A Phase I trial for the evaluation of the two-way pharmacokinetic-pharmacodynamic interaction of gender affirming exogenous estrogen (with testosterone suppression) on TDF/FTC PrEP in transgender women (TGW)

Funded by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
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DAIDS Program Officer: Roberta Black

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Transaminase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
ART	Antiretroviral Therapy
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BLQ	Below the Limit of Quantitation
BMD	Bone Mineral Density
BP	Blood Pressure
BUN	Blood Urea Nitrogen
°C	Celsius
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CBC	Complete Blood Count
CCR5	Chemokine Receptor 5
CD4	Cluster of Differentiation 4
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CGM	Cisgender men
CGW	Cisgender women
CT	Chlamydia
Cl	Chloride
CLIA	Clinical Laboratory Improvement Amendments
C_{max}	Maximum Serum Concentration
CMP	Comprehensive Metabolic Panel
CO2	Carbon Dioxide
C-PMPA	Radiolabeled Tenofovir
Cr	Creatinine
CRF	Case Report Form
CRS	Clinical Research Site
CT	*Chlamydia trachomatis*
CVL	Cervicovaginal Lavage
CXCR4	CXC Chemokine Receptor 4
d4T	Stavudine
DAIDS	Division of AIDS
DNA	Deoxyribonucleic Acid
DP	Diphosphate
DSMB	Data and Safety Monitoring Board
ELISA	Enzyme-Linked Immunosorbent Assay
EAE	Expedited Adverse Event
ES	Endocrine Society
ET	Eastern Time
FDA	Food and Drug Administration
FTC	Emtricitabine

FSH	Follicle Stimulating Hormone
g	Gram
GAHT	Gender-affirming hormonal therapy
GC	*Neisseria gonorrhoeae*
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
HBsAg	Hepatitis B Surface Antigen
HHS	Health and Human Services
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
HR	Heart Rate
hr	Hour
HSV	Herpes Simplex Virus
IATA	International Air Transport Association
IB	Investigator's Brochure
IDS	Investigational Drug Service
IoR	Investigator of Record
IPCP	Integrated Pre-Clinical/Clinical Program
iPrEx	Pre-Exposure Prophylaxis Initiative
IRB	Institutional Review Board
IGF	Insulin-like Growth Factor
IV	Intravenous
JHU	Johns Hopkins University
K	Potassium
kg	Kilogram
LC-MS	Liquid Chromatography-Mass Spectroscopy
LC-UV	Liquid Chromatography-Ultraviolet
LH	Leutinizing Hormone
LL	Local Laboratory
LLOQ	Lower Limit of Quantification
mg	Milligram
µg	Micrograms
min	Minute
mITT	Modified Intent To Treat Analysis
mL	Milliliter
MMC	Mucosal Mononuclear Cells
mOsmol	MilliOsmole, or one-thousandth of an osmole
MO	Medical Officer
MSM	Men who have Sex with Men
MTN	Microbicide Trials Network
MTT	[1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan]
N-9	Nonoxynol-9
Na	Sodium
NAAT	Nucleic acid amplification test
NIAID	National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health
NL	Network Laboratory
NOAEL	No Observed Adverse Effect Level
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor

NRTI	Nucleoside Reverse Transcriptase Inhibitor
O.D.	Optical density
OHRP	Office for Human Research Protections
Osmol	Osmole; one osmole is defined as a unit of osmotic pressure equivalent to the amount of solute that dissociates in solution to form one mole (Avogadro's number) of particles (molecules and ions)
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic(s)
PEP	Post-Exposure Prophylaxis
PIV	Peripheral IV
PK	Pharmacokinetic(s)
PMPA	9-[(R)-2-(phosphonomethoxy)propyl] adenine monohydrate
PO_4	Phosphate
PoR	Pharmacist of Record
PrEP	Pre-Exposure Prophylaxis
PRO	Protocol Registration Office
PSRT	Protocol Safety Review Team
PTID	Participant Identification Number
PRN	As needed
(qc)-RT PCR	Quantitative Competitive
QD	Daily
RAI	Receptive Anal Intercourse (refers to coitus only, does not include manual stimulation or the use of sex toys or purgatives)
RMP	Rectal Microbicide Program
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagin
RRI	Rabbit Rectal Irritation
RSC	Regulatory Support Center
RRR	Relative Risk Reduction
RT	Reverse Transcriptase
RU	Research Unit
SAE	Serious Adverse Experience
SADR	Suspected Adverse Drug Reaction
SHIV	Simian/Human Immunodeficiency Virus
SIV	Simian Immunodeficiency Virus
SMC	Safety Monitoring Committee
SMS	Short Message Service
SOP	Standard Operating Procedure
sRAI	Simulated RAI
SSP	Study Specific Procedures
SPECT/CT	Single Photon Computed Tomography/Computed Tomography
STD/ STI	Sexually Transmitted Disease/Infection
SUSAR	Suspected and Unexpected Serious Adverse Reactions
^{99m}Tc-DTPA	Technetium-99m-DTPA
T_{max}	The time at which maximal concentration is reached
TAF	Tenofovir Alafenamide Fumarate (formerly GS7340)
TDF	Tenofovir Disoproxil Fumarate (oral tenofovir)
Temp	Temperature

TFV	Tenofovir
TFV-DP	Tenofovir Diphosphate
TER	Transepithelial Resistance
TGW	Transgender Women
UA	Urinalysis
ULN	Upper Limit of Normal
ULOQ	Upper Limit of Quantification
URAI	Unprotected Receptive Anal Intercourse
USP	United States Pharmacopeia
UTI	Urinary tract infection
VI	Virus Isolation
vRNA	Viral Ribonucleic Acid
WPATH	World Professional Association for Transgender Health

PROTOCOL TEAM ROSTER

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Improving PrEP protection of transgender women through mechanistic pharmacokinetic understanding

INVESTIGATOR SIGNATURE FORM

Version 3.0
26 April 2023

Funded by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institutes of Health

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

I have read and understand the information in the Investigator's Brochure(s), including the potential risks and side effects of the products under investigation, and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____
Name of Investigator of Record

_____ _____
Signature of Investigator of Record Date

A Phase I trial for the evaluation of the two-way pharmacokinetic-pharmacodynamic interaction of gender affirming exogenous estrogen (with testosterone suppression) on TDF/FTC PrEP in transgender women (TGW).

PROTOCOL SUMMARY

Short Title: PrEP-GAHT Interactions in TGW

Clinical Phase: Phase 1

Sponsor: NIH/Division of AIDS

Protocol Chair: Mark A. Marzinke (JHU)

Study Site Investigators: Craig Hendrix, MD
Adrian Dobs, MD

Santiago Alvarez Arango, MD
Jennifer Hoffman, CRNP

Sample Size: 20 evaluable, self-identifying transgender women (TGW)

Study Population: HIV-negative, self-identifying TGW who are not currently on any form of GAHT (including estrogen-based therapies, spironolactone, and progesterone), or who are willing to abstain from feminizing therapies until serum total testosterone concentrations exceed 2 ng/mL (cisgender male reference interval).

Participating Clinical Research Site (CRS): Johns Hopkins University (JHU)

Study Rationale and Objectives: TGW frequently use estrogen-based gender affirming hormonal therapies (GAHT) to promote feminization and decrease gender dysphoria. A highly vulnerable population, TGW are 49-times more likely to acquire HIV as compared to cisgender men and women; therefore, pre-exposure prophylaxis (PrEP, tenofovir disoproxil fumarate/emtricitabine), which prevents HIV acquisition in high-risk populations, is greatly needed by TGW. Because the interaction between GAHT and PrEP is poorly understood, an investigation on the impact of exogenous hormonal therapies on PrEP effectiveness (and vice versa) is required to optimize dosing regimens for HIV protection, while also not impacting the effects of GAHT on reducing gender dysphoria.

