Study of Truvada for HIV Pre Exposure Prophylaxis Using Daily Directly Observed Therapy to Look at Potential Interactions Between Truvada and Hormone Therapy

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I-BrEATHe - Interactions BEtween Antiretrovirals And Transgender Hormones:

A Pharmacokinetic sub study of the TRIUMPH study

Protocol Number:	N/A
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Approval:

PI/Sponsor Signature (Name and Title)

Date

This confidential information about the protocol is provided for the exclusive use of investigators of this protocol and is participant to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Participant to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing Drs. Robert Grant and Madeline Deutsch with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: N/A

Protocol Title: **I-BrEATHe** - Interactions BEtween Antiretrovirals And Transgender Hormones.

Protocol Date: 22Mar2017

_____ _____

Investigator Signature *Date*

_____

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ART	Antiretroviral Therapy
CoE-T	Center of Excellence for Transgender Health
CFR	Code of Federal Regulations
CRF	Case Report Form
DMC	Data Monitoring Committee
DOT	Directly Observed Therapy
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
FTC	Emtricitabine
FTC/TDF	Emtricitabine/ Tenofovir disoproxil fumarate
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
HT	Hormone Therapy
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
iPrEx	iniciativa Profilaxis Pre-Exposición
IRB	Institutional Review Board
MSM	Men who have Sex with Men
PI	Principal Investigator
PK	Pharmacokinetic
PrEP	Pre-Exposure Prophylaxis
RCT	randomized controlled trial
SAE	Serious Adverse Event
TDF	Tenofovir disoproxil fumarate
TFV-DP	Tenofovir diphosphate
UCSF	University of California, San Francisco
ULN	upper limit of normal

PROTOCOL SYNOPSIS

TITLE	**I-BrEATHe** - Interactions BEtween Antiretrovirals And Transgender Hormones.
SPONSOR	Robert M. Grant and Madeline B. Deutsch
FUNDING ORGANIZATION	University of California Office of the President via the California HIV/AIDS Research Program
NUMBER OF SITES	1
RATIONALE	Drug concentrations after receipt of oral Truvada Pre Exposure Prophylaxis (or PrEP) appeared to be lower in transgender women compared to Men who Have Sex with Men (MSM) in the iPrEx study. Reasons for the lower drug concentrations may be behavioral or biomedical, or a combination of both. There are few to no studies which investigate the potential drug to drug interactions between the components of Truvada (emtricitabine or FTC and tenofovir disproxil fumarate or TDF) and feminizing hormones. In addition, no PrEP studies have ever included transgender men; therefore no knowledge exists on the potential interactions between FTC/TDF and masculinizing hormones. The pharmacokinetic study, using directly observed therapy (DOT), is designed to address the question whether feminizing or masculinizing hormones affect FTC/TDF levels in transgender women or transgender men respectively.
STUDY DESIGN	This is a pharmacokinetic study
PRIMARY OBJECTIVE	Assess pharmacokinetic drug-drug interactions between tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) and cross-sex hormone therapy.
SECONDARY OBJECTIVES	Assess Adverse Events related to TDF/FTC in transgender men and women and compare to historical controls in non trangender MSM
NUMBER OF PARTICIPANTS	48 (24 transgender women and 24 transgender men)
PARTICIPANT SELECTION CRITERIA	Inclusion Criteria: All subjects: • HIV antibody seronegative (negative HIV rapid test), • 18 years or older, • Has a smart phone with access to two-way video call capability, • Willingness to be contacted for a short call every day for 4 weeks,

	• Adequate renal function (creatinine clearance ≥ 60 ml/min estimated by the Cockcroft Creatinine Clearance Formula), • Provides written informed consent, For Transgender women: • Assigned male sex at birth, and self-reported current gender identity as "woman" or "transgender women", or other trans-feminine spectrum identity, • Current feminizing Hormone Therapy (HT) use for at least 6 months For Transgender men: • Assigned female sex at birth, and self-reported current gender identity as "man" or "transgender man", or other trans-masculine spectrum identity, • Current masculinizing Hormone Therapy (HT) use for at least 6 months with testosterone. <u>Exclusion Criteria</u>: • Expects to change or discontinue current HT use during the 4 weeks study period, • Signs of symptoms of acute viral syndrome, • Use of FTC or TDF in the past 90 days • Receiving ongoing therapy with any of the following: ART, including nucleoside analogs, non-nucleoside reverse transcriptase inhibitors, protease inhibitors or investigational antiretroviral agents, interferon (alpha, beta, or gamma) or interleukin (e.g., IL-2) therapy, aminoglycoside antibiotics, amphotericin B, cidofovir, systemic chemotherapeutic agents, other agents with significant nephrotoxic potential, other agents that may inhibit or compete for elimination via active renal tubular secretion (e.g., probenecid), and/or other investigational agents • Renal insufficiency documented as Creatinine Clearance < 60 ml/min • For masculine-spectrum identifying persons, positive pregnancy test at screening • At enrollment, has any other condition that, based on the opinion of the investigator or designee, would preclude provision of informed consent; make participation in the study

	unsafe; complicate interpretation of study outcome data; or otherwise interfere with achieving the study objectives.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	FDA approved Truvada (one tablet containing 200 mg of emtricitabine or FTC and 300 mg of tenofovir disoproxil fumarate or TDF) once daily taken orally with or without food. Gilead Sciences will dispense medication to through their FDA approved commercial packaging of 30 tablets per bottle with an included desiccant.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	*N/A*
DURATION OF PARTICIPANT PARTICIPATION AND DURATION OF STUDY	Participants will be on study for up to 56 days **Screening:** up to 28 days **Treatment:** 4 weeks or 28 days (participants come to clinic weekly and DOT via video calls) **Follow-up:** n/a The total duration of the study is expected to be 7-9 months: 1-3 months for participant recruitment and 6 for final participant follow-up.
CONCOMMITANT MEDICATIONS	Allowed: Testosterone, estradiol, spironolactone, Prohibited: ART, including nucleoside analogs, non-nucleoside reverse transcriptase inhibitors, protease inhibitors or investigational antiretroviral agents, interferon (alpha, beta, or gamma) or interleukin (e.g., IL-2) therapy, aminoglycoside antibiotics, amphotericin B, cidofovir, systemic chemotherapeutic agents, other agents with significant nephrotoxic potential, other agents that may inhibit or compete for elimination via active renal tubular secretion (e.g., probenecid), and/or other investigational agents
EFFICACY EVALUATIONS	*N/A*
PRIMARY ENDPOINT	Concentrations of FTC triphosphate and tenofovir diphosphate (TFV-DP) in Dried Blood Spots (DBS)
SECONDARY ENDPOINTS	*N/A*
OTHER EVALUATIONS	*N/A*

