Project ADHERE: Clinical Proof-of-Concept of a Tenofovir (TFV) Aptamer-Based Biosensor for Determing Adherence Using Different Dosing Regimens of Disoproxil Fumarate/Emtricitabine (TDF/FTC)

VERSION 2.0

29 AUG 2019

Prepared By:

Terry Jacot PhD and Andrea Thurman MD

INVESTIGATOR'S AGREEMENT

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This document may contain confidential information. It is understood that persons to whom this information is disclosed will not disclose it further without permission from Dr. Jacot, unless such information is published or otherwise becomes public knowledge.

I have received and read:

- Package insert for Emtricitabine/ Tenofovir disoproxil fumarate (Truvada) tablets. I have read the protocol and agree to conduct the study as outlined. I will comply with all requirements regarding the obligations of clinical investigators as fully outlined in 21 Code of Federal Regulations (CFR) Parts 50, 56, and 312.60 and in the Statement of Investigator (1572), which I have also signed. I will ensure that all associates, colleagues, and employees assisting in the conduct of this study are informed about the obligations incurred by their contribution to the study. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

EMERGENCY CONTACTS

Table 1: Emergency Contact Information

Study Role	Name	Email and Telephone number
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R21 Principal Investigator and Laboratory Lead	Terry Jacot PhD	jacotta@evms.edu 757-446-7056

1. SYNOPSIS

Name of Sponsor: National Institutes of Allergy and Infectious Disease (R21) RFA PA-19-054	
Name of Study Products: TDF/FTC [tenofovir disoproxil fumarate / emtricitabine] (Truvada)	
Name of Active Ingredients: emtricitabine; tenofovir disoproxil fumarate	
Title of Study: Project ADHERE: Clinical Proof-of-Concept of a Tenofovir (TFV) Aptamer-Based Biosensor for Determining Adherence Using Different Regimens of Disoproxil Fumarate/Emtricitabine	
Principal Investigators and Study Centers: • Terry A. Jacot, PhD, CONRAD, Eastern Virginia Medical School, Norfolk, VA, US (R21 Grant PI) • Andrea Thurman, MD, Clinical Research Center, Eastern Virginia Medical School, Norfolk, VA, USA (Clinical Site PI)	
Study period: Estimated date first patient enrolled: Q4 2020 Estimated date last patient completed: Q2 2021	**Phase of development:** Pilot

Background and Rationale: Truvada®, is an oral pill comprised of two anti-retroviral compounds, emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF). Gilead Sciences reported a total of nearly 100,00 individuals in the US starting Truvada for pre-exposure prophylaxis (PrEP) between the years 2012-2016 [1], and this number is growing in the US and globally. Adherence to the one pill per day regimen is crucial for its effectiveness in reducing the risk for sexual transmission of human immunodeficiency virus (HIV) infection by 90%, and by 70% from injection drug use [2]. Two landmark safety and effectiveness trials which supported the FDA approval of Truvada for PrEP were the iPrEX trial, which investigated Truvada use in HIV-negative men or transgender women, and the Partners PrEP trial, conducted in serodiscordant couples [3, 4]. Both showed Truvada reduced risk of HIV infection by 42% (iPrEX) and 72% (Partners PrEP), noting that risk reduction was highly correlated with drug adherence. Another important study supporting PrEP was the TDF2 trial, which showed an HIV risk reduction of 62% [5]. Although other clinical studies did not show efficacy, the results correlating poor drug efficacy with low plasma drug levels did confirm the importance of adherence [6, 7]. Adherence measured by self-report did not correlate with effectiveness [6, 7]. Although adherence is key to effectiveness, physicians and other health care providers (HCPs) do not have objective tools to monitor drug adherence and be able to tailor counselling to the actual needs of their patients and clients. The evidence supporting the association between adherence and effectiveness is strong, but only objective assessment of adherence is reliable and correlates with effectiveness. The scientific premise supporting our work is based on this evidence, as well as the absence of a point-of-care objective diagnostic test for drug adherence. The most objective measure of adherence currently available to detect and quantify drug levels in plasma is liquid chromatography tandem mass spectrometry (LC-MS/MS). This method, however, requires a specialized lab and does not qualify as a point-of-care (POC) test. *Therefore, our project is aimed at filling this gap by developing and validating an aptasensor (aptamer-based biosensor), capable of detecting tenofovir (TFV) in various biological fluids at a point-of-care/clinic with a simple-to-use procedure, which will empower HCPs to provide real-time adherence feedback with each prescription renewal.* Ultimately, real-time monitoring of adherence will lead to better counselling, resulting in higher effectiveness and improved success rates for treatment and prevention of HIV infection.

Given the importance of adherence to the efficacy of TDF/FTC it is of clinical significance to have a reliable metric for adherence levels. Self-reporting of adherence, a non-invasive and simple method of assessment does not reliably correlate with effectiveness and blood serum levels. Currently, the most objective measure of adherence available is to detect and quantify plasma levels of tenofovir diphosphate (TFV-DP), the active metabolite of TDF, using LC-MS/MS. Although this method of quantification is reliable, it requires a specialized lab and equipment which comes at a significant cost and does not qualify as POC test. Other recently developed methods of measuring TFV levels in urine, ELISA and lateral flow assays, have shown promise, but are limited by inadequate sensitivity to detect levels observed in plasma and the inability to distinguish high adherence from white coat effects. The lack of a POC test to measure adherence leaves a critical gap for physicians and other HCPs when tailoring counseling to improve adherence and ultimately HIV prevention of their patients and communities.

As such, there is an opportunity to evaluate a new method of measuring adherence in a reliable, objective, and cost-effective manner. Aptamers are DNA- or RNA-based ligands capable of binding practically any molecular target with high specificity, including small molecules such as antiretroviral drugs [8]. Aptamers have advantages over antibodies. They are more stable and made through a process that is more economical and reproducible, which increases the aptamer's potential capability of being more specific than its antibody counterpart. Therefore, aptamers are being used as capture molecules for biosensor platforms, which are becoming smaller than previous models, more portable, more sensitive and quantitative (rivaling LC-MS/MS), require less sample volume, and simpler to use. Therefore, aptamer-based biosensors or "aptasensors", have great potential for real-

time therapeutic drug monitoring. We will be utilizing a previously validated DNA aptamer specific for TFV and applying it to a biosensor platform.

This study will focus on determining the sensitivity and specificity of the TFV aptasensor and assess its ability to distinguish TFV concentrations associated with low and high adherence using biological fluids collected from women taking TDF/FTC at different dosing regimens.

Objectives:

Compare plasma, urine, and vaginal fluid TFV concentrations measured by the aptasensor with those values quantitated by LC-MS/MS to determine agreement between the two methodologies, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV).