Study Objectives: This is a Non-IND study designed to more closely evaluate the two-way pharmacokinetic-pharmacodynamic (PK-PD) interaction of GAHT (estrogen with testosterone suppression) on TDF/FTC PrEP in TGW through the completion of a five-phase PK-PD study, in which TGW take daily PrEP, followed by a testosterone suppressing agent alone and in conjunction with low- and high-dose estrogen therapy (Table 1). To reduce variability, observed PrEP administration will be conducted for 7 days prior to each intensive PK visit while on PrEP. PrEP and hormone doses will be directly observed by study personnel either in person, by live video streaming, or with a timestamped video. In the high-dose estrogen phase of the clinical trial, estrogen dosing may be split to a morning and an evening dose; only one dose per day will be monitored using one of the aforementioned strategies. Study objectives including safety, pharmacokinetics (PK) and pharmacodynamics (PD), are essential in understanding the impact of co-administration of PrEP and GAHT on downstream drug and hormone concentrations (Table 2).

Table 1: Drug administration during each phase of the PK-PD clinical study

PK Phase	PrEP	GnRH Agonist	Low-Dose Estrogen	High-Dose Estrogen
1	X			
2	X	X		
3	X	X	X	
4	X	X		X
5		X		X

Study Design: The **PrEP-GAHT Interactions in TGW** protocol is a phase 1, open label study to compare the safety, PK and PD of five sequential phases of PrEP administration in the presence or absence of testosterone-reducing therapies or dose-escalated estrogen therapy. Each participant will undergo a Screening Visit to evaluate eligibility. Following Baseline evaluation, eligible participants will receive 300 mg TDF/200 mg FTC (Truvada®, Gilead Sciences) once daily for seven days, using the aforementioned direct observation approaches, to achieve steady state drug PrEP concentrations. After one week of therapy, participants will undergo intensive PK analysis as well as collection of colorectal biopsies for PD testing (PK1). During the PK-intensive day, iohexol will be administered intravenously for the empirical determination of renal function and measured glomerular filtration rate (mGFR). While concurrently on PrEP, participants will then be intramuscularly administered depot leuprolide acetate (11.25 mg Lupron®). Two weeks post-injection, when testosterone concentrations are far below the lower limit of normal of total testosterone in cisgender men (typically < 200 ng/dL, or < 2 ng/mL), sampling for PK, PD, and renal function will be performed (PK2). Participants will then immediately begin low-dose oral estrogen therapy (1 mg 17β-estradiol) in conjunction with PrEP for one week, at which time samples will be collected for the analyses described above (PK3). While still on PrEP, participants will then transition to high-dose estrogen therapy (6 mg 17β-estradiol) for the remainder of the study. One-week post-high dose estrogen therapy in the presence of PrEP, pharmacologic and renal samples will be collected for analysis (PK4). PrEP will then be discontinued, and two weeks later, samples will be collected to assess renal function and hormonal concentrations, and evaluate the presence of any remaining PrEP in plasma, PBMC, or colorectal tissue (though it should be near undetectable levels for most analytes according to our prior data) (PK5). The schema is summarized in **Figure 1.** Safety assessments, including history/physical, chemistry/hematology labs at screening and interim history will be performed at each study-intensive visit. Additionally, periodic assessments of gender dysphoria will be conducted at baseline and a convenient time during PK visits throughout the study. To ensure compliance, participants will undergo direct observation of dosing each day of the week prior to PK visits, using the aforementioned strategies.

Table 2: Study Objectives and Endpoints

*as defined by the *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected*

Objectives	Endpoints & Data Analysis
• Describe the impact of GAHT on TDF/FTC PrEP PK-PD • Describe the bi-directional impact of TDF/FTC & GAHT combinations	• Non-compartmental analysis of TFV/FTC in plasma and urine • Concentrations of all analytes in blood, PBMC, & colorectal tissue • Compare PK1 to PK2, PK3, PK4 for GAHT impact on PrEP • Compare PK5 to PK2-4 for PrEP impact on GAHT
• Describe the impact of GAHT on PrEP suppressed HIV susceptibility	• Describe changes in explant p24 antigen under all PK conditions • Correlation and E_{max} model of drug concentration-p24 relationship
• Explain the mechanism of estrogen impact on PrEP PK	• Mechanistic modeling of PK change • Renal changes with addition of GAHT
• Describe adverse effects associated with PrEP & GAHT	• Grade 2 or higher AEs*
• Assess gender dysphoria will participants are on study product	• Gender dysphoria evaluations at baseline and during PK1 through PK5

Version 2.1 - July 2017, and Addendum 3 (Rectal Grading Tables for Use in Microbicide Studies)