SAFETY EVALUATIONS	Incidence of adverse events *(AEs will be collected as part of the weekly visit if participants report AEs)*
PLANNED INTERIM ANALYSES	*N/A*
STATISTICS **Primary Analysis Plan**	We will compare levels of Tenofovir DiPhosphate (TFV-DP), the phosphorylated active tenofovir molecule in dried blood spots and rates of adverse events between trans men and trans women and a group of MSM historical controls from the iPrEx OLE study. We will compare log transformed TFV-DP levels using a linear mixed effects model with factors for group (trans men v. trans women v. MSM) and times (1, 2, 3 and 4 weeks after initiation) and their interaction with an unstructured covariance across visits and test for differences at between groups at each time point. If values appear to violate the normal distribution, we will obtain p-values and confidence intervals using the bootstrap. All p-values will be two-sided with p-value < 0.05 considered statistically significant. Adverse events will be compared using the Fisher exact test.
Rationale for Number of Participants	A sample size of 48 (24 trans women and 24 trans men) allows detection of a difference of 0.52 log difference (1.68 fold difference) in TFV-DP from dried blood spots with 81% power on a two-sided 0.05 level test at any time point between our trans participants and MSM historical controls. A difference of 0.70 log (2.0 fold difference) can be detected between either group (trans men or women), and MSM historical controls with 80% power on a two-sided 0.05 level test.

1 PROTOCOL TEAM AND SITE

1.1 Protocol Team Roster

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TBN as needed to support study site

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1.5 Institutional Review Board

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2 BACKGROUND

The TRIUMPH study, or trans Research-Informed communities United in the Mobilization for the Prevention of HIV, is a new study responding to a pressing need for culturally-relevant community-led pre-exposure prophylaxis (PrEP) programs for transgender communities, especially for trans women of color who are most affected by the HIV epidemic. The TRIUMPH study will strive to identify the most effective PrEP service provision strategies which take into account the particular facilitators and barriers that transgender communities encounter in the health care system. To date, transgender women included in HIV prevention studies have been lumped into the larger aggregate of Men who have sex with Men (MSM) without taking into account the unique sociocultural context of transgender women. The TRIUMPH study will also include transgender men and other members of various transgender communities. The TRIUMPH project's goal is to identify the best methods to deliver PrEP safely and effectively to transgender communities while achieving the highest levels of adherence possible.

PrEP is the newest and most promising biomedical HIV prevention intervention yet developed and tested. The first clinical trial of PrEP ('iPrEx' or iniciativa Profilaxis Pre-Exposición) included MSM and transgender women who were at higher risk for HIV infection, and found that daily oral Truvada PrEP reduced the risk of HIV acquisition by 44% on an intention to treat basis [1]. However, a sub-analysis of the iPrEx data conducted by Deutsch *et al* , found zero effectiveness on an intention to treat basis among the small subgroup of transwomen in the study [2]. Further analyses revealed that the subgroup of trans women in iPrEx who were using hormones were less likely to have protective drug levels than those trans women not on hormones (OR 0.14, $p<0.001$). It is unclear if this difference is behavioral (less drug uptake) or biomedical (drug-drug interactions) in nature, or due to some combination of these factors, given the lack of pharmacokinetic (PK) studies testing for interactions between cross-sex hormone therapy (HT) and TDF/FTC, the active ingredients of Truvada. While there are studies of systemic drug-drug interactions between oral contraceptives and TDF, no such studies have been done with FTC, and no studies have been done with gender affirming HT, which differs from hormonal contraception in several respects. Drug-drug interactions with bioidentical 17-beta estradiol used for gender affirming hormone therapy among transgender women have not been studied.

The Interactions BEtween Antiretrovirals And Transgender Hormones, or I-BrEATHe study, described in this protocol is a sub study of the TRIUMPH study. I-BrEATHe will address one of the secondary aims of the TRIUMPH study which is to assess the pharmacokinetic drug-drug interactions between cross-sex HT and daily oral FTC/TDF.

Given that there are no PrEP studies to date that have specifically enrolled trans identified men, or have looked at the interactions between masculinizing HT and FTC/TDF, I-BrEATHe will enroll both transgender women and men. Transgender men report sexual rapports without condoms both vaginally and anally (range 26-60%), exhibit patterns of STIs transmission and unwanted pregnancies. Transgender men also report use of sex for gender affirmation. Small convenience samples show that HIV prevalence is between 2-5% [3, 4]. To date, there are no programs or PrEP recommendations specific for transgender men.

2.1 Overview of Non-Clinical Studies

N/A

2.2 Overview of Clinical Studies

There have been a large number of trials conducted with Truvada PrEP, including several demonstration projects. While there is no evidence to suggest that TDF/FTC interacts with commonly used feminizing or masculinizing hormone regimens, and evidence from studies of ARV interactions with hormonal contraceptives have been reassuring, no direct study of these interactions has been conducted to date.

Our team led the only trans-specific sub analysis to date of the iPrEx randomized controlled trial (RCT) data as well as the open label extension (OLE) study, which included periodic dried blood spot (DBS) drug level monitoring [2]. In iPrEx, gender identity data was not collected using current best practices ("two-step" method [5]), making the identification of transgender participants in this multinational, multicultural and multilingual trial difficult. As all iPrEx participants had been assigned male sex at birth, a transgender subgroup of 339/2499 participants (14% of overall sample) was constructed comprised of the 29 (1%) who identified as women, 296 (12%) who identified as "trans", and 14 (1%) who reported use of feminizing hormones. None had undergone gender affirming genital surgery. Compared with MSM, transgender women more frequently reported transactional sex, receptive anal intercourse without a condom, or more than 5 partners in the past 3 months. Excluding those with a male identity who also used feminizing hormones, trans- and woman-identified participants were less likely to have TDF levels detected on random testing in the RCT (OR=0.39, 95% CI = 0.16 to 0.96, p=0.04). While there was no effectiveness of PrEP for transgender women on an intention-to-treat basis, none of the transgender participants who seroconverted had detectible drug levels at the time of HIV diagnosis. In comparison to MSM in the OLE, transgender participants had less time with protective drug concentrations (17% vs 35%, p<0.001) and were less likely to have concentrations indicating > 3 tablets/week (OR 0.71, 95% CI 0.49 to 1.03, p=0.07). Of the two transgender women who seroconverted during the OLE, both had drug levels below 350 fmol/punch in DBS, the level associated use of 2 tablets per week at the time of seroconversion. The lower concentrations were more likely if the transgender women used feminizing hormones.