Primary Objectives:

- Compare plasma TFV concentrations measured by the aptasensor with those values quantitated by LC-MS/MS to determine agreement between the two methodologies, sensitivity, specificity, NPV and PPV
- Determine if the TFV aptasensor can detect differences in the plasma TFV concentrations between high and low adherence dosing regimens and compare the mean difference to the difference calculated from values generated by LC-MS/MS

Secondary Objectives:

- Compare urine TFV concentration measured by the aptasensor with those values quantitated by LC-MS/MS to determine agreement between the two methodologies, sensitivity, specificity, NPV, and PPV
- Determine if the TFV aptasensor can detect differences in the urine concentrations of TFV between high and low adherence dosing regimens and whether these levels correlate with adherence
- Compare vaginal fluid TFV concentrations measured by the aptasensor with preclinical determinations of the range of detectable TFV levels and the aptasensor's limit of detection

- Determine if differences in adherence dosing regimens can be correlated to differences in vaginal fluid TFV levels

Endpoints:

Primary Endpoints:

- TFV concentrations in *plasma* at baseline (pre-dose), 24 hours, 7 days and 14 days after dosing

Secondary Endpoints:

- TFV concentrations in *urine* and *vaginal fluid* at baseline (pre-dose), 24 hours, 7 days, 14 days after dosing

Study Design:

This pilot, prospective, randomized study will screen approximately 20 healthy, non-pregnant, HIV negative, premenopausal women (aged 18-50) at Eastern Virginia Medical School (EVMS) who are not at risk of pregnancy and are at low risk for sexually transmitted infections (STIs) in order to have approximately 14 women complete all study visits. This study will examine the plasma, urine, and vaginal fluid from women in two dosing regimens of oral tablets: High adherence (HA): TDF/FTC (300/200 mg), 7 pills per week versus low adherence (LA): TDF/FTC (300/200mg), 3 pills per week. Participants will be randomized to regimen as per the following schemata:

Randomization Assignments

Regimen	N	Dose
LA	7	TDF/FTC (300/2200 mg), 3 pills/week. Total of 6 pills
HA	7	TDF/FTC (300/200 mg), 7 pills/week. Total of 14 pills

Participants will undergo the low adherence (LA) regimen or a high adherence (HA) regimen for two weeks, consisting of 5 visits.

Visit 1: At screening/enrollment, volunteers will be consented and undergo procedures to confirm they are eligible to continue in the study. Eligible participants will proceed to Visit 2.

Visit 2: Baseline blood, urine, and vaginal fluid will be collected. Participants will be randomized to dosing regimen and the first dose of TDF/TFC will be ingested in the clinic. Participants will be instructed to take subsequent doses at home.

Dosing Contact: Participants will be sent reminder text messages to facilitate dosing occurring at the same time of day. Participants will text message the study coordinator to confirm ingestion of tablet. If the participant does not respond to a text within approximately 4 – 6 hours after it is sent, a reminder text will be sent.

Visits 3 - 5: Participants will return at 24 hours, 7 days, and 14 days after visit 2 for the pre-dose collection of blood, urine, and vaginal samples.

Follow Up Contact: A final follow up site contact will be conducted approximately 1 – 2 weeks after the last visit to ask the participant about adverse events and concomitant medications. The participant will be exited unless there are symptoms that require follow up.
Number of patients (planned): Approximately 20 women may be consented and undergo assessment procedures in order to have approximately 14 women who meet eligibility criteria complete the study. All 14 women will be randomly assigned to undergo either LA dosing or HA dosing. All randomized participants will be included in the analysis.
Diagnosis and main criteria for inclusion: Healthy, non-pregnant, premenopausal, HIV-uninfected women aged 18-50 (showing regular menstrual cycles), with a BMI $\geq$18 and <35 kg/m^2, and either sexually abstinent or in a monogamous relationship with a healthy partner. Protected from pregnancy by effective contraception, same-sex relationship, or abstinence.
Study product, dosage and mode of administration: Study Products: tenofovir disoproxil fumarate / emtricitabine (TDF/FTC) tablet Dosage: tenofovir disoproxil fumarate (300 mg) with emtricitabine (200 mg) Mode of administration: oral
Control therapy, dosage and mode of administration: HA regimen (7 pills/week) versus LA regimen (3 pills/week).
Duration of treatment: Low Adherence (LA) Group: 14 days consisting of 3 pills/week (one tablet/dose) for a total of 6 pills ingested High Adherence (HA) Group: 14 days consisting of 7 pills/week (one tablet/dose) for a total of 14 pills ingested
Criteria for evaluation: ***Safety:*** It is expected that there will be no clinically significant concerns.
Statistical methods: Sample size is based on feasibility rather than statistical considerations; thus, analysis will be primarily descriptive. Participant flowchart (e.g. number screened, number with sampling) will be provided. The Full Analysis Set (FAS) will consist of all subjects who are randomized and have at least one post-baseline assessment. All populations will be defined based on the adherence regimen. *Evaluation of Study Objectives:* Study objectives will be evaluated by presentation of descriptive summaries and graphical displays.

Primary analysis of this study will evaluate the performance of the TFV aptasensor by comparing its measurements of the concentrations of TFV in clinical samples from all women with those values obtained from LC-MS/MS. Correlation and agreement between the TFV levels measured by the aptasensor and LC-MS/MS will be analyzed using scatterplot and linear regression. Continuous variables will be compared between values measured with the aptasensor versus LC-MS/MS using paired comparisons. From this comparison, sensitivity, specificity, NPV, and PPV of the aptasensor will also be determined. Confidence intervals will be calculated to assess precision and margin of error of those measures. Using the14 baseline pre-dose samples, each woman will be her own control, and these pre-dose samples will be used for the assessment of specificity and NPV.

The secondary analysis of this study will evaluate whether the aptasensor can detect differences in TFV levels between the two independent cohorts of women taking TDF/FTC at different dosing regimens representing high (7 pills/week) and low adherence (3 pills/week). We will compare the two independent cohorts using an independent samples t test for normally distributed data or a Wilcoxon-Mann-Whitney test for non-parametric data. TFV concentrations from the LA and HA cohorts will be characterized using descriptive statistics (mean, median, interquartile range, minimum and maximum). The values will be compared between the high adherence and low adherence groups as well as comparing the mean difference between groups generated by the aptasensor to that difference generated by LC-MS/ MS. Distributions will be checked for normality so as to determine the appropriate statistical test.

Descriptive Statistics: Categorical variables will be summarized by frequencies and percentages. Continuous variables will be summarized by means, standard deviations, medians, quartiles, minima and maxima.

Control of Type I Error: This pilot study is descriptive in nature. P-values and confidence intervals around estimates of treatment differences will not be adjusted for multiplicity and are descriptive in nature. These statistics will be provided to guide further future development of the aptasensor; caution must be used as these statistics will not have a controlled Type I error.

Definition of Baseline: For both adherence regimens baseline is defined as the Visit 2 pre-dose measurement.

2. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

LIST OF TABLES

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Table 2: Abbreviations

AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Act
STI	Case report form
CV	Cervicovaginal
EVMS	Eastern Virginia Medical School
FAS	Full analysis set
FDA	U.S. Food and Drug Administration
TDF/FTC	Emtricitabine + TDF (Truvada®)
GCP	Good Clinical Practice
HA	High Adherence
HCPs	Health Care Providers
HIPAA	The Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IRB	Institutional Review Board
LA	Low Adherence
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometry
LLOQ	Lower limit of quantification
NIH	National Institutes of Health
NPV	Negative Predictive Value
PI	Principal Investigator
PK	Pharmacokinetic
POC	Point of Care
PPV	Positive Predictive Value
PrEP	Pre-exposure prophylaxis
PSA	Prostate Specific Antigen
SAE	Serious adverse event
SAP	Statistical analysis plan

STI	Sexually transmitted infection
TFV	Tenofovir
TP	Treated Population

4. INTRODUCTION

Nearly 35 million people worldwide have died from acquired immunodeficiency syndrome (AIDS) - related causes, and approximately 77 million people have become infected with HIV since the epidemic began [9]. This epidemic continues to grow with almost 2 million new infections occurring every year, and significant increases in youth populations in developing countries will only compound the problem. Tenofovir disoproxil fumarate / Emtricitabine (TDF/FTC - Truvada®) is the only oral pill that is approved for prevention of HIV acquisition in the United States [10]. Two landmark safety and effectiveness trials which supported the FDA approval of TDF/FTC showed TDF/FTC reduced risk of HIV infection by 42% (iPrEX) and 72% (Partners PrEP), noting that risk reduction was highly correlated with drug adherence [4, 5]. Although other clinical studies did not show efficacy, the results correlating poor drug efficacy with low plasma drug levels did confirm the importance of adherence [6, 7]. The approved one pill per day regimen TDF/FTC reduces the risk for sexual transmission of HIV by 90% suggesting that with proper adherence a much greater reduction in risk of infection is achievable.

Given the importance of adherence to the efficacy of TDF/FTC it is of clinical significance to have a reliable metric for adherence levels. Self-reporting of adherence, a non-invasive and simple method of assessment, does not reliably correlate with effectiveness and blood serum levels [6, 7]. Currently, the most objective measure of adherence available is to detect and quantify plasma levels of tenofovir (TFV), the first metabolite of TDF, using liquid chromatography tandem mass spectrometry (LC-MS/MS). Although this method of quantification is reliable, it requires a specialized lab and equipment which comes at a significant cost and does not qualify as point-of-care (POC) test. Other recently developed methods of measuring TFV levels in urine, ELISA and lateral flow assays, have shown promise, but are limited by inadequate sensitivity to detect levels observed in plasma and the inability to distinguish high adherence from white coat effects. This lack of a POC test to measure adherence leaves a critical gap for physicians and other health care providers (HCPs) when tailoring counseling to improve adherence and ultimately HIV prevention of their patients and communities.

As such, there is an opportunity to evaluate a new method of measuring adherence in a reliable, objective, and cost-effective manner. Aptamers are DNA- or RNA-based ligands capable of binding practically any molecular target with high specificity, including small molecules such as antiretroviral drugs [8]. Aptamers have advantages over antibodies. They are more stable and made through a process that is more economical and reproducible, which increases the aptamer's potential capability of being more specific than its antibody counterpart. Therefore, aptamers are being used as capture molecules for biosensor platforms, which are becoming smaller than previous models, more portable, more sensitive and quantitative (rivaling LC-MS/MS), require less sample volume, and simpler to use. Therefore, aptamer-based biosensors or "aptasensors," have great potential for real-time therapeutic drug monitoring. We will be utilizing a previously validated DNA aptamer specific for TFV and applying it to a biosensor platform.

This study will focus on determining the sensitivity and specificity of the TFV aptasensor and assess its ability to distinguish TFV levels associated with low and high adherence using biological fluids collected from women taking TDF/FTC at different dosing regimens.

5. TRIAL PURPOSE, OBJECTIVES AND ENDPOINTS

5.1. Purpose

The purpose of this study is to assess the sensitivity and specificity of the TFV aptamer-based biosensor measuring TFV levels in plasma, urine, and vaginal fluid and its ability to monitor adherence to antiretroviral therapy. The concentrations measured with aptamer-based biosensor will be compared to concentrations measured via LC-MS/MS, which is currently the gold standard for pharmacokinetic analysis of TFV.

5.2. Objectives

Compare plasma, urine, and vaginal fluid TFV concentrations measured by the aptasensor with those values quantitated by LC-MS/MS to determine agreement between the two methodologies, sensitivity, specificity, NPV, and PPV.

5.2.1. Primary Objectives

- Compare plasma TFV concentrations measured by the aptasensor with those values quantitated by LC-MS/MS to determine agreement between the two methodologies, sensitivity, specificity, NPV, and PPV
- Determine if the TFV aptasensor can detect differences in the plasma TFV concentrations between high and low adherence dosing regimens and compare the mean difference to the difference calculated from values generated by LC-MS/MS

5.2.2. Secondary Objectives

- Compare urine TFV concentration measured by the aptasensor with those values quantitated by LC-MS/MS to determine agreement between the two methodologies, sensitivity, specificity, NPV, and PPV
- Determine if the TFV aptasensor can detect differences in the urine concentrations of TFV between high and low adherence dosing regimens and whether these levels correlate with adherence
- Compare vaginal fluid TFV concentrations measured by the aptasensor with preclinical determinations of the range of detectable TFV levels and the aptasensor's limit of detection
- Determine if differences in adherence dosing regimens can be correlated to differences in vaginal fluid TFV levels

5.3. Endpoints

5.3.1. Primary Endpoint

- TFV concentrations in *plasma* at baseline (pre-dose), 24 hours, 7 days and 14 days after dosing

5.3.2. Secondary Endpoint

- TFV concentrations in *urine* and *vaginal fluid* at baseline (pre-dose), 24 hours, 7 days and 14 days after dosing

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This pilot, prospective, randomized study will screen approximately 20 healthy, non-pregnant, HIV negative, premenopausal women (aged 18-50) at EVMS who are not at risk of pregnancy and are at low risk for sexually transmitted infections (STIs) in order to have approximately 14 women complete all study visits. This study will examine the plasma, urine, and vaginal fluid from women in two dosing regimens of oral tablets: TDF/FTC (300/200 mg), 7 pills per week and TDF/FTC (300/200mg), 3 pills per week. Participants will be randomized to regimen as per the following schemata:

Table 3: Randomization Assignments

Regimen	N	Dose
Low Adherence (LA) Regimen	7	TDF/FTC (300/2200 mg), 3 pills/week, for a total of 6 ingested pills
High Adherence (HA) REgimen	7	TDF/FTC (300/200 mg), 7 pills/week, for a total of 14 ingested pills

Participants will undergo the low adherence (LA) regimen or a high adherence (HA) regimen for two weeks, consisting of 5 visits.

Visit 1: At screening/enrollment, volunteers will be consented and undergo procedures to confirm they are eligible to continue in the study. Eligible participants will proceed to Visit 2.

Visit 2: Baseline blood, urine, and vaginal fluid will be collected. Participants will be randomized to dosing regimen and the first dose of TDF/TFC will be ingested in the clinic under direct observation. Participants will be instructed to take subsequent doses at home.

Dosing Contact: Participants will be sent reminder text messages to facilitate dosing occurring at the same time of day. Participants will text message the study coordinator to confirm ingestion of tablet. If the participant does not respond to a text within approximately 4 – 6 hours after it is sent, a reminder text will be sent.

