CCTG 605

F/TAF Switch Study for Transgender Individuals for HIV Pre-exposure Prophylaxis (TAF4TRANS)

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A Clinical Study of the California Collaborative Treatment Group (CCTG)

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SCHEMA

Design: This is an open-label, single-arm switch study to evaluate the efficacy of emtricitabine/tenofovir alafenamide (F/TAF) as pre-exposure prophylaxis for the prevention of HIV infection in transgender and non-binary people taking versus not taking gender-affirming hormone therapy (HT).

Duration:	This is a two-phased study. In Phase I, participants will be on emtricitabine/tenofovir disoproxil fumarate (F/TDF) for a minimum 12-week lead-in period prior to switching to F/TAF. In Phase II, participants will switch to F/TAF with a 48-week follow-up.
Sample Size:	Up to 60 transgender or non-binary people.
Study Population:	Eligible participants will include HIV-uninfected people identifying as transgender or non-binary, at least 18 years of age, are currently on F/TDF for PrEP, and who are willing to switch to F/TAF for 48 weeks. Participants may co-enroll and roll off from an existing PrEP in transgender/non-binary people study (CCTG 603; HRPP# 161807).
Intervention:	Following Phase I lead-in, all participants will continue PrEP with fixed dose combination daily oral F/TAF substituting for F/TDF in Phase II. Participants will continue to receive PrEP in accordance with standardized comprehensive methods of prescribing, which includes risk reduction counseling, adherence counseling, and clinical assessments with safety monitoring, as well as HIV and STI screening. Participants will receive daily adherence-supporting text message reminders through 12 weeks after F/TAF initiation.
Regimen:	Phase I: daily oral fixed-dose FTC 200 mg / TDF 300 mg Phase II: daily oral fixed-dose FTC 200 mg / TAF 25 mg
Outcomes:	The primary outcome is intracellular tenofovir diphosphate (TFV-DP) concentrations, compared between individuals taking gender-affirming HT versus individuals not on HT. Secondary outcomes include safety, tolerability, and self-reported adherence to F/TAF in transgender individuals compared to F/TDF. Safety and tolerability will be measured through reported adverse events (AEs), serum creatinine concentrations and creatinine clearance estimates, and self-report. Adherence will be defined as TFV-DP concentrations commensurate with 4 and 7 doses per week at Week 12 on F/TAF compared to last TFV-DP concentrations on F/TDF. All participants that remain on study drug through Week 12 will be included in the analysis. Additional adherence assessments include self-reported adherence by text message responses on F/TDF compared to on F/TAF.

1.0 STUDY OBJECTIVES AND HYPOTHESES

1.1 Primary Objective:

To evaluate the impact of gender-affirming HT on TFV-DP concentrations with F/TAF-use in transgender/non-binary individuals taking versus individuals not taking gender-affirming HT.

Primary Hypothesis: TFV-DP concentrations in participants taking HT will differ no greater than 25% to TFV-DP concentrations in participants not taking HT.

1.2 Secondary Objectives

1.2.1 Safety and Tolerability

To compare the safety and tolerability of F/TAF versus F/TDF in transgender/non-binary individuals.

Hypothesis 2a: The proportion of participants reporting any adverse event taking F/TAF over 48 weeks will not differ from the proportion of participants reporting any adverse event taking F/TDF over 48 weeks.

Hypothesis 2b: Self-reported tolerability to F/TAF will not differ from the self-reported tolerability to F/TDF.

Hypothesis 2c: Hormone concentrations while participants are taking F/TAF will not differ from the hormone concentrations while participants are taking F/TDF.

Hypothesis 2d: Serum creatinine concentrations will decrease in transgender/non-binary people taking F/TAF for 48 weeks compared to serum creatinine concentrations while taking F/TDF over 48 weeks.

1.2.2 Adherence

To compare adherence to F/TAF versus adherence to F/TDF in transgender individuals, as measured by intracellular TFV-DP concentrations

Hypothesis 2a/b: The proportion of participants adherent to F/TAF will not differ to the proportion of participants adherent to F/TDF as measured by TFV-DP concentrations commensurate with a) seven doses taken in the past week and b) four doses in the past week.

Hypothesis 2c: The mean percent days of self-reported adherence by daily text message in the first 12 weeks on F/TAF will not differ from the mean percent days of self-reported adherence in the last 12 weeks on F/TDF.

2.0 INTRODUCTION

2.1 Study Background

There is an immediate need to apply all modes of HIV prevention in the transgender and non-binary populations.[1] Transgender women (TGW) are considered to be one of the highest risk groups for HIV infection, with a recent systemic review estimating a summary HIV prevalence of 21.6% (95% CI 18.8-24.3) in high-income countries.[2] Since the review, additional studies have

documented prevalence as high as 40%.[3] High rates of infection are related to multiple HIV risk factors including biological, behavioral, social/sexual networks, and environmental/societal.[3] Although significant structural changes are needed to address many of these factors, the uptake of pre-exposure prophylaxis (PrEP) would have an immediate impact at the individual and, possibly, population level.

PrEP studies with the first FDA-approved drug, emtricitabine/ tenofovir disoproxil fumarate (F/TDF, trade name Truvada®), have demonstrated that over 90% protection against HIV infection can be achieved by as little as four doses per week.[4,5] Transgender individuals, however, were underrepresented and insufficient numbers were enrolled in these studies to show efficacy; transgender individuals who acquired HIV while on study did not have drug concentrations commensurate with adequate adherence. In the iPrEx,OLE study, transgender participants had 30% lower TFV-DP concentrations compared to MSM for reasons that were not determined but may have been due to poorer adherence, different pharmacokinetics (PK), or both.[6]

At the 2018 AIDS Conference in Amsterdam, two low-powered studies suggested that tenofovir in plasma and rectal tissues appeared to be lower in transgender individuals taking feminizing hormones. A study conducted in Thailand showed tenofovir concentrations in N=20 TGW were 17% less after 24 hours of initiating feminizing hormones.[7] Clinical significance is still uncertain and further evaluation is needed due to the study's small sample size. For example, if transgender individuals maintain high adherence, then a 17% TFV-DP concentration reduction would not matter. However, if there are many who are moderately non-adherent it creates potential for failure. Moreover, drug quantification by DBS measurement, the best predictor of effectiveness, was not utilized in the Thai study and it is still not clear if DBS concentrations suffer during hormone therapy.

Emtricitabine/tenofovir alafenamide (F/TAF) is an antiretroviral formulation found to have generally better toxicity and bone safety profiles as compared to TDF-based regimens in HIV-positive people.[8] Given this, F/TAF is a promising alternative to F/TDF as PrEP. In a study of rhesus macaques, no macaque (n=0/6) exposed to chimeric simian/human immunodeficiency virus (SHIV) and given daily oral F/TAF became infected.[9] Additionally, preliminary results presented at CROI 2019 from a phase 3, randomized, double-blinded study of N=5387 adults in the US, the EU, and Canada showed F/TAF as non-inferior to F/TDF in preventing HIV infection.[10] However, while the intended population for this study was high-risk men who have sex with men (MSM) and TGW, only n=74 (2%) were TGW and no conclusions should be made regarding F/TAF safety/efficacy in the transgender/non-binary populations.

A supplemental New Drug Application was submitted to the FDA in April 2019 for F/TAF for PrEP and approved for use as PrEP except for those having receptive vaginal sex in October 2019.

Adherence in a large cohort of transgender/non-binary individuals has not been studied. Adherence may especially differ among transgender persons due to: i) individual psycho-social factors (e.g., sense of stigma, finances); ii) beliefs about medication (e.g., real or perceived side effects, concerns and uncertainty about PrEP efficacy and interactions with hormones); iii) barriers to adherence (e.g., irregular routines, life crises, drug and alcohol use, mental illness); and iv) informational deficits (e.g., lack of competent medical providers, mistrust of the medical system, negative feedback from social networks).[11]

Currently, the CCTG 603 study (HRPP#161807) is enrolling N=300 transgender and non-binary individuals for F/TDF PrEP initiation at five sites in Los Angeles and San Diego counties. The primary objective of the CCTG 603 study is to compare the effectiveness of a text-messaging intervention to improve daily PrEP adherence with vs. without motivational interviewing. Intracellular TFV-DP concentrations will be used as biomarkers for adherence and the study intends to examine the impact of HIV risk factors and factors specific to the transgender/non-binary populations on PrEP adherence. To address ongoing concerns regarding PrEP and hormone therapy (HT) drug-drug interactions, a key secondary objective is to quantify and examine differential TFV-DP concentrations in persons taking vs. not taking gender-affirming HT.

The current study will leverage the CCTG 603 study to enroll and initiate F/TAF as PrEP in transgender/non-binary individuals. Participants will have a 12-week minimum lead-in period on F/TDF and will switch to F/TAF for 48 weeks. The primary objective of the current study is to examine differential TFV-DP concentrations in persons taking vs. not taking HT, but will also provide additional PK, safety, adherence, and efficacy data for supporting the application for F/TAF PrEP indication in these vulnerable and understudied populations.

3.0 STUDY DESIGN

3.1 Study Design

This is a two-phased prospective switch study of 60 transgender individuals who are eligible for F/TDF PrEP and who are willing to switch to F/TAF for 48 weeks. The purpose of the current study is to contribute safety and efficacy data to support the use of F/TAF in transgender/non-binary individuals. The current study leverages existing studies in trans/non-binary populations (e.g. CCTG 603; HRPP#161807) funded by the California HIV/AIDS Research Program (CHRP).