Of the 7 clinical trials of Truvada PrEP for HIV prevention conducted to date, iPrEx is the only one with confirmed enrollment of trans women [6]. No clinical studies or demonstration projects were conducted with transgender men.

For more detail regarding PrEP clinical trials, please refer to the Prescribing information for Truvada.

3 STUDY RATIONALE

The fact that concentrations of FTC/TDF were especially low among transgender women reporting use of feminizing hormones in the iPrEx study, which may reflect less PrEP use or a drug-drug interaction, is of high interest given that concerns about the impact of PrEP

on gender affirming hormone therapy is the main barrier for uptake of PrEP among transgender women [7]. While transgender men as a group have also been shown to be at elevated risk for HIV infection [4, 8], no information about PrEP use and drug levels is available for transgender men since they have never been formally included in PrEP research. Therefore, there are no data on the potential effects of testosterone on FTC/TDF drug concentrations. FTC/TDF drug concentrations have emerged as strong correlates of protection in PrEP trials, accounting for the majority of the variation in PrEP benefits across trials and across individual participants in trials. For example, while oral FTC/TDF PrEP had 44% efficacy on an intention to treat basis in iPrEx, the protective effect was more than 90% among those with detectable drug and negligible among those with no detectable drug in blood [1, 9].

The pharmacokinetic study of PrEP metabolites concentrations in association with feminizing and masculinizing hormones using directly observed therapy (DOT) will allow to establish whether interactions exist between these drugs. I-BrEATHe also provides an opportunity to include transgender men in PrEP research. Though transgender men are likely to be at lower risk for HIV acquisition than transgender women, the lack of available data regarding use of PrEP in transgender men must be remediated. I-BrEATHe will recruit equal numbers of transgender women who are using estradiol plus spironolactone (the most common HT regimen) and transgender men who are using testosterone. The primary aim will be to measure the intracellular active metabolites of TDF/FTC, tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP), levels in DBS collected after 1, 2, 3, and 4 weeks of video-observed Truvada for PrEP daily dosing, and comparing drug levels in the two groups receiving HT to previously acquired DBS drug levels data in MSM [9-11].

3.1 Risk / Benefit Assessment

Oral Truvada for PrEP has been FDA approved since 2012. At this time, there is plentiful evidence to support the use of daily oral FTC/TDF in HIV-1-uninfected heterosexual biological men and women, and men who have sex with men for HIV prevention [12]. The toxicity and tolerability of daily oral FTC/TDF in HIV-uninfected individuals have been established. Side effects that may arise in the first weeks of PrEP use -- nausea, abdominal cramping, vomiting, dizziness, headache, and fatigue usually go away without treatment interruption (see Truvada Prescribing information for more details). Known potential risks can be effectively managed by experienced PrEP providers. Participants enrolled in this study will be provided Truvada for PrEP within the confines of an established PrEP clinic, and will benefit from the expertise of trained staff. Individuals who may be susceptible to the possible nephrotoxic effect of FTC/TDF as determined by the measure of creatinine clearance (see exclusion criteria) will not be enrolled in the study. Long term effect of Truvada use (such as bone density loss or kidney dysfunction) are unlikely to occur over the course of this short study in otherwise healthy participants [13-15]. Participants will be seen weekly by a clinician, and any adverse event immediately addressed, in addition participants will be interacting daily with study staff via phone, providing an opportunity to address any questions or concerns. Moreover, the daily interactions with study staff will provide support for adherence. Concerns about risks of combining gender-affirming hormone therapy and PrEP will be alleviated by trained providers with expertise in the management of both antiretroviral and hormone

therapy. Guidelines for these matters will be provided by the UCSF Center of Excellence for Transgender Health (CoE-T).

4 STUDY OBJECTIVES

4.1 Primary Objective

To assess pharmacokinetics for daily oral FTC/TDF in transgender women and transgender men, and to determine if FTC/TDF drug concentrations are lower among transgender women who are using hormone therapy and in transgender men who use testosterone compared to historical controls in non-transgender MSM.

4.2 Secondary Objectives

To determine if daily oral FTC/TDF is associated with comparable rates of adverse events (AEs) in transgender women and in transgender men compared to historical controls in non-transgender MSM.

5 STUDY DESIGN

5.1 Study Overview

This is a single center pharmacokinetic study of daily oral Truvada at the following FDA approved dose of one tablet containing 200 mg of emtricitabine (FTC) and 300 mg of tenofovir disoproxil fumarate (TDF) for PrEP using daily directly observed therapy (DOT).

The study will include 48 participants will be divided into two groups: one group will include 24 participants who currently describe their gender identity as “woman” or “transgender women”, or other transfeminine spectrum identity, and the second group will enroll 24 participants who identify as “man” or “transgender man”, or other transmasculine spectrum identity.

Each participant will be taking a single daily dose of study drug in DOT performed either via video calling, or at study visit, for a total study duration of 28 to 30 days (4 weeks).

Laboratory evaluations for HT (including testosterone, or estradiol) drug levels will be performed at enrolment, and after 4 weeks on study drug. Study drug levels will be monitored weekly in DBS while participants are on study.

Screening data will be reviewed to determine participant eligibility. Participants who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The treatment regimen that will be used:

- Truvada at the following FDA approved dose of one tablet containing 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate

Total duration of participant participation will be up to 4 weeks, plus the 28 days of screening window period between screening visit and on study daily observed therapy. Total duration of the study is expected to be 9 -10 months.

