STUDY PROTOCOL

Quantification of Estradiol's Impact on Nucleotides in Cellular Populations of the Lower GI Tract

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Quantification of Estradiol's Impact on Nucleotides in Different Cellular Populations of the Lower Gastrointestinal Tract

Protocol Number IRB # 19-0333

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1. List of Abbreviations and Acronyms

ACTU	AIDS Clinical Trials Unit
AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome
aPTT	activated partial thromboplastin time
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the time vs. concentration curve
BP	blood pressure
CBC	complete blood count
CPAC	Clinical Pharmacology Analytical Chemistry
CPT	cell preparation tube
CRF	case report form
CT	Chlamydia (Chlamydia trachomatis)
CTRC	Clinical and Translational Research Center
DAIDS	Division of AIDS
DBS	dried blood spots
DNA	deoxyribonucleic acid
EAE	expedited adverse event (reporting)
EC	ethics committee
eCcr	estimated calculated creatinine clearance
FDA	(United States) Food and Drug Administration
FHT	feminizing hormone therapy
FTC	emtricitabine
FTC-TP	emtricitabine triphosphate
GC	gonorrhea (Neisseria Gonorrhoeae)
GCP	good clinical practices
GI	gastrointestinal
HepBsAg	hepatitis B surface antigen
Hep C Ab	hepatitis C antibody
HIV	Human Immunodeficiency Virus

HR	heart rate
ICH	International Conference on Harmonisation
IDS	investigational drug service
IRB	institutional review board
IUD	intrauterine device
LLN	lower limit of normal
LLOQ	lower limit of quantification
MRN	medical record number
MSM	men who have sex with men
NC TraCS	North Carolina Translational and Clinical Sciences Institute
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health
NRTI	nucleos(t)ide reverse transcriptase inhibitor
OHRP	Office for Human Research Protections
PD	pharmacodynamics
PI	principal investigator
PK	pharmacokinetics
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
RR	respiration rate
SAE	serious adverse event
SOP	standard operating procedures
SST	serum separator tube
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
TGW	transgender women
TraCS	The North Carolina Translational and Clinical Sciences Institute
ULN	upper limit of normal
UNC	University of North Carolina

2. List of Figures and Tables

3. Terminology for Study Drug

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

4. Protocol Team Roster

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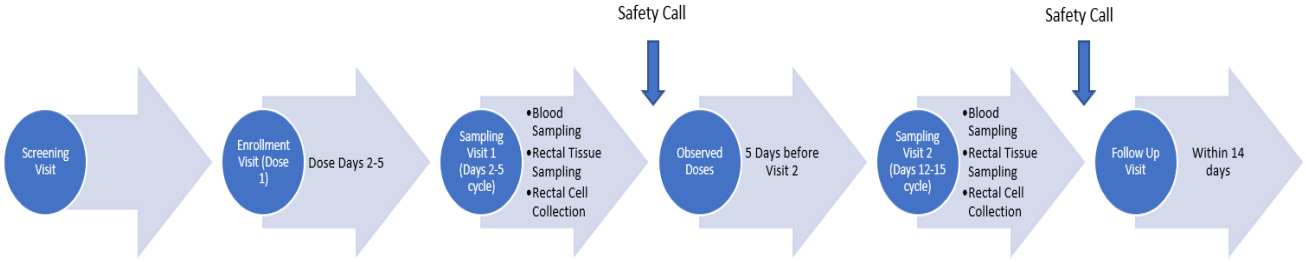
5. Protocol Summary

Protocol Chair:	Mackenzie Cottrell, PharmD, MS
Sample Size:	10 cisgender females
Study Population:	Premenopausal healthy volunteer cisgender women between 18-49 years of age with an intact gastrointestinal tract
Study Site(s):	NC TraCS Clinical Translational Research Center at the University of North Carolina, Chapel Hill, NC USA
Study Design:	Single-center, open-label PK sampling study
Study Duration:	Women will be enrolled in the study within 42 days of screening, depending on the timing of their menstrual cycle, and then will be on active study for two weeks. A follow-up visit will occur within 14 days.
Study Products:	Truvada®
Study Regimen:	Five witnessed doses daily prior to each sampling visit
Primary Objectives:	To assess the impact of high and low *in vivo* estradiol exposure on PrEP nucleotide concentrations in different cellular populations of the lower GI tract
Hypothesis:	PrEP nucleotide concentrations in total rectal cells collected via cytobrush will significantly correlate with concentrations in $CD4^+$ T cells isolated from rectal tissue biopsies regardless of *in vivo* estradiol exposure.
Secondary Objectives:	To quantify the relationship between estradiol, progesterone and testosterone on PrEP nucleotide concentrations in rectal and peripheral blood mononuclear cells To quantify the relationship between estradiol, progesterone and testosterone on PrEP concentrations in plasma

6. Overview of Study Design and Allocation Scheme

A) Allocation and Tissue Sampling Scheme:

Figure 1. Sampling Schema Pictorial



B) Study Procedure Time and Event**:**

Time Period → Activity ↓	Screen (-42 to 0 Days)	Enrollment /Dose 1	Dosing Days	Sampling Visit 1	Dosing Days	Sampling Visit 2	Follow-up (Within 14 days)
Eligibility	X	X					
Informed Consent	X						
Review of Medical History	X	X					
History of Menses	X						
Vital Signs [a]	X			X		X	X
Complete Physical Examination	X						
Targeted Physical Examination				X		X	X
Virology Tests [b]	X						X
Pregnancy Test [c]	X	X		X		X	X
STD Tests [d]	X						
Urine Drug Test	X						
Safety Labs [e]	X	X					X
Medication Dosing [f]		X	X		X		
Blood Sampling				X		X	
Rectal Cell Collection by cytobrush				X		X	
Rectal Tissue Biopsy				X		X	
Adverse Event Assessment		X		X		X	X

a. Vital signs include: blood pressure, pulse, respiratory rate, temperature, and weight. Height should be documented at the screening visit.

b. Virology tests will include HIV Ag/Ab test, HbsAg, and Hepatitis C antibody at screening visit. HIV testing will be repeated at follow up, as well as anytime infection is suspected.

c. Serum pregnancy test will be obtained at screening visit. Urine pregnancy test will be obtained at enrollment and follow up. Particpants will also have urine pregnancy test prior to sampling on assigned biopsy days.
d. Participants will be tested for the following sexually transmitted diseases: syphilis, gonorrhea, and chlamydia.
e. Safety labs include: CBC with differential, urine drug screen, and the following serum chemistries: Na, K, Cl, CO2, BUN, SCr, glucose, Alb, total protein, AST, ALT, Alkaline phosphatase, total bilirubin. PT/INR and aPTT will be obtained at screening. CBC without differential at follow up.
f. Member of study team will witness dose administration.