The dosage for Phase I is FTC 200 mg / TDF 300 mg. The dosage for Phase II is FTC 200 mg / TAF 25 mg. TDF/FTC is FDA approved for use as PrEP in adults and adolescents at risk for HIV-1. F/TAF has received FDA approval for use as PrEP as of October 2019 except for those

having receptive vaginal sex. Participants who are capable of receptive vaginal sex will be informed of the FDA restrictions and advised to use an additional method of protection such as condoms.

Eligibility criteria will be based on the CDC guidance for PrEP use in transgender persons. All participants must be confirmed HIV-negative (either by rapid test or Ag/Ab test), must have acceptable safety laboratory values, and must currently be taking or will initiate F/TDF by the Screening/Baseline visit. Participants will have a 12-week lead-in period prior to switching to F/TAF. At the Switch Visit, participants will be instructed to substitute F/TDF with F/TAF for PrEP for 48 weeks. Regular follow-up evaluations will occur at Weeks 12, 24, 36, and 48.

Participants will receive health education, clinical assessments, laboratory safety monitoring, STI and HIV screening, standard HIV risk reduction and adherence counseling, assessment of psychosocial barriers, and completion of a computer-based self-report survey that includes assessments of adherence and risk behaviors. Intracellular TFV-DP quantification will be performed in retrospect on batched, banked samples and additional banked specimens will be frozen for future use. Adherence by TFV-DP concentrations and self-reported measures will be used for the primary and secondary analyses of the study. Additional outcomes for the study will include changes in risk behavior and determinants of PrEP adherence.

This study will continue iTAB, a daily text message support system for adherence up until 12 weeks after F/TAF switch. As needed, the study will provide a) subject reimbursement to pay for unlimited text messaging and/or b) an appropriate cell phone if a subject does not have one. Daily dosing text message reminders will be sent for the duration of the study. Both reminder timing and content can be individualized. Participants have selected 15 personal reminders from a list of pre-determined reminders that cover various themes shown to be effective in improving adherence (e.g., social support, loss frame, health gain, etc.) as developed through focus groups and targeted group feedback. These messages can be modified and the patient can choose to create their own reminders if they prefer. These reminder times can vary for different days of the week to accommodate for changes in schedule (e.g., 8 M-F, and 10 AM on Sat/Sun). Once the time is identified, the text reminder system is automated. Patients will confirm medication taking via text responses to the personalized reminders.

Quantitative Plasma Concentration of TFV-DP

In the pivotal PrEP randomized clinical trials, the reduction of HIV acquisition was dependent on adherence to TDF or F/TDF.[4-6] A sub-study from iPrEx correlated intracellular drug concentrations with PrEP efficacy and found that the risk of HIV infection was reduced by ≥90% relative to placebo among subjects with TFV-DP concentration ≥16 fmol/M viable cells.[5,6] Using pharmacokinetic modeling of TFV-DP, the authors estimated that ≥ 4 doses of F/TDF per week would lower the risk of HIV acquisition by more than 90%.[6]

Intracellular RBC TFV-DP levels measured from dried blood spots are becoming the gold standard method to measure PrEP adherence, not only because levels correlate with efficacy but also because levels provide information on adherence over the past week.[5] Studies of directly observed F/TDF dosing found that TFV-DF levels were associated with the number of doses taken per week; median levels increased from 228 fmol/ punch with 1/7 ingested doses to 1560 fmol/punch with 7/7 doses.[5] For F/TDF we will define adequate adherence based on TFV-DP levels corresponding with subject ingestion of 4 doses or more per week and perfect adherence i.e. 7/7 doses according to TFV-DP ≥719 fmol/punch and ≥1246 fmol/punch,

respectively. These levels represent the lower limit of the interquartile range observed in these participants.[5] For F/TAF current levels commensurate with adherence are being determined by our collaborator Peter Anderson who developed the DBS sampling methodology and is currently working with Gilead on this project. Prior studies have suggested that intracellular TFV-DP with TAF use are seven times higher at the 25 mg dose so the expected levels of TFV-DP commensurate with 4 or 7 dose as well may be significantly higher.[12]

F/TAF has been well tolerated and in fact can result in improvement in kidney function as determined by creatinine clearance. Notably in a switch study for HIV infected individuals randomized to F/TAF regimen compared to F/TDF regimen saw a significant difference in lowering of the creatinine, a mean change of -0.08 compared to -0.04 mg/dl.[13] In this switch study we would therefore expect that reported adverse events would be unchanged and there would be improvement in creatinine levels.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

It is the responsibility of the study investigator to ensure that a participant is eligible for the study prior to any study procedures.

4.1 Study Inclusion Criteria

- 4.1.1 Transgender or non-binary, defined as identifying with a gender differently from sex assigned at birth
- 4.1.2 Age 18 years or older
- 4.1.3 Currently eligible to take F/TDF for PrEP and willing to switch to F/TAF
- 4.1.4 Negative for HIV infection
- 4.1.5 Acceptable renal function as measured by calculated creatinine clearance of at least 60 mL/min by the Cockcroft-Gault formula in the past 30 days

4.2 Study Exclusion Criteria

- 4.2.1 Unable to give informed consent

4.2.2 Active hepatitis B defined by a positive hepatitis B surface antigen (HBsAg)

4.2.3 Substantial medical condition that, in the opinion of the investigator, would preclude participation, as defined by

- gastrointestinal condition that would impair absorption of study drugs
- known condition of reduced bone density (e.g. osteoporosis or osteogenesis imperfect) that significantly elevate the risk of bone fracture
- neurological or severe psychiatric condition that would significantly impair the ability to adhere to PrEP
- tubular or glomerular kidney disease that could be exacerbated by tenofovir
- other medical condition that would unacceptably increase the risk of harm from study drug or significantly impair the ability to adhere to PrEP

4.2.4 Suspected sensitivity or allergy to the study drug or any of its components

4.2.5 Currently using an essential product or medication that interacts with the study drug such as the following:

- other antiretroviral agent other than F/TDF (including nucleoside analogs, non-nucleoside reverse transcriptase inhibitors, integrase inhibitors, protease inhibitors or investigational antiretroviral agents)
- agents with known nephrotoxic potential:
  - aminoglycoside antibiotics (including gentamicin)
  - IV amphotericin B
  - cidofovir
  - cisplatin
  - foscarnet
  - IV pentamidine
  - IV vancomycin
  - oral or IV gancyclovir
  - other agents with significant nephrotoxic potential
- drugs that slow renal excretion
  - probenecid
- immune system modulators
  - systemic chemotherapeutic agents (i.e. cancer treatment medications)
  - ongoing systemic corticosteroids (with the exception of short courses of tapering steroid doses for asthma or other self- limited condition).
  - interleukin-2 (IL-2)
  - interferon (alpha, beta, or gamma)
- other agent known to have a significant interaction with TDF, TAF, or FTC
- the following table includes medications/herbal supplements to be excluded or should be used with

caution while participants are taking study drugs due to potential drug-drug interactions with F/TAF.

Table 4.2.5 – Prior and Concomitant Medications to be prohibited or to be used with caution		
Medication Class	Use with caution	Prohibited
Antiarrhythmics	amiodarone, quinidine: may increase concentrations of TAF and/or TFV	
Anticonvulsants		carbamazepine, oxcarbazepine, phenolbarbital, phenytoin
Antimycobacterials	clarithromycin: may increase concentrations of TAF and/or TFV	rifapentine, rifabutin, rifampin
Antifungals	itraconazole, ketoconazole, voriconazole: may increase concentrations of TAF and/or TFV	
Calcium channel blockers	diltiazem, felodipine, verapamil: may increase concentrations of TAF and/or TFV	
Digoxin	concomitant use may result in an increased or decreased digoxin concentration; use with caution and with appropriate monitoring of serum digoxin concentrations	

Herbal / natural supplements		St. John's Wort, echinacea, milk thistle (e.g. silymarin) Chinese herb sho-saiko-to (or Xiao-Shai-Hu-Tang)
Hepatitis C therapies	ledipasvir / sofosbuvir: has been shown to increase tenofovir exposure	boceprevir, telaprevir
Nephrotoxic medications	high dose or multiple NSAIDS	systemic chemotherapeutic agents, aminoglycoside antibiotics, amphotericin B, cidofovir, cisplatin, foscarnet, IV pentamidine, or other agents with significant nephrotoxic potential
Systemic glucocorticoids		dexamethasone (more than 1 dose) or chronic use of other systemic glucocorticoids
Other		probenecid

4.2.6 Proteinuria 2+ or greater by urine dipstick

4.2.7 Pregnancy (if applicable)

4.2.8 Other condition that, in the opinion of the investigator, would put the participant at risk, complicate interpretation of study outcome data, or would otherwise interfere with participation or achieving the study objectives

5.0 STUDY TREATMENT

5.1 Regimens, Administration, and Duration

5.1.1 Regimens

Phase I: F/TDF 200/300 mg once daily

Phase II: F/TAF 200/25 mg once daily

5.1.2 Administering and Dispensing

Phase I: F/TDF fixed-dose combination containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate will be administered orally as one tablet once daily with or without food.