Visits 3-5: Participants will return at 24 hours, 7 days, and 14 days after visit 2 for the pre-dose (for visits 3 and 4) collection of blood, urine, and vaginal samples.

Follow Up Contact: A final follow up site contact will be conducted approximately 1 – 2 weeks after the last visit to ask the participant about adverse events and concomitant medications. The participant will be exited unless there are symptoms that require follow up.

Table 4: Schedule of Evaluations

Single Dose Phase (EVMS only)					
		Visit 1 Screening	**Visit 2** Baseline	**Visit 3-5** 24h, 7 days, and 14 days after Visit 2	**Follow Up Contact**
Informed consent		✓			
Review eligibility criteria, interval history		✓	✓		
Demographic info/medical history		✓			
Height, weight, and blood pressure		✓			
Ask about compliance with study instructions/restrictions			✓	✓	✓
Ask about AEs, CMs			✓	✓	✓
Directed Physical Exam, pelvic exam		(✓)	(✓)	(✓)	(✓)
Urine	Urine Pregnancy test	✓	(✓)	(✓)	(✓)
	PK (Urine): TFV		✓	✓	✓
Blood and Genital Fluids	Safety: HIV, HBsAg and serum creatinine	✓			
	PK (Plasma): TFV		✓	✓	✓
	PK (CV Fluid): TFV			✓	
Randomize to dosing regimen (HA versus LA)			✓		
Single Dose in clinic			✓	(✓)	
Provide relevant instructions (e.g., adverse events)		✓	✓	✓	✓

(✓) = if indicated

6.2. Anticipated Length of Study

Recruitment for the study is expected to take approximately 2-3 months. Each participant is expected to complete the study in approximately 2 months from time of enrollment with all participants complete within a total of 6 months. Lab assays will begin once the first participant completes all visits and will continue until the last participant. Data analysis is expected to take approximately 3 months and preparation of the final report 2 additional months.

6.3. Number of Subjects

Approximately 20 women may be consented and undergo assessment procedures in order to have approximately 14 women who meet eligibility criteria complete the study. All 14 women will be randomly assigned to undergo either a low adherence dosing or high adherence dosing. All randomized participants will be included in the analysis.

6.4. Treatment Assignment

Women will be randomized to one of two in two dosing regimens of oral tablets: TDF/FTC (300/200 mg), 7 pills per week and TDF/FTC (300/200mg), 3 pills per week.

6.5. Criteria for Study Termination

Recruitment for the study or a treatment arm may be stopped if, in the opinion of the grant principle investigator:

- The site has failed to enroll participants at an acceptable rate
- The protocol requirements have not been adhered to, and/or
- Administrative reasons

7. SELECTION AND WITHDRAWAL OF PARTICIPANTS

The volunteers for this study will be recruited primarily through word of mouth and by using existing databases of previous study participants (as protected health information permits.)

Only women will be recruited since the endpoints require vaginal sampling. The NIH has mandated that children, defined as younger than 18 years old, 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"). As such, this study does not plan to enroll children. Efforts will be made by the sites to recruit participants so that the racial and ethnic characteristics of the subject population will reflect the demographics of the study sites. No selection criteria shall be based on race or ethnicity.

7.1. Inclusion Criteria

Volunteers must meet all of the following criteria prior to baseline sampling at visit 2:

1. Age 18 to 50 years, inclusive
2. General good health (by volunteer history and per investigator judgment) without any clinically significant systemic disease (including, but not limited to significant liver disease/hepatitis, gastrointestinal disease, kidney disease, thyroid disease, osteoporosis or bone disease, and diabetes) and with an intact gastrointestinal tract, uterus and cervix.
3. Estimated calculated creatinine clearance (eCcr) of at least 80 mL/min
4. Body Mass Index (BMI) of ≥18 and <35kg/m^2; and a total body weight >45 kg (99.2 lbs)
5. Willing to give voluntary consent and sign an informed consent form
6. Willing and able to comply with protocol requirements, including swallowing tablets
7. Must be protected from pregnancy by:
    - Condoms
    - Hormonal contraceptives
    - Copper or Levonorgestrel IUD
    - Sterilization of either partner
    - Heterosexual abstinence
    - Same sex relationship
8. If in a relationship, must be in a mutually monogamous relationship with a partner who is not known to be HIV positive and has no known risk of STIs

7.2. Exclusion Criteria

Volunteers must not meet any of the following criteria prior to baseline sampling:

1. Currently pregnant
2. Currently breastfeeding or planning to breastfeed during the course of the study
3. In the last three months, diagnosed with or treated for any STI

4. Positive test for HIV, or Hepatitis B surface antigen (HBsAg)

5. Systemic use in the last two weeks or anticipated use during the study of any of the following: antiretrovirals (e.g. Viread®, Atripla®, Emtriva®, or Complera®), or drugs that may interact with TFV (e.g., protease inhibitors, anticonvulsants, antimycobacterials, St. John's Wort).
6. Participation in any other investigational trial with use of a drug/device within the last 30 days or planned participation in any other investigational trial with use of a drug/device during the study
7. Grade 2 or higher laboratory abnormality, per the 2014 update of the Division of AIDS, National Institute of Allergy and Infectious Disease (DAIDS) Table for Grading the Severity of Adverse Events, or clinically significant laboratory abnormality as determined by the clinician
8. Abnormal finding on laboratory or physical examination or a social or medical condition in the volunteer which, in the opinion of the investigator, would make participation in the study unsafe or would complicate interpretation of data

7.3. Participant Withdrawal

7.3.1. Withdrawal Criteria

Participants who sign the informed consent and agree to participate in the study, but do not meet eligibility criteria will not undergo baseline sampling and will not continue in the study. Once a participant undergoes baseline genital/rectal sampling, she may be withdrawn from the study for the following reasons:

- Failure to follow protocol requirements that is judged severe enough by the investigator to significantly affect study outcomes
- Pregnancy or desire to become pregnant
- Medical reasons, including diagnosis of an STI
- Personal reasons (participant request)
- Discontinuation of treatment adherence arm, or of entire study (Section 6.5)

7.3.2. Target Enrollment

Participants who initiate product use but discontinue the study prior to completion may not re-enroll. Participants will be screened and enrolled so that approximately 14 women (7 per adherence cohort) complete all study visits.

7.3.3. Follow-up

If a participant chooses to discontinue the study after baseline sampling, the site may ask the participant to return for the next visit's study procedures, if she is willing and if relevant. She will be asked about medications taken and adverse events (AEs) since the last visit. She will be exited from the study during her last contact with the site.

8. STUDY PROCEDURES

Prospective participants may be pre-screened by telephone or in person: the study will be explained, the inclusion/exclusion criteria reviewed, volunteers' questions answered, and Visit 1 scheduled.

8.1. Visit 1: Screening/Enrolment

The following will take place to confirm the volunteer is eligible to continue in the study. Note that if the participant is not able to complete any of the study procedures at this visit, she may return for an unscheduled visit prior to Visit 2.