7. Key Roles

7.1 Protocol identification

Protocol title: Quantification of Estradiol's Impact on Nucleotides in Different Cellular Populations of the Lower Gastrointestinal Tract

7.2 Clinical Laboratories

McLendon Labs

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

1. Urine drug screen
2. Serum pregnancy test
3. Syphilis (RPR) titer
4. HIV diagnostic testing
5. Hepatitis testing
6. Safety labs
    a. CBC (with differential)
    b. Coagulation tests (PT/INR and aPTT)
    c. Serum Chemistries

UNC CFAR Virology, Immunology and Microbiology (VIM) Core

The following samples/tests will be processed at UNC STD-CRC Core Lab:

1. Chlamydia test
2. Gonorrhea test

Reproductive Endocrinology Laboratory

The following analyses will be performed in a core lab directed by Dr. Frank Stanczyk at the Keck School of Medicine of the University of Sothern California:

1. Estradiol, Progesterone, Testosterone serum concentrations

UNC CFAR Clinical Pharmacology and Analytical Chemistry (CPAC) Core

The following analyses will be performed at the CPAC lab:

1. Plasma PrEP (TFV and FTC) concentrations
2. PBMC PrEP nucleotide (TFVdp, FTCtp, dATP and dCTP) concentrations
3. Rectal tissue and total rectal cells PrEP nucleotide (TFVdp, FTCtp, dATP and dCTP) concentrations

This laboratory, directed by Angela Kashuba, was created to provide clinical pharmacology expertise and laboratory support to facilitate clinical and preclinical HIV/AIDS research. The Core has been CLIA certified since December 2004 and CAP certified since 2008 and participates in four proficiency testing activities per year. The Core has > 90% accuracy for all antiretrovirals tested, has been awarded certificates of excellence and is approved to perform TDM for all PIs and NNRTIs. Sample storage, processing, and HPLC methods are performed in approximately 6000 square feet of space in in the UNC Eshelman School of Pharmacy Genetic Medicine Building. This laboratory has validated analytical methods for all currently marketed antiretrovirals.

7.3 Data Management Center

Data management will be maintained by the research coordinator with oversight from the principle investigator. The study records will be stored in a locked filing cabinet in a locked office located at 1100 Genetic Medicine Building, 120 Mason Farm Road,

Chapel Hill, NC 27599. Data will be transferred by the investigators to a password protected Microsoft Access database and stored on the UNC Secure Network Attached Storage (Secure Nas) server, which is administered and maintained by UNC IT staff, and has custom port configurations (Windows firewall) which block all unused ports and limit access to open ports based on IP address. Security logging and auditing follow industry best practices. Access to the server is restricted to key personnel including investigators, and computer support staff. User access to server resources via network connection is limited via firewall configuration, IP address filtering, and encrypted user authentication protocols. Access to the study data will be further restricted via user roles. Application security for data entry will be protected using firewalling and IP address filtering. User authentication will be managed by a single-sign-on model of log in. Passwords for access to server resources are managed in a Microsoft Active Directory repository. Passwords for applications are managed via a single-sign-on (SSO) application which stores passwords using a one-way 128 bit hashing algorithm (MD5). Password policies force users to employ “strong” passwords, which use numbers, letters and special characters. Passwords must change at least every 90 days. All data will be de-identified and assigned a nonsense random unique identifier.

7.4 Study Operations

This study will be conducted in accordance with ICH/GCP, The Code of Federal Regulations, Office for Human Research Protections (OHRP) and FDA regulations and guidance, in addition to department Standard Operating Procedures

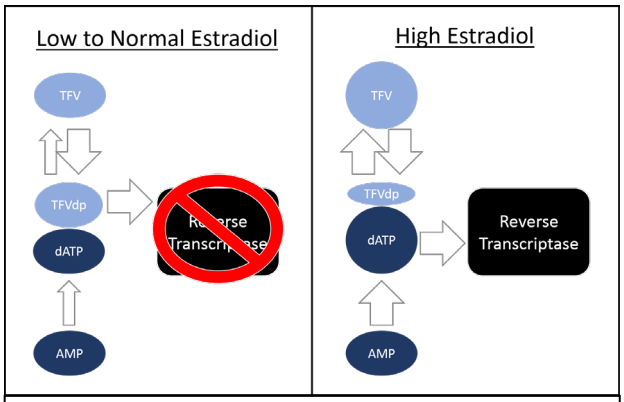
The Principal Investigator (PI) accepts ultimate responsibility for the quality of the data, subject safety and protocol adherence for all research conducted through the UNC NC TraCS CTRC. She also accepts responsibility for the overall functioning of the site.

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

8. Scientific Rationale

Female steroid sex hormones modulate enzymes involved in maintaining PrEP nucleotide balance. Currently only a fixed dose combination of 2 nucleos(t)ide reverse transcriptase inhibitors (NRTIs), tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC), is FDA approved for HIV pre-exposure prophylaxis (PrEP). TDF and FTC are intracellularly phosphorylated to their active forms (TFVdp and FTCtp), which compete with the host cells' naturally occurring deoxynucleotides (dATP and dCTP) for incorporation into the proviral DNA strand to block reverse

Figure 2 Theoretical Framework



Tenofovir (TFV) is intracellularly phosphorylated to its active form (TFVdp), which is chemically similar to deoxyadenosine triphosphate (dATP). TFVdp competes for incorporation into proviral HIV DNA by reverse transcriptase (RT), which results in chain termination. Estradiol reduces TFVdp and increases dATP minimizing the likelihood that RT will incorporate TFVdp.

transcription. The female sex steroid hormone, estradiol, increases expression of 5'nucleotidase enzymes involved in maintaining intracellular nucleotide balance.[1] Particularly, ecto-5'nucleotidase (located on the extracellular surface of the plasma membrane) catalyzes AMP to ADP, which is a precursor in the biochemical formation of dATP. Conversely, intracellularly expressed cytosolic 5'nucleotidase may dephosphorylate and deactivate PrEP's active metabolites (TFVdp and FTCtp; Figure 2).