Phase II: F/TAF fixed-dose combination containing 200 mg of emtricitabine and 25 mg tenofovir alafenamide will be administered orally as one tablet once daily with or without food.

Enough study product should be dispensed to last until the participant's next scheduled in-person visit.

5.1.3 Duration

Phase I: Participants will continue or initiate F/TDF for PrEP for a minimum 12-week lead-in period prior to switching to F/TAF.

Phase II: Participants will be switched to study-provided F/TAF for PrEP until 48 weeks after initiation. Participants will receive study treatment for the duration of the study unless they meet criteria for discontinuation.

5.2 Study Product Formulation

Emtricitabine/tenofovir disoproxil fumarate (F/TDF, Truvada®): 200mg / 300mg coformulated tablet. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F) [see USP Controlled Room Temperature].

Emtricitabine/tenofovir alafenamide (F/TAF, Descovy®): 200 mg/25 mg coformulated tablet. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F) [see USP Controlled Room Temperature].

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Supply/Distribution

F/TDF and F/TAF will be supplied by Gilead Sciences.

The site pharmacist can obtain study product for this protocol by following the instructions in the Pharmacy Manual.

5.3.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study product received from the UCSD/AVRC Research Pharmacy. All unused study product must be returned to the sponsor (or as otherwise directed by the sponsor) after the study is completed or terminated. The procedures to be followed are provided in the Pharmacy Manual.

5.4 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose is changed, site investigators must review the participant's concomitant medications and study agent's most recent package inserts or Investigator's Brochure to obtain the most current information on drug interactions, contraindications, and precautions.

5.4.1 Prohibited Medications (Refer to Section 4.2.5 for additional information on prohibited medications).

5.4.1.1 All other investigational drugs

5.4.1.2 All HIV vaccines

5.4.1.3 Any immunomodulators or cytotoxic chemotherapy

5.4.1.5 All other antiretroviral medications except TDF/FTC

5.4.1.6 Drugs with known nephrotoxicity

6.0 CLINICAL AND LABORATORY EVALUATION

6.1 Schedule of Evaluations

Schedule of Evaluations	Study Weeks of Follow-up			
	Phase I	Phase II		
	Screen/Baseline	Switch Visit	Wks 12, 24, 36	Wk 48 / EOS
Window	+/- 2 weeks			
Informed Consent	X			
Inclusion / Exclusion	X			
iTAB setup	X			
Targeted medical/medication history and physical exam	X	X	X	X
HIV risk-reduction counseling	X	X	X	
Adherence counseling	X	X	X	
F/TDF dispensed	X			
F/TAF dispensed		X	X	
Adverse events assessment	X	X	X	X
Computer assisted self-interview (CASI) surveys	X	X	X	X
Laboratory				
Rapid HIV test[1]	X	X	X	X
Creatinine and calculated CrCl	X	X	X	

Schedule of Evaluations	Study Weeks of Follow-up			
	Phase I	Phase II		
	Screen/Baseline	Switch Visit	Wks 12, 24, 36	Wk 48 / EOS
Urinalysis	X	X	X	
Urine β-HCG[2]	X	X	X	
STI screen[3]: GC, CT, syphilis		X	X[4]	X
HBV	X[5]			
HCV	X			X
Plasma for hormone cocns.	X	X	X	
DBS collection for TFV-DP cocns.	X	X	X	X
Banked whole blood, serum, plasma	X	X	X	X
Banked urine	X	X	X	X
HIV confirmatory test if HIV+	(X)	(X)	(X)	(X)
HIV RNA and HIV genotype if HIV+ after baseline	(X)	(X)	(X)	(X)

[1] Participants with medication interruption greater than 14 days will require confirmation of HIV seronegative status using a 4th generation HIV antigen/antibody (Ag/Ab) test prior to re-initiation of PrEP. Re-initiation must occur prior to the Phase II Week 36 visit.
[2] Only for participants capable of becoming pregnant
[3] Includes additional x2 banked rectal swabs collection if rectal swabs are being collected for STI screening
[4] Wk 24 only
[5] If never evaluated

6.2 Site Initiation

Before implementing this study the site must have the protocol and informed consent form approved by the local institutional review board (IRB). Upon approval, the site will conduct an initiation visit to brief study staff on the protocol objectives. Mock enrollment and in-clinic visits will be conducted to train and familiarize study staff of the protocol procedures.

6.3 Phase I

6.3.1 Screen/Baseline Visit (approximately one to two hours)

The Screen/Baseline Visit, consenting process, and study enrollment may only be initiated in-person; to enroll, a participant must present to the clinical site.

Participants co-enrolled in CCTG 603 may have their Screen/Baseline Visit overlap with the CCTG 603 Week 36 visit. Evaluations completed as part of their regular CCTG 603 Week 36 study procedures may satisfy requirements for their Screen/Baseline Visit.

<u>Registration</u>

Each participant will be assigned a patient identification number (PID) and a study identification number (SID). Both PIDs and SIDs must be included on every source document and laboratory sample for that participant. The

site is responsible for maintaining a master list of PIDs and SIDs in a central location.

- A pre-generated list of PIDs will be developed prior to study initiation. Each unique person will be assigned only one PID that will be used across all studies; if a person has been assigned a PID from a previous study, a new PID will not be generated.
- SIDs will be assigned using the site's SID assignment list and will be used for that study only. SIDs will not be reassigned should a participant screen fail, withdraws from the study, or ultimately does not provide written consent.

<u>During Screen/Baseline Visit</u>

All participants interested in participating in CCTG 605 will sign an Informed Consent Form (ICF) that had been approved by the local IRB.

Screening/Baseline procedures will include:

- Assessment of inclusion and exclusion criteria
  - Confirmation of HIV status via Rapid Test.
  - If the participant has had PrEP interruption greater than 14 days prior to Screen/Baseline, a 4th generation HIV-1 Ag/Ab test will be performed in addition to the Rapid Test.
- Laboratory evaluations
  - HBV and HCV
  - Calculated creatinine clearance (Cockroft-Gault)
  - Urinalysis
  - Urine for β-HCG, if applicable
  - Banked whole blood, plasma, serum, urine, and DBS
- Reassessment of HIV acquisition risk
- Review of detailed medical history including medical insurance status, and current medical and psychiatric conditions.
- Review of detailed medication history including gender-affirming hormone therapy (HT), if applicable, and other concomitant medications.
- Collection of height and weight
- Computer-assisted self-interview (CASI) assessments
- iTAB will be setup or configured for an additional 24 weeks.
- Medication dispensation if HIV negative via Rapid Test. Participants will be provided enough medication to last until the next scheduled visit through the investigational pharmacy.
  - If a participant is receiving F/TDF through their provider, they may choose not to use the study-provided F/TDF.
- Risk reduction and medication adherence counseling. Health educators will provide HIV transmission risk reduction counseling and detailed information about the use of PrEP, including the risks, potential adverse events and the critical importance of drug adherence.
  - An informational pamphlet on F/TDF will be provided that will summarize the label package insert information for patients (in

English or Spanish) with changes made to tailor the information for someone that is not HIV infected.

- Participants will be directed to take one pill once a day routinely at a convenient time.
    - If there are missed doses, participants will be told to take the next scheduled dose on time and not to take any additional pills to catch up.
    - Study-provided bottles with any remaining tablets will be returned to the pharmacy at the time of medication renewal and the number of untaken doses will be recorded.

6.4 Phase II

6.4.1 Switch Visit (approximately 1 to 2 hours)

Participants will be asked to return to the clinic for the Switch Visit after the 12-week lead-in period on F/TDF.

Participants co-enrolled in CCTG 603 may have their Switch Visit overlap with the CCTG 603 Week 48 visit. Evaluations completed as part of their regular CCTG 603 Week 48 study procedures may satisfy requirements for their Switch Visit.

Study visit procedures include:

- Assessment of adverse events since last contact
- Targeted medical history and physical examination, including collecting weight
- Review of concomitant medications, including detailed HT use
- Laboratory evaluations
    - Rapid HIV screening
    - 3-site (throat, rectal, urine) STI NAAT screening for gonorrhea and chlamydia
    - Syphilis RPR
    - Calculated creatinine clearance (Cockroft-Gault)
    - Urinalysis
    - Urine for β-HCG, if applicable
    - Banked whole blood, plasma, serum, and DBS
- CASI assessments
- Medication dispensation. Participants will be instructed to stop taking F/TDF and will initiate study-provided F/TAF at this visit. Participants will be provided enough medication to last until the next scheduled visit through the investigational pharmacy.
    - An informational pamphlet on F/TAF will be provided that will summarize the label package insert information for patients (in English or Spanish) with changes made to tailor the information for someone that is not HIV infected.
- Participants will be directed to take one pill once a day routinely at a convenient time.

        - If there are missed doses, participants will be told to take the next scheduled dose on time and not to take any additional pills to catch up.
        - Study-provided bottles with any remaining tablets will be returned to the pharmacy at the time of medication renewal and the number of untaken doses will be recorded.
    - Risk reduction and medication adherence counseling.

6.4.2 Weeks 12, 24, 36, and 48 Follow-up Visits (approximately 1 hour)

Participants will be asked to return to the clinic every 12 weeks up to 48 weeks for regular follow-up and safety evaluations.

