participant who becomes HIV-infected during the study. HIV resistance testing will be performed at the time of HIV diagnosis (see Appendix ID). HIV resistance test results from that study visit (time of HIV

diagnosis) will be provided to study sites at study closure. Results from that testing will be provided to study sites prior to study closure with approval of the HPTN NL and the Protocol Chair. That testing may be performed at a commercial laboratory (e.g., Monogram Biosciences). Additional testing may be performed for research purposes, either at the HPTN NL, or at an outside laboratory designated by the HPTN NL. This may include testing with assays used for analysis of HIV incidence testing to evaluate the fading of resistant variants (including minority variants assays) HIV subtyping, and other testing to characterize HIV viruses and/or the host response to HIV infection. Results from those tests will not be returned to study sites or study participants.

STI Testing

The HPTN NL will perform HSV-2 PCR testing and GC/CT nucleic acid amplification testing (NAAT). Results from these tests will be returned to study participants.

Pharmacology

The HPTN NL will perform all drug testing for this protocol. Drugs will be measured in serum, PBMCs, CD4+ blood cells, total tissue cells, CD4+ tissue cells, tissue homogenate, and luminal (vaginal and rectosigmoid) fluid using assays that have an assay validation report (AVR) and SOP approved by the CPQA. Results from these tests will not be returned to study participants.

As described in Section 5, the following types of specimens will be collected for testing at the HPTN NL:

Blood

- Serum for drug levels
- PBMC for drug levels
- CD4+ blood cells for drug levels
- Plasma for storage (for QC of HIV testing, and for viral characterization in any participant who acquires HIV infection during the study)

Rectosigmoid Sampling

- Tissue biopsy intact (for homogenate)
- Tissue total cells lysate
- Tissue CD4+ cells lysate
- Luminal fluid

Vaginal Sampling

- Tissue biopsy intact (for homogenate)
- Tissue total cells lysate
- Tissue CD4+ cells lysate
- Luminal fluid

- Cervical cells (from cytobrush)

Note: Vaginal tissues will be processed in the priority listed above and will be dependent upon the amount of tissue collected during the biopsy. Sites will collect up to 5 biopsies; however, the upper limits will be dependent upon site specific IRB approvals.

Genital swabs

- For HSV-2 testing (only if active lesions are present)

Seminal Fluid

- Drug levels

Rectal swabs

- For GC/CT testing

All specimen collection and specimen processing procedures will be outlined in the study specific procedures manual.

Each study site will adhere to standards of good laboratory practice and the HPTN Network Laboratory Manual for proper collection, processing, labeling, and transport of specimens for the NL. All specimens will be shipped in accordance with IATA specimen shipping regulations. All shipments will be documented using the HPTN LDMS as described in the SSP manual.

9.3 Quality Control and Quality Assurance Procedures

The clinical sites will document that their clinical laboratories are CLIA-certified and/or participate in DAIDS sponsored EQA programs. NL staff will conduct periodic visits to each site to assess the implementation of on-site laboratory QC procedures, including proper maintenance of laboratory testing equipment and use of appropriate reagents. NL staff will follow-up directly with site staff to resolve any QC or QA problems identified through proficiency testing and/or on-site assessments. Throughout the course of the study, the HPTN NL will select a random sample of stored specimens to test for QA purposes. NL staff will follow-up directly with site staff to resolve any QA problems identified through this process.

QC for HIV Diagnostic Testing

Before performing HIV diagnostic testing, all sites must validate their testing algorithm, and the validation study must be approved by the HPTN NL. Local laboratories will perform testing for HIV diagnosis at screening, enrollment, and other scheduled visits. Algorithms for HIV diagnostic testing are provided in the SSP.

QC for HIV RNA Monitoring
Quantitative HIV RNA (viral load) testing will be performed at local laboratories for any participant with confirmed HIV infection. Note that this is distinct from use of qualitative HIV RNA testing that may be performed to determine HIV infection status in selected cases. Local laboratories must participate in the DAIDS Virology QA program, with EQA results that are deemed satisfactory by the HPTN NL.

QC for CD4 Cell Count Determination
CD4 cell count testing will be performed at local laboratories for any participant with confirmed HIV infection. U.S. laboratories performing CD4 cell count testing must be CLIA-certified; enrollment in the DAIDS Immunology QA (IQA) program is preferred.

9.4 Specimen Storage and Possible Future Research Testing

Study site staff will store all plasma specimens collected in this study until all protocol-related testing has been completed, including QC testing and other testing performed at or coordinated by the HPTN NL. Study staff will also store all samples collected for drug testing as directed by the NL. The study site will be informed by SCHARP when shipments to the NL are required, and will be instructed which samples to ship. In addition, study participants will be asked to provide written informed consent for their specimens to be stored after the end of the study for possible future testing. The specimens of participants who do not consent to long-term storage and additional testing will be destroyed at the end of the study.

9.5 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the U.S. Centers for Disease Control and Prevention. All infectious specimens will be transported in accordance with U.S. regulations (42 CFR 72).

10.0 ADMINISTRATIVE PROCEDURES

10.1 Study Activation

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) *WILL* be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

Pending successful protocol registration and submission of all required documents, CORE staff will "activate" the site to begin study operations. Study implementation may not be initiated until a study activation notice is provided to the site.

10.2 Study Coordination

The United States Food and Drug Administration (U.S. FDA) has been informed of this study and has provided an IND-exemption.

Assignment of all sponsor responsibilities for this study will be specified in a Clinical Trials Agreement executed by DAIDS and Gilead Sciences.

Study implementation will be directed by this protocol as well as the SSP manual. The SSP manual — which will contain reference copies of the *Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials*, as well as the DAIDS Manual for Expedited Reporting of Adverse Events to DAIDS, Version 2.0, dates January 2010 and the DAIDS Toxicity Tables — will outline procedures for conducting study visits; data and forms processing; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations.

Study CRFs will be developed by the study team and HPTN SDMC. Data will be transferred to the HPTN SDMC, entered, and cleaned using the SDMC DataFax data management system.

Quality control reports and queries routinely will be generated and distributed to the study sites for verification and resolution.

Close coordination between protocol team members will be necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, adherence, follow-up, and AE incidence will be monitored closely by the team as well as the HPTN Study Monitoring Committee. The Protocol Chair, DAIDS Medical Officer, Protocol Biostatistician, and CORE Protocol Specialist will address issues related to study eligibility and AE management and reporting as needed to assure consistent case management, documentation, and information-sharing across sites.

10.3 Study Monitoring

On-site study monitoring may be performed in accordance with DAIDS policies. Study monitors will visit the site to:

- Verify compliance with human participants and other research regulations and guidelines;
- Assess adherence to the study protocol, study-specific procedures manual, and local counseling practices; and
- Confirm the quality and accuracy of information collected at the study site and entered into the study database.

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the HPTN CORE, SDMC, NL, NIAID, Gilead Sciences, FDA and other U.S. government and regulatory authorities. A site visit log will be maintained at the study site to document all visits.

10.4 Protocol Compliance

The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chair and NIAID Medical Officer. All protocol amendments must be submitted to and approved by the relevant IRB(s)/EC(s) and the DAIDS Regulatory Support Center (RSC) prior to implementing the amendment.

10.5 Investigator's Records

The study site investigator will maintain, and store in a secure manner, complete, accurate and current study records throughout the study. The investigator will retain all study records for at least three years after the completion of the study, unless directed otherwise by DAIDS. Study records include administrative documentation, including site registration documents and all reports and correspondence related to the study, as well as documentation related to each participant screened and/or enrolled in the study, including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents.

10.6 Use of Information and Publications

Publication of the results of this study will be governed by HPTN policies. Any presentation, abstract, or manuscript will be submitted by the Investigator to the HPTN Manuscript Review Committee, DAIDS, and Gilead Sciences for review prior to submission.

11.0 REFERENCES

Barditch-Crovo, P., S.G. Deeks, A. Collier, S. Safrin, D.F. Coakley, M. Miller, B.P. Kearney, R.L. Coleman, P.D. Lamy, J.O. Kahn, I. McGowan, and P.S. Lietman. 2001. Phase i/ii trial of the pharmacokinetics, safety, and antiretroviral activity of tenofovir disoproxil fumarate in human immunodeficiency virus-infected adults. *Antimicrob Agents Chemother*. 45:2733-9.

Cardo, D.M., D.H. Culver, C.A. Ciesielski, P.U. Srivastava, R. Marcus, D. Abiteboul, J. Heptonstall, G. Ippolito, F. Lot, P.S. McKibben, and D.M. Bell. 1997. A case-control study of HIV seroconversion in health care workers after percutaneous exposure. Centers for Disease Control and Prevention Needlestick Surveillance Group. *N Engl J Med*. 337:1485-90.