- The study and informed consent form will be reviewed and all volunteer questions will be answered. If the volunteer is eligible and wishes to participate in the study, she will be asked to sign an informed consent form. The Principal Investigator (PI) or designee will sign the form and offer a copy to the participant. Permission may be requested as part of the informed consent process for storage of the biologic samples obtained during the study for possible future testing, as allowed by the site.
- The informed consent process will include an explanation of adherence support (e.g., text message reminders) and a request for permission to contact the participant for this purpose.
- The participant will be interviewed to obtain medical history and demographic information.
- Height, weight, and blood pressure will be measured and recorded.
- A urine specimen will be obtained for a urine pregnancy test. If the pregnancy test is positive, the participant will be referred as necessary and will not continue in the study.
- If the participant's history is significant for a medical condition, a directed physical exam and or pelvic exam will be performed as necessary.
- Blood samples (approximately 4 teaspoons) will be collected for HIV and HBsAg testing as well as serum creatinine to calculate a creatinine clearance. Participants who test HIV or HBsAg positive at screening are not eligible to continue in the study. They will be counseled and given referrals for medical and social services.

Eligible participants will be scheduled for the next visit and directed to refrain from vaginal intercourse for 48 hours prior to visit 2.

8.2. Visit 2: Baseline Pre-Dose Sample Collection

This visit will be scheduled for the early follicular phase of the menstrual cycle (for cycling women).

- An interval history will be collected.
- Eligibility criteria will be reviewed. If any lab tests reveal that the participant is not eligible, the visit should not proceed and the participant will not continue in the study.
- If indicated by signs or symptoms or history, a directed physical exam and/or urine pregnancy test will be performed. If pregnancy is diagnosed, the participant will be referred as necessary and will be discontinued.

- A vaginal swab for prostate specific antigen (PSA) will be obtained, if the PSA test indicates semen exposure, the visit will be re-scheduled.
- A urine sample will be obtained.
- Vaginal swabs will be obtained
- A blood sample (approximately 2 teaspoons) will be obtained.
- The participant will be randomized to the HA versus LA regimen and will take her first does of TDF/FTC in the clinic under direct observation and the time of product ingestion will be recorded in the source document. The pill will be ingested after urine, vaginal and blood samples are obtained.
- The participant will confirm her contact information for the medication text reminder.
- Participants will be instructed to refrain from vaginal intercourse for 48 hours prior to visits 3 – 5.

8.3. Visits 3 – 5: 24 hours , 7 days, and 14 days after first dose

Visits 3, 4, and 5 will be scheduled for 24 hours, 7 days, and 14 days after initial dosing at Visit 2, respectively. The time of the visit will assure that it has been at least 24 hours since the participant's last dose of TDF/FTC. At each visit, the following procedures will be performed:

- The study staff will ask the participant if she followed the instructions from the previous visit, and ask about adverse events and concomitant medications.
- If indicated, a directed physical exam, pelvic exam and/or pregnancy test will be performed. If pregnancy is diagnosed, the participant will be referred as necessary and will be discontinued.
- Visits 4 and 5: Study staff will count the number of pills remaining and query the participant on compliance with her assigned dosing regimen.
- A vaginal swab for PSA will be obtained, if the PSA test indicates semen exposure, the participant will be reminded of the restrictions on vaginal intercourse, but the visit will proceed.
- A urine sample will be obtained.
- Vaginal swabs will be obtained
- A blood sample (approximately 2 teaspoons) will be obtained.
- Visits 3 and 4: The participant will take her dose of medication in the clinic, under direct observation, if applicable to her dosing regimen, after urine, vaginal and blood samples are obtained.
- Visits 3 and 4: Study staff will dispense the appropriate number of TDF/FTC pills in a pill carrying case, so that the participant has enough pills to take her to before her next scheduled visit.

8.4. Final Follow Up Contact

The site will contact the participant approximately 1 - 2 weeks after the last visit. The participant will be asked about any AEs and concomitant medications since her last visit. The participant will be exited from the study unless symptoms require further follow up or she is appropriately referred.

8.5. Unscheduled Visits

The participant will be instructed to contact the site if she experiences moderate to severe symptoms (e.g. gastrointestinal symptoms).

Unscheduled visits or procedures (e.g. urinalysis, vital sign measurements) may be performed at the participant's request or as deemed necessary by the investigator at any time during the study. The participant will be asked to come in for an evaluation, as indicated. Unscheduled visits that require examination or interview due to symptoms will be in the study database.

When an unscheduled visit occurs in response to an AE experienced by a study participant, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care, as necessary. All AEs will be evaluated and follow-up of any observed abnormalities will proceed according to the study manual.

8.6. Early Discontinuation

If the participant discontinues from the study prior to the final visit, the site may ask the participant to return for study procedures and/or sample collection, if she is willing and if relevant. At a minimum, the site may ask participants to return for safety assessments. She will be asked about medications taken and adverse events including symptoms since the last visit. She will be exited from the study during her last contact with the site.

9. TREATMENT OF PARTICIPANTS

9.1. Study Products

Table 5 provides a summary of the treatment to be administered, including the doses and route of administration.

Table 5: Emtricitabine/tenofovir disoproxil fumarate (FTC/TDF 200/300 mg)

Product Name	emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) (Truvada®)
Unit Dose	200 mg FTC / 300 mg TDF
Dosage Form	Tablet
Route of Administration	Oral
Physical Description	The tablets are blue, capsule-shaped, film-coated, and debossed with "GILEAD" on one side and with "701" on the other side
Manufacturer	Gilead Sciences, Inc. (Foster City, CA USA)

9.2. Concomitant Medications

All concomitant medications will be recorded on source documents and the study database for participants who have undergone baseline genital/rectal sampling.

Antriretrovirals (e.g., Viread®, Atripla®, Emtriva®, or Complera®), or drugs that may interact with TFV (e.g., protease inhibitors, anticonvulsants, antimycobacterials, St. John's Wort) should not be used during the study (see Section 7.2).

9.3. Treatment Compliance

Treatment compliance will be evaluated by directly observed therapy (in-clinic dosing) at visits 2, 3 and 4, as appropriate to the participant's dosing regimen, participant report and returned tablet count.

9.4. Randomization and Blinding

This is a prospective, randomized trial. Participants and clinical study staff will not be blinded to the adherence dosing regimen. The laboratories and statistical/data analysts will be blinded to study treatment. Participants will be randomized 1:1 to the HA versus LA regimen, by simple randomization.

10. STUDY DRUG MATERIALS AND MANAGEMENT

10.1. Description of Study Drug

10.1.1. Emtricitabine/tenofovir disoproxil fumarate (F/TDF)

The F/TDF tablet is marketed as Truvada®. Each tablet contains 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate (which is equivalent to 245 mg of tenofovir disoproxil). The tablets are blue, capsule-shaped, film-coated with a length of 19 mm and a width of 8.5 mm, and are debossed with "GILEAD" on one side and with "701" on the other side. Excipients include: [Tablet Core] croscarmellose sodium, lactose monohydrate, magnesium stearate, microcrystalline cellulose, and [Film Coating] polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, and iron oxide black. Thirty (30) tablets are packaged in 75 ml, white, high density polyethylene bottles with a three-gram desiccant canister (without patent number) present in each bottle. Each bottle is enclosed with a white, continuous thread, child-resistant polypropylene screw cap with an induction-sealed, aluminum-faced liner. Participants will receive enough study drug at visits 3 and 4 to take them to their next follow up appointment. Study drug will be dispensed in accordance with the Clinical Research Center standard operating procedure.