Study visit procedures include:

- Assessment of adverse events since last contact
- Targeted medical history and physical examination, including collecting weight
- Review of concomitant medications, including detailed HT use
- Laboratory evaluations
    - Rapid HIV screening
    - 3-site (throat, rectal, urine) STI NAAT screening for gonorrhea and chlamydia (Weeks 24 and 48 only)
    - Syphilis RPR (Weeks 24 and 48 only)
    - HCV (Week 48 only)
    - Calculated creatinine clearance (Cockroft-Gault)
    - Urinalysis
    - Urine for β-HCG, if applicable
    - Banked whole blood, plasma, serum, and DBS
- CASI assessments
- Medication dispensation, except for Week 48
- Risk reduction and medication adherence counseling, except for Week 48
- End of Study assessment (Week 48 only). This survey will assess participants' experiences over the course of the study, including changes in their medical insurance status, access to other healthcare services, changes in perceptions of PrEP and of their own health, and their opinions about F/TAF.
    - Participants that express the desire to continue PrEP after completing the study will be provided information regarding access and payment strategies. Considerations for transitioning should begin at Week 36.

6.5 Schedule of Evaluations for Subjects On-study, Off-medications

At any point of the study, participants may elect to stop study medication but remain on study. Participants not on study medication will follow the same schedule as defined in the schedule of evaluations until the end of study, with the following exceptions:

- Medication will not be dispensed
- Adherence counseling and monitoring of medications will not be performed
- DBS will not be collected

- iTAB account for participant will be switched to “inactive”

Participants who stay on study but stop study medication will be allowed to resume study medication again if they re-initiate before completing the Week 36 visit. If the amount of time since last dosing of PrEP exceeds 14 days, a negative rapid HIV EIA and HIV VL or Ag/Ab test should be documented prior to PrEP re-initiation.

Approximately 300 mL (20 tablespoons) of blood will be drawn across 6 scheduled study visits over 48 weeks. An additional 60 mL (4 tablespoons) will be drawn for each additional, unscheduled visit.

Stored Specimens: Plasma, serum, whole blood, and DBS (if on PrEP) will be collected at Screen/Baseline and every subsequent visit for storage, and at every seroconversion visit.

6.6 Special Instructions and Definitions of Evaluations

6.6.1 Documentation of HIV infected status at screening

The lack of HIV-1 infection will be documented by NAT or Ab/Ag test. A positive HIV test will be confirmed by a second confirmatory test per the routine of the site.

6.6.2 Laboratory Assessments for Safety

At screening and follow-up clinic visits, participant will have blood drawn for estimated creatinine clearance and results documented in the source documentation. During follow-up, if CrCl is <50 mL/min a second, confirmatory test will be scheduled within 7 days. The participant will be advised to discontinue PrEP immediately if the second CrCl is <50 mL/min.

Adverse events will be review at every visit after a participant initiates PrEP. If a Grade 3 or 4 adverse event is reported and thought to be related to study medication (see Section 7.0 Toxicity Management), the participant will be advised to discontinue PrEP immediately.

Any laboratory toxicity, regardless of grade, leads to a change in treatment, the toxicity must be reported as an AE.

Refer to the Division of AIDS Table for Grading Adult Adverse Experiences.

6.6.3 Study Drug Modifications

No modifications of PrEP regimen will be allowed on this study. F/TAF may be temporarily held for adverse events as per Section 7.0 Toxicity Management. F/TAF should be discontinued if any criteria for stopping are met (see Section 8.0). Discontinuation will be documented in the source documentation.

6.6.4 HIV Seroconversion

Rapid test for HIV will be performed at every follow-up clinic visit and documented in the source documentation. Results will be shared with participant before the end of the clinic visit.

If HIV seroconversion is detected, the participant will be advised to discontinue PrEP immediately. Any positive rapid test will be confirmed by another confirmatory test (eg IFA, second rapid). For any seroconversion, an HIV RNA, CD4 cell count, and HIV genotype to detected drug resistance will be performed. Newly diagnosed HIV participants will be counseled and linked to HIV clinical care within the existing clinic or to another provider designated by the participant.

Participants may schedule an interim visit with the study coordinator to screen for HIV if they experience symptoms or encountered high-risk exposure.

6.6.5 HIV-1 RNA, CD4 T-Cell Counts, and HIV Genotype

An HIV-1 RNA viral load will be performed at the site's local laboratory within 7 days of a new positive antibody test or NAT after Baseline.

CD4 T-cell enumeration will be performed to obtain absolute CD4+/CD8+ count and percentages within 7 days of new HIV diagnosis after Baseline.

An HIV drug resistance test should be performed with detectable viral load of greater than 500 copies/mL after Baseline.

6.6.6 Chlamydia, gonorrhea, and syphilis testing

Participants will have STI screening assessments at the Switch Visit, and Weeks 24 and 48. Screening includes collection of urine and swabs from the rectum and throat for chlamydia and gonorrhea NAT testing. Blood will be collected for syphilis RPR. Results will be documented in the source documentation.

All new STI cases will be referred to their provider or the Department of Health for treatment. Participants will be asked to confirm receiving treatment for their STI at their next study visit. All new STI cases will also be counseled on the need for partner therapy and will suggest that they receive assessment and treatment of partner delivered therapy.

Participants may schedule an interim visit with the study coordinator to screen for STIs if they experience symptoms or encountered high-risk exposure.

6.6.7 Adherence and Behavioral Assessments

Adherence will be measured by using self-reported daily text messages, CASI survey instruments (CCTG 605 CASI Questions (English version)),

and through participant interviews with study coordinators. The CASI utilizes standardized surveys including the SCID Screen, DAST-10, Ira Wilson, PHQ9, PHQ4 and AUDIT in addition to non-standardized survey questions about adherence and behavioral assessments.

Self-reported daily text message reminders will include a personalized lead-in reminder message (e.g., “Those who are closest to you care about your health”) and participants will be asked “Did you take your dose today? Reply: Y) Yes N) No P) Postpone. If “Postpone” is selected, participants will receive a follow-up message 1 hour later asking them if they took their medication (Yes or No). Participants will also have the option of determining the word used to describe the study drug (e.g. “med”, “dose”, or other personal choice). Nonresponse or a “No” response will be counted as not taking their dose.

CASI survey instruments will capture several sub-domains of PrEP adherence including a participant’s willingness to take PrEP and ability to take PrEP, as prescribed, over the past several days. If a participant has missed doses, CASI instruments will also capture barriers and reasons for non-adherence.

Lastly, in the standard study visit, the study coordinator will perform HIV testing and blood work, review test results, provide PrEP prescriptions, review adverse events, counsel on risk reduction strategies, and discuss medication adherence and troubleshooting any possible barriers. Adherence will be collected through a 3-day recall of the most recent doses taken.

6.6.8 TFV Quantitative Dried Blood Spot Intracellular Concentration

Dried blood spots will be collected at every visit starting Screen/Baseline for TFV concentrations. Samples will not be run in real time and will not be available for clinical use.

6.6.9 Plasma hormone levels

Blood will be collected at every visit up to and including Week 36 to measure estradiol and testosterone plasma concentrations.

6.6.10 Medical History

At screening, a medical history will be obtained and will be recorded in the source documentation. The medical history should include any previous medical and psychiatric diagnoses, other routine medical conditions, history of STI, medical insurance status, and access to other healthcare services.

6.6.11 Medication History

At screening, a medication history (only of those taken within the last 30 days prior to entry) with actual or estimated start and stop dates will be

obtained and recorded in the source documentation. Medications to be recorded include:

- All antibiotics
- All prescription medications.
- Non-prescription medications.
- Alternative therapies and/or dietary supplements.
- Allergies to any medications and their formulations must be documented.
- Gender-affirming hormone therapy

Detailed hormone therapy use, including formulation, route, and frequency, will be recorded in the source documentation.

During study visits, study coordinators will review changes in concomitant medications taken since the last visit and will record changes in the source documentation.

6.6.12 Clinical Assessments

Targeted Physical Exam

A targeted physical examination will be based on any signs or symptoms previously identified that the participant experienced since the last visit. This examination will be performed at all visits when indicated. Documentation must include any symptoms consistent with acute HIV infection.

Weight

Weight will be measured at each regular study visit.

Signs and Symptoms

All signs, symptoms, toxicities, hospitalizations, and deaths since the last study visit will be documented in the participant's record. All Grade > 1 signs and symptoms since the last visit will be recorded in the source documentation, including dates of onset and resolution. Any signs or symptoms that lead to a change in treatment, regardless of Grade, will also be recorded.

Refer to the ACTG Table for Grading Adult Adverse Experiences.

Diagnoses

The following should be recorded on the CRFs: HIV and STI diagnoses, malignancies, new medical conditions and death. The source document must include date of diagnosis and date of resolution.

6.6.13 Interim Visits

A reason for an interim visit would be for a change in medical status that is of importance to study such as adverse event, pregnancy, high risk for HIV exposure while not taking PrEP for more than a week or symptomatic for acute HIV or STI. If a participant misses a study visit and are past the study window but not yet in the next study visit window, then an interim visit should be performed for the missed study visit and will be documented as a protocol deviation. If a participant is in the next study visit window, then the previous visit is a missed visit and they can continue on schedule. Missed visits should be documented as a protocol deviation.

6.6.14 Stored Specimens

Plasma, serum, whole blood, and DBS (if on PrEP) will be collected at Screen/Baseline and every subsequent visit for storage, and at every seroconversion visit.