Garcia-Lerma, G., M.-E. Cong, and J. Mitchell. 2009. Prevention of Rectal Simian HIV Transmission in Macaques by Intermittent Pre-exposure Prophylaxis with Oral Truvada (Abstract 47). *In* 16th Conference on Retroviruses and Opportunistic Infections (CROI 2009), Montreal, Canada.

Garcia-Lerma, J.G., R.A. Otten, S.H. Qari, E. Jackson, M.E. Cong, S. Masciotra, W. Luo, C. Kim, D.R. Adams, M. Monsour, J. Lipscomb, J.A. Johnson, D. Delinsky, R.F. Schinazi, R. Janssen, T.M. Folks, and W. Heneine. 2008. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. *PLoS Med*. 5:e28.

Gish, R.G., N.W. Leung, T.L. Wright, H. Trinh, W. Lang, H.A. Kessler, L. Fang, L.H. Wang, J. Delehanty, A. Rigney, E. Mondou, A. Snow, and F. Rousseau. 2002. Dose range study of pharmacokinetics, safety, and preliminary antiviral activity of emtricitabine in adults with hepatitis B virus infection. *Antimicrob Agents Chemother*. 46:1734-40.

Gough, K., M. Hutchinson, and O. Keene. 1995. Assessment of dose proportionality: report from the pharmaceutical industry. *Drug Information Journal*. 29:1039-1048.

Hawkins, T., W. Veikley, R.L. St Claire, 3rd, B. Guyer, N. Clark, and B.P. Kearney. 2005. Intracellular pharmacokinetics of tenofovir diphosphate, carbovir triphosphate, and lamivudine triphosphate in patients receiving triple-nucleoside regimens. *J Acquir Immune Defic Syndr*. 39:406-11.

Hummel, J., S. McKendrick, C. Brindley, and R. French. 2009. Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. *Pharm Stat*. 8:38-49.

Kearney, B.P., J. Zong, J. Begley, and J. Shah. 2004. Bioequivalence of combination tenofovir DF/emtricitabine tablets for one-pill once daily administration [poster no. 7.3]. *In* 5th International Workshop on Clinical Pharmacology of HIV Therapy, Rome, Italy.

Pruvost, A., E. Negredo, H. Benech, F. Theodoro, J. Puig, E. Grau, E. Garcia, J. Molto, J. Grassi, and B. Clotet. 2005. Measurement of intracellular didanosine and tenofovir phosphorylated metabolites and possible interaction of the two drugs in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother*. 49:1907-14.

Pruvost, A., E. Negredo, F. Theodoro, J. Puig, M. Levi, R. Ayen, J. Grassi, and B. Clotet. 2009. Pilot pharmacokinetic study of human immunodeficiency virus-infected patients receiving tenofovir disoproxil fumarate (TDF): investigation of systemic and intracellular interactions between TDF and abacavir, lamivudine, or lopinavir-ritonavir. *Antimicrob Agents Chemother*. 53:1937-43.

Rousseau, C.M., R.W. Nduati, B.A. Richardson, M.S. Steele, G.C. John-Stewart, D.A. Mbori-Ngacha, J.K. Kreiss, and J. Overbaugh. 2003. Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and of its relationship to infant infection and maternal disease. *J Infect Dis*. 187:741-7.

Rousseau, F.S., J.O. Kahn, M. Thompson, D. Mildvan, D. Shepp, J.P. Sommadossi, J. Delehanty, J.N. Simpson, L.H. Wang, J.B. Quinn, C. Wakeford, and C. van der Horst. 2001. Prototype trial design for rapid dose selection of antiretroviral drugs: an example using emtricitabine (Coviracil). *J Antimicrob Chemother*. 48:507-13.

Schechter, M., R.F. do Lago, A.B. Mendelsohn, R.I. Moreira, L.H. Moulton, and L.H. Harrison. 2004. Behavioral impact, acceptability, and HIV incidence among homosexual men with access to postexposure chemoprophylaxis for HIV. *J Acquir Immune Defic Syndr*. 35:519-25.

Smith, B.P., F.R. Vandenhende, K.A. DeSante, N.A. Farid, P.A. Welch, J.T. Callaghan, and S.T. Forgue. 2000. Confidence interval criteria for assessment of dose proportionality. *Pharm Res*. 17:1278-83.

Subbarao, S., A. Ramos, C. Kim, D. Adams, M. Monsour, S. Butera, T. Folks, and R.A. Otten. 2007. Direct stringency comparison of two macaque models (single-high vs. repeat-low) for mucosal HIV transmission using an identical anti-HIV chemoprophylaxis intervention. *J Med Primatol*. 36:238-43.

UNAIDS. 2008. Report on the Global AIDS Epidemic.

Van Belle, G., and D.C. Martin. 1993. Sample Size as a Function of Coefficient of Variation and Ratio of Means. *American Statistician*. 47:165-167.

Veazey, R.S., M.S. Springer, P.A. Marx, J. Dufour, P.J. Klasse, and J.P. Moore. 2005. Protection of macaques from vaginal SHIV challenge by an orally delivered CCR5 inhibitor. *Nat Med*. 11:1293-4.

Wang, L.H., J. Begley, R.L. St Claire, 3rd, J. Harris, C. Wakeford, and F.S. Rousseau. 2004. Pharmacokinetic and pharmacodynamic characteristics of emtricitabine support its once daily dosing for the treatment of HIV infection. *AIDS Res Hum Retroviruses*. 20:1173-82.

Zhou, J., J. Li, and B. Coate. 2006. Empirical Power Estimation for Phase I Dose Proportionality Studies Based on Power-Law Model Using Confidence Interval Criteria. *In* SUGI 31, San Francisco, California.

APPENDICES

APPENDIX I: SCHEDULE OF EVALUATIONS AND PROCEDURES

APPENDIX IA: SCHEDULE OF EVALUATIONS AND PROCEDURES - GENERAL PROCEDURES

Phase	Screening	Enrollment	Accumulation																									Steady-State							Decay																				
Study Week			Week 1							Week 2							Week 3							Week 4							Week 5							Week 6							Week 7							
Study Day		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Treatment Arm and Regimen																																																						
1. FTC 200 mg/TDF 300 mg one tablet orally once weekly for 5 weeks		X							X							X							X							X																						
2. FTC 200 mg/TDF 300 mg one tablet orally twice weekly for 5 weeks		X			X				X			X				X			X				X			X				X			X																		
3. FTC 200 mg/TDF 300 mg two tablets orally twice weekly for 5 weeks		X			X				X			X				X			X				X			X				X			X																		
4. FTC 200 mg/TDF 300 mg C one tablet once daily for 5 weeks		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X															
Administrative and Behavioral Procedures																																																				
Informed Consent	X																																																			
Locator Information	X	X							X							X							X							X							X																X
Demographic Information	X																																																		
Medical History Questionnaire	X																																																		

APPENDIX IB: SCHEDULE OF EVALUATIONS AND PROCEDURES - CLINICAL PROCEDURES AND LABORATORY TESTS

Phase	SCR	ENR	Accumulation																							
Study Week		Week 1							Week 2							Week 3							Week 4			
Study Day		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Clinical Procedures and Laboratory Tests																										
Blood (Serum/Plasma)																										
HIV Diagnostic Testing[a]	X	X																								
Safety testing[b]	X															X										
Coagulation testing[c]	X																									
HBsAg[*]	X																									
Syphilis Testing[k, *]	X																									
Plasma storage	X	X																								
Urine																										
Pregnancy test[j] (women)	X	X																								
Dipstick urinalysis[d, *]	X																									
GC/CT testing [k, *]	X																									

Phase				Steady-State							Decay														
Study Week				Week 5							Week 6							Week 7							
Study Day	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Clinical Procedures and Laboratory Tests																									
Blood (Serum/Plasma)																									
HIV Diagnostic Testing[a]																									X
Safety testing[b]				X							X														X
Coagulation testing[c]				X																					
HBsAg[*]																									X
Syphilis Testing[k, *]																									
Plasma storage				X																					X
Urine																									
Pregnancy test[j] (women)				X							X														X
Dipstick urinalysis[d, *]																									
GC/CT testing [k, *]																									

Phase	SCR	ENR	Accumulation																									Steady-State							Decay														
Study Week			Week 1							Week 2							Week 3							Week 4							Week 5							Week 6							Week 7							
Study Day			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Pelvic (women) (intensive cohort only)																																																		
Wet mount[e]	X																																																
Vaginal fluid pH[f]	X																																																
Pap Smear[g]	X																																																
Genital lesions (if present; (intensive cohort only)																																																	
HSV-2 testing[h, *]	X																																																
Rectal (intensive cohort only)																																																	
GC/CT testing[i, *]	X																																																

FOOTNOTES FOR APPENDIX IB

[a] Perform testing as described in the SSP. Participants with one or more HIV rapid test results are not eligible for enrollment, even if HIV infection is not confirmed. HIV diagnostic testing should be repeated at any study visit if a participant expresses a concern that he/she may have acquired HIV infection.