Study Schema:

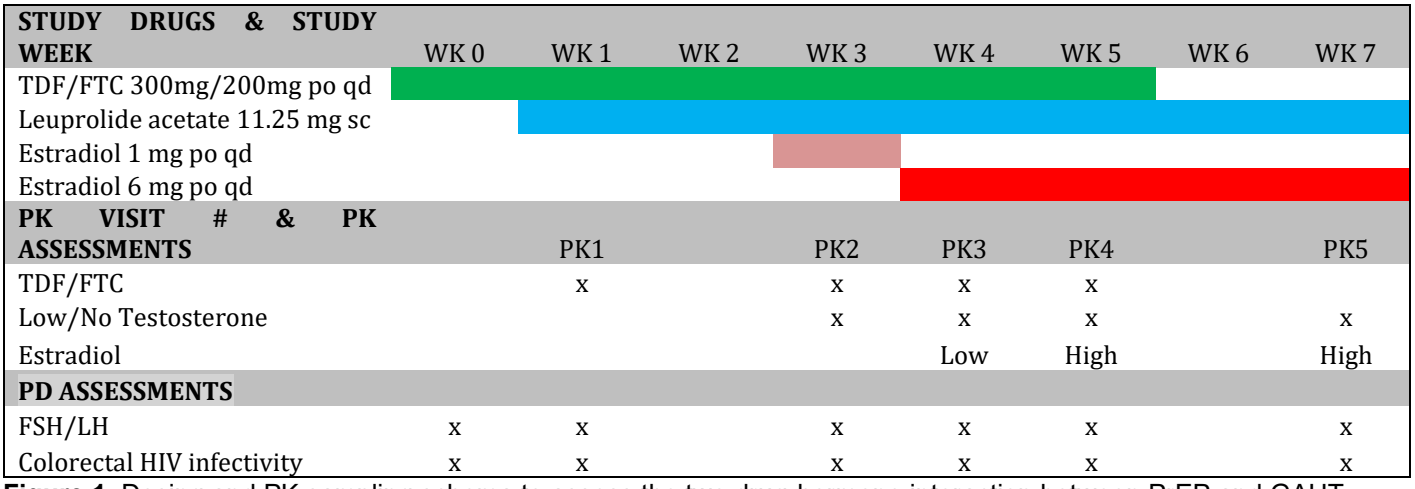

Figure 1. Dosing and PK sampling schema to assess the two drug-hormone interaction between PrEP and GAHT.

Study Products:

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

Study Product Acquisition. 17β-estradiol (1 x 1 mg or 3 x 2 mg) is manufactured and marketed by Allergan, Inc. Leuprolide acetate is formulated as an 11.25 mg depot, and is manufactured and

marketed by AbbVie, Inc. The study product will be purchased and dispensed per investigator prescription by the Investigational Drug Service (IDS) Pharmacy at The Johns Hopkins Hospital.

Study Duration:
Participant accrual will take approximately 3 years and each participant will be on study for approximately 4 months (up to 2 months in screening and 56 days under observationon study drugs). Total study duration is about 4 years.

1 KEY ROLES

1.1 Protocol Identification

Protocol Title: **A Phase I trial for the evaluation of the two-way pharmacokinetic-pharmacodynamic interaction of gender affirming exogenous estrogen (with testosterone suppression) on TDF/FTC PrEP in transgender women (TGW).**

Short Title: PrEP-GAHT Interactions in TGW

Date: 26 April 2023

1.2 Funding Agency, Sponsor, and Monitor Identification

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2 INTRODUCTION

More than 1.3 million adults in the U.S. identify as transgender, with the highest percentage (2.78%) of transgender individuals living in the Washington DC area[1]. The term transgender describes persons whose gender identity is not congruent with the sex they were assigned at birth and those associated sexual characteristics[2]. Due to this gender dysphoria, many transgender individuals experience distress and face significant physiological, social, and psychological challenges. All of these factors greatly impact the quality of life within the community[3]. Further, transgender individuals face significant barriers in terms of employment and healthcare, with up to 28% and 48% of transgender persons postponing healthcare due to discrimination or financial constraints, respectively[4,5]. Organizations such as the World Professional Association for Transgender Health (WPATH), a multidisciplinary society with expertise in multiple facets of transgender health, provide guidelines in the culturally competent care to transgender persons, including gender affirming hormonal therapy (GAHT)[6]. Despite these guidelines, significant knowledge gaps still exist in transgender care, especially where it overlaps with other health needs of this vulnerable population. For example, with increased risk of HIV acquisition, the transgender community needs access to effective HIV pre-exposure prophylaxis (PrEP) with the confidence that drugs for PrEP and GAHT do not interfere with each other's efficacy[7]. The focus of this proposal is to better characterize the pharmacologic interactions between estrogen-based GAHT and PrEP drugs.