Female sex steroid hormones may disproportionately impact PrEP pharmacology in different cellular populations. Previously, investigators have utilized several different sampling strategies from which to draw conclusions about PrEP pharmacology in HIV transmission sites. These surrogate measures include 1) collecting biopsies for measurement in isolated $CD4^+$ T cells – generally considered gold standard; 2) tissue homogenization for measurement in mixed cell populations; 3) collecting swabs and cytobrush for measurement in fluid or mixed cell populations. A previous clinical study noted a predictive relationship between parent TFV measured in rectal fluids versus tissue homogenates in individuals taking oral TDF.[2] Additionally, Yang et al. demonstrated a predictive correlation between TFVdp in mixed cell populations of homogenized rectal tissue vs $CD4^+$ T cells isolated from rectal tissue following topical application of TFV gel.[3] Yet the impact of estradiol on these surrogate relationships has not been well characterized. One *in vitro* study utilizing very high concentrations of TFV (1mg/ml) suggests that estradiol could disproportionately impact TFVdp formation in epithelial cells (enhanced formation) vs $CD4^+$ T cells (diminished formation)[4], which could confound the relationship between surrogate measures of mixed vs isolated cell populations.

We aim to quantify estradiol's impact on PrEP nucleotide concentrations in mixed cell populations obtained via cytobrush sampling vs $CD4^+$ T cells isolated from rectal tissue biopsies in order to understand whether cytobrush is a valid PrEP pharmacology surrogate in people taking exogenous estradiol.

9. Study Objectives

9.1 Primary Objective:

To assess the impact of high and low *in vivo* estradiol exposure on PrEP nucleotide concentrations in different cellular populations of the lower GI tract

9.2 Secondary Objective(s):

To quantify the relationship between estradiol, progesterone and testosterone on PrEP nucleotide concentrations in rectal and peripheral blood mononuclear cells

To quantify the relationship between estradiol, progesterone and testosterone on PrEP concentrations in plasma

10. Study Design

10.1 Identification of Study Design

This is a single center, open-label, multi-matrix sampling study of PrEP nucleotides in mucosal tissue concentrations measured after multiple doses of Truvada® (TDF/FTC). Participants will take a single daily dose of study drug for five days before each sampling visit. The visits will be scheduled during the early follicular phase of the menstrual cycle (approximately Days 2-5 after the first day of menses, Visit 1) when estradiol is predicted to be the lowest; and the late follicular phase (approximately Days 12-15 after the first day of the menses, Visit 2) when estradiol is predicted to be highest. After participant education, informed consent, and screening for study eligibility, participants will be evaluated at baseline.

10.2 Description of Study Population

Healthy, HIV-uninfected premenopausal women (N=10) will be enrolled based on the following inclusion criteria: ≥18 years of age, no clinical evidence of sexually transmitted infections (STIs), unimpaired renal function (>60ml/min; as estimated by Cockcroft-Gault), a regular menstrual cycle (defined as at least 1 day of menses occurring every 21-35 days), being willing to refrain from inserting anything rectally for 3 days prior and 7 days post biopsy visits. We have carefully considered allowance of different forms of contraception in this study and their impact on endogenous hormonal fluctuation (See section 14.7 for details).

10.3 Clinical Trial Experience

The UNC Chapel Hill Center for AIDS Research has considerable prior experience with the conduct of cervical, vaginal, and gastrointestinal biopsy studies in women. We recently enrolled 48 women in a phase I dose ranging study PK study (NCT01330199, clinicaltrials.gov) requiring all three types of biopsies to be performed. Prior to this study, UNC enrolled the majority of women in HPTN 066 (NCT01276600, clinicaltrials.gov) which required all three types of biopsies to be performed within 60 minutes of each other. In both cases, these biopsies were performed successfully and UNC enrolled to completion. Approximately 10 women who participated in the aforementioned study (NCT01330199), have expressed interest in participating in additional studies of the similar nature. As part of the screening and informed consent process, every participant will receive a detailed description of the procedures involved and the intensity of the protocol.

10.4 Study Sampling

At both sampling visits, the following samples will be collected in all participants:

1. Blood plasma
2. Serum

3. PBMCs
4. Rectal tissue biopsies
5. Rectal cell collection by cytobrush

10.5 Primary Endpoints:

TFVdp, FTCtp, dATP and dCTP concentrations in mixed rectal cells collected via cytobrush vs $CD4^+$ T cells isolated from rectal tissue biopsies during the early (low estradiol) and late (high estradiol) follicular phases of the menstrual cycle

10.6 Secondary Endpoints:

1. Estradiol, progesterone and testosterone concentrations in serum
2. TFVdp, FTCtp, dATP and dCTP concentrations in peripheral blood mononuclear cells
3. TFV and FTC concentrations in plasma

Table 1 Sample collection and bioanalytical measurement

Matched Visit 1 and 2 Measurements	
Collection Matrix	*Bioanalytical assay*
PBMCs	TFVdp FTCtp dATP dCTP
Total rectal cells	TFVdp FTCtp dATP dCTP
Rectal CD4 cells	TFVdp FTCtp dATP dCTP
Plasma	TFV FTC
Serum	Estradiol progesterone testosterone
TFVdp=tenofovir diphosphate; FTCtp=emtricitabine triphosphate; dATP=deoxyadenosine triphosphate; dCTP=deoxycytidine triphosphate; TFV=tenofovir; FTC=emtricitabine	

10.7 Time to Complete Accrual

Based on our prior experience with several other pharmacokinetic studies that collected rectal tissue and samples from other matrices, we anticipate an accrual rate of two participants per month for a total recruitment time of 5 months.