6.6.15 Pharmacokinetic Studies

At each visit, record the time and date of the last 3 doses of PrEP in the source documentation. Dried blood spots will be stored at -80 at each visit for possible future use for plasma and intracellular TFV and FTC pharmacology.

6.6.16 Computer Assisted Self-Interview (CASI) Survey Instruments

At each visit, the subject should complete all CASI surveys.

6.6.17 Pregnancy

Any participant who should become pregnant while on study will be discontinued.

6.6.18 Social Harms

Although study sites will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could be perceived as being HIV-infected or at risk" for HIV infection). For example, participants could be treated unfairly, or could have problems being accepted by their families and/or communities. Social harms that are judged by the core investigators to be serious or unexpected will be reported to the IRB(s) at least annually, or according to their individual requirements. Social harms will be collected and reported on CRFs during regular visits. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant.

All In Vitro Diagnostics used in the study are FDA approved. A summary of each device and FDA approval date is as follows.

Test Name:	FDA Approval Date:
Rapid HIV test- INSTI Rapid HIV2 by bioLytical Labs:	FDA Approved BP090032/7 01/28/2015
RPR Syphilis- Carbon Antigen RPR Test for Syphilis (ASI Evolution) by Arlington Scientific:	K182391 11/30/2018 (ASI evolution by Arlington Scientific, prior approvals: K173376 06/14/2018 and BK170114 12/28/2017)
TP-PA- Serodia TP-PA by Fujirebio Inc:	FDA approved K971502 11/13/1997
HCV- Cobas 6000 series e601 analyzer by Roche Diagnostics: Cobas 8000 series e602 analyzer by Roche Diagnostics:	FDA Approved K060372 03/13/2006 FDA Approved K100853 09/09/2010
STI NAAT- Aptima Combo 2 Assay (Panther System) by Hologic, Inc:	FDA Approved K190515 05/23/2019

7.0 TOXICITY MANAGEMENT

The management of medication-related toxicities should be undertaken by the local investigators with guidance available from the protocol team and pharmaceutical sponsor, to ensure the optimal safety and efficacy for the individual subject.

7.1 General Management for Grade 1-4 Events

7.1.1 Grade 1 or 2

Subjects who develop a Grade 1 or 2 adverse event or toxicity may continue PrEP without alteration of the dosage, except as noted below. Persistent grade 1 or 2 toxicity should be discussed with the protocol team. Those subjects experiencing Grades 1 or 2 adverse events which results in discontinuation of the PrEP should continue to be followed on study, but off study medication.

7.1.2 Grade 3

Management of Grade 3 toxicities should be discussed with the protocol team via email. Please refer to the subsequent sections for management of specific events.

In the event that a subject develops a symptomatic Grade 3 reaction considered to be PrEP-related, the study drug should be discontinued and the subject should be followed weekly until resolution of the adverse event.

Once Grade 3 is resolved the subject should be followed on-study, but off study medication. For subjects with asymptomatic Grade 3 toxicity or laboratory abnormalities, the protocol team should be consulted for possible re-introduction of PrEP.

7.1.3 Grade 4

Subjects who develop a Grade 4 adverse event or toxicity judged to be PrEP-related will have the study drug permanently discontinued. For other Grade 4 events, if the toxicity or laboratory elevation is thought not to be due to PrEP, PrEP may be continued after consultation with the study team and laboratories repeated within 2 weeks (for example for asymptomatic elevation of CPK or triglycerides). If the investigator cannot identify another potential causative agent/ condition or if PrEP could be the causative agent, then PrEP must be discontinued. Subjects experiencing Grade 4 adverse events requiring permanent discontinuation of PrEP therapy should be followed weekly until resolution of the adverse event. Once Grade 4 is resolved the subject should be followed on-study, but off study medication.

7.2 Management of Specific Adverse Events

7.2.1 Rash

Grade 1 or 2

PrEP may be continued without interruption. Subjects with a Grade 1 or 2 rash may be treated symptomatically with permitted antipyretic, antihistamine and/or non-steroidal anti-inflammatory medications, but should be monitored closely by the local investigator.

Grade 3 or 4

Grade 3 or 4 rash necessitates that PrEP be held unless the rash is determined to be unrelated to PrEP. The rash should be followed closely for resolution and the subject followed on-study, off study medication.

7.2.2 Nausea and Vomiting

Grade 1 or 2

F/TAF may be continued without interruption. Subjects with Grade 1 and 2 nausea or vomiting may be treated symptomatically with oral antiemetic therapies or antiemetic suppositories. Subjects will be instructed to take medications with food.

Grade 3 or 4

Subjects with Grade 3 PrEP-related nausea and vomiting should interrupt PrEP until the toxicity grade returns to Grade $\leq$ 2 or to baseline and be treated symptomatically. After discussion with the protocol team and if the subject is willing, the PrEP may be resumed when symptoms have resolved. If Grade 3 nausea and vomiting recurs upon the resumption of PrEP despite symptomatic treatment, PrEP should be discontinued. Grade 4 nausea or vomiting will lead to permanent discontinuation of drug. Once resolved, the subject should be followed on-study, but off study drug.

7.2.3 Diarrhea

Grade 1 or 2

PrEP may be continued without interruption. Subjects with diarrhea of any toxicity grade may be treated symptomatically with permitted antimotility agents.

Grade 3 or 4

For grade 3 diarrhea that is unresponsive to antimotility agents and for which an alternative etiology (e.g., infectious diarrhea) is not established, PrEP should be interrupted until resolution of diarrhea to Grade $\leq$ 2 or baseline. If Grade $\geq$ 3 diarrhea recurs upon the resumption of study medications, PrEP should be permanently discontinued. Grade 4 will lead to permanent discontinuation of study medication.

7.2.4 Creatinine Elevations

PrEP will be discontinued if the creatinine clearance is confirmed to be < 50 mL/min. Subjects should be followed as medically indicated until the creatinine returns to baseline. The protocol team will be notified within 48 hours of any permanent therapy discontinuations due to change in creatinine clearance. Sites should consider additional testing including serum phosphate concentration, urine dipstick for proteinuria and glycosuria, and urine phosphate and creatinine concentration for calculation of fractional excretion of phosphate in consultation with the protocol team.

If the serum creatinine increases more than 0.3 from baseline, the level should be repeated in 2 weeks. Elevations in creatinine will be monitored regularly on study drug, and further intervals of repeated laboratory testing will be at the discretion of the site PI.

8.0 CRITERIA FOR TREATMENT AND STUDY DISCONTINUATION

8.1. Temporary Treatment Discontinuation

Attempts should be made to avoid temporary interruptions of study drug.

Symptoms should be managed in an attempt to avoid discontinuation provided that symptoms are either Grade 1 or 2 and there is no laboratory evidence of toxicity. In the event of symptomatic Grade 3 or any Grade 4 toxicity attributable to study drug, PrEP should be discontinued immediately, with the exception being if toxicity is unlikely to be attributable to PrEP administration. Permission of exception should be obtained from the protocol PI. See Sections 7.1 and 7.2 for general and specific toxicity management guidelines.

In the event of inadvertent discontinuation due to missed appointments, loss of medication, or other unforeseen circumstances, treatment should be resumed as quickly as possible. If a participant discontinues PrEP on their own they may restart up until week 36 visit.

Treatment should ONLY be reinstituted in the absence of signs/symptoms of acute infection. If the amount of time since last dosing of PrEP exceeds 14 days when the participant wishes to reinitiate PrEP, a negative rapid HIV EIA and HIV VL or Ag/Ab test should be documented PRIOR to PrEP re-initiation.

8.2 Permanent Discontinuation of Study Drugs

Permanent treatment discontinuation prior to 48 weeks of treatment should be limited to participants with the reasons listed above. Such participants should be followed per the protocol SOE even though they are no longer taking study medication. HIV seroconversions do not have follow up visits once the appropriate End of Study visit has been completed. Assistance as needed should be given to transition the seroconverted subject into HIV treatment.

8.3 Criteria for Study Discontinuation:

Participants should be discontinued from the study under the following circumstances:

8.3.1 Diagnosis of HIV infection.

8.3.2 Request by the participant to withdraw.

8.3.3 Opinion of the primary care provider in consultation with the participant that discontinuation of the study is in the best interest of the participant.

8.3.4 Serious problems not addressed in the toxicity management of the protocol that are decided by the study team.

8.3.5 Participant judged by the investigator to be at significant risk of harm, to self or others, or seriously interfere with the validity of the study results.

8.3.6 Decision of CCTG, FDA, CHRP, or pharmaceutical sponsors.

9.0 STATISTICAL CONSIDERATIONS

A formal Statistical Analysis Plan (SAP) will be drafted and finalized prior to database lock. The SAP will contain a more detailed and/or comprehensive presentation of statistical methods; attention to any changes of substance to planned analysis procedures relative to those indicated in the protocol will be submitted as an amendment. The SAP is the final authority for all statistical analyses. The following section briefly describes the planned statistical analyses. In case the language in this section differs from the language in the SAP, the SAP takes precedence.

9.1 Endpoints

The primary endpoint will be dried blood spot intracellular tenofovir diphosphate (TFV-DP) concentrations. TFV-DP will be compared between participants taking versus not taking gender-affirming HT. Additional analysis will be by type of HT and for those on HT with strata the correlation of HT levels with TFV-DP levels.