5.2 Pharmacogenetic testing

Pharmacogenetics (PGx) studies the variability in drug response due to hereditary factors in populations. There is increasing evidence that individual's genetic background may impact the pharmacokinetics (absorption, distribution, metabolism and elimination) of drugs. The goal of the PGx analyses is to investigate a relationship between genetic factors and PK measures in I-BrEATHe, for example if there is potential unexpected or unexplained variation in drug levels. Blood samples collected for drug levels on DBS may be used for DNA extraction and PGx assessments. Participants will be specifically asked if they wish to participate in the genetic testing in the informed consent form.

6 CRITERIA FOR EVALUATION

6.1 Primary Endpoint

The primary endpoints for this study are the pharmacokinetics measures. These PK analyses are dependent on adherence and will be assessed at the end of study completion.

6.2 Secondary Endpoints

N/A

6.3 Safety Evaluations

AEs: Grade 1 and above as reported side effects on a structured instrument

7 PARTICIPANT SELECTION

7.1 Study Population

I-BrEATHe is a sub study of the TRIUMPH study with an anticipated overall cohort of 188 transgender participants. Volunteers who are participants in the larger TRIUMPH project may be recruited to participate in this sub study, however the I-BrEATHe sub study may also enroll volunteers outside of the TRIUMH study.

Up to 48 volunteers who meet the inclusion and exclusion criteria will be eligible for participation.

7.2 Inclusion Criteria:

All subjects:

- HIV antibody seronegative (negative HIV rapid test),
- 18 years or older,
- Has a smart phone with access to two-way video call capability,
- Willingness to be contacted for a short call every day for 4 weeks,
- Adequate renal function (creatinine clearance ≥ 60 ml/min estimated by the Cockcroft Creatinine Clearance Formula),

- Provides written informed consent,

For Transgender women:

- Male assigned sex at birth, and self-reported current gender identity as “woman” or “transgender women”, or other trans-feminine spectrum identity,
- Current feminizing Hormone Therapy (HT) use for at least 6 months,

For Transgender men:

- Female assigned sex at birth, and self-reported current gender identity as “man” or “transgender man”, or other trans-masculine spectrum identity,
- Current masculinizing Hormone Therapy (HT) use for at least 6 months with testosterone

7.3 Exclusion Criteria

- Expects to change or discontinue current HT use during the 4 weeks study period,
- Signs of symptoms of acute viral syndrome,
- Use of FTC or TDF in the past 90 days
- Receiving ongoing therapy with any of the following:

  ART, including nucleoside analogs, non-nucleoside reverse transcriptase inhibitors, protease inhibitors or investigational antiretroviral agents interferon (alpha, beta, or gamma) or interleukin (e.g., IL-2) therapy, aminoglycoside antibiotics, amphotericin B, cidofovir, systemic chemotherapeutic agents, other agents with significant nephrotoxic potential, other agents that may inhibit or compete for elimination via active renal tubular secretion (e.g., probenecid), and/or other investigational agents

- Renal insufficiency documented as Creatinine Clearance < 60 ml/min
- For masculine-spectrum identifying persons, positive pregnancy test at screening
- At enrollment, has any other condition or factor that, based on the opinion of the investigator or designee, would preclude provision of informed consent; make participation in the study unsafe; complicate interpretation of study outcome data; or otherwise interfere with achieving the study objectives.

7.4 Eligibility determination

Eligibility criteria must be carefully assessed at the screening visit and confirmed at the enrolment visit prior to drug dispensation. Eligibility for recruitment will be determined by the study team based on the data collected at the screening visit. Eligibility assessment will happen between the screen and enrolment visits, and will include reviewing medical record with medical exam, medical history and ongoing medications, reviewing testing results for HIV and chemistries. All clinical and laboratory assessments of eligibility must be performed and reviewed within 28 days of initiating the screening process and all screen results available prior to enrolment. If all inclusion criteria are met, and participant

is deemed likely to adhere to the daily observed therapy regimen, participants will be confirmed for their pre-scheduled enrolment visit.

At the enrolment visit, an HIV rapid test will be repeated. If the rapid HIV test results are negative, and no other exclusion criteria are identified, participant will be enrolled into the study, and study medication will be dispensed.

7.5 Recruitment and retention

7.5.1 Study site

The study site will have the capacity to comply with the protocol, study-specific procedures, and all applicable regulations. The site will have a reception area or a waiting area, a physical examination room, and/or counseling room, a study storage area or pharmacy, a data management area with locked cabinets or equivalent, computer access, laboratory facilities, and/or a phlebotomy area.

7.5.2 Recruitment

The current recruitment aim for this study is 48 participants, as described in sections 5.1 and 7.1. To reach the target enrolment number, up to 80 potential participants may be screened.

The recruitment strategies may include one or several of the following:

- Sending a “Dear Doctor” letter to healthcare providers who have a large number of transgender patients and asking for provider referral,
- Advertising the I-BrEATHe study on social media plate-forms,
- Using principal investigators’ database of participants who were enrolled in studies previously conducted at the UCSF CoE-T. Volunteers in this CoE-T database have given consent to be contacted for future research,
- Asking participants who have enrolled in the main TRIUMPH study,
- Inviting USCF CoE-T staff who may be good candidates,
- Using word of mouth,
- Posting flyers in targeted areas, agencies, clinics, Community Based Organizations, or Triumph community mobilization activities

These approaches, along with input from our community advisory boards and our current programs that serve transgender communities, will be used in recruitment efforts.

Volunteers interested in I-BrEATHe will be able to contact a special phone number created especially for the study. Callers to this number will be routed to the study team. If a study staff member is unable to answer the phone, there will be an outgoing message which will allow leaving a voicemail for a call back, as well as information on clinic drop-in hours for those with unreliable phone service. Study team members may also be available at community mobilization activities to recruit. Efforts will be made to pre-screen inquiries by phone, at events, or at the referring clinics for eligibility based on inclusion criteria. Participants will then be referred to the study site for screening.

7.5.3 Retention

Given to the lack of evidence to guide trans-specific recruitment and retention strategies or trans-inclusive implementation practices, we will use the expertise of the CoE-T to engage with our transgender identified participants. The lack of attention to the sociocultural context of trans women's lives especially and adaptations from other populations (i.e. MSM) have proven insufficient and unacceptable by the trans communities, evidenced by low recruitment and retention in clinical trials. Our study team has an understanding of the pervasive role of trauma in transgender people's lives, specifically trauma that is often enacted in medical settings and can lead to the avoidance of medical care, including HIV testing and prevention. For transgender people, feeling safe and being able to trust one's provider is a pre-requisite to successful engagement and retention in healthcare. Trust must be established within and throughout the community, as transgender social networks are tightly knit.