10.2. Study Drug Packaging and Labeling

The site will manage the distribution of TDF/FTC according to the CRC SOP. The study drug will be purchased from a local licensed pharmacy and stored in a secure, temperature controlled space.

10.3. Study Drug Storage

Study products will be stored at room temperature (15° - 30°C) (59° - 86°F) in a locked, secure, temperature monitored area in the clinic prior to dispensing.

10.4. Study Drug Preparation

These study products are ready to use and require no preparation other than packaging as described in Section 10.2.

10.5. Administration

Each of the doses is to be administered orally by the participant. In-clinic doses will include the dose on Day 1 (visit 2), Day 2 (visit 3) and Day 7 (visit 4). Participants will be asked to self-administer the other doses at home; participants may also come to the clinic to take their daily doses. The time of visits will be scheduled so it has been at least 24 hours since the participant's last dose.

10.6. Study Drug Accountability

Dispensation of doses, doses taken by the participant, and returned unused doses will be recorded in the source documents.

10.7. Study Drug Handling and Disposal

All un-dispensed and returned study drug remaining at the end of the study will be recorded by the site and reconciled by the investigator. After reconciliation, any remaining study drug will be properly disposed of.

11. BIOANALYTICAL AND BIOCHEMICAL ASSESSMENTS

Blood, Urine and CV swabs will be taken to PK as detailed below in Tables 6 and 7.

Table 6: Example of Schedule for PK Samples for High Adherence Dosing Regimen (total of 14 doses)

Day of Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	Mon
Visit	2	3						4							5
Dose	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
24 hour Post Dose Procedures	Blood, Urine, Vaginal Fluid	Blood, Urine, Vaginal Fluid						Blood, Urine, Vaginal Fluid							Blood, Urine, Vaginal Fluid
Day of Study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Table 7: Example of Schedule for PK Samples for Low Adherence Dosing Regimen (Total of 6 doses)

Day of Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	Mon
Visit	2	3						4							5
Dose	1		2		3			4		5		6			
24 hour Post Dose Procedures	Blood, Urine, Vaginal Fluid	Blood, Urine, Vaginal Fluid						Blood, Urine, Vaginal Fluid							Blood, Urine, Vaginal Fluid
Day of Study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

12. ASSESSMENT OF SAFETY

12.1. Medical History

A medical history will be completed at Visit 1. Height, weight, and blood pressure will be measured.

As part of AE data collection, participant AEs will be assessed at each study visit starting after baseline sample collection at Visit 2 as applicable; participant report of symptoms will be documented and may result in follow-up assessments.

12.2. Physical Examination

A physical exam and pelvic exam will be performed at Visit 1 as necessary, based on participant history, signs and symptoms. If indicated, a directed physical exam will be performed at each study visit, with changes on physical exam documented.

12.3. Laboratory Assessments

12.3.1. Blood Samples

HIV and HBsAg tests will be performed at Visit 1. A serum creatinine will be performed to calculate a creatinine clearance at visit 1.

12.4. Pregnancy Screen

A pregnancy test is required at Visit 1. Note that participants must be protected from pregnancy as described in Section 7.1 in order to be eligible for study participation. A pregnancy test will be done at subsequent visits, if indicated by participant signs and symptoms.

13. ADVERSE AND SERIOUS ADVERSE EVENTS

13.1. Definition of Adverse Event (AE)

An AE is any untoward medical occurrence associated with the use of an investigational or approved product in humans, whether or not considered product related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. Pre-existing events that increase in frequency or severity in nature during or as a consequence of use of an investigational product in human clinical trials will also be considered as AEs. Any AE with an onset date after the first date of study product administration is considered to be treatment-emergent.

All AEs with onset at or after baseline sampling, whether or not related to the study product, will be recorded in the study database.

According to 21 CFR 312.32 "IND Safety Reports" the following definitions of terms apply to AEs occurring in clinical studies involving drugs.

13.1.1. Suspected Adverse Reaction

A suspected adverse reaction is "any AE for which there is a reasonable possibility that the study product caused the event." An AE is considered to be a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the AE. Examples of a causal relationship include:

- A single occurrence of an event that is uncommon and known to be strongly associated with product exposure (e.g., not necessarily applicable to this study, include angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event that is not commonly associated with product exposure, but is otherwise uncommon in the population exposed to the study product (e.g., tendon rupture)
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of product exposure) that indicates those events occur more frequently in the product exposure group than in a concurrent or historical control group.

13.1.2. Adverse Reaction

An adverse reaction is any AE caused by the study product. Adverse reactions are a *subset* of suspected adverse reactions where there is reason to conclude that the product caused the event.

13.1.3. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it:

- Results in death

- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

All SAEs starting from baseline until the last contact with the site, whether or not they are related to the study product, must be recorded on in the source documents and study database. Reporting of SAEs is outlined in Section 13.4.

13.1.4. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the drug packet insert, is not listed at the specificity or severity that has been observed. For example (not necessarily applicable to this study), under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the package referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the package insert listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

13.1.5. Serious and Unexpected Suspected Adverse Reaction

The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the product and the AE as described above.

13.2. Relationship to Study Product

An Investigator who is qualified in medicine must make the determination of relationship to the investigational product for each AE. 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. Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, January 2010), the relationship categories that will be used for this study are:

- **Related:** There is a reasonable possibility that the AE is related to the study product(s)
- **Not related:** There is not a reasonable possibility that the AE is related to the study product(s)

13.3. Recording and Grading Adverse Events for Severity

Adverse events spontaneously reported by the participant and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the clinical site. The AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the date of onset, date of resolution, severity, relatedness, action

taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study. Each AE will be graded for severity using the applicable DAIDs tables for grading the severity of adverse events (see Table 8), which can also be found at http://rsc.tech-res.com/safetyandpharmacovigilance/.

Table 8: Grading the Severity of Adverse Events

Adult and Pediatric AEs	http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_Grading_Table_v2_NOV2014.pdf
Female Genital AEs	http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Addendum_1_Female_Genital_Grading_Table_v1_Nov_2007.pdf
Rectal Grading Table	http://rcc.tech-res.com/docs/default-source/safety/rectal-tox-table-updates-05-11-2012.pdf?sfvrsn=6

For clinical AEs NOT identified in the DAIDS AE Grading Tables, the following scale (as listed in the DAIDS Table for Grading the Severity of Adult and Pediatric AEs) should be used to grade severity:

- Mild: Symptoms causing no or minimal interference with usual social and functional activities
- Moderate: Symptoms causing greater than minimal interference with usual social and functional activities
- Severe: Symptoms causing inability to perform usual social and functional activities
- Potentially Life-Threatening: Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 13.1.3. An AE of severe intensity may not necessarily be considered serious.