[b] Safety testing includes CBC, ALT/AST, phosphate, creatinine (for estimated creatinine clearance), electrolytes (HCO3, K, Cl, Na), Ca, glucose.

[c] Coagulation testing includes INR, PT and PTT. Intensive sampling cohort only.

[d] If urine dipstick is positive for leukocyte esterase or nitrates, perform urinalysis along with urine culture.

[e] Wet mount includes testing for BV, candida, trichomonas.

[f] Vaginal pH testing is only required if Amstel criteria are used for diagnosis of BV.

[g] A Pap smear will be performed if a normal test result cannot be documented within the previous 12 months.

[h] A PCR-based test for HSV-2 will be performed at the HPTN NL.

[i] Rectal GC/CT NAAT testing will be performed at the HPTN NL.

[j] Pregnancy testing will be done before any vaginal study procedures are performed. On Day 35, pregnancy testing will only be performed on women in the intensive sampling cohort.

[k] These tests are only for participants in the intensive sampling cohort

[*] Individuals with a positive result for any of these tests are not eligible for enrollment, and will be referred for care. Confirmatory testing should be performed as needed according to local testing guidelines. Testing should be repeated at any study visit if a participant expresses a concern that he/she may have acquired hepatitis B or an STI.

APPENDIX IC: SCHEDULE OF EVALUATIONS AND PROCEDURES - SAMPLE COLLECTION FOR PK EVALUATION (DRUG TESTING)

Phase	SCR	ENR	Accumulation																							Steady-State							Decay																			
Study Week			Week 1							Week 2							Week 3							Week 4							Week 5							Week 6							Week 7							
Study Day		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
PK Sampling																																																				
Blood serum		X	4		2 3				X 1							X							X							X							X															X
Blood PBMC		X	4		2 3				X 1							X							X							X							X															X
Blood CD4+ cells		X	4		2 3				X 1							X							X							X							X															X
Rectosigmoid tissue homogenate																																					X[a]															X[a]
Rectosigmoid tissue total cells																																					X[a]															X[a]
Rectosigmoid tissue CD4+ cells																																					X[a]															X[a]
Rectosigmoid luminal fluid																																					X[a]															X[a]
Vaginal tissue homogenate																																					X[a]															X[a]
Vaginal tissue total cells																																					X[a]															X[a]
Vaginal tissue CD4+ cells																																					X[a]															X[a]
Vaginal fluid																																					X[a]															X[a]
Semen																																					X[a]															X[a]

In rows for Blood Serum, Blood PBMC, and Blood CD4 cells, days 1-7, the number indicates a sample collection for each cohort (indicated by cohort number, as listed in the protocol schema) no more than one hour before the second dose for each cohort. These will occur on day 7 (Cohort 1), Day 3 (Cohort 2 and 3), and Day 1 (Cohort 4).

[a] Sixteen participants who agree to intensive tissue sampling will be enrolled in a balanced fashion across dosing cohorts such that two men and two women per cohort are enrolled. For other participants, they will only have blood and blood cells collected on Day 35 and 49.

APPENDIX 1D: ADDITIONAL PROCEDURES FROM STUDY PARTICIPANTS WITH CONFIRMED HIV INFECTION

	Time of diagnosis	All subsequent visits[4]
CD4 cell count testing	X	
HIV viral load testing	X	
Shipment of samples to the HPTN NL[1]	X	X
HIV resistance testing [2]	X	
Other testing [3]	X	X
Additional plasma storage	X	X

[1] All stored samples from HIV seroconverters will be centralized at the HPTN NL.

[2] The HPTN NL will coordinate HIV resistance testing using samples shipped to the HPTN NL. HIV resistance test results from this study visit (time of HIV diagnosis) will be provided to study sites at study closure. Results from this testing may be provided to study sites prior to study closure with approval of the HPTN NL and the Protocol Chair.

[3] Additional testing may be performed at the HPTN NL or at another laboratory designated by the HPTN NL for research purposes; those results will not be returned to study sites or study participants. This testing may include HIV subtyping, minority variants assays, other assays to characterize HIV viruses and/or the host response to HIV infection. Additional safety testing will also be performed on all participants who seroconvert during the study.

[4] Participants who seroconvert during study participation will have a final safety visit (visit may be split if necessary) after confirmation of their HIV status.

APPENDIX II: SCREENING/ENROLLMENT SAMPLE INFORMED CONSENT FORM (Intensive Sampling Cohort)

SAMPLE INFORMED CONSENT DOCUMENT (Intensive Sampling Cohort)

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

29 NOVEMBER 2010
VERSION 2.0

PRINCIPAL INVESTIGATOR: [Insert Name]
PHONE: [Insert Number]

GENERAL OVERVIEW

You are being asked to take part in a research study. Joining this study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives information about the study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign your name on this form. You will be given a copy of this form to keep.

Before you learn about the study, it is important that you know the following:

- Your participation is voluntary. You do not have to take part in the screening tests or in the study.
- You may decide not to take part in the study or to leave the study at any time without losing your regular medical care.
- If you decide not to take part in the study, you can still join another study at a later time if there is one available and you qualify.
- You cannot join this study if you are taking part in another study of drugs, medical devices or vaginal/rectal products. You are asked to tell the study staff about any other studies you are taking part in or thinking of taking part in. This is very important for your safety.

PURPOSE OF THE STUDY

This study is being conducted to look at how the body handles the drug Truvada® when it is given in different amounts and at different time schedules. This study will also look at the difference of medication amount in men and women.

The study will look at levels of Truvada® in the blood, reproductive tract and gastrointestinal tract of female and male volunteers. The aim is to establish how much of the drug (taken by mouth) reaches the reproductive and gastrointestinal tracts when given at different doses and at different dose schedules. It is believed that the presence of this drug in the reproductive and gastrointestinal tracts may be beneficial in the prevention of the transmission of the AIDS virus.

About 32 participants will be enrolled in the study at 2 different sites in the United States. About 16 people will be enrolled in the study here at [study site]. The study should take approximately 12 months to complete. Each participant will be followed for approximately 2 months.

The study will take samples of blood once a week for the first 5 weeks after taking Truvada® according to the dose you are assigned to. After that time, blood samples and tissue samples from the gastrointestinal tract (from men and women) will be collected. Samples will also be collected from the vagina in women. Men will be asked to donate a semen sample. All of these samples are to look at drug concentrations. You will then have a second set of blood sample and tissue biopsies after you have stopped taking the study drug for 2 weeks. The study will also collect information on the safety of the medication.

Only 16 of the 32 participants will provide tissue/semen/vaginal samples as part of study participation. The remaining participants will only provide blood samples. Potential participants will decide with study staff which group they will enroll in before they are consented for the study.

Truvada® is a tablet that is a combination of two medications—emtricitabine and tenofovir. This medication is used to treat people who have HIV. The tablets are generally safe when used as treatment for HIV. These tablets are not a cure for HIV/AIDS, but they are very effective for improving the health of people who have HIV/AIDS. Although these tablets improve the health of people with HIV, **we do not know if they work to protect against HIV.** The results of this study may be used in the future to determine if this medication can be taken by healthy people to prevent them from getting HIV.

The United States Food and Drug Administration (U.S. FDA) has been informed of this study. Truvada has not been approved by the U.S. FDA for the prevention of HIV.

Before you decide whether to take part in the study, we would like to explain the purpose of the study, the risks and benefits, what is expected of you, and what to expect from us. This consent form might contain some words that are unfamiliar. Please ask questions about anything you do not understand or want to learn more about.

STUDY GROUPS
There are four study groups. Each group will have 8 people. There will be 4 women and 4 men in each group. If you decide to take part in the study, you will be randomly assigned to one of the four groups, like flipping a coin. Once you are assigned to a group, you cannot change to another group. The four groups will each be given the same medication, but it will be provided in different doses.

- One group will take one Truvada® tablet one time each week for five weeks
- One group will take one Truvada® tablet two times each week for five weeks
- One group will take two Truvada® tablets two times each week for five weeks
- One group will take one Truvada® tablet each day for five weeks

All four groups are very important to the study. It is very important that each person takes his/her medication as provided. Because of this, all participants will be observed by a study employee when taking the medication so that we can be sure that the participant has taken the medication on the right day and at the right time. This means that some people will be required to come to the clinic every day (including Saturday and Sunday) to get their medication.