HIV infection remains a public health concern. Current estimates indicate that there are nearly 37 million individuals worldwide living with the disease, with nearly 2 million new infections occurring annually[8]. The primary modality for the prevention of HIV in high-risk populations is the administration of antiretroviral agents for PrEP. Currently, two fixed-dose formulations containing both tenofovir (TFV) and emtricitabine (FTC) - 300 mg TFV disoproxil fumarate (TDF)/200 mg FTC (Truvada®, Gilead Sciences) and 25 mg TFV alafenamide fumarate (TAF)/200 mg FTC (Descovy®, Gilead Sciences) are the only FDA-approved regimens to prevent HIV acquisition. TFV and FTC are nucleotide and nucleoside reverse transcriptase inhibitors (NRTIs), respectively, that achieve their antiviral effects as the intracellular phosphorylated derivatives, TFV-diphosphate (TFV-DP) and FTC triphosphate (FTC-TP). With excellent adherence to the daily TDF/FTC regimen, protection from HIV acquisition can be as high as 100%[9]. However, small reductions in adherence can result in significant reductions of protective efficacy, indicating that HIV protection is sensitive to drug concentration[10-13].

Among those most affected by the disease, transgender communities, in particular transgender women (TGW), are disproportionately burdened by HIV infection. A recent meta-analysis reported an estimated HIV prevalence of 19.1% in TGW, with a 49-times greater risk for HIV acquisition when compared to adult cisgender men (CGM) and women (CGW) of reproductive age[14]. Based on clinical trial data, HIV prevalence amongst TGW was highest in the United States, South America, Indonesia, and India[14]. Further, compared to their cisgender counterparts, TGW sex workers are four times more likely to be living with HIV. Due to the disproportionate risk for HIV acquisition in TGW, PrEP uptake and efficacy is critical. However, the impact of GAHT on PrEP pharmacology, and vice versa, has not been extensively characterized. Therefore, a thorough pharmacological evaluation of the relationship between GAHT and PrEP is needed to ensure sufficient protection against HIV acquisition and to understand the potential impact of PrEP on hormonal therapies.

The PrEP-GAHT Interactions in TGW protocol will allow for the closer evaluation of the two-way pharmacokinetic-pharmacodynamic (PK-PD) interaction of GAHT (estrogen with testosterone suppression) on TDF/FTC PrEP in TGW through the completion of a five-phase PK-PD study, in

which TGW take daily PrEP, followed by a testosterone suppressing agent alone and in conjunction with low- and high-dose estrogen therapy.

2.1 Background on gender affirming estrogen-based therapy in TGW

Based on a survey of more than 7,000 transgender and gender non-conforming individuals in the U.S., 72% of TGW reported accessing GAHT, with higher percentages of hormonal use in transgender individuals undertaking transition-related surgeries[4]. The WPATH and the Endocrine Society have published transgender-focused guidelines that include estrogen therapy as the foundation for achieving feminization in TGW[6,15]. Via a negative feedback mechanism, elevated concentrations of estrogens can inhibit the hypothalamic release of gonadotropin releasing hormone (GnRH), thereby disrupting the hypothalamic-pituitary-testis axis, resulting in the reduction of testosterone concentrations[16-20]. Estrogen therapy, through interactions with the estrogen receptor, can stimulate feminizing characteristics, including changes in fat distribution, reduction of male-pattern hair growth, and dermatological changes, as well as decreased frequencies of erections and sexual desire[21]. However, estrogen therapy alone is typically insufficient to achieve maximal androgen suppression, and consequently, adjunctive therapies, including anti-androgenic medications or GnRH agonists, are co-administered as part of gender affirming feminizing therapeutic regimens. Physical changes may occur in the first 3-12 months post-initiation of hormonal replacement therapy.