Therefore, the I-BrEATHe study staff will be selected based on heightened sensitivity to transgender issues, appropriate training in transgender competency, and will possibly be members of the transgender communities themselves. Staff will be in close contact with the participants. Staff will remind participants by e-mail and/or telephone of their upcoming appointments. The participants will be interacting daily via video calls which can help create a relationship of trust. Participants will receive monetary incentive at their visit to the study site to encourage the video calls participations during the previous week. These incentives will be reviewed by the UCSF IRB to ensure that they are not excessive and coercive. At the end of the study, the participants may be invited to an appreciation event.

8 CONCURRENT MEDICATIONS

All participants should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

8.1 Allowed Medications and Treatments

Standard therapy is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

8.2 Prohibited Medications and Treatments

The following medications are prohibited during the study and administration will be considered a protocol violation:

ART, including nucleoside analogs, non-nucleoside reverse transcriptase inhibitors, protease inhibitors or investigational antiretroviral agents, interferon (alpha, beta, or gamma) or interleukin (e.g., IL-2) therapy, aminoglycoside antibiotics, amphotericin B, cidofovir, systemic chemotherapeutic agents, other agents with significant nephrotoxic potential, other agents that may inhibit or compete for elimination via active renal tubular secretion (e.g., probenecid), and/or other investigational agents

9 STUDY TREATMENT

9.1 Method of Assigning Participants to Treatment Groups

Participants will be separated into two groups based on their gender identity, and all participants will undergo the same study procedures, to the exception of a pregnancy test for female assigned at birth transmasculine people, when appropriate (see study procedures below).

9.2 FTC/TDF

9.2.1 FTC/TDF Formulation

FTC/TDF is a fixed-dose co-formulation of FTC and TDF disproxil fumarate (TDF). Each FTC/TDF tablet contains of 200 mg of FTC and 300 mg of TDF (equivalent to 245 mg of tenofovir disoproxil). Please refer to the full prescribing information for FTC/TDF for more information regarding drug information, side effects, and storage conditions. The Truvada pill is a blue oblong pill to be swallowed with some liquid.

9.2.2 FTC/TDF and pregnancy

FTC/TDF is categorized as pregnancy category B by the FDA. For the study population assigned male at birth, the risks to pregnancy will be limited. For the population assigned female at birth, risks associated with pregnancy will be shared, and a positive pregnancy test at enrolment will be an exclusion criterion. Study participants will be counselled that study drug should not be shared with any individual.

9.2.3 Packaging and Labeling

Gilead Sciences will dispense medication to the study team through their FDA approved commercial packaging of 30 tablets per bottle with a child-resistant screw cap. In addition to the tablets, each bottle contains a silica gel desiccant to protect the product from humidity and polyester packing material that cushions it during handling and shipping. Gilead will prepare bottles of study agent that are each labeled with the active agents, the expiry date and instructions for use as well as for storage. Each bottle will be assigned the participant's individual identification number or PID at the site for tracking purposes.

9.3 Supply of Study Drug at the Site

Gilead will ship Study Drug to the investigational site at study start up. The initial study drug shipment will be shipped after site activation (i.e., all required regulatory documentation has been received and approved by the UCSF IRB, and the study is ready to begin). Subsequent study drug shipments will be made after site request for resupply. Replacement study drug will be shipped at the investigator's request.

9.3.1 Storage

The study drug will be stored in accordance with the drug manufacturer's recommendations. Storage area will be locked by a secure door. The study area and storage facility will have climate-controlled environments, with temperature to remain within limits allowed by the manufacturer for drug storage. The drug will be stored at room temperature, between 68 and 77 degrees F (20 and 25 degrees C). Brief storage at temperatures between 59 and 86 degrees F (15 and 30 degrees C) is permitted. Drug will be stored away from heat, moisture, and light. If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the investigators and captured as a deviation. Participants will be instructed to store the medication in original packaging (bottle and protected from light) at room temperature according to the instructions outlined on the drug packaged insert.

9.4 Dispensation of Study Drug at the Site

9.4.1 Dosage/Dosage Regimen

The treatment is FTC/TDF orally once daily via DOT, with or without food for a duration of 28 days. Optimal timing between doses is 24 hours.

9.4.2 Dispensing

Study clinicians (PrEP clinician, or Research Nurse Practitioner, or physician) or nurses will be responsible for dispensing study medication to participants. Only participants who meet enrolment criteria will be dispensed study medication. Upon study drug dispensation, the study staff will document dispensation and date of dispensation in a Drug Dispensation Log.

9.4.3 Administration Instructions

Participants who enroll in the study will schedule a face to face video calls plan with the study clinician. Calls will occur daily at a time that is convenient for the participant. Calls will last 60 seconds in duration and will be focused on watching the FTC/TDF tablet be placed into the mouth, swallowed, and the mouth opened to verify the table is no longer present. Before the table is ingested, the participant will be asked a question about food ingestion in the prior hour or intent to eat in the next hour.

Participant are encouraged not to take the study drug outside of the video call setting. However, if participant cannot perform a video call for the DOT (no Wi-Fi, cell phone dies…), the pill taking can happen over an audio phone call, or back up documentation of the pill taking such as filming oneself taking the pill may be done. The time stamped video will be shared with study team at the next visit. In last resort, if none of these options are possible, a simple text such as "Hi *study clinician's name*, dosing at hh.mm on dd-mmm, Thanks" could be sent to the team. If the daily DOT call is not documented, it will be considered a missed dose.

9.4.4 Participant Replacement Instructions

Participants who withdraw from the study prior to completion or who fail to adhere to the DOT regimen may be replaced.

9.5 Study Drug Accountability

An accurate and current accounting of study drug for each participant will be maintained on an ongoing basis via the DOT video calls and the on-site visits, by a member of the study site staff. The number of study drug dispensed and returned by the participant will be recorded on the Drug Dispensation and DOT Log. The study staff will verify these documents throughout the course of the study. If participant misplace the study medication, replacement will be allowed once.