CRITERIA FOR STUDY PARTICIPATION
In order to participate in the study, you must meet all of the following criteria:

- Be 18 to 44 years of age, inclusive on the date of screening.
- Provide informed consent for the study.
- Provide information on how to contact and locate you
- Be willing to be tested for HIV infection and receive your test results.
- Be HIV negative.
- Have good kidney function.
- Be sexually active (at least one sex act - vaginal or anal intercourse - in the previous 30 days).
- Agree to use condoms.
- Women must:
    - Be pre-menopausal
    - Have regular menstrual cycles with at least 21 days between menses (unless on contraception that causes lack of or irregular menses)
    - Have a cervix
    - Have a negative urine pregnancy test
    - Have documentation of a normal Pap smear within 12 months
    - Be utilizing an alternative method of birth control in addition to condoms (hormonal contraceptive, diaphragm or have undergone surgical sterilization) or have a vasectomized exclusive male partner.
    - Not using spermicide as a means of birth control (with a condom or a diaphragm)

REASONS WHY YOU WOULD NOT BE ABLE TO PARTICIPATE
You will not be able to participate in the study if any of the following things apply to you:

- Blood tests that show the following:
  - Poor liver function
  - Anemia (low blood counts)
  - Abnormal laboratory values
- Co-enrollment in any other HIV research study that involves an experimental drug or prior enrollment in the active arm of a HIV vaccine trial.
- Active skin disorders.
- Excessive use of alcohol (more than 4 drinks a day on a regular basis).
- Any procedure that has altered the stomach or intestines or drug absorption.
- Abnormalities that will not allow the biopsies to be taken.
- Pelvic exam abnormalities.
- Women who have had cervical procedures within the previous 6 months.
- A positive test for syphilis, gonorrhea, chlamydia, hepatitis B, or HSV-2 (individuals with active lesions only).
- Bacterial vaginosis, vaginal candidiasis, or trichomonas (women only).
- Sexually transmitted infection within the last 3 months
- Women who are pregnant or breastfeeding.
- A reactive or positive HIV test result at screening or enrollment, even if you are found to be HIV-uninfected based on further testing.
- Medications that prolong blood clotting time.
- Medications that affect the kidney or liver.
- Anything else in the judgment of the study clinician that would make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

STUDY PROCEDURES

Screening and Enrollment Procedures:

If you agree to have the screening tests, the screening visits will be performed in one to two visits, but may be done in more than two if necessary. However, if a person agrees to be enrolled, the enrollment visit must take place within 30 days of the very first screening visit.

Screening Visit:
Your screening visit will happen after you read, discuss, understand, and sign this form. Study staff will help you understand the form and answer your questions before you sign this form. The procedures done at this visit will take about 2-3 hours.

The study staff will:

- Ask you where you live and other questions about you, your medical health, and your sexual practices.
- Discuss the details of "directly observed therapy" and identify how you will be able to come to the clinic (or other identified designation) to receive your medication. You will need to understand that you have a 1 in 4 chance of having to come to the clinic every day to receive your medication.
- If these questions show that you may be eligible for the study and you are a woman, you will give urine for a pregnancy test. If you are pregnant, you are not eligible for the study. The study staff will refer you to available sources of medical care and other services you may need. If the study is still open after your pregnancy, you can come back here to find out if you are eligible then.
- If you are a woman who is not pregnant or breastfeeding or a man, you will talk with study staff about HIV, HIV testing, and ways to avoid HIV and other infections passed through sex. You will be asked to give a blood sample (up to 20 mL or 4 teaspoons) for HIV testing. You will be told your result as soon as it is available. You will talk with the study staff about the meaning of your results and how you feel about them. Sometimes HIV tests are not clearly positive, but also not clearly negative. In that case, we will do more tests until we know your status for sure. You must receive your HIV test results to be in the study. If the test shows that you have HIV, you will not be eligible for the study. The study staff will tell you about other studies you may be eligible for, if any. They will refer you to available sources of medical care and other services you may need. Your partner(s) may also have access to free HIV counseling and testing if needed.
- Measure your weight.
- Perform a complete physical examination including your genital area.
- Examine inside your vagina (if you are a woman).
    - The study staff will collect fluid from your vagina to test for infections. These infections are called trichomoniasis, candidiasis, and bacterial vaginosis (BV).
- Talk with you about contraception and ways to prevent sexually transmitted diseases.
- Test your urine for:
    - Chlamydia and gonorrhea. These are sexually transmitted infections.
    - Common urine infections and the health of your kidneys
- Collect 40 mL (about 8 teaspoons) of blood for :
    - Syphilis testing. This is a sexually transmitted infection.
    - To check the health of your blood, liver and kidneys.
    - Hepatitis B testing. This is an infection of the liver that can be passed from mother to baby, through sex, or through body fluids infected with hepatitis B. If the tests show that you have hepatitis B that is active in your liver, you will not be eligible for the study. If the tests show that you have had hepatitis B in the past, but it is no longer active in your liver, or that you are immune to hepatitis B because of a prior immunization, you may be eligible for the study.
    - Plasma storage for study related testing and long-term storage (if you provide consent)
- Give you condoms.

The results of the tests listed above will be available within 1-2 weeks after your visit. You will be contacted about the results of your tests when they are available. A small amount of plasma will be stored from this visit. No other samples collected at the time of screening will be kept or used for any other tests other than those listed above.

Confirmation of Eligibility:
Once all the results of the screening tests are known, the study staff will:

- Tell you your test results from your screening visit and what they mean. If the results show that you might have some health problems, you may not be eligible for the study. Study staff will refer you to available sources of medical care and other services you may need. Later, if these problems resolve, you can come back to find out if you are eligible at that time.
- Refer you for treatment of a urinary tract infections and sexually transmitted infections, if you need it. If you need this treatment, you will not be eligible for the study.
- If you have a reactive or positive HIV test or a test that shows that you have active hepatitis B infection, you will not be eligible for the study, but you will be referred to the appropriate clinic or other medical resource.
- Give you referrals for other health services if you need them.
- Identify a clear plan for directly observed therapy.

Enrollment Visit:
If you are eligible for this study and decide to take part in the study, you will be asked to return for the enrollment visit. At that time, the study staff will:

- Perform a urine pregnancy test (women only)
- Collect up to 50 mL (about 10 teaspoons) of blood for:
    - HIV diagnostic testing
    - To check the amount of medication (Truvada®) in your blood.
- Review and update your contact details.
- Review and update your medical history.
- Talk with you again about HIV and other infections passed through sex and how to avoid these.
- Identify your study group.
- Give you condoms.
- Administer your first dose of study medication to you and review the procedures for all future doses to be observed by study personnel.

Before your second dose:
Before you take your second dose of study medication, you will have blood drawn (up to 40 mL or about 8 teaspoons).

At Visits on Days 7, 14, 21, and 28, you will:

- Tell study staff if you had any health problems since your last visit.
- Tell study staff about any medications, herbal treatments and supplements you are taking.
- Tell study staff any new information on where you live and how to keep in contact with you. They will use this information to remind you of visits. If you miss a visit, the study

staff will try to contact you by [*site-specific methods*]. They also may visit your home. They will try to reach you through the contact people that you list. If they talk to these people, they will not say why they want to reach you.
- Talk with study staff about ways to avoid HIV and other infections passed through sex.
- Talk to study staff about adherence to the directly observed therapy regimen (if necessary).
- Talk with study staff about contraception.
- Give a urine sample for a pregnancy test (women only, Day 28 only).
- Give blood (up to 65 mL or about 13 teaspoons):
    - To check the amount of medication (Truvada®) in your blood.
    - To confirm that the medication is not causing problems for certain parts of your body such as the liver and kidneys and to check the health of your blood (Days 14 and 28 only).
    - Plasma storage for study related testing and long-term storage (if you provide consent; Days 28).
- Get condoms.

The Enrollment and Days 7, 14, 21, and 28 visits will take 1-2 hours each.

At Visits on Days 35 and 49, you will:
- Participate in the same activities as the previous visits.
- Have an exam of the rectum and colon and small samples of your rectal tissue will be taken to measure the amount of the medications in your rectal tissue. For this, you will be taken to the endoscopy area where a doctor will perform a flexible sigmoidoscopy (also known as an endoscopy). To do this, the doctor will use an endoscope to look inside your rectum. An endoscope is a long, thin tube with a camera in it. The doctor will use a long wire with a tiny pincher to take up to 30 small biopsies of your intestinal lining. Each biopsy is a small piece of tissue measuring smaller than the surface of a pencil eraser and about the thickness of a dime.
- Have a clear, plastic, lubricated tube gently inserted into the rectum (no more than 4 inches) in order to collect the rectal luminal fluid with a swab.
- Have a vaginal exam (women only), and vaginal fluids, vaginal cells and small samples of vaginal tissue will be collected to measure the medication levels in your vaginal fluids, cells and tissue. (These samples will not be taken during menstruation so the timing of your menstrual cycle will have to be considered before you take the first study medication dose. Pregnancy tests will be performed just prior to any vaginal sampling and confirmed to be negative prior to any vaginal procedure.)
- Provide a semen sample (men only).
- Provide 50 mL (about 10 teaspoons) of blood for:
    - Tests to confirm that the medication is not causing problems for certain parts of your body such as the liver and kidneys,
    - HIV test and Hepatitis B test and (Day 49 only)
    - Plasma storage for study related testing and long-term storage (if you provide consent; Day 49 only).
- Urine for a pregnancy test (women only).