10.8 Expected Duration of Participation

Once enrolled in the study, participants will participate in the study for approximately 2 weeks and then have a follow up visit approximately 1-14 days after the last sample is collected. No study data will be collected after the follow-up visit unless the participant has an adverse event (AE), which will be followed until resolution.

10.9 Clinical Study Site

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

11. Study Population

11.1 Recruitment

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

1. Listing our study with NC TraCS's UNC Clinical Trials Searchable Database and on the Research Match Searchable Database
2. Sending IRB approved emails to the UNC-CH campus listserv
3. Posting IRB approved fliers across campus
4. Contacting a list of subjects who have expressed interest in any of our previous healthy volunteer studies

Potential participants will be given an email address or a phone number to call if they are interested, and the study coordinator conducts a brief initial IRB-approved screening interview over the phone in a private area to determine further eligibility. If the subject is potentially eligible, a screening visit is scheduled; if one or more of

the basic inclusion criteria are not met, the subject is informed that they are not eligible.

11.2 Subject Withdrawal

There is a low chance of participant withdrawal from the study due to the relatively short study period. If a participant does withdraw before completion of all biopsies and samples, it will be necessary to replace them in order to have a complete set of data. If a subject completes all biopsies but misses more than 1 outpatient dose it will be necessary to replace the subject in order to have a complete set of data. If a subject is unable to provide all of the serum samples because of poor vascular access or other issues it will be necessary to replace the subject in order to have a complete set of data.

11.3 Inclusion Criteria

1. Healthy cisgender pre-menopausal female participants between the ages of 18 and 49 years, inclusive on the date of screening (Healthy is defined as no irregular menstrual cycles or clinically relevant abnormalities identified by a detailed medical history, full physical examination, including blood pressure and pulse rate measurement, and clinical laboratory tests.
2. Regular menstrual cycles defined as at least 1 day of menses occurring every 21-35 days)
3. Estimated calculated creatinine clearance (eCcr) of at least 60 mL/min by the Cockcroft-Gault formula where: eCcr (female) in mL/min = [(140 - age in years) x (weight in kg) x 0.85] / (72x serum creatinine in mg/dL).
4. Negative serum pregnancy test at screening
5. All participants should be using at least one of the following methods of contraception* from the screening visit through 72 hours prior to inpatient admission (at which time the women will be asked to remain abstinent until after their follow-up visit):

a. Non continuous systemic hormonal contraceptives that permit intermittent menstruation
b. IUD (non-hormonal) placed at least 1 month prior to study enrollment
c. Bilateral tubal ligation (Sterilization)
d. Vasectomized male partners
e. Condom + Spermicide
f. *Unless engaged in sexual activity with female only sex partners or abstinent for at least 3 months prior with no intention of becoming sexually active during the study period. Any history of recent or present concomitant male sex partners will be addressed and ruled out in the context of screening participants for eligibility for the protocol

6. Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the trial.
7. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other trial procedures.
8. Subject must be willing to abstain from sexual intercourse, and all and intrarectal objects and products for at least 72 hours prior to Sampling #1 until study completion.
9. Subject must be HIV-1 and Hepatitis B and C negative as documented on screening labs.
10. Subject must not be actively involved in the conception process and must be non-lactating.
11. Subject must be able to swallow pills and have no allergies to any component of the study product

11.4 Exclusion Criteria

1. Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic,

or allergic disease (including documented drug allergies, but excluding untreated, asymptomatic, seasonal allergies at time of dosing).

2. Participants with a history of hysterectomy
3. Participants who are pregnant, possibly pregnant or lactating
4. History of febrile illness within five days prior to first dose.
5. Any condition possibly affecting drug absorption (eg, gastrectomy or other significant alterations of the gastrointestinal tract)
6. A positive urine drug screen.
7. An untreated-positive test for syphilis, gonorrhea, or Chlamydia at screening.
8. Any clinically relevant laboratory chemistry or hematology result Grade 2 or greater according to the DAIDS Laboratory Grading Tables
9. Treatment with an investigational drug within 4 months preceding the first dose of study product.
10. History of regular alcohol consumption exceeding 14 drinks (1 drink = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of spirits) per week.
11. Participation in a clinical trial involving rectal biopsies within 6 months preceding the first dose of trial medication.
12. Blood donation of approximately 1 pint (500 mL) within 56 days prior to dosing.
13. Any condition which, in the opinion of the investigator, is likely to interfere with follow-up or ability to take the study medication appropriately.
14. Unwilling or unable to comply with the dietary and concomitant drug restrictions in regard to study drug administration as outlined in the study procedures and prohibited medications sections.
15. Women utilizing continuous hormonal contraception options such as Seasonique, injectables, implants, and hormonal IUDs

11.5 Co-enrollment Guidelines

Participants cannot have participated in a clinical trial in which they received treatment with an investigational drug within 4 months preceding the first dose of trial medication or have participated in a clinical trial involving rectal biopsies within 6 months preceding the first dose of trial medication.

Figure 3 Truvada® Identification

12. Study Product

12.1 Study Product Description

Truvada® (Figure 3) is an FDA-approved pill that contains two different HIV medicines combined in one pill: emtricitabine and tenofovir disoproxil fumarate. Both medicines belong to the group of HIV drugs called nucleoside reverse transcriptase inhibitors (NRTIs) made by Gilead, Inc.

12.2 Administration

The participant will take a single oral daily dose of Truvada for the five days before Sampling Visit #1 and Sampling Visit #2 as scheduled. Doses can be given in person, or through video conferencing programs, such as SKYPE. A study team member will witness all doses and record the times of administration. The drug name and dose will be confirmed with visual inspection during a time-out procedure prior to dose administration.

12.3 Supply and Accountability

The medications will be purchased and dispensed by the Investigational Drug Service at UNC Hospitals.