9.6 Measures of Treatment Adherence

Daily adherence will be directly observed by participants taking part in real time DOT video calls, or witnessed by study staff at the study visit if the drug is taken at the clinic. Study staff will monitor the FTC/TDF tablet be placed into the mouth, swallowed, and the mouth opened to verify the table is no longer present. Alternate documentations of pill taking as described in 9.4.1 may be used as back up measures if access to video capacity is impossible for participant. DOT will be recorded daily by the study team to document on the Drug Dispensation, Hormones and DOT log. Repeated failure to adhere to the DOT regimen will lead to discontinuation from study participation.

If participant fails to connect with study staff for the call, study staff will make every possible attempt to connect with participant. If participant misses the call and does not provide back up evidence of pill taking, it will be considered a missed dose.

If participant misses two or more study drug doses, the participant will be excluded from study participation.

DOT calls will capture hormone therapy adherence as well, which may be either directly observed if feasible, or verbal self-report. Hormone adherence will be captured on the Drug Dispensation, Hormones and DOT log.

10 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization will be signed and dated by the participant or participant's legal representative.

10.1 Clinical Assessments

10.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening, Enrolment, weeks 1, 2, 3 and 4, and at early termination when applicable. Dose, route,

unit frequency of administration, and indication for administration and dates of medication will be captured in a Concomitant Medications Log.

10.1.2 Demographics

Demographic information (i.e. age, birth assigned sex, gender identity, race, ethnicity, education, employment…) will be recorded at screening.

10.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent history, and information regarding underlying diseases will be recorded at Screening.

10.1.4 Physical Examination

Qualified clinician (Research Nurse Practitioner, or physician) or nurse will complete a brief targeted physical exam at enrolment, including weight and height. Abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit. Special attention will be given to signs of symptoms of acute viral infection.

10.1.5 Vital Signs

Body temperature, blood pressure, pulse will be performed after resting for 5 minutes at screening and enrolment. In addition, weight and height will be collected at enrolment and exit.

10.1.6 HIV Rapid Test

At the screening, enrolment and end of study visits, an FDA approved HIV rapid test will be performed and results provided back to the participant. Rapid HIV test will only be performed once. Participants with positive results will be referred to the PHAST (SFDPH HIV Surveillance Unit phone #: 415/554-9050) or other equivalent program for participants outside of SF. Cases must be reported within 7 days to the Health Officer or other equivalent program for community resources provision and to initiate HIV care. Study team will ensure that participant is linked to care.

10.1.7 Counselling around daily DOT

At screening, at enrolment, when initial study drug is dispensed, and at every weekly visit when the blood is drawn for DBS, participants will meet briefly with their study clinician or nurse, to remind participant to the importance of participating in the daily DOT for the success of the pharmacokinetic study

10.1.8 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded via interviewer administered symptoms and side effects questionnaires and on an AE log, as appropriate. Study participants can also prompt study team during daily video calls to potential concerns, and these concerns may be addressed on a return phone call or at next visit by a clinician or a nurse.

10.1.9 Drug and Alcohol Use assessment

Information regarding the use of drug and alcohol will be captured at the screening visit using the AUDIT 10-items questionnaire for screening alcohol use disorders and the DAST-10, a 10-items for screening drug use. Each takes approximately 5 minutes to complete. In addition, participants will be asked weekly if, and what type, substances were used in the past week.

10.1.10 Pregnancy test

Participants who were assigned female at birth will have a urine pregnancy test at enrolment, unless they affidavit that they are engaging in NO sexual activity with anyone with a penis, and/or have no ovaries and/or uterus.

10.2 Clinical Laboratory Measurements

10.2.1 Blood Chemistry Profile

Blood will be obtained for determination of creatinine, and creatinine clearance estimated by the Cockcroft Creatinine Clearance Formula, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubine.

10.2.2 Kidney function Profile

Urine and blood will be obtained for kidney function biomarkers measurements, including, but not limited to, uromoduline and cystatin C, at enrolment and every on-study visit week.

10.2.3 Hormonal Therapy Profile

At screening, hormone levels may be extracted from participant's existing health records, from referring clinic or physician.

10.2.4 Serology testing

Hepatitis B immunity status will be either documented from other reliable sources proving previous testing (health records, referring provider) or blood may be used for Hepatitis B Surface Antigen testing as part of the PrEP delivery standard of care at the study site. This testing will be done as an off protocol testing, see 10.2.4.

10.2.5 HIV infection testing

The I-BrEATHe protocol may conduct a series of testing related to HIV seroconversion (viral load, genotype, western blot …) if a participant is suspected of HIV acquisition.

10.2.6 Off protocol testing

If the need occurs to answer specific questions critical to study participant's well-being in the I-BrEATHe study, at the discretion of the clinician, participant may have additional tests performed at the study site clinic or be referred to his or her physician for further laboratory testing.

10.3 Research Laboratory Measurements

10.3.1 Pharmacokinetic Measurements

Blood will be drawn for DBS at weeks 0, 1, 2, 3 and 4. DBS are readily accessible, require minimal processing, are easy to store and ship, and have been demonstrated to be suitable for monitoring active drug metabolites concentrations among PrEP users in iPrEx OLE. Analysis of the active phosphorylated moieties of tenofovir has a large dynamic range, capable of detecting as little as 2 tablets per month. DBS will be processed in accordance with procedures developed for the iPrEx OLE study [9].

10.3.2 Hormonal Therapy Profile

Blood will be drawn at enrolment and at study exit for quantification of estradiol, and total and free testosterone levels, as well as sex hormone binding globulin (SHBG) and dihydrotestosterone (DHT) using Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) and/or equilibrium dialysis. These measures will be used for the outcome analyses.

10.3.3 Genetic testing

In accordance with UCSF IRB approval, study participants will be asked if they wish to provide written informed consent for genetic testing. If participant consent, DBS may be used for DNA extraction and pharmacogenetic studies.

10.4 Video dosing Assessments

The participant will be witnessed by study personnel to take the study drug dose by mouth every single day. Study personnel will ensure the tablet was swallowed by asking the participant to show that their mouth is empty. This will be accomplished in person at visits at the study site, as allowed and if feasible for participant, and by visual video-call on other days, including holidays, and weekends. However, if participant cannot perform a video call for the DOT, the pill taking can happen over an audio phone call, or back up documentation of the pill taking such as filming oneself taking the pill may be done. The time stamped video will be shared at the next visit. In last resort, if none of these options are possible, a simple text such as “Hi *study clinician’s name*, dosing at hh.mm on dd-mmm, Thanks” could be sent. Study personnel will use the Drug Dispensation, Hormones and DOT (DDHD) Log to document directly observed doses. Participants will be given a detailed calendar that shows scheduled dosing days. This schedule will also be reinforced when study personnel interact with participants. The video assessments will last approximately 1 minute and study personnel will not discuss study details for privacy reasons. General study questions will be discussed, such as, “how do you feel”, “do you have any concerns”, etc. In this way, adverse events may be monitored on all dose witnessing days. During the video assessment, staff will confirm with study participant uptake of HT, which may or may not be directly observed.