The Days 35 and 49 visits may take 2-4 hours each. In some cases, due to scheduling limitations, these visits may require visiting the clinic on 2 different days.

Men will be asked to abstain from sex for 24-48 hours before semen collection.

All participants should start a low fiber diet 3 days before each rectal biopsy procedure and have a clear liquid diet 12 hours prior to the procedure. They should also refrain from meals (or coffee) the day of the procedure. Enemas will be administered before the biopsy procedures take place. An enema is a procedure that introduces liquids into the rectum and colon through the anus to cleanse the lower intestinal tract.

One day after the biopsies, a study team member will contact you by phone to see how you're doing.

AT ANY TIME IN THE STUDY

If you have health problems that may be caused by sexually transmitted infections, you will:

- Have an exam of your genital area and inside your vagina.
- Give blood, urine, and/or vaginal fluid to test for infections passed through sex or other vaginal infections
- Be referred for treatment of any sexually transmitted infection.

Any time HIV or STI testing is done during the study, study staff will contact you to provide you with the test results as soon as they become available. Study staff will also provide you with information related to your health that is discovered at any study visit.

If you have to leave the study for any reason, you will have one additional follow-up visit where you will have the same activities as at the Day 7 through Day 21 visits as well as blood tests to confirm the drug has not caused any problems in certain parts of your body such as the liver and kidneys. You will also have an HIV and hepatitis b test and a urine pregnancy test (women only).

Study staff will review and assess any medical conditions that you have during the study. Study staff may contact you after the study has ended to be sure that you do not have any continuing medical problems that require medical attention.

POSSIBLE FUTURE TESTS

Some of the blood that you give during this study may be left over after all of the study tests are completed. The study staff also would like to keep your leftover blood. Future tests on stored blood may be unrelated to this study. You will be asked to sign at the end of this consent form to give permission for that. Even if you do not give permission to store your blood after the study, you can still be in this study. You may also withdraw your consent for specimen storage at any time.

RISKS AND/OR DISCOMFORTS

Blood Draws and Sexually Transmitted Infection Testing

Whenever your blood is drawn, you may:

- Feel discomfort or pain.
- Feel dizzy or faint.
- Have a bruise, swelling, small clot, or infection where the needle goes in your arm or finger.

When you have genital exams, you may:
- Feel discomfort in your genital area and/or inside your vagina.
- Women may have a small amount of vaginal bleeding which will stop shortly after the exam.

Rectal Exam and Tissue Samples

Whenever you have a rectal exam and tissue samples are taken, you may:
- Have mild discomfort
- Have the feeling of a "bloated stomach"

On extremely rare occasions, you may have:
- Pain
- Infection
- Bleeding or
- Perforation of the gastrointestinal tract (occurs about once out of every 100,000 procedures and may require hospitalization and surgical management)

When rectal fluid is collected via insertion of the plastic tube into the anus, on rare occasion you may:
- Experience mild discomfort
- Have pain (should you have another condition that is already causing pain in the area)

When you have an enema, you may experience:
- Temporary discomfort caused by a bloated or cramping feeling
- Some air may be pumped into the rectum causing gas
- On extremely rare occasion, the enema tube could make a hole in the rectum requiring surgery

You should not have anal sexual intercourse or insert anything into your rectum for at least 72 hours after the tissue sampling.

Vaginal Exam and Tissue Samples

Whenever you have a vaginal exam and tissue samples are taken, you may:
- Have discomfort or pain during the procedure and for a few hours after
- Have mild vaginal spotting (bleeding) for one or two days
- Have temporary discomfort with sexual intercourse

There is a very small risk that you may:
- Get an infection

- Have heavy bleeding

You should not have vaginal sexual intercourse or insert anything into our vagina for at least 72 hours after the tissue sampling.

Study Medications

About 5 out of 100 people with HIV taking Truvada® tablets have these occasional side effects which usually go away after stopping the drug:

- Upset stomach, vomiting, gas, loose or watery stools
- Dizziness or headache
- Abdominal pain
- Lack of energy/general body weakness
- Mild problems of kidney function that are only detected by laboratory tests
- Shortness of breath or cough
- Rash, including allergic reaction
- Anxiety
- Joint pain, muscle pain, or other pain syndrome
- Fever

Fewer than 5 out of 100 people with HIV taking Truvada® tablets have:

- Skin discoloration/darkening of the palms and/or soles of the feet

Potentially serious side effects are rare, but include:

- Liver function problems
- Serious kidney damage or failure
- Low phosphate levels (a chemical in the blood) or protein or sugar in the urine
- Inflammation or swelling and possible damage to the pancreas
- Bone pain and bone changes such as thinning and softening which may increase the risk of breakage
- Allergic reaction
- Lactic acidosis and severe hepatomegaly (enlarged liver) with steatosis (fatty liver) that may result in liver failure, other complications and death have been reported with the use of antiretroviral nucleoside analogues alone or in combination. The liver complications and death have been seen more often in women on these drug regimens. Some nonspecific symptoms that might indicate lactic acidosis include: unexplained weight loss, stomach discomfort, nausea, vomiting, fatigue, weakness, and shortness of breath.
- If you are infected with both Hepatitis B and HIV, you should be aware that your liver function tests may increase, and symptoms associated with hepatitis (an acute inflammation of the liver) may worsen if emtricitabine or tenofovir is stopped.

Some people taking emtricitabine, one of the ingredients in Truvada®, experienced the following side effects:

- Inability to sleep, unusual dreams
- Runny nose

- Rash, itching, which sometimes can be a sign of an allergic reaction
- Increases in pancreatic enzyme (substances in the blood), which could mean a problem with the pancreas
- Increased triglycerides
- Increased creatine phosphokinase (CPK), which could mean muscle damage

Some people taking tenofovir, one of the ingredients in Truvada®, experienced the following side effects:

- Depression
- Inflammation or swelling and possible damage to the pancreas and liver
- Allergic reaction: symptoms may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, a general feeling of illness or a potentially serious swelling of the face, lips, and/or tongue

You could have these side effects or other side effects that we do not know about.

Other Possible Risks

We do not know if there are other risks if you use herbal treatments or supplements while you are using the tablets. Please tell study staff if you are using any herbal treatments or supplements.

We will perform an HIV test, which is routinely done before HIV drugs are tested in non-HIV subjects. You will be counseled before and after this test is done. In accordance with some state laws, all positive tests must be reported to the state health department. In the event that you test positive for HIV, you will be notified and provided with counseling. If you do test positive, the test results will become part of public health records. A positive result will be placed in your hospital chart in order to provide you with the best of care. You are not required to make these results available to your insurance company or employer.

We will perform screening for common sexually transmitted diseases, including bacterial vaginosis, syphilis, gonorrhea, Chlamydia, HSV-2 and trichomonas. This is being done because infections could affect the measurements of drug concentrations in cervicovaginal fluid or vaginal tissue that will be taken in this study. Syphilis, gonorrhea, and Chlamydia are reportable sexually transmitted infections in some states, and positive results must be reported with your name to the local health department. With any positive result for any of these tests, you will be referred to an infectious diseases provider for confirmation (if needed) and treatment.
In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers immediately.

There may also be some social risks to participating in this study. You may feel embarrassed or uncomfortable with some of the questions you will be asked, some of the procedures that will be done, or some of the test results that you will receive. You may also experience stigma as a result of being involved in an HIV study because people may assume that you are HIV-infected.

Pregnancy and Breastfeeding

If you become pregnant during the study, or within 28 days after completing the study, you should notify the study personnel right away for safety monitoring. If you become pregnant during the study, you will stop using the tablets right away. The study staff will refer you to available sources of medical care and other services you or your baby may need. The study does not pay for this care.

Please tell your obstetrician about your study participation. Your study doctor will ask that you, or your obstetrician, provide updates on the progress of your pregnancy and its outcome. The study doctor will make this information available to the study sponsor for safety monitoring follow-up.