9.1.1 Secondary Safety Endpoints

Safety and tolerability outcomes will be primarily assessed by comparing incidence rates of Grade 1 or higher AEs between F/TDF time period and F/TAF period within group. Additional safety outcomes will be measured by self-reported tolerability and by changes in creatinine concentrations.

9.1.2 Secondary Adherence Endpoints

The proportion of participants on F/TDF with intracellular TFV-DP concentrations $\geq$ 1246 fmol/punch (commensurate with 7 doses in the past week) will be compared to the proportion of participants on F/TAF with TFV-DP concentrations commensurate with 7 doses of F/TAF in the past week, as determined by Dr. Peter Anderson (University of Colorado). Only participants reporting ongoing F/TAF use at Weeks 12 and 48 will be included in the modified intent-to-treat analysis.

The proportion of participants on F/TDF with TFV-DP concentrations $\geq$ 719 fmol/punch (4 doses/past week) will be compared to the proportion of participants on F/TAF with TFV-DP concentrations commensurate with 4 doses of F/TAF in the past week, determined by Dr. Anderson.

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Self-reported adherence by iTAB text message responses between Screen/Baseline to Week 12 on F/TDF will be compared to self-reported adherence between Week 12 to Week 24 on F/TAF.

9.2 Study Power and Sample Size Justification

Primary Outcome: The current study is powered to identify non-inferior TFV-DP concentrations in participants on HT compared to those not on HT. In our previous study, mean TFV-DP concentrations at Week 48 in MSM participants taking F/TDF and receiving the iTAB intervention was 1241 fmol/punch (standard deviation 535 fmol/punch). Based on a non-inferiority level of 25% with a one-sided alpha of 0.05 and 0.70 power we will be able to show non-inferiority with 23 participants per arm. We will aim to enroll 60 participants to adjust for attrition at Week 12.

Adherence: In our previous study, adherence to F/TDF was high among MSM and transwomen: 320/361 (88.6%) participants retained at Week 12 and 264/320 (82.5%) participants at Week 48 achieved TFV-DP concentrations ≥ 719 fmol/punch, while 171 (47.4%) Week 12 participants and 141 (44.1%) Week 48 participants achieved TFV-DP ≥ 1246 fmol/punch. Although adherence may not be as high in transgender individuals compared to MSM, we will use these numbers as the basis for our power calculation. Based on the Week 48 expected adherence to F/TDF we can show non-inferiority with 15% difference in concentrations with 0.77 power.

9.3 Monitoring

The study team will review all adverse events during PrEP therapy as cumulative reports in both arms combined, on monthly team calls. Adverse events will be graded using the ACTG toxicity grading scale and recorded using standard CCTG AE electronic data capture. An independent Data Safety and Monitoring Board (DSMB) will not be used for this study because the F/TDF is licensed for this indication, F/TAF is in the process of FDA review and approval, and both formulations are relatively non-toxic.

9.4 Analyses

9.4.1 Statistical Analysis Plan

In general, analyses of efficacy will incorporate the modified intent-to-treat (mITT) principle, namely, all randomized participants dispensed study medication will be included in the analysis. For all secondary analyses of efficacy, no adjustments for multiple comparisons will be made and a p-value of 0.05 will be considered statistically significant. Analyses of safety will be focused on “as treated” populations.

Demographic and baseline measurement variables will be summarized via standard descriptive statistics. Interim analyses for futility or efficacy will not be conducted.

9.4.2 Analysis of Primary Outcome

Analysis will be performed on the primary outcome of TFV-DP levels by HT status. Comparison of those on and off HT will be done be t-test. Additional sub-analysis of the primary will stratify by HT type and using both concentrations of TFV-DP and estradiol levels test for correlation. Also cut offs for adherence consistent with 4 doses a week and 7 doses a week will be compared between strata.

9.4.3 Analysis of additional secondary outcomes

Descriptive analysis will be performed for all the secondary outcomes. For group comparisons, Fisher’s exact test will be used for categorical outcomes; t-test or Wilcoxon rank sum test will be used for continuous outcomes. Multivariable logistic regression model will be used to study factors associated with poor adherence. Factors will include demographics, substance use, untreated mental illness, socioeconomic

status, low health/HIV and system literacy, fear of disclosure and non-English primary language.

Safety data will be reported for all participants during use of study drug and immediately after (30 days) completion (as treated analysis). Tables will summarize the number of serious adverse events, adverse events (Grade 1 or higher) over 48 weeks, and PrEP discontinuation due to adverse events. Tolerability will be assessed using Likert scales for those that found favorable tolerability and greater between week 12 F/TDF and week 12 F/TAF. Serum creatinine concentrations and calculated creatinine clearance will be compared between Week 12 of F/TDF and Week 12 on F/TAF.

Adherence will be assessed by comparing TFV-DP concentrations commensurate with i) 7 doses/week and ii) 4 doses/week; iii) TFV-DP levels as a continuous measure; and iv) self-reported adherence by text message over 48 weeks between participants on F/TDF versus participants on F/TAF

10.0 PHARMACOLOGY PLAN

TFV-DP and FTC-TP levels will be determined by standardized methods at the lab of Peter Anderson at the University of Colorado. Estradiol and testosterone quantification will be performed at UCSD.

11.0 DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

11.1 Retention of records

Case report forms (CRFs) will be completed for each participant on which participants will be identified using only their PID and SID (provided by the CCTG Data Unit upon registration). A confidential ID Assignment Log will link ID's to participant names but will be stored in a locked cabinet in a secure office at the study site, available only to the site investigators.

Self-reported surveys that are performed electronically will be automatically stored in the electronic database secured by the CCTG Data Core.

11.2 Role of Data Management

11.2.1 Instructions concerning the recording of study data on CRFs will be provided by the CCTG Data Core.

11.2.2 CCTG Data Core will assure the quality of computerized data for this study.

11.3 Serious Adverse Experience (SAE) Reporting

Serious adverse events are not expected in this study. All SAEs must be documented on the SAE Reporting Form within 5 working days of site awareness of the event and submitted to the CCTG Data Core.

12.0 HUMAN SUBJECTS

12.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for the oversight of the study. A consent form describing the purpose of the study, the procedures to be followed, the risks and benefits of participation, and contact information for reaching the study staff will be

read, reviewed with the study staff, and signed by each subject. A copy of the consent form will be given to the subject.

12.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be stripped of any patient identifiers (name, birthdate, medical record number) and only identified by the coded PID in order to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only and analyzed centrally without any possibility of linking subject identity with subject data. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB and governmental agencies.

12.3 Study Discontinuation

The study may be discontinued at any time by the IRB or other government agencies as part of their duties to ensure that research subjects are protected

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APPENDIX I: RENAL EFFECTS OF HORMONES/BIOMARKERS IN TRANSGENDER PREP RECIPIENTS SUB-STUDY

SCHEMA

Design:	This cross-sectional sub-study will use a subset of the main study cohort of transgender (TG) or non-binary (NB) individuals to evaluate the relationships between self-reported exogenous hormone use, endogenous hormone values (serum estradiol, estrone, free/total testosterone), renal biomarkers, drug levels (measured by tenofovir diphosphate, TFV-DP, and emtricitabine triphosphate, FTC-TP, on dried blood spot cards and urine) and directly measured renal function (iohexol clearance).
Duration:	Each subject enrolled in this sub-study will be evaluated on two study visits: once while taking emtricitabine/tenofovir disoproxil fumarate (F/TDF) or within 1 week of initiating F/TAF and again after switching to emtricitabine/tenofovir alafenamide (F/TAF) for a minimum of 12 weeks. Each study visit is expected to last 3.5 hours.
Sample Size:	Forty subjects from the main study cohort will be included in the sub-study. The composition will be TG/NB individuals: i) assigned male at birth taking exogenous feminizing hormones (n = 10), ii) assigned male at birth and not taking exogenous feminizing hormones (n = 10), iii) assigned female at birth and taking exogenous androgenizing hormones (n = 10) and iv) assigned female at birth and not taking exogenous androgenizing hormones.
Study Population:	Eligible subjects will recruited from the main study which includes HIV-uninfected, TG/NB adults at risk of acquiring HIV with a calculated creatinine clearance (CRCL) ≥ 60 mL/minute, taking F/TDF and anticipate switching to F/TAF. The only additional inclusion criteria for the sub-study are a willingness to: i) receive a small dose of iohexol, ii) provide four blood and one urine sample during each sub-study visit. The only additional exclusion criteria are: i) allergy to iohexol, ii) use of concurrent medications that may interfere with iohexol such as metformin, amiodarone or beta-blockers iii) anuric or unable to produce 30 mL of urine and iv) other condition that, in the opinion of the investigator, would put the participant at risk, complicate interpretation of study outcome data, or would otherwise interfere with participation or achieving the study objectives
Outcomes:	The primary outcome of the sub-study is a direct measure of renal function. The gold standard for directly measured renal function is iohexol clearance (IHX-CL). During each sub-study visit occurring up to 1 week past F/TAF initiation for Visit #1 and at least 12 weeks past F/TAF initiation for Visit #2, each participant will have one measured concentration of IHX. The concentration from this sample will be used to compute IHX-CL using the Jacobsson equation where IHX-CL = (1/(t/V + 0.0016))* ln (Dose/(V *C1)

(in mL/min)) and $V_{\text{male assignment at birth}}$: 166 *W+ 2490, $V_{\text{female assignment at birth}}$: 95*W+ 6170, Ct is the sample concentration (µg/mL) at time t (minute), V is the apparent volume of distribution (mL) and W is the weight (kg).(1,2)

14.1 Sub-study Primary Objective

14.1.1. Primary Objective

To evaluate the relationship between exogenous hormone use, *in vivo* hormonal measures, renal biomarkers (RBM), renal function (IHX-CL), and drug levels (urine/blood TFV) in TG/NB subjects taking PrEP.