11 EVALUATIONS BY VISIT

11.1 Phone or referring clinic pre screening

A number of questions will be asked from potential participants over the phone, at recruiting events or at the referring provider's clinic, to prescreen volunteers. These questions include capability to do two-ways daily video call, ability to go to the study site weekly for blood draws, gender identity, and current trans hormone therapy.

11.2 Visit 1 (Screening visit)

1. Review the study with the participant (or participant's legal representative) and obtain written informed consent and HIPAA authorization, if applicable.
2. Assign the participant a unique screening number. The screen number will be the Participant ID or PID for the duration of the study if enrolled.
3. Record demographics data.
4. Record brief targeted medical history, including a history of diseases, diagnosis date, and prior treatments.
5. Record concomitant medications.
6. Perform and record vital signs.
7. Conduct the drug and alcohol use scales
8. Collect blood for clinical laboratory tests (chemistry and serology, as applicable). If hormone levels and/or serology are available from participant's regular health management, collect from existing records.
9. Collect blood for HIV rapid test, perform test and provide results.
10. If HIV rapid test is negative, and inclusion criteria are met, tentatively schedule participant for Visit 2 (week 0).
11. Provide visit reimbursement.

11.3 Visit 2 (week 0 or enrolment, within 28 days of screening)

1. Review results from screening visit tests with participant
2. Concomitant medications review.
3. Perform and record vital signs, including weight and height.
4. Perform brief targeted physical exam, which includes an assessment of signs and symptoms of an acute viral syndrome, including fever, pharyngitis, rash, myalgias, arthralgias, diarrhea, or headache.
5. Perform and record blood HIV rapid testing
6. If female assigned at birth, perform pregnancy test as indicated
7. Confirm eligibility
8. Draw blood for hormone levels, DBS, kidney function
9. Collect urine for kidney function markers

10. Provide symptoms and side effect questionnaires and ask about potential substance use
11. Dispense study medication and instruct participant to take daily
12. Record study medication bottle number on the Drug Dispensation Log
13. Review importance of study procedures (DOT/weekly visit), using client centered counselling for adherence
14. Coordinate video calling schedule with participant and provide calendar of calls
15. Provide visit reimbursement.

11.4 Visit 3 (Week 1, 2 and 3, visit window plus or minus 2 days)

1. Record any Adverse Experiences, using PrEP and a hormones specific symptoms and side effects questionnaires, and the AE log
2. Assessment for acute viral infection
3. Review participant daily observed therapy log, discuss adherence challenges if any, provide support if necessary for DOT.
4. Ask about potential substance use
5. Record changes to concomitant medications
6. Draw blood for DBS and kidney function markers
7. Collect urine for kidney function markers
8. Provide visit reimbursement

11.5 Visit 4 (Week 4 or end of study, visit window plus or minus 2 days)

1. Record any Adverse Experiences (including the symptoms and side effects questionnaires) and review participant daily observed therapy log
2. Ask about potential substance use
3. Record changes to concomitant medications.
4. Perform vital signs, including height and weight
5. Asses for symptoms of acute viral infection
6. Collect blood for chemistries, HIV rapid testing, hormone levels, and kidney function markers as well as for DBS
7. Collect urine for kidney function biomarkers
8. Collect all unused study drug.
9. Provide visit reimbursement
10. Thanks participant for participation
11. Referral to a clinical PrEP service if the participant chooses to continue PrEP

11.6 Early Withdrawal Visit

1. Record any Adverse Experiences and review participant DOT log. Discuss adherence challenges if any.
2. Administer symptoms and side effects questionnaires
3. Ask about potential substance use
4. Capture reasons why participant is dropping out of the study
5. Record changes to concomitant medications.
6. Collect all unused study drug
7. Perform vital signs, including height and weight
8. Collect blood for chemistries, HIV rapid testing, hormone levels, kidney function and for DBS
9. Collect urine for kidney function biomarkers
10. Provide visit reimbursement
11. Thanks participant for participation
12. Referral to a clinical PrEP service if the participant chooses to continue PrEP.

12 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

12.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the participant, for the occurrence of AEs during each participant visit and record the information in the site's source documents. Adverse events will be recorded in the patient chart, in an AE log and/or CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

A side effect structured questionnaire will be used to track AE.

AE Severity

The U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. [November 2014] should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The guidelines shown in

Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the participant's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

12.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity

- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the participant or require intervention to prevent one of the outcomes listed.

12.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

12.2.2 Suicidal ideation

Suicidal ideation-related events will be reported to the IRB as indicated by the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. [November 2014] described above. In addition, participants will be offered support and access to mental health services available at the study site. Study staff will ensure study participants are linked to care.

12.3 Medical Monitoring

Dr. Robert Grant should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: *415-350-8909*

13 DISCONTINUATION AND REPLACEMENT OF PARTICIPANTS

13.1 Early Discontinuation of Study Drug

A participant may be discontinued from study treatment at any time if the participant, or the investigator, feels that it is not in the participant's best interest to continue. The following is a non-exclusive list of possible reasons for study treatment discontinuation:

- Participant withdrawal of consent
- Participant is not adhering with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the participant to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment; including change or interruption of the HT regimen
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (performed outside of the study settings)

If a participant is withdrawn from treatment due to an adverse event, the participant will be referred to care.

All participants who discontinue study treatment will be withdrawn from the study and every effort will be made to welcome participant for an early withdrawal visit as soon as possible at which the procedures listed in section 10.5 should be performed. At this visit, reasons for stopping the study will be documented, if participant is willing to share.

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents Refer to Section 10.5 for early termination procedures.

12.3 Withdrawal of Participants from the Study

A participant may be withdrawn from the study at any time if the participant, or the investigator feels that it is not in the participant's best interest to continue.