12.4 Dietary Restrictions and Prohibited Medications and Practices

1. Participants must comply with a low fiber diet for 3 days prior to all rectal biopsies.
2. Participants must consume only clear liquids 12 hours prior to all rectal biopsies, refraining from anything containing red or purple dyes.

12.5 Toxicity Management

Laboratory tests will be taken before study drug administration to look for baseline abnormalities and these will be compared to the laboratory tests to be taken at the follow-up visit after study drug exposure. Any side effects will be managed symptomatically on a case by case basis. Participants will be advised to contact study staff if any adverse effects occur at any time during the study period and to seek medical attention as they see appropriate. A follow up call will be made by the research coordinator to assess for adverse events the day following any visits where a biopsy was performed.

12.6 Adverse Effects Associated with Use for Treatment of HIV Infection

Emtricitabine

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

Tenofovir disoproxil fumarate

A phase I single-dose study with tenofovir 300 mg showed it was generally well tolerated. The most frequently reported adverse events were headache (19%) and

dizziness (13%). Flatulence (7%), hot flush (3%), and an increase in alanine aminotransferase (3%) were also reported.

13. Study Procedures

Study specific procedures are presented in table format in the Schedule of Events. All patients will attend an outpatient screening visit, an enrollment visit, two outpatient biopsy visits, and a follow-up visit. Dosing visits will occur in person or through videoconferencing. All on-site visits will be conducted at the UNC NC TraCS Clinical Translational Research Center. During the outpatient biopsy visits tissue will be collected by insertion of an anoscope for the rectal biopsies and rectal cells. We currently have a list of approximately 10 women who have expressed interest in participating in this study after having participated in a previous pharmacokinetic study where biopsies of this nature were collected. As part of the screening and informed consent process, every participant will receive a detailed description of the procedures involved and the intensity of the protocol.

13.1 Pre-screening

Persons interested in participating will be pre-screened by telephone interview using a standardized IRB-approved questionnaire to assess general eligibility prior to scheduling a screening visit. Participants may be provided a copy of the informed consent prior to the screening visit, if requested.

13.2 Screening Visit

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

The screening visit will consist of the following activities:

1. Review study overview and obtain written informed consent

2. Review approved contraception options to be used during the entire study period
3. Assign Participant ID
4. Collect demographic information
5. Collect medical/menstrual history and review contraception history
6. Perform physical exam
7. Vitals (blood pressure, heart rate, respiratory rate, height, weight (Safety vitals obtained on arrival to research clinic by nursing staff may be used)
8. Obtain safety labs:
    a. CBC with differential (includes hemoglobin, hematocrit, WBC and differential count, platelets)
    b. Coagulation studies (PT/INR and aPTT)
    c. Serum Chemistry Panel (Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, serum glucose, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin)
9. Serum Pregnancy Test
10. Urine Drug Test (amphetamine, barbiturate, benzodiazepine, cannabinoid cocaine, methadone, opiates)
11. Virology tests (HIV antibody combo, HBsAg, Hep C Ab)
12. STI tests (syphilis, gonorrhea, chlamydia)

13.3 Enrollment Visit

Within 42 days of the screening visit, all participants will present on their scheduled date for their enrollment visit. A repeat urine pregnancy test will be performed. If participant still meets eligibility criteria, the first dose of study drug will be administered by study staff, and they will be provided with 4 more doses to take home.

13.4 Biopsy Visit 1 and 2

Patients will be admitted to the Center for Translational and Clinical Research (CTRC) at University of North Carolina Chapel Hill Hospital at approximately Days 2-5 and 12-15 of their menstrual cycle, after 5 days of dosing study product.

This visit will consist of the following activities:

1. Targeted physical examination, as indicated
2. Vitals (BP, HR, RR, weight)
3. Urine Pregnancy test
4. Blood draw
    a. Blood to be collected in one 3 mL K_2 EDTA (purple top tube) and stored on ice for no more than 1 hour prior to processing blood plasma by centrifuging at 1700rcf (converts to 2800rpm) for 10 minutes at 4°C. Blood plasma will be aliquoted into an appropriately labeled cryovial and temporarily stored at -20°C until transferred to permanent storage at -80°C in the CPAC laboratory.
    b. Blood to be collected in one 8 mL Cell Preparation tube (CPT) with Sodium Citrate and stored upright at room temperature for no more than 4 hours before transferring to the CPAC laboratory and initiating processing procedures for PBMCs as according to CPAC SOP0345.
    c. Blood to be collected into one 6mL Serum Separator Tube (SST) and processed to serum by allowing to coagulate for 30 minutes at room temperature then centrifuging at 1700rcf (converts to 2800rpm) for 10 minutes at 4° C. Serum will be aliquoted into an appropriately labeled cryovial and temporarily stored at -20°C until transferred to permanent storage at -80°C in the CPAC laboratory.
5. Tissue samples will be obtained:
    a. Digital exam will be performed prior to insertion of the anoscope. KY-jelly lubricant will be used for digital exam and anoscope insertion, but no anesthetic will be applied. Rectal cells will first be

collected with a cytobrush, placed immediately into a Falcon tube, and transported fresh back to the CPAC lab on ice. 10 biopsies will be collected from the rectal mucosa using 2.4mm radial jaw, single use, biopsy forceps by a board-certified gastroenterologist. Tissue will be transferred to two appropriately labeled, cryovials with 5 pieces of tissue in each cryovial. The samples will be transported fresh back to the CPAC lab on ice.

6. Adverse event assessment using a standard adverse events questionnaire will be conducted after the last sample is collected, prior to discharge to home.
7. Discharge to home

The patient will be offered a meal prior to discharge.
During Visit 1, the participant will be given a pill bottle containing the 5 doses of study product that they will take for the five days prior to Biopsy Visit 2, and dosing appointments will be scheduled.

The day following biopsies, each subject will be contacted in person/phone by a study team member to assess for adverse events. Additional study visits will be scheduled for any subject experiencing an adverse event.