We do not know if Truvada® tablets have any effect on pregnancy, on the fetuses of women who use tablets when pregnant, or on the babies of women who use tablets when breastfeeding. Because of this, pregnant women and women who are breastfeeding will not be allowed to participate in this study. Women who join the study must use condoms in addition to an effective contraception and method must have pregnancy tests at the screening visit, before starting the study, and at visits 4, 5, and 6 while in the study. Effective contraception includes hormonal methods (such as the birth control pill or shot), intrauterine contraceptive device (IUCD); and sterilization. You should not use spermicides as a method of contraception while participating in this study.

BENEFITS

You will not receive any direct benefit from being in this study. You or others may benefit in the future from information learned in this study.

NEW INFORMATION

You will be told any new information learned during this study that might affect your willingness to stay in the study. For example, if information becomes available that shows that the medication may be causing bad effects, you will be told about this. You will also be told when the results of the study may be available, and how to learn about them.

WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be withdrawn from the study without your consent if any of the following occur:

- Become pregnant.
- Are breastfeeding.
- Are taking certain medications that affect your kidneys.
- Are unable or unwilling to follow all of the study procedures or instructions.
- Could be harmed by continuing to take tablets.
- The study is stopped or canceled.
- The study staff feels that staying in the study would be harmful to you.
- You are not able to attend clinic visits or complete all of the study procedures.
- You are not able to have directly observed therapy according to study instructions.

- Other reasons, as decided by the study staff.
- You become HIV positive

If you become HIV positive during the course of the study or are withdrawn for any reason, you will have a final study visit for safety testing before you are withdrawn from study participation.

ALTERNATIVES TO PARTICIPATION
[*Sites to include/amend the following if applicable:* There may be other studies going on here or in the community that you may be eligible for. If you wish, we will tell you about other studies that we know about. There also may be other places where you can go for HIV counseling and testing and contraception. We will tell you about those places if you wish.]

COSTS TO YOU
There is no cost to you for being in this study.

REIMBURSEMENT
[*Sites to insert information about local reimbursement amount and schedule:*]
You will receive [$xx] for your time, effort, and travel to and from the clinic.

CONFIDENTIALITY
We will do everything we can to keep your personal information confidential. To help us keep your personal information confidential, we have obtained a Certificate of Confidentiality from the US Federal Government. This certificate protects researchers from being forced to tell people who are not connected with this study, such as the court system, about your participation. However, it is not possible to guarantee confidentiality. Your personal information may be disclosed if required by law.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

The study staff will also use your personal information, if needed, to verify that you are not taking part in any other research studies. This includes other studies conducted by [site name] and studies conducted by other researchers that study staff know about. Any publication of this study will not use your name or identify you personally.

Your records may be reviewed by:
- the United States Food and Drug Administration (FDA)
- the United States National Institutes of Health (NIH)
- the United States Department of Heath and Human Services (DHHS), Office of Human Research Protection (OHRP)
- [*insert names of applicable IRBs/ECs*]
- study staff
- study monitors
- the company that makes Truvada® tablets (Gilead Sciences)

[Sites to include/amend the following if applicable:] [*Local/state/national*] regulations require study staff to report the names of people who test positive for [*HIV and other infections*] passed during sex to the [*local health authority*]. Outreach workers from the [*health authority*] may then contact you about informing your partners, since they also should be tested. If you do not want to inform your partners yourself, the outreach workers will contact them, according to the confidentiality guidelines of the [*health authority*].

RESEARCH-RELATED INJURY

[Sites to specify institutional policy:] It is unlikely that you will be injured as a result of study participation. If you are injured, the [*institution*] will give you immediate necessary treatment for your injuries. You [*will/will not*] have to pay for this treatment. You will be told where you can get additional treatment for your injuries. There is no program to pay money or give other forms of compensation for such injuries either through this institution or the NIH. You do not give up any legal rights by signing this consent form.

PROBLEMS OR QUESTIONS

If you ever have any questions about the study, or if you have a research-related injury, you should contact [*insert name of the investigator or other study staff*] at [*insert telephone number and/or physical address*].

If you have questions about your rights as a research participant, you should contact [*insert name or title of person on the IRB/EC or other organization appropriate for the site*] at [*insert physical address and telephone number*].

If you have questions about whom to contact at the research site, you should contact [*insert name of the investigator or community educator or CAB member*] at [*insert physical address and telephone number*].

SIGNATURES

[Insert signature blocks as required by the local IRB/EC:] If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to join the study, please sign your name or make your mark below. Also, please indicate if you are willing to allow your leftover samples to be stored for future testing.

Specimens Stored for Future Testing

_____ My initials indicate that any left-over samples may be stored for future testing:

_____ I do not agree to allow leftover samples to be saved for long term storage and future testing after the study

_____	_____	_____
Participant Name (print)	Participant Signature	Date
_____	_____	_____
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
_____	_____	_____
Witness Name (print)	Witness Signature	Date

APPENDIX III: SCREENING/ENROLLMENT SAMPLE INFORMED CONSENT FORM (Non-Intensive Sampling Cohort)

SAMPLE INFORMED CONSENT DOCUMENT (NON-Intensive Sampling Cohort)

HPTN 066

DOSE-PROPORTIONALITY AND INTRA-INDIVIDUAL VARIABILITY OF INTRACELLULAR TENOFOVIR DIPHOSPHATE AND EMTRICITABINE TRIPHOSPHATE IN HEALTHY VOLUNTEERS

29 NOVEMBER 2010
VERSION 2.0

PRINCIPAL INVESTIGATOR: [Insert Name]
PHONE: [Insert Number]

GENERAL OVERVIEW

You are being asked to take part in a research study. Joining this study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives information about the study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign your name on this form. You will be given a copy of this form to keep.

Before you learn about the study, it is important that you know the following:

- Your participation is voluntary. You do not have to take part in the screening tests or in the study.
- You may decide not to take part in the study or to leave the study at any time without losing your regular medical care.
- If you decide not to take part in the study, you can still join another study at a later time if there is one available and you qualify.
- You cannot join this study if you are taking part in another study of drugs, medical devices or vaginal/rectal products. You are asked to tell the study staff about any other studies you are taking part in or thinking of taking part in. This is very important for your safety.

PURPOSE OF THE STUDY
This study is being conducted to look at how the body handles the drug Truvada® when it is given in different amounts and at different time schedules. This study will also look at the difference of medication amount in men and women.

The study will look at levels of Truvada® in the blood, reproductive tract and gastrointestinal tract of female and male volunteers. The aim is to establish how much of the drug (taken by mouth) reaches the reproductive and gastrointestinal tracts when given at different doses and at different dose schedules. It is believed that the presence of this drug in the reproductive and gastrointestinal tracts may be beneficial in the prevention of the transmission of the AIDS virus. However, half of the study participants will only participate in the blood portion of the study. That is the group that you are being evaluated for. Potential participants will decide with study staff which group they will enroll in before they are consented for the study.

The study will take samples of blood once a week for the first 5 weeks after taking Truvada® according to the dose you are assigned to. All of these samples are to look at drug concentrations. The study will also collect information on the safety of the medication.

Truvada® is a tablet that is a combination of two medications—emtricitabine and tenofovir. This medication is used to treat people who have HIV. The tablets are generally safe when used as treatment for HIV. These tablets are not a cure for HIV/AIDS, but they are very effective for improving the health of people who have HIV/AIDS. Although these tablets improve the health of people with HIV, **we do not know if they work to protect against HIV.** The results of this study may be used in the future to determine if this medication can be taken by healthy people to prevent them from getting HIV.

The United States Food and Drug Administration (U.S. FDA) has been informed of this study. Truvada has not been approved by the U.S. FDA for the prevention of HIV.

About 32 participants will be enrolled in the study at 2 different sites in the United States. About 16 people will be enrolled in the study here at [study site]. The study should take approximately 12 months to complete. Each participant will be followed for approximately 2 months.

Before you decide whether to take part in the study, we would like to explain the purpose of the study, the risks and benefits, what is expected of you, and what to expect from us. This consent form might contain some words that are unfamiliar. Please ask questions about anything you do not understand or want to learn more about.

STUDY GROUPS
There are four study groups. Each group will have 8 people. There will be 4 women and 4 men in each group. If you decide to take part in the study, you will be randomly assigned to one of the four groups, like flipping a coin. Once you are assigned to a group, you cannot change to another group. The four groups will each be given the same medication, but it will be provided in different doses.

- One group will take one Truvada® tablet one time each week for five weeks
- One group will take one Truvada® tablet two times each week for five weeks
- One group will take two Truvada® tablets two times each week for five weeks
- One group will take one Truvada® tablet each day for five weeks

All four groups are very important to the study. It is very important that each person takes his/her medication as provided. Because of this, all participants will be observed by a study employee when taking the medication so that we can be sure that the participant has taken the medication on the right day and at the right time. This means that some people will be required to come to the clinic every day (including Saturday and Sunday) to get their medication.