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents. As noted above, participants who discontinue study treatment early (i.e., they withdraw prior to week 4) should have an early withdrawal visit. Refer to Section 10.5 for early termination procedures. Participants who withdraw after week 0 but prior to week 1 should be encouraged to come in for a withdrawal visit.

12.4 Replacement of Participants

Participants who withdraw from the study treatment may be replaced.

14 PROTOCOL VIOLATIONS

A protocol violation occurs when the participant, or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, participant safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to adhere to study drug regimen
- Failure to participate in the daily directly observed therapy (2 or more failed calls with no back up documentation of pill taking)
- Change or interruption of HT regimen

- Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor, in this case the principal investigators, will determine if a protocol violation will result in withdrawal of a participant.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated and submitted to the IRB. A copy of the form will be filed in the site's regulatory binder.

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible and dosed participants who are enrolled into the study and receive all doses of the study drug will be included in the pharmacokinetic analysis.

15.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized: race, ethnicity, age, gender identity, birth sex.

15.3 Analysis of Primary Endpoint

We will compare levels of TFV-DP in dried blood spots and rates of adverse events between trans men and trans women and a group of MSM historical controls from the iPrEx OLE study. We will compare log transformed TFV-DP levels using a linear mixed effects model with factors for group (trans men v. trans women v. MSM) and times (1, 2, 3 and 4 weeks after initiation) and their interaction with an unstructured covariance across visits and test for differences at between groups at each time point. If values appear to violate the normal distribution, we will obtain p-values and confidence intervals using the bootstrap. All p-values will be two-sided with p-value < 0.05 considered statistically significant. Adverse events will be compared using the Fisher exact test.

Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug.

15.4 Sample Size

A sample size of 48 (24 trans women and 24 trans men) allows detection of a difference of 0.52 log difference (1.68 fold difference) in TFV-DP from dried blood spots with 81% power on a two-sided 0.05 level test at any time point between our trans participants and MSM historical controls. A difference of 0.70 log (2.0 fold difference) can be detected between either group (trans men or women) and MSM historical controls with 80% power on a two-sided 0.05 level test. This is sufficient to determine if the differences in drug

concentrations observed in iPrEx OLE between MSM and trans women were due to interactions with feminizing hormone drug-drug interactions.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each participant treated with the study drug.

Study personnel at study site will enter data from source documents corresponding to a participant's visit into the protocol-specific database when the information corresponding to that visit is available. Participants will not be identified by name in the study database or on any study documents to be collected by the investigators, but will be identified by a site number, participant identification number (PID).

For paper source documents: If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator or his representative. A copy of the CRF and/or source documents will remain at the Investigator's site at the completion of the study for a period of 2 years after final data analysis.

16.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of data validation checks will be implemented and applied to the database on a regular basis. For example, query reports pertaining to data omissions and discrepancies may be forwarded to the study site for resolution, as applicable. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of funding agencies, IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. For each participant, the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that participant will be maintained. Data will be kept separate from documents containing personal identifiers. The Investigator must ensure the reliability and availability of source documents from which the information in the database was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, source logs and check lists, etc.) must be kept secured for a period of 2 years following study results analysis is done.

16.6 Monitoring

Monitoring visits at the study site may be conducted by representatives of the investigators according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the investigators grant permission to the appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Participant Confidentiality

In order to maintain participant confidentiality, only a PID (personal identifying number) will identify all study participants on CRFs and other documentation, specimens send for testing on site or at remote laboratories and with the data sent to data management team for analyses. Some information may be extracted from existing medical charts, in the case of hormonal therapy for referred participants, for example; in this case, data will be transferred to the I-BrEATHe study document and participant's chart, associated with his or her PID and no personal identifiers. For the video calling, every effort will be made to protect the privacy or participants. Video conference platforms that encrypt data, password protection and study specific phone will be used. Privacy for video-dosing will be stressed by study personnel, and all meetings will occur in a mutually agreed upon location. Study details will not be discussed by study personnel during these sessions, unless at participant's specific request. These procedures and risks will be described in the informed consent document.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, CRFs, logs, reports and other source records, as well as the results database will be identified by a coded PID only. All study

records will be kept in a locked file cabinet and informed consent forms linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without documented permission of the participant, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the investigators, who are the sponsors for this study. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of the participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to study drug supplier prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Participants (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, and research HIPAA authorization form. Investigators will provide the documents to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will keep an IRB/IEC-approved copy of the Informed Consent Form for the study file.

A properly executed, written, informed consent will be obtained from each participant prior to entering the participant into the study. Information should be given in both oral and written form and participants (or their legal representatives) must be given ample opportunity to inquire about details of the study. If a participant is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the participant. A copy of the signed consent form will be given to the participant or legal representative of the participant and the original will be maintained with the participant's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the IRB, except when to protect the safety, rights or welfare of participants.
2. Personally conduct or supervise the study.
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).

7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to participants or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/participants.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

	VISIT 1 (Screen)	VISIT 2 (Week 0/enrollment) [a]	VISIT 3 (Week 1, 2, 3)[b]	VISIT 4 (Week 4)[b]	VISIT 5 (Early withdrawal)
Informed Consent	X				
Demographics	X				
Medical History	X	X			
Abbreviated Physical Exam		X			
Height		X		X	X
Weight		X		X	X
Vital Signs	X	X		X	X
HIV Rapid test	X	X		X	X
Symptoms of acute viral infection		X	X	X	X
Pharmacokinetics (DBS)		X	X	X	X
Chemistry[c]	X			X	X
Kidney biomarkers (blood/urine)		X	X	X	X
Hormone Therapy levels		X		X	X
Pregnancy Test (Urine)		X[d]			
AUDIT and DAST scale	X				
Dispensing of Study Drug		X			
Counting of Returned Study Drug				X	X
Schedule DOT		X			
Review DOT log		X	X	X	X
Concomitant Medication Review	X	X	X	X	X
Questions (PrEP, HT, drug use)		X	X	X	X
Condom distribution (optional)		X	X	X	X
Refer to PrEP program (optional)				X	X

[a] within 28 days
[b] ±2 days
[c] Creatinine and creatinine clearance, AST, ALT, total bilirubine. Blood may be used for Hepatitis B surface antigen testing if data not available from medical chart.
[d] Only when indicated, see section 10.1.11 pge 27

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