Participants must be protected from pregnancy to participate in the study. Should a pregnancy occur after baseline sampling, it must be reported as soon as possible to the study investigators. The participant will be discontinued from the study and appropriate exit procedures will be followed. Note that pregnancy in itself is not regarded as an AE. If the participant has been exposed to study product, the course of the pregnancy should be followed until it has an outcome (e.g., spontaneous miscarriage, elective termination, normal birth, congenital abnormality). If the participant seeks care outside the site, every effort should be made to obtain her consent for the site to receive a copy of her medical records related to the pregnancy, its outcome and the health of the neonate, if applicable.

Reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be reported as AEs.

13.4. Reporting Serious Adverse Events

Any SAE, including those listed in the protocol or package insert must be reported to the EVMS IRB within 72 hours of discovery. If there is any question whether the event meets the criteria for "serious" it should be reported anyway. In addition, a completed SAE form must be filled out as soon as possible. The investigator must complete, sign and date the SAE pages, verifying the accuracy of the information recorded on the SAE pages and the corresponding source documents.

Additional follow-up information, if required or available, should all be sent to the EVMS IRB within one business day of receipt and this should be completed on an SAE form and placed with the original SAE information and kept with the appropriate section of the study database and/or study file.

The investigator will notify the EVMS IRB of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible, but in no case later than 7 calendar days after she initially receives the information.

The site will promptly investigate and follow up on all safety information it receives, with the cooperation of the investigator.

The investigator is responsible for complying with IRB requirements for AE reporting.

13.5. Temporary Product Hold/Permanent Discontinuation in Response to Adverse Events

13.5.1. Grade 1 or 2

In general, a participant who develops a Grade 1 or 2 AE, regardless of relationship to study product, may continue product use. If the PI/designee opts to temporarily hold study product, reasons for product hold must be documented in the study source documents.

Follow-up testing for Grade 2 laboratory test results should be performed at scheduled study visits, as clinically relevant. More frequent testing may be performed at any time if required to properly monitor and/or manage participant safety, at the discretion of the PI/designee.

13.5.2. Grade 3 or 4

A participant who develops a Grade 3 or a Grade 4 AE that is product-related should have the product temporarily withheld. If a participant develops a Grade 3 or Grade 4 AE that is not product-related, the PI/designee may temporarily withhold product. In either case, the PI/designee must continue the temporary product hold until resolution of the AE. If product use is resumed and the same AE recurs at the same (or worse) grade level at any time during the study, study product must then be permanently discontinued.

14. STATISTICS

14.1. Sample Size Justification

Sample size is based on feasibility rather than statistical considerations; thus, analysis will be primarily descriptive and graphical. Participant flowchart (e.g. number screened, number with genital sampling) will be provided.

14.2. General Statistical Issues

All attempts will be made to avoid missing data. However, missing values will remain as missing, i.e., no attempt will be made to impute. In general, only observed values will be used in data analyses and presentations.

14.3. Analysis Populations

The population includes healthy, non-pregnant, HIV negative women. The analysis population will include all participants who undergo baseline genital/rectal sampling.

The Full Analysis Set (FAS) will consist of all subjects who are randomized and have at least one post-baseline assessment. The FAS will be used to summarize study disposition, baseline characteristics, and all non-safety endpoints.

Evaluation of safety endpoints will be conducted using the Treated Population (TP), consisting of women with at least one dose of product use. If all subjects in the TP have at least one post-baseline assessment, then the TP will be identical to the FAS.

All populations will be defined based on the adherence regimen assigned (HA versus LA).

Additional populations or refinements to these definitions, if needed due to unanticipated circumstances, will be described in the SAP or statistical report.

14.4. Statistical Analysis

Sample size is based on feasibility rather than statistical considerations; thus, analysis will be primarily descriptive. Participant flowchart (e.g. number screened, number with sampling) will be provided. All populations will be defined based on the adherence regimen.

Evaluation of Study Objectives: Study objectives will be evaluated by clinical review of descriptive summaries and graphical displays.

Primary analysis of this study will evaluate the performance of the TFV aptasensor by comparing its measurements of the concentrations of TFV in clinical samples from all women with those values obtained from LC-MS/MS. Correlation and agreement between the TFV levels measured by the aptasensor and LC-MS/MS will be analyzed using scatterplot and linear regression. Continuous variables will be compared between values measured with the aptasensor versus LC-MS/MS using paired comparisons. From this comparison, sensitivity, specificity, NPV, and PPV of the aptasensor will also be determined. Confidence intervals will be calculated to assess precision and margin of error of those

measures. Using the14 baseline pre-dose samples, each woman will be her own control, and these pre-dose samples will be used for the assessment of specificity and NPV.

The secondary analysis of this study will evaluate whether the aptasensor can detect differences in TFV levels between the two independent cohorts of women taking TDF/FTC at different dosing regimens representing high (7 pills/week) and low adherence (3 pills/week). We will compare the two independent cohorts using an independent samples t test for normally distributed data or a Wilcoxon-Mann-Whitney test for non-parametric data. TFV concentrations from the LA and HA cohorts will be characterized using descriptive statistics (mean, median, interquartile range, minimum and maximum). The values will be compared between the high adherence and low adherence groups as well as comparing the mean difference between groups generated by the aptasensor to that difference generated by LC-MS/ MS. Distributions will be checked to determine normality so as to determine the appropriate statistical test.

Descriptive Statistics: Categorical variables will be summarized by frequencies and percentages. Continuous variables will be summarized by means, standard deviations, medians, quartiles, minima and maxima.

Control of Type I Error: This pilot study is descriptive in nature. P-values and confidence intervals around estimates of treatment differences will not be adjusted for multiplicity and are descriptive in nature. These statistics will be provided to guide clinical judgment; caution must be used as these statistics will not have a controlled Type I error.

Definition of Baseline: For both adherence regimens baseline is defined as the Visit 2 pre-dose measurement.

14.5. Interim Analysis

No interim analysis will be conducted.

15. MANAGEMENT OF INTERCURRENT EVENTS

15.1. Loss to Follow-up

If a participant fails to appear for a scheduled visit, at least three attempts to contact her should be made over the subsequent 30 days. These attempts should be documented in the participant's study file. The final attempt must be a certified letter to the participant with return-receipt requested, or an outreach attempt. A copy of this letter or documentation of the final outreach attempt should be in her file. After these three attempts, no further efforts need to be made to find her, but her file should remain open until study closeout.

If the participant does not contact the clinic before the study is closed, her final disposition will be recorded at the time of study closeout, to indicate that she was lost to follow-up. The lost to follow-up designation cannot be made for any participant until study close out.

15.2. Protocol Adherence and Sampling Windows

Visits 3, 4 and 5 should be scheduled so that it has been at least 24 hours since the participant's last dose of study medication. Visit 3 should be conducted 24 hours + 4 hours from the ingestion of the first dose at Visit 2. Visit 4 should be conducted 7 days + 6 hours from the ingestion of the first dose at visit 2. Visit 5 should be conducted 14 days + 6 hours from the ingestion of the first dose at visit 2.