13.5 Follow-up Visit

The follow up visit will consist of the following activities:

1. Targeted physical examination, as indicated
2. Vitals (BP, HR, RR, Temp and weight)
3. Safety labs:
    a. CBC
    b. Serum Chemistry Panel (Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin)

c. HIV test

4. Urine Pregnancy test

13.6 Behavioral Assessment

Adherence to behavior guidelines, approved contraception and medications taken will be assessed prior to the biopsies by a series of questions to be asked prior to proceeding. Women who do not have sex that could result in pregnancy, will only be tested at screening for pregnancy.

13.7 Clinical Evaluations and Procedures

The extent of examination for the brief physical exam will be determined by clinical judgment based on the interim medical history.

14. Assessment of Safety

14.1 Safety Monitoring

Adverse events will be monitored throughout the study. Adverse events will be assessed formally before discharge to home at the inpatient visit, after each outpatient visit and at the follow-up visit during which participants will answer standardized questions regarding to prompt reporting of adverse events associated with the study medication and procedures. The research coordinator will contact participants the day following all biopsy procedures.

14.2 Clinical Data and Safety Review

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are the first layer of this tiered system and are responsible

for the initial evaluation and reporting of safety information at the participant level, and for alerting the Protocol Safety Review Team (PSRT) if unexpected concerns arise. The PSRT will consist of the following study site investigators: Cynthia Gay, MD (Study Physician), Heather Prince, PA-C (Study Physician Assistant), Mackenzie Cottrell, PharmD (Principal Investigator).

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

14.3 Adverse Events Definition and Reporting Requirements

An adverse event (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. This definition is applied to all the study groups and is applied to all groups beginning from the time of consent. The term “investigational product” for this study refers to all study products. Study participants will be instructed to contact the study site staff to report any AEs they may experience at any time between consent and follow-up. In the case of a life-threatening event, they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. Study staff will obtain written permission from the participant to obtain and use records from non-study medical providers to complete any missing data element on a CRF related to an adverse event. All participants reporting an untoward medical occurrence will be followed clinically,

until the occurrence resolves (returns to baseline) or stabilizes.

The study physician will determine AE resolution or stabilization in their best clinical judgment. Study site staff will document in source documents all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. Study staff will record all AEs on case report forms.

14.4 Serious Adverse Events

Serious adverse events (SAEs) will be defined by the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010) as AEs occurring at any dose that:

1. Results in death
2. Is life-threatening
3. Results in persistent or significant disability/incapacity
4. Is a congenital anomaly/birth defect
5. Requires inpatient hospitalization or prolongation of existing hospitalization
6. The following are examples of hospitalization that will not be considered to be AEs:
    a. Protocol-specified admission (e.g. for procedure required by study protocol)
    b. Admission for treatment of target disease of the study, or for pre-existing condition (unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator)
    c. Diagnostic admission (e.g. for a work-up of an existing condition such as persistent pretreatment lab abnormality)
    d. Administrative admission (e.g. for annual physical)
    e. Social admission (e.g. placement for lack of place to sleep)
    f. Elective admission (e.g. for elective surgery)

Important medical events that may not result in death, be life-threatening, or require

hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Study staff will report information on all AEs and SAEs to the IRB in accordance with all applicable regulations and requirements.

14.5 Adverse Event Relationships

The relationship of all AEs to study product will be assessed per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010). Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010), the relationship categories that will be used for this study are:

1. *Related*: AE and administration of study product are related in time, and a direct association can be demonstrated

2. *Possibly related*: There is a reasonable possibility that the adverse event, incident, experience or outcome may have been caused by the procedures involved in the research.

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

14.6 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1, March 2017 will be the primary tools for grading adverse events for this protocol.

14.7 Pregnancy and Pregnancy Outcomes

Participation in this study requires taking a Pregnancy Category B medication outside of routine clinical care; therefore only women not intending to become pregnant will be enrolled into this study. The duration of medication dosing is short (5 days before each sampling visit) and scheduled during the early phases of the menstrual cycle (pre-ovulation); therefore the risk of unintentional *in utero* drug exposure is low. Women will be tested at screening using a serum pregnancy test (human chorionic gonadotropin; hCG) and immediately prior to enrollment and each sampling visit using a urine pregnancy test. They will be required to use at least 1 pregnancy prevention strategy during their participation in the study. These may include abstinence for 3 months preceding; monogamy with a vasectomized cisgender male or a cisgender female partner; condoms with spermicide; a non-hormonal IUD; and hormonal contraception. Because the goal of this study is to characterize nucleotide concentrations within an individual during high and low estradiol exposure, only hormonal contraception options that do not impede cycle fluctuation will be allowed. Estradiol has been well characterized in women taking traditional 21/7 hormonal contraception regimens that allow for monthly menses. While peak estradiol concentrations are slightly diminished (1.6-1.8 fold) after initiating this contraceptive regimen, estradiol's overall concentration vs time profile follows a predictable pattern similar to the pre-contraception pattern.[5,6] Furthermore, peak estradiol concentrations are still 6-9 fold greater than trough concentrations during these contraceptive cycles. Therefore, we will include women utilizing a traditional 21/7 hormonal contraception regimen in this study. Since estradiol fluctuations over the contraceptive cycle are dramatically reduced by shortening the on/off interval to 23/5 days[5], women utilizing continuous hormonal contraception options such as Seasonique, injectables, implants, and hormonal IUDs will be excluded from this study.

Pregnancy-related data will be collected using the pregnancy CRFs for all pregnancies detected during the study. After the participant's follow-up study visit, the study team will make every attempt to follow the participant until an outcome of the pregnancy can be ascertained to allow reporting of these outcomes meeting EAE criteria. Pregnancy outcomes that meet criteria for EAE reporting as described above

(e.g., maternal complications, congenital anomalies) occurring among participants known to be pregnant at the Final Study Visit will continue to be expeditiously reported.

14.8 Social Harms Reporting

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

15. Clinical Management

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

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

15.1 Dose Modification Instructions

Study dose will not be modified.