14.1.2. Primary Hypothesis

Among TG/NB individuals, IHX-CL and TFV values will be heterogeneous based on use of exogenous hormones, *in vivo* hormonal measures and presence/absence of various renal biomarkers.

14.2 Sub-study Secondary Objectives

14.2.1.a. To assess if incorporation of gender identity, exogenous hormone use/*in vivo* hormonal measures, and RBM into a predictive equation improves accuracy of renal function estimation relative to traditionally-employed renal function estimating equations (i.e. Cockcroft-Gault, Jelliffe, Modification of Diet in Renal Disease (MDRD), or modified MDRD (adjusted to individual body surface area)).(3)

14.2.1.b. Hypothesis: A new gender coefficient in the Cockcroft-Gault equation will improve approximation of IHX-CL.

Hypothesis: Incorporation of cystatin C and use of exogenous hormones or *in vivo* hormonal measures into an equation derived from the linear regression model will better approximate IHX-CL than the standard methods of estimating renal function.

14.2.2.a. To compare IHX-CL between users and non-users of exogenous androgenizing hormones.

14.2.2.b. Hypothesis: The use of exogenous testosterone will be associated with decreased IHX-CL.

14.2.3.a. To compare IHX-CL between users and non-users of exogenous feminizing hormones.

14.2.3.b. Hypothesis: The of exogenous estrogens will be associated with increased IHX-CL.

14.2.4.a. To compare IHX-CL between individuals with high/low levels of free/unbound testosterone.

14.2.4.b. Hypothesis: Higher levels of measured free/unbound testosterone will be associated with decreased IHX-CL.

14.2.5.a. To compare IHX-CL between individuals with high/low levels of estradiol/estrone.

14.2.5.b. Hypothesis: Higher levels of measure estradiol/estrone will be associated with increased IHX-CL.

14.2.6.a. To compare IHX-CL between individuals with and without various renal biomarkers associated with kidney injury (i.e. beta-2 microglobulin, neutrophil gelatinase associated lipocalin (NGAL), osteopontin, epidermal growth factor (EGF), and uromodulin).

14.2.6.b. Hypothesis: The presence of any injury-related renal biomarker (RBM) will be associated with decreased IHX-CL.

14.2.7.a. To determine the relationship between presence of multiple RBMs and IHX-CL.

14.2.7.b. Hypothesis: The relationship between RBM and IHX-CL will be monotonic. As the number of injury-related RBMs increases, there will be a corresponding decrease in IHX-CL.

14.2.8.a. To compare TFV values between users and non-users of exogenous androgenizing hormones.

14.2.8.b. Hypothesis: The use of exogenous testosterone will be associated with increased TFV values.

14.2.9.a. To compare TFV values between users and non-users of exogenous feminizing hormones.

14.2.9.b. Hypothesis: The of exogenous estrogens will be associated with decreased TFV values.

14.2.10.a. To compare TFV between individuals with high/low levels of free/unbound testosterone.

14.2.10.b. Hypothesis: Higher levels of measured free/unbound testosterone will be associated with increased TFV.

14.2.11.a. To compare TFV between individuals with high/low levels of estradiol/estrone.

14.2.11.b. Hypothesis: Higher levels of measure estradiol/estrone will be associated with decreased TFV.

14.2.12.a. To compare TFV values between individuals with and without various RBM.

14.2.12.b. Hypothesis: The presence of any RBM will be associated with increased TFV.

14.2.13.a. To determine the relationship between presence of multiple RBMs and TFV.

14.2.13.b. Hypothesis: The relationship between RBM and TFV will be monotonic. As the number of RBMs increases, there will be a corresponding increase in TFV value.

14.3 Background and Significance

The incidence of HIV infection in the transgender/non-binary (TG/NB) population is 3 times the national average and nearly 14% of TG/NB patients are living with HIV infection.(4,5) This vulnerable population may benefit greatly from pre-exposure prophylaxis (PrEP), a regimen which has shown great promise in clinical trials.(6, 7, 8) Currently, tenofovir disoproxil fumarate (TDF) coformulated with emtricitabine (F) as a single tablet regimen (F/TDF) and F in combination with tenofovir alafenamide (F/TAF) are the only products approved by the United States Food and Drug Administration (FDA) for PrEP.(9) While F/TDF was first approved for PrEP, recent clinical trials have evaluated the efficacy of F/TAF, a salt of TDF that achieves higher concentrations in lymphocytes.(10) These clinical trials have demonstrated promising results as measured by an extremely low number of new HIV infections when compared to F/TDF.(6)

While both TDF/FTC and TAF/FTC are highly efficacious, these medications are cleared by the kidneys and their dosing relies on accurate estimation of renal function to initiate PrEP and monitor for toxicity.(9, 11, 12) Inaccurate estimation of renal function among TGNB individuals complicates prevention efforts in 3 ways: i) missed opportunities to initiate PrEP, ii) early discontinuation of PrEP after starting therapy, and iii) missed or delayed identification of acute kidney injury (AKI) and other toxicities associated with PrEP. From a public health perspective, the first 2 issues reduce the availability of effective HIV prevention tools for TG/NB individuals, problematic given that this population is already at elevated risk of infection. The third issue, AKI, is associated with increased morbidity (increased risk of developing chronic kidney disease, accelerated progression to end-stage renal disease, etc.), mortality, and increased healthcare utilization/expenditures (hospitalizations, hemodialysis, etc.).(13) Thus, accurate estimation of renal function is critical.

The most prevalent method of estimating renal function involves calculating creatinine clearance (CRCL) using the Cockcroft-Gault (CG) equation.(3) While a variety of factors can alter renal function, the CG equation considers only age, weight, sex and serum creatinine; the computation does not delineate sex assigned at birth versus current gender identity.(3) This distinction can affect renal function estimation by a scalar of 15%.(3) The CG equation relies on serum creatinine, a biomarker that has been used to approximate kidney function but is imperfect. Specifically, serum creatinine is variable and can fluctuate based on small changes to muscle mass.(14) There is also a temporal lag (~48h) between measured serum creatinine and actual renal function (glomerular filtration rate, GFR).(15, 16) Finally, the CG equation ignores the contribution of inflammation and hormones, both of which are known to affect renal function.(17-19)

Within the TG/NB population, there is a high use of exogenous hormones, like estrogen and testosterone, and certain hormones have been associated with changes in muscle mass and an

increased presence of inflammatory biomarkers.(20) Despite the existence of several renal biomarkers associated with inflammation, it is important to differentiate that some are associated with kidney ***injury*** (i.e. beta-2 microglobulin, neutrophil gelatinase associated lipocalin (NGAL), osteopontin, epidermal growth factor (EGF), and uromodulin) and whereas other renal biomarkers (i.e. creatinine and cystatin C) reflect the ***functional*** status of the kidneys.(21) Both types of inflammatory renal biomarkers are important for TGNB individuals on PrEP since dosing relies on kidney function and both TDF and TAF can cause renal injury.(4, 9, 11) Unfortunately, very little is known about how high dose exogenous hormone use, common in the TGNB population, affects both functional and injury-related renal biomarkers.

In addition to clarifying the appropriateness of PrEP therapy, accurate estimation of renal function among the TGNB population can improve the delivery of other therapeutic regimens, such as those living with HIV infection receiving combination antiretroviral therapy. In addition, several of the most commonly used medication classes (acid suppressants, anticoagulants, anticonvulsants, antidiabetic medications, antihypertensives, antimicrobials including antiretrovirals for HIV infection, lipid lowering therapies, etc.) require dose adjustment based on an individual's renal function.(22) Failure to accurately quantify renal function and make the appropriate dose adjustments may lead to increased medication exposure and toxicity.(22)

Preliminary Data: This is a pilot study. To date, there have been no integrated evaluations of hormones, RBMs, kidney function as measured by IHX-CL and drug concentrations. Classification as female vs male using the CG method will always result in 15% lower CRCL because of a 0.85 multiplier built into the equation.(3) The appropriateness of this multiplier is not understood in the TGNB population. In a retrospective study of TGNB patients initiating hormonal treatment, changes in serum creatinine, the only RBM included in the CG equation, were observed.(23) Among the 33 patients who were assigned male at birth and transitioning to female (MtF), serum creatinine improved by 7.7% within 18 months of commencing feminizing therapy with estrogen. Conversely, in the 19 patients who were assigned female at birth and transitioning to male, serum creatinine worsened by 19.2% after initiating androgenizing therapy after only 6 months. These data support the hypothesis that use of exogenous hormones alters serum creatinine and accurate estimation of renal function .(23)

14.4 Sub-study Procedures

After obtaining informed consent, patients will be scheduled for a study visit.

A full list of study procedures are described in Table 1.