CRITERIA FOR STUDY PARTICIPATION
In order to participate in the study, you must meet all of the following criteria:

- Be 18 to 44 years of age, inclusive on the date of screening.
- Provide informed consent for the study.
- Provide information on how to contact and locate you
- Be willing to be tested for HIV infection and receive your test results.
- Be HIV negative.
- Have good kidney function.
- Be sexually active (at least one sex act - vaginal or anal intercourse - in the previous 30 days).
- Agree to use condoms.
- Women must:
    - Be pre-menopausal
    - Have regular menstrual cycles with at least 21 days between menses (unless on contraception that causes lack of or irregular menses)
    - Have a negative urine pregnancy test
    - Be utilizing an alternative method of birth control in addition to condoms (hormonal contraceptive, diaphragm or have undergone surgical sterilization) or have a vasectomized exclusive male partner.

REASONS WHY YOU WOULD NOT BE ABLE TO PARTICIPATE
You will not be able to participate in the study if any of the following things apply to you:

- Blood tests that show the following:
    - Poor liver function
    - Anemia (low blood counts)
    - Abnormal laboratory values
- Co-enrollment in any other HIV research study that involves an experimental drug or prior enrollment in the active arm of a HIV vaccine trial.

- Active skin disorders.
- Positive test for Hepatitis B
- Excessive use of alcohol (more than 4 drinks a day on a regular basis).
- Women who are pregnant or breastfeeding.
- A reactive or positive HIV test result at screening or enrollment, even if you are found to be HIV-uninfected based on further testing.
- Medications that affect the kidney or liver.
- Anything else in the judgment of the study clinician that would make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

STUDY PROCEDURES

Screening and Enrollment Procedures:

If you agree to have the screening tests, the screening visits will be performed in one to two visits, but may be done in more than two if necessary. However, if a person agrees to be enrolled, the enrollment visit must take place within 30 days of the very first screening visit.

Screening Visit:
Your screening visit will happen after you read, discuss, understand, and sign this form. Study staff will help you understand the form and answer your questions before you sign this form. The procedures done at this visit will take about 2-3 hours.

The study staff will:

- Ask you where you live and other questions about you, your medical health, and your sexual practices.
- Discuss the details of "directly observed therapy" and identify how you will be able to come to the clinic (or other identified designation) to receive your medication. You will need to understand that you have a 1 in 4 chance of having to come to the clinic every day to receive your medication.
- If these questions show that you may be eligible for the study and you are a woman, you will give urine for a pregnancy test. If you are pregnant, you are not eligible for the study. The study staff will refer you to available sources of medical care and other services you may need. If the study is still open after your pregnancy, you can come back here to find out if you are eligible then.
- If you are a woman who is not pregnant or breastfeeding or a man, you will talk with study staff about HIV, HIV testing, and ways to avoid HIV and other infections passed through sex. You will be asked to give a blood sample (up to 20 mL or 4 teaspoons) for HIV testing. You will be told your result as soon as it is available. You will talk with the study staff about the meaning of your results and how you feel about them. Sometimes HIV tests are not clearly positive, but also not clearly negative. In that case, we will do more tests until we know your status for sure. You must receive your HIV test results to be in the study. If the test shows that you have HIV, you will not be eligible for the study.

The study staff will tell you about other studies you may be eligible for, if any. They will refer you to available sources of medical care and other services you may need. Your partner(s) may also have access to free HIV counseling and testing if needed.

- Measure your weight.
- Perform a complete physical examination.
- Talk with you about contraception and ways to prevent sexually transmitted diseases.
- Test your urine for:
  - The health of your kidneys
- Collect 35 mL (about 7 teaspoons) of blood:
  - To check the health of your blood, liver and kidneys.
  - Hepatitis B testing. This is an infection of the liver that can be passed from mother to baby, through sex, or through body fluids infected with hepatitis B. If the tests show that you have hepatitis B that is active in your liver, you will not be eligible for the study. If the tests show that you have had hepatitis B in the past, but it is no longer active in your liver, or that you are immune to hepatitis B because of a prior immunization, you may be eligible for the study.
  - Plasma storage for study related testing and long-term storage (if you provide consent)
- Give you condoms.

The results of the tests listed above will be available within 1-2 weeks after your visit. You will be contacted about the results of your tests when they are available. A small amount of plasma will be stored from this visit. No other samples collected at the time of screening will be kept or used for any other tests other than those listed above.

Confirmation of Eligibility:
Once all the results of the screening tests are known, the study staff will:

- Tell you your test results from your screening visit and what they mean. If the results show that you might have some health problems, you may not be eligible for the study. Study staff will refer you to available sources of medical care and other services you may need. Later, if these problems resolve, you can come back to find out if you are eligible at that time.
- Refer you for treatment of a urinary tract infections and sexually transmitted infections, if you need it. If you need this treatment, you will not be eligible for the study.
- If you have a reactive or positive HIV test or a test that shows that you have active hepatitis B infection, you will not be eligible for the study, but you will be referred to the appropriate clinic or other medical resource.
- Give you referrals for other health services if you need them.
- Identify a clear plan for directly observed therapy.

Enrollment Visit:
If you are eligible for this study and decide to take part in the study, you will be asked to return for the enrollment visit. At that time, the study staff will:

- Perform a urine pregnancy test (women only)
- Collect up to 50 mL of blood (about 10 teaspoons) for:

    - HIV diagnostic testing and
    - To check the amount of medication (Truvada®) in your blood.
- Review and update your contact details.
- Review and update your medical history.
- Talk with you again about HIV and other infections passed through sex and how to avoid these.
- Identify your study group.
- Give you condoms.
- Administer your first dose of study medication to you and review the procedures for all future doses to be observed by study personnel.

Before your second dose:
Before you take your second dose of study medication, you will have blood drawn (40 mL or about 8 teaspoons).

At Visits on Days 7, 14, 21, 28, 35, and 49 you will:
- Tell study staff if you had any health problems since your last visit.
- Tell study staff about any medications, herbal treatments and supplements you are taking.
- Tell study staff any new information on where you live and how to keep in contact with you. They will use this information to remind you of visits. If you miss a visit, the study staff will try to contact you by [*site-specific methods*]. They also may visit your home. They will try to reach you through the contact people that you list. If they talk to these people, they will not say why they want to reach you.
- Talk with study staff about ways to avoid HIV and other infections passed through sex.
- Talk to study staff about adherence to the directly observed therapy regimen (if necessary).
- Talk with study staff about contraception.
- Give blood (up to 65 mL or about 13 teaspoons):
    - To check the health of your blood, liver and kidneys.
    - To check the amount of medication (Truvada®) in your blood.
    - To confirm that the medication is not causing problems for certain parts of your body such as the liver and kidneys (Day 14, 28, 35, and 49 only).
    - For an HIV test and Hepatitis test (Day 49 only).
    - Plasma storage for study related testing and long-term storage (if you provide consent; Days 28 and 49 only).
- Get condoms.
- Give a urine sample for a pregnancy test (Women only; Day 28 and 49 only).

The Enrollment and all subsequent study visits will take 1-2 hours each.

AT ANY TIME IN THE STUDY

If you have health problems that may be caused by sexually transmitted infections, you will:

- Have an exam of your genital area and inside your vagina.
- Give blood, urine, and/or vaginal fluid to test for infections passed through sex or other vaginal infections
- Be referred for treatment of any sexually transmitted infection.

Any time HIV or STI testing is done during the study, study staff will contact you to provide you with the test results as soon as they become available. Study staff will also provide you with information related to your health that is discovered at any study visit.

If you have to leave the study for any reason, you will have one additional follow-up visit where you will have the same activities as at the Day 7 through Day 21 visits as well as blood tests to confirm the drug has not caused any problems in certain parts of your body such as the liver and kidneys. You will also have an HIV and hepatitis b test and a urine pregnancy test (women only).

Study staff will review and assess any medical conditions that you have during the study. Study staff may contact you after the study has ended to be sure that you do not have any continuing medical problems that require medical attention.

POSSIBLE FUTURE TESTS

Some of the blood that you give during this study may be left over after all of the study tests are completed. The study staff also would like to keep your leftover blood. Future tests on stored blood may be unrelated to this study. You will be asked to sign at the end of this consent form to give permission for that. Even if you do not give permission to store your blood after the study, you can still be in this study. You may also withdraw your consent for specimen storage at any time.

RISKS AND/OR DISCOMFORTS

Blood Draws and Sexually Transmitted Infection Testing

Whenever your blood is drawn, you may:

- Feel discomfort or pain.
- Feel dizzy or faint.
- Have a bruise, swelling, small clot, or infection where the needle goes in your arm or finger.