15.2 Discontinuation of Study Product in the Presence of Toxicity

Stopping rules have been created to protect the health and safety of study participants. If a single subject experiences a Serious Adverse Event, enrollment will be held while the protocol undergoes review. If 2 or more participants experience Grade 3 or Grade 4 adverse events, enrollment will be held while the protocol undergoes review.

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

15.3 Pregnancy

Pregnant and lactating women are excluded from this study. Routine serum pregnancy testing is being performed at the screening visit and routine urine testing is being performed at other study visits as noted. If a participant becomes pregnant at any time during the course of the study, study treatment and interventions would be terminated, but she will continue with follow-up visits and safety lab assessments.

Pregnancy-related data will be collected using the Antiretroviral Pregnancy Registry forms for all pregnancies detected during the study. The study team will make a very strong attempt to maintain contact with the pregnant participant until the outcome of the pregnancy is determined or it becomes clear that it will be impossible to obtain outcome information.

15.4 Criteria for Early Termination of Study Participation

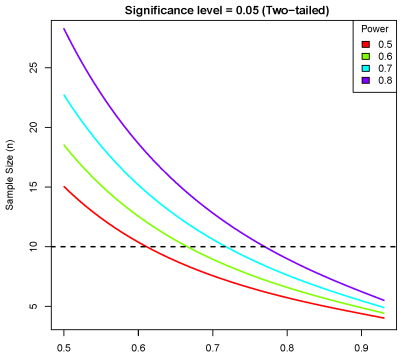
Participants may voluntarily withdraw from the study for any reason at any time. The site investigator also may withdraw participants from the study to protect their safety and/or if they are unwilling or unable to comply with required study procedures, after consultation with the PSRT. Participants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

16. Statistical Consideration

16.1 Statistical analysis.

We hypothesize that PrEP nucleotide concentrations in total rectal cells obtained via cytobrush will significantly correlate with isolated CD4+ T cells regardless of estradiol exposure. To test this hypothesis TFVdp and dATP concentrations in total rectal cells collected by cytobrush at each visit will be compared to CD4+ T cells isolated from rectal biopsies using Pearson's correlation. We expect strong correlations (>0.8) to exist during both visits. Linear regression will be performed for log10 concentrations of total rectal cells (X) vs isolated CD4+ T cells (Y) to describe the relationship between these sampling matrices within each visit. These analyses will also be performed for FTCtp and dCTP.

Figure 4 Power Determination



Sample size required to detect a positive correlation given various scenarios of r and beta values.

16.2 Sample Size and Power Calculations

Power determination for the correlation analysis was carried out using the R-package pwr.[7] This package

implements the power calculation methods described in Cohen, 1988.[8] Assuming r is >0.78, a sample size of 10 women achieves 80% power (Figure 2). This assumed r value is more conservative than our previous observation of TFV concentration in rectal fluid versus tissue homogenate where r was 0.944.

16.3 Participant Accrual, Follow-up and Retention

A total of 10 evaluable women will be enrolled over a period of approximately 5 months. Participants who complete all visits but the follow-up visit will still be considered evaluable. All unevaluable participants will be replaced.

17. Data and Safety Monitoring and Analysis

17.1 Clinical Data Monitoring

The study physician will meet with the PA for screen and entry reviews. The key indicators that will be assessed include informed consents, eligibility criteria, drug dosing, protocol required evaluations, review of subject responsibilities, and contraception counseling. Additionally, laboratory results, concomitant medications, proper error documentation, adherence to unit specific SOPs, and CRF completion will be assessed. When meeting for "on-study" reviews, the key indicators that will be assessed are SAE reporting, adverse event monitoring, and implementation of new protocol versions and amendments. Additionally, missed visits, proper symptom grading, laboratory results, concomitant medications, proper error documentation, adherence to unit specific SOPs, and CRF completion will be reassessed. Additional training on source documentation and good clinical practices will be addressed as needed.

17.2 Sample and Data Analysis

All blood, cells, and rectal tissue samples will be analyzed by the Clinical Pharmacology and Analytical Chemistry Laboratory (CPAC) at the UNC School of Pharmacy, which is directed by Angela Kashuba, PharmD. TFV and FTC will be quantified in plasma by validated LC-MS/MS methods (lower limit of quantification; LLOQ=1ng/ml). TFVdp, FTCtp, dATP and dCTP will be quantified in PBMCs and rectal cell lysates by validated LC-MS/MS methods (LLOQ=0.02ng/ml). These analyses will be performed at the UNC CFAR Clinical Pharmacology and Analytical Chemistry Core (see Kashuba letter).

18. Data Handling and Record Keeping

18.1 Data Management Responsibilities

The investigator will maintain, and store securely, complete, accurate and current study records throughout the study. The investigator will retain all study records for at least three years following the completion of the study. Study records must be maintained on site for the entire period of study implementation.

18.2 Quality Control and Quality Assurance

The quality control and assurance of clinical study data will be the responsibility of the study physician assistant, Heather Prince. All clinical charts prepared by study staff will be reviewed by Heather Prince for quality assurance within 7 days of the subject visit. Quality assurance for analytical methods and drug concentration data will be performed by the quality control officer Hannah Bryan.

18.3 Study Coordination

The coordination of subject visits will be the responsibility of the clinical team, led by the study Physician Assistant.

19. Clinical Site Monitoring

All clinical and experimental activities related to this study will be conducted at the University of North Carolina at Chapel Hill. There will be no enrollment outside this institution. The investigators listed in the team roster have affiliations with UNC Eshelman School of Pharmacy and UNC School of Medicine/Center of Infectious Disease. Site quality management will be the responsibility of the principal investigator and co-investigators, and this process will follow the guidelines and operating policies already established by the UNC ACTU. The Clinical Pharmacology and Analytical Chemistry Core (CPAC) and its Director, will provide oversight for laboratory personnel who are not governed by the ACTU policies.

The principal investigator will hold weekly meetings to discuss clinical research and laboratory activities. Concerns in these matters will be addressed during these meetings.