The study site should carefully record the date and time of each collection of blood, urine and CV swabs. The site should carefully record the date and time of each ingestion of medication in the clinic.

15.3. Protocol Violations

If a protocol violation is required to protect the life or physical well-being of a participant in an emergency, it may be carried out without prior approval from the IRB. The investigator must, however, report the violation to the IRB as soon as possible, no later than 5 working days after the emergency occurred.

Other violations from the protocol may not be carried out without prior approval from the IRB if the change involves the rights, safety, or welfare of participants; and without prior approval from the grant PI (Terry Jacot, PhD) if the change involves the validity of the data, the study's scientific soundness or the rights, safety, or welfare of participants.

All protocol violations should be recorded in a protocol violation log.

15.4. Modification of Protocol

No modification of this protocol may be made without written approval of Dr. Jacot.

16. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

16.1. Study Monitoring

The study site may be monitored by the EVMS IRB at any time. This monitoring may include:

- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the CRFs, and that investigational product accountability checks are being performed
- Confirm all volunteers have been properly consented
- Record and report any protocol violations not previously sent to the IRB
- Confirm AEs and SAEs have been properly documented and confirm any SAEs have been forwarded to the IRB and those SAEs that met criteria for reporting have been forwarded to the IRB

16.2. Institutional Review Board (IRB)

The Principal Investigator (PI) must obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

17. ETHICS

17.1. Ethics Review

The final study protocol, including the final version of the informed consent form, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. The study must be conducted in accordance with all conditions of approval by the IRB.

The PI is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

17.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH GCP, and applicable regulatory requirements. In addition, the PI will follow U.S. Department of Health and Human Services regulations regarding the Health Information Portability and Accountability Act (HIPAA 45, CFR 164). The PI will ensure that appropriate health care or referral is provided for the participants throughout the study.

17.3. Written Informed Consent

17.3.1. Procedure for Obtaining Informed Consent

No volunteer may be admitted into this study until the PI (or designee) has obtained her legally effective informed consent. The PI (or designee) shall seek such consent only under circumstances that provide the prospective participant with sufficient opportunity to consider whether or not to participate in the study. Informed consent must be obtained without coercion, undue influence, or misrepresentation of the potential benefits or risks that might be associated with participation in the study.

Informed consent encompasses all oral and written information given to the volunteer about the study and the study materials. This includes the consent form signed by the participant, the instructions for use of study materials that are provided to the participant, recruitment advertising, and any other information provided to the participant. All such information that is given to the participant will be in a language that is understandable to her. The information will not include any language in which the participant is made to waive any of her rights or which releases or appears to release the PI or the PI's institution from liability for negligence.

Informed consent will be documented by the use of a written consent form that is signed by the participant and the PI (or designee). A copy of the consent form will be offered to each participant. The original signed consent form for each participant will be kept at the site. The consent form must include each of the basic and additional elements of informed consent described in 21 CFR Part 50.25 and must describe each of the risks or discomforts to the participant that have been identified as reasonably foreseeable.

17.3.2. Subject Confidentiality

The confidentiality of all subjects consented into this clinical study will be protected to the fullest extent possible. Study subjects will not be identified by name on any database, or on any other documentation sent to the IRB or other organizations involved in this study, and will not be reported by name in any report or publication resulting from data collected in this study.

18. DATA HANDLING AND RECORDKEEPING

18.1. Method of Data Capture

Clinical data will initially be recorded on source documents at the clinical site. The source documents, including signed informed consent forms, laboratory reports, and participant records, should be maintained at the site, and should be available for review during monitoring visits.

Information from the source documents will then be entered in to an electronic study database. The electronic database will be stored on a password protected computer in a locked office.

18.2. Inspection of Records

The IRB or funding agency (NIH) will be allowed to conduct site visits to the clinical site for the purpose of monitoring any aspect of the study. The PI agrees to allow the monitor or designee to inspect the storage area, inventory, and accountability records for study product; subject charts and study source documents, and other records relative to study conduct.

18.3. Retention of Records

The signed original informed consent documents for each participant and originals of all study documentation (e.g., study product inventory forms, participant clinic records, original laboratory reports) will be retained by the PI for a minimum of 2 years at the site. No records may be destroyed without written permission from the grant PI (Terry Jacot PhD).

19. INVESTIGATOR RESPONSIBILITIES

19.1. Prior to Starting Study

19.1.1. Signing of Investigator's Agreement and Amendments

Prior to study start the clinical site PI is responsible for signing and dating the Investigator's Agreement for this study protocol. The signed and dated original must be submitted to Dr. Jacot, and a copy must be maintained by the PI at the site with the study files.

All protocol amendments must be signed and dated by the PI. The signed and dated original must be submitted to Dr. Jacot, and a copy must be maintained by the PI at the site. Amendments must be approved by Dr. Jacot and the IRB before implementation.

19.1.2. Forms and Records

Prior to study activation, Dr. Jacot, the grant PI, will review the electronic database and source documents with the site PI, Dr. Thurman. The following forms will be kept in a regulatory binder for the study:

- Statement of the Investigator (FDA Form 1572)
- Financial Disclosure Statement (completed by each staff member listed on FDA Form 1572). Note that this form will also need to be provided for each staff person at the end of the study and one year following completion of the study, as possible.
- IRB information consisting of:
    - Copy of IRB approval letter for protocol, consent form, and other written materials provided to participants
    - Copy of IRB-approved consent form, and other written materials provided to participants, as applicable
- Laboratory information including:
    - Name of laboratories to be used to process study specimens
    - Curriculum vitae of laboratory director(s)
    - Current license(s) and/or laboratory certification (such as the Clinical Laboratory Improvement Act [CLIA] certification), with expiration date
    - Copy of normal values for tests done by each laboratory for this study

19.2. During the Study: Forms and Records

The following forms and records will be maintained at the study site, including but not limited to:

- Package insert for TDF/FTC
- Subject Status Log(s)
- Subject Identification Code List
- Delegation of Responsibilities Log
- Study Supplies Accountability Logs and packing slips
- Protocol Violation Log

- Source documents
- Signed and dated informed consent forms
- IRB documents:
  - Submission and approval letters for protocol and any protocol amendments
  - Submission and approval letters for original and any revised consent forms and any other written material provided to participants
  - Annual submission and approval letters
  - Other IRB correspondence
- Updates on curricula vitae and laboratory information
- General correspondence

Copies of all correspondence between the site and its IRB should be sent to Dr. Jacot.

The PI is responsible for obtaining any updates to these documents, including certification renewals, and sending them to Dr. Jacot in a timely fashion.

19.3. During the Study: Progress Reports

During the study, a monthly report will be sent to Dr. Jacot and will include:

- Participant status
- Protocol violations
- AEs that are serious and/or related to product use

Annual progress reports and a final report will be submitted by the site to the IRB or IEC according to local regulations or guidelines.

20. PUBLICATION POLICY

All information concerning the study supplied by the PI and not previously published is considered confidential.

No data collected in this study will be presented or published without prior approval from the laboratory lead.

21. REFERENCES

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