When you have genital exams, you may:

- Feel discomfort in your genital area and/or inside your vagina.
- Women may have a small amount of vaginal bleeding which will stop shortly after the exam.

Study Medications

About 5 out of 100 people with HIV taking Truvada® tablets have these occasional side effects which usually go away after stopping the drug:

- Upset stomach, vomiting, gas, loose or watery stools

- Dizziness or headache
- Abdominal pain
- Lack of energy/general body weakness
- Mild problems of kidney function that are only detected by laboratory tests
- Shortness of breath or cough
- Rash, including allergic reaction
- Anxiety
- Joint pain, muscle pain, or other pain syndrome
- Fever

Fewer than 5 out of 100 people with HIV taking Truvada® tablets have:

- Skin discoloration/darkening of the palms and/or soles of the feet

Potentially serious side effects are rare, but include:

- Liver function problems
- Serious kidney damage or failure
- Low phosphate levels (a chemical in the blood) or protein or sugar in the urine
- Inflammation or swelling and possible damage to the pancreas
- Bone pain and bone changes such as thinning and softening which may increase the risk of breakage
- Allergic reaction
- Lactic acidosis and severe hepatomegaly (enlarged liver) with steatosis (fatty liver) that may result in liver failure, other complications and death have been reported with the use of antiretroviral nucleoside analogues alone or in combination. The liver complications and death have been seen more often in women on these drug regimens. Some nonspecific symptoms that might indicate lactic acidosis include: unexplained weight loss, stomach discomfort, nausea, vomiting, fatigue, weakness, and shortness of breath.
- <u>If you are infected with both Hepatitis B and HIV, you should be aware that your liver function tests may increase, and symptoms associated with hepatitis (an acute inflammation of the liver) may worsen if emtricitabine or tenofovir is stopped</u>.

Some people taking emtricitabine, one of the ingredients in Truvada®, experienced the following side effects:

- Inability to sleep, unusual dreams
- Runny nose
- Rash, itching, which sometimes can be a sign of an allergic reaction
- Increases in pancreatic enzyme (substances in the blood), which could mean a problem with the pancreas
- Increased triglycerides
- Increased creatine phosphokinase (CPK), which could mean muscle damage

Some people taking tenofovir, one of the ingredients in Truvada®, experienced the following side effects:

- Depression
- Inflammation or swelling and possible damage to the pancreas and liver

- Allergic reaction: symptoms may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, a general feeling of illness or a potentially serious swelling of the face, lips, and/or tongue

You could have these side effects or other side effects that we do not know about.

Other Possible Risks

We do not know if there are other risks if you use herbal treatments or supplements while you are using the tablets. Please tell study staff if you are using any herbal treatments or supplements.

We will perform an HIV test, which is routinely done before HIV drugs are tested in non-HIV subjects. You will be counseled before and after this test is done. In accordance with some state laws, all positive tests must be reported to the state health department. In the event that you test positive for HIV, you will be notified and provided with counseling. If you do test positive, the test results will become part of public health records. A positive result will be placed in your hospital chart in order to provide you with the best of care. You are not required to make these results available to your insurance company or employer.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers immediately.

There may also be some social risks to participating in this study. You may feel embarrassed or uncomfortable with some of the questions you will be asked, some of the procedures that will be done, or some of the test results that you will receive. You may also experience stigma as a result of being involved in an HIV study because people may assume that you are HIV-infected.

Pregnancy and Breastfeeding

If you become pregnant during the study, or within 28 days after completing the study, you should notify the study personnel right away for safety monitoring. If you become pregnant during the study, you will stop using the tablets right away. The study staff will refer you to available sources of medical care and other services you or your baby may need. The study does not pay for this care.

Please tell your obstetrician about your study participation. Your study doctor will ask that you, or your obstetrician, provide updates on the progress of your pregnancy and its outcome. The study doctor will make this information available to the study sponsor for safety monitoring follow-up.

We do not know if Truvada® tablets have any effect on pregnancy, on the fetuses of women who use tablets when pregnant, or on the babies of women who use tablets when breastfeeding. Because of this, pregnant women and women who are breastfeeding will not be allowed to participate in this study. Women who join the study must use condoms in addition to an effective contraception method and must have pregnancy tests at the screening visit, before starting the study, and at visit 4 while in the study. Effective contraception includes hormonal methods (such as the birth control pill or shot), intrauterine contraceptive device (IUCD); and sterilization. You should not use spermicides as a method of contraception while participating in this study.

BENEFITS

You will not receive any direct benefit from being in this study. You or others may benefit in the future from information learned in this study.

NEW INFORMATION
You will be told any new information learned during this study that might affect your willingness to stay in the study. For example, if information becomes available that shows that the medication may be causing bad effects, you will be told about this. You will also be told when the results of the study may be available, and how to learn about them.

WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT
You may be withdrawn from the study without your consent if any of the following occur:

- Become pregnant.
- Are breastfeeding.
- Are taking certain medications that affect your kidneys.
- Are unable or unwilling to follow all of the study procedures or instructions.
- Could be harmed by continuing to take tablets.
- The study is stopped or canceled.
- The study staff feels that staying in the study would be harmful to you.
- You are not able to attend clinic visits or complete all of the study procedures.
- You are not able to have directly observed therapy according to study instructions.
- Other reasons, as decided by the study staff.
- You become HIV positive

If you become HIV positive during the course of the study or are withdrawn for any reason, you will have a final study visit for safety testing before you are withdrawn from study participation.

ALTERNATIVES TO PARTICIPATION
[*Sites to include/amend the following if applicable:* There may be other studies going on here or in the community that you may be eligible for. If you wish, we will tell you about other studies that we know about. There also may be other places where you can go for HIV counseling and testing and contraception. We will tell you about those places if you wish.]

COSTS TO YOU
There is no cost to you for being in this study.

REIMBURSEMENT
[*Sites to insert information about local reimbursement amount and schedule:*]
You will receive [$xx] for your time, effort, and travel to and from the clinic.

CONFIDENTIALITY
We will do everything we can to keep your personal information confidential. To help us keep your personal information confidential, we have obtained a Certificate of Confidentiality from the US Federal Government. This certificate protects researchers from being forced to tell people who are not connected with this study, such as the court system, about your participation. However, it is not possible to guarantee confidentiality. Your personal information may be disclosed if required by law.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

The study staff will also use your personal information, if needed, to verify that you are not taking part in any other research studies. This includes other studies conducted by [site name] and studies conducted by other researchers that study staff know about. Any publication of this study will not use your name or identify you personally.

Your records may be reviewed by:
- the United States Food and Drug Administration (FDA)
- the United States National Institutes of Health (NIH)
- the United States Department of Heath and Human Services (DHHS), Office of Human Research Protection (OHRP)
- [*insert names of applicable IRBs/ECs*]
- study staff
- study monitors
- the company that makes Truvada® tablets (Gilead Sciences)

[Sites to include/amend the following if applicable:] [*Local/state/national*] regulations require study staff to report the names of people who test positive for [*HIV and other infections*] passed during sex to the [*local health authority*]. Outreach workers from the [*health authority*] may then contact you about informing your partners, since they also should be tested. If you do not want to inform your partners yourself, the outreach workers will contact them, according to the confidentiality guidelines of the [*health authority*].

RESEARCH-RELATED INJURY
[Sites to specify institutional policy:] It is unlikely that you will be injured as a result of study participation. If you are injured, the [*institution*] will give you immediate necessary treatment for your injuries. You [*will/will not*] have to pay for this treatment. You will be told where you can

get additional treatment for your injuries. There is no program to pay money or give other forms of compensation for such injuries either through this institution or the NIH. You do not give up any legal rights by signing this consent form.

PROBLEMS OR QUESTIONS
If you ever have any questions about the study, or if you have a research-related injury, you should contact [*insert name of the investigator or other study staff*] at [*insert telephone number and/or physical address*].

If you have questions about your rights as a research participant, you should contact [*insert name or title of person on the IRB/EC or other organization appropriate for the site*] at [*insert physical address and telephone number*].

If you have questions about whom to contact at the research site, you should contact [*insert name of the investigator or community educator or CAB member*] at [*insert physical address and telephone number*].

SIGNATURES

[Insert signature blocks as required by the local IRB/EC:] If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to join the study, please sign your name or make your mark below. Also, please indicate if you are willing to allow your leftover samples to be stored for future testing.

Specimens Stored for Future Testing

_____ My initials indicate that any left-over samples may be stored for future testing:

_____ I do not agree to allow leftover samples to be saved for long term storage and future testing after the study

_____	_____	_____
Participant Name (print)	Participant Signature	Date
_____	_____	_____
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
_____	_____	_____
Witness Name (print)	Witness Signature	Date