19.1 Human Participants Protection

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

19.2 Institutional Review Boards

The final protocol (version 1.0) will be submitted along with the informed consent to the UNC IRB. Modifications will be made to version 1.0 after initial review for final approval by the UNC IRB. Subsequent to initial review and approval, the IRB will review the protocol at least annually. The Investigator will make safety and progress reports to the IRB at least annually. These reports will include the total number of participants enrolled in the study, the number of participants who completed the

study, all changes in the research activity, and all unanticipated problems involving risks to human participants or others.

19.3 Risk Benefits Statement

This research study is designed to gain new knowledge for society. Participants participating in this research will not receive any direct benefit from their participation. Certain risks are associated with the study procedures and are listed below.

19.4 Risks

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

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

3. **Anoscopy with rectal biopsy and cells**:
   This is a commonly practiced medical procedure and the 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." Anoscopic biopsies are painless and heal quickly (usually within three days). On extremely rare occasions, the 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 perforation may be necessary.

4. **Confidentiality**:

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

5. **Study Drug**:

Truvada® tablets are fixed-dose combination tablets containing emtricitabine (FTC) 200mg and tenofovir disoproxil fumarate (TDF) 300mg in each table. There is some experience in healthy volunteers taking Truvada®. No new or unexpected side effects are observed with the fixed-dose tablet than those observed when each drug is given separately. The dosing regimens will be consistent with package insert recommendations. Individual daily doses of this magnitude were studied during development of both TDF and FTC and these were well tolerated. In our previous studies using the fixed-dose tablet in healthy volunteer research projects, there were no serious adverse advents attributed to study drug.

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/soles, increased cough, runny nose, and abnormal liver function tests.

TDF. The most common side effects associated with oral TDF in patients with HIV infection are nausea, headache, diarrhea, vomiting, flatulence, asthenia, abdominal distension 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 tables for 24-48 weeks.

19.5 Benefits

Participants in this study will experience no direct benefit. 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 safe and effective interventions to prevent HIV transmission. Additionally, participants will be referred for treatment for any incidental findings detected during screening and other examinations.

19.6 Informed Consent Process

The investigators will obtain informed consent from each subject before starting any study procedures according to the standards set forth in the ICH Good Clinical Practice guidelines and per unit SOPs. The process will include reviewing consent forms with potential participants in a confidential setting and explaining all risks and benefits associated with participation of the study. This involves reading over the IRB-approved consent form with the subject in a private space, soliciting questions from the subject, allowing the subject ample time alone to review the form, soliciting questions again, and then offering the subject the opportunity to sign the consent form. To ensure understanding, study staff may ask questions of the participants regarding study procedures. The consent forms will use language that is sufficiently simple for lay persons to comprehend. Participants will not be coerced into participating. Children under the age of 18 years, decisionally impaired adults and

non-English speakers will not be enrolled in this study. Each subject will be provided with a photocopy of all documents that she signs. The informed consent process will cover all elements of informed consent required by research regulations. In addition, the process specifically will address the following topics of importance to this study:

1. The unknown safety and unproven efficacy of the study products
2. The need to practice safer sex behaviors regardless of study treatment group
3. The importance of adherence to the study visit and procedures schedule
4. The potential medical risks of study participation (and what to do if such risks are experienced)
5. The potential social harms associated with study participation (and what to do if such harms are experienced)
6. The real yet limited benefits of study participation
7. The distinction between research and clinical care
8. The right to withdraw from the study at any time

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

19.7 Participation Confidentiality

Confidentiality will be maintained by storing all specimens for current and future use with a unique identifying number, which will be linked to the subject's name, social security number, address, telephone number, and hospital medical record (MR) number. The principal investigators and study staff will be the only people with

access to the identifying information. Any information provided to other people working on this study will be given with the study ID number, not other identifying information. The records will be secured in a locked file cabinet in the locked office specifically designated for study record storage purposes.

All electronic data for this study will be stored on a dedicated University server which contains extensive protections and securities. The server is housed and administered at the server farm at the Manning Data Center, located at 211 Manning Drive, Chapel Hill, NC 27599-3420.

19.8 Special Populations

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

19.9 Pregnant Women

Women who test positive for pregnancy at the screening visit or prior to study drug administration at the inpatient pharmacokinetic visit, will not be eligible to participate in this study. A serum pregnancy test will be performed at the screening visit and urine pregnancy tests at all study visits, and additionally if clinically indicated. During the informed consent process, women will be informed that none of the study products are methods of contraception and about the current knowledge of effects of these products on a developing human fetus.

19.10 Children

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

19.11 Compensation

Participants will be compensated for their time and effort in this study. Participants will be provided with parking voucher to cover the cost of parking in the UNC Memorial Hospital's designated visitor parking deck. Meals will be provided when having a biopsy. Participants will be given $40 for the screening, and $25 for the follow-up visit. They will be compensated $5 for each dose, for $50 total. They will receive $225 for each biopsy day ($200 for biopsy and $25 for blood). A participant may be compensated $565 for completing all aspects of the study. Stipends will be provided at screening and follow-up.

Pro-rated amounts will be provided to participants who are unable or unwilling to complete the protocol-specified visits.

19.12 Communicable Disease Reporting

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

19.13 Access to HIV-related Care

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

19.14 HIV Counseling and Testing

HIV test-related counseling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study, and to all enrolled participants at each follow-up HIV testing time point. Testing will be

performed in accordance with the algorithm in Appendix 3. Counseling will be provided in accordance with standard HIV counseling policies and methods at the site and additionally will emphasize the unknown efficacy of the study products in preventing HIV infection. In accordance with the policies of the US NIH, participants must receive their HIV test results to take part in this study.

19.15 Care for Participants Identified as HIV-infected

Participants will be provided with their HIV test results in the context of post-test counseling. Participants found to be HIV-infected will be referred to available sources of medical and psychosocial care and support, and local research studies for HIV-infected adults. Our medical officer, Dr. Cynthia Gay, is an attending physician in the UNC Infectious Disease clinic and can provide ready access to multi-disciplinary care for any participant who tests positive.

19.16 Study Discontinuation

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

20. Publication Policy

Any presentation, abstract, or manuscript will be made available to Gilead Sciences prior to publication, with the final decision remaining with the PI.

21. References

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