Participants will be asked to abstain from eating their morning meal and drinking any caffeinated beverages prior to arriving to the study site. Upon arrival at the UCSD AntiViral Research Center (AVRC), the study coordinator/personnel will weigh the patient and measure the patient's height. Patients will be asked about concomitant medications, with an emphasis on exogenous hormone use. For each medication, the following will be recorded: drug name, dose, frequency, route of administration, and duration of use. The date/time of last three doses of PrEP will be recorded.

Before administration of iohexol, the patient will provide one urine and three blood samples (5 mL each; total 15 mL). The urine sample will be divided into five aliquots. One urine aliquot will be used for kidney injury molecule 1 (KIM-1) measurement. The second urine aliquot will measure 7-plex MesoScale Discovery® Electrochemiluminescent multiplex immunoassay

platform (MSD). The items measured in the 7-plex MSD will be albumin, beta-2 microglobulin, cystatin C, NGAL, osteopontin, EGF, and uromodulin. The third urine aliquot will be used for urine TFV measurement. The fourth urine aliquot will be used for urine creatinine determination. The fifth urine aliquot will be frozen in the event that any of the aforementioned tests need to be re-run. Those capable of becoming pregnant will complete a urine pregnancy test. The three blood samples will be centrifuged and serum or plasma will be divided into five 1mL aliquots. The first serum aliquot will be used for a complete metabolic panel (CMP). The second serum aliquot will be used to measure estradiol. The third serum aliquot will be used to measure estrone. The fourth serum aliquot will measure sex hormone binding globulin and free/total testosterone concentrations. The fifth serum aliquot will be frozen in the event that that any of the aforementioned tests need to be re-run.

Sub-Study Procedures	Visit #1	Switch from TDF/FTC to TAF/FTC for ≥ 3 months	Visit #2
Screening/consent	X		
Drug concentrations via DBS	X		X
Iohexol injection	X		X
Blood samples:			
• Complete metabolic panel	X		X
• Iohexol	X		X
• Estradiol	X		X
• Estrone	X		X
• Sex hormone binding globulin	X		X
• Free/total testosterone	X		X
Urine samples:			
• Urine β-HCG[1]			
• Urinary KIM-1	X		X
• 7-plex MesoScale Discovery®	X		X
• Urine tenofovir	X		X
• Urine creatinine (x 2 samples)	X		X
• Stored sample	X		X

DBS: dried blood spot, KIM-1: kidney injury molecule
1: β-HCG will be collected in those capable of becoming pregnant

The research pharmacist will prepare the iohexol injection in a 1cc tuberculin syringe, consisting of 0.5cc sterile water for injection (SWFI) and 0.5cc iohexol (Omnipaque 300). The study coordinator/personnel (licensed RN) will inject the iohexol dose into the subcutaneous tissue on the opposite arm used for blood sampling; the time will be recorded. The patient will be instructed to return to the waiting area and will be offered a standardized meal.

After 180 minutes (3h:0m post-iohexol injection), the patient will provide a blood sample (5mL) for iohexol measurement, a urine sample for urine creatinine determination (1mL) and the time will be recorded. An aliquot of urine will be frozen in the event the urine creatinine determination needs to be re-run. After this, the patient will be dismissed. The study coordinator will centrifuge the blood sample and aliquot 1mL of plasma into a vial for iohexol determination. The remaining serum will be frozen as a back-up sample.

Sub-study Visit 1 will be scheduled within 1 week of screen/baseline visit after initiation of F/TAF has occurred.

Example: If the participant has been taking F/TDF for 12 weeks or more at the date of enrollment, Visit #1 will be scheduled within 1 week of the screen/baseline visit. If the participant has been taking F/TDF for less than 12 weeks prior to enrollment, Visit #1 will be scheduled between screen/baseline visit up to 1 week past the initiation of F/TAF.

The entire procedure will be repeated in the second phase of the sub-study after patient has switched from F/TDF to F/TAF for at least 12 weeks, the time point at which steady state intracellular concentrations will be achieved.

14.5 Statistical Analyses and Sample Size Considerations

Sample Size and Power: Assuming a type 1 error of 5%, power of 80% and pooled SD of 10mL/min, a minimum of 7 patients per group would be needed to detect a clinically meaningful difference of 15mL/min between any two comparison groups (e.g., 85mL/min for hormone users versus 70mL/min for non-hormone users). This study seeks to enroll 10 patients per group (n = 40 total) in the event that the observed effect size is smaller than anticipated.

Data Analysis Plan:
Aim 1: Relationship between exogenous hormone use, in vivo hormonal measures, inflammatory renal biomarkers (RBMs) and IHX clearance.
The distribution of measured IHX-CL values and other continuous variables will be assessed to ensure a parametric distribution. If a parametric distribution is not observed, the variables will be log-transformed to fit a normal distribution. To assess the bivariate relationship between continuous variables and IHX-CL, Pearson's correlation coefficient will be calculated. To assess the bivariate relationship between dichotomous variables (e.g. RBM present/absent), the mean ± standard deviation (SD) IHX-CL values will be compared between patients with the variable present and absent using the Student's t test. Given the sample size of the study population and to prevent undue influence of any extreme values on the mean ± SD, the median (interquartile range, IQR) IHX-CL values will be compared between patients with and without each dichotomous variable present using the Mann-Whitney *U* test.

To examine the relationship between exogenous hormone use and IHX-CL, the population will be partitioned based on sex assignment at birth. Among patients assigned male at birth, mean and median IHX-CL values will be compared between those using and not using exogenous feminizing hormones with the Student's t and Mann-Whitney U tests, respectively. Similarly, among patients assigned female at birth, mean and median IHX-CL values will be compared between those using and not using exogenous androgenizing hormones with the aforementioned tests. Concentrations of estradiol, estrone, sex hormone binding globulin, and bound/unbound testosterone are continuous measures and Pearson's correlation coefficient will be calculated to determine if any of these values are associated with IHX-CL.

To ensure all associations and potential interaction variables are identified, we will evaluate all of the variables simultaneously in a log-linear regression model. The specific variables to be entered into the log-linear regression model will be the use of exogenous feminizing/androgenizing hormones, *in vivo* concentrations of hormones (estradiol, estrone, sex hormone binding globulin and bound/unbound testosterone), sex assigned at birth versus current gender identity, each of the renal biomarkers (albumin, beta-2 microglobulin, cystatin C, NGAL, osteopontin, EGF, uromodulin and KIM-1), renal components of the CMP (creatinine, total protein, etc.) and IHX-CL.

Aim 2: Incorporate exogenous hormone use, RBMs and gender identity into creatinine clearance calculation
The traditional elements of the CG equation will be entered into a linear regression model. A new gender variable will be created. The new gender covariate will be categorized into 4 discrete categories: a) assigned male at birth not on hormones, b) assigned male at birth on hormones, c) assigned female at birth not on hormones and d) assigned female at birth not on hormones. The new gender covariate in the linear regression model will be compared to the traditional dichotomous gender covariate (male/female assignment at birth) to determine if a new coefficient improves accuracy in predicting IHX-CL.

A second linear regression model will be developed to predict IHX-CL. This model will include

patient demographics (age, weight, sex assigned at birth versus current gender identity), clinical factors (use of exogenous feminizing/androgenizing hormones) and laboratory parameters (serum creatinine, total protein, blood urea nitrogen, albumin, beta-2 microglobulin, cystatin C, NGAL, osteopontin, EGF, uromodulin, KIM-1, estradiol, estrone, sex hormone binding globulin and bound/unbound testosterone). Prior to model entry, each of the covariates will be plotted against IHX-CL to ensure linear regression assumptions are satisfied (homoscedasticity, equal variances, independence, linearity in the regression parameters and Gaussian distribution). To prevent collinearity of the model, covariates demonstrating strong associations with one another in the log-linear regression (Aim 1) will not entered into the linear regression model simultaneously. Using a backwards, stepwise approach, variables will be individually removed from the model if they do not substantively contribute to predicting IHX-CL until the most parsimonious model is achieved. The residuals of the model will be assessed for goodness of fit. Outliers/extreme values will be jack-knifed in/out to determine model stability.

For each patient, the predicted IHX-CL will be calculated using the equation derived from the linear regression analyses. The $IHX\text{-}CL_{predicted}$ will be compared to the actual $IHX\text{-}CL_{measured}$ by computing the percent difference ($|IHX\text{-}CL_{measured} - IHX\text{-}CL_{predicted}| / IHX\text{-}CL_{measured}$). Similarly, we will compute $CRCL_{CGmethod}$ for all patients. We will compare calculated $CRCL_{CGmethod}$ to actual $IHX\text{-}CL_{measured}$ by examining the percent difference ($|IHX\text{-}CL_{measured} - CRCL_{CGmethod}| / IHX\text{-}CL_{measured}$). The mean ± SD percent differences will be compared using the paired T-test. If the mean ± SD percent differences are significantly ($p<0.05$) lower for the $IHX\text{-}CL_{predicted}$ than $CRCL_{CGmethod}$, we will conclude that the extra covariates enhanced precision of renal function estimation ($IHX\text{-}CL_{measured}$).

Aim 3: Effect of exogenous hormones, in vivo hormonal measures and RBMs on concentrations of TDF/FTC and TAF/FTC.
The data analysis plan for this Aim is identical to that of Aim 1. The only difference is the dependent variable will be concentrations of F/TDF and F/TAF instead of IHX-CL.

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