Estrogen-based regimens used for feminization in TGW are administered at dosages higher than those used in the treatment of CGW for pre- and post-menopausal symptoms. Oral estrogen doses range from 1 mg/daily to 8 mg/daily for transition management[15,22,23]. Transdermal estradiol patches release from 0.025 to 0.2 mg/day; intramuscular injectable doses range from 2-10 mg of estrogen conjugates weekly[24]. Estrogens can be administered as the “bioidentical” human 17β-estradiol (orally or transdermally), as synthetic or semisynthetic hormone conjugates (such as estradiol valerate and estradiol cypionate), or as equine estrogens. Supraphysiological doses of estradiol are associated with significant adverse effects, including venous thromboembolic disease (VTE); van Kesteren and colleagues observed a 20-fold increase in VTE in TGW using the semisynthetic ethinyl estradiol[24-28]. Ethinyl estradiol, consequently, is not recommended by the Endocrine Society in the treatment and management of TGW[15]. However, VTE is likely a risk with all formulations. Estrogens are also associated with a moderate risk for macroprolactinoma, coronary artery disease, hypertriglyceridemia, cholelithiasis, and hypertension[15]. A summary of recommended estrogen-based hormone preparations and dosing has been published by the Center of Excellence for Transgender Health (**Table 3**).

Table 3. Estrogen preparations and dosing recommendations by the Center of Excellence for Transgender Health[29] (modified).

Estrogen	Initial-low	Initial	Maximum
Estradiol oral/sublingual	1 mg/day	2-4 mg/day	8 mg/day
Estradiol transdermal	50 mcg	100 mcg	100-400 mcg
Estradiul valerate intramuscular	<20 mg IM Q2W	20 mg IM Q2W	40 mg IM Q2W

Estradiol cypionate intramuscular	<2 mg IM Q2W	2 mg IM Q2W	5 mg IM Q2W

2.2 Rationale for Gonadotropin Releasing Hormone Agonists for Androgen Suppression

Adjunctive medications are typically required to reduce testosterone concentrations to reflect levels observed in CGW[30]. Commonly used in feminizing GAHT, the mineralcorticoid receptor antagonist spironolactone has a weak affinity for the androgen receptor, resulting in reduced testosterone binding through receptor competition[31]. However, the efficacy of spironolactone in decreasing the effects of testosterone in TGW is suboptimal[32,33]. Therefore, other drugs have been used to further promote feminization in TGW. GnRH agonists disrupt the hypothalamus-pituitary-testis axis via initial stimulation and subsequent desensitization of the hypothalamic GnRH receptor; suppression of GnRH results in decreased production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland[34,35]. The terminal effect of chronic administration of GnRH agonists is decreased testosterone production, and they are commonly used in the treatment of prostate cancer. While these agonists have been used to suppress pubertal development, they have also shown efficacy in demasculinization in TGW[22,36–38]. In a cohort of 60 TGW treated with monthly doses of the GnRH agonist goserelin acetate (3.8 mg) and 6 mg/daily 17β-estradiol valerate, serum total and free testosterone concentrations decreased from 18.75 to 0.53 nmol/L and 0.396 to 0.004 nmol/L, respectively[22]. There was also an 8.9-fold increase in serum estradiol concentrations, with no significant adverse effects observed. Retrospective studies have also demonstrated that the GnRH agonists cyproterone acetate and leuprolide acetate are also effective in the reduction of LH, FSH, and testosterone concentrations[39]. Notably, adverse effects were not observed when these agents were used in conjunction with low- to medium-dose estrogen therapies. Also, while cyproterone acetate is not approved by the FDA for use in the United States, long-acting leuprolide acetate is, and is marketed as a monthly 3.75 mg or three-month 11.25 mg intramuscular depot Lupron™. Previous studies in a prostate cancer cohort demonstrated that one injection of Lupron™ resulted in an initial increase of testosterone concentrations 3 days post-injection, but by 21 days post-administration, 78.7% of CGM achieved medical castration (testosterone concentrations <0.2 ng/mL; **Figure 1**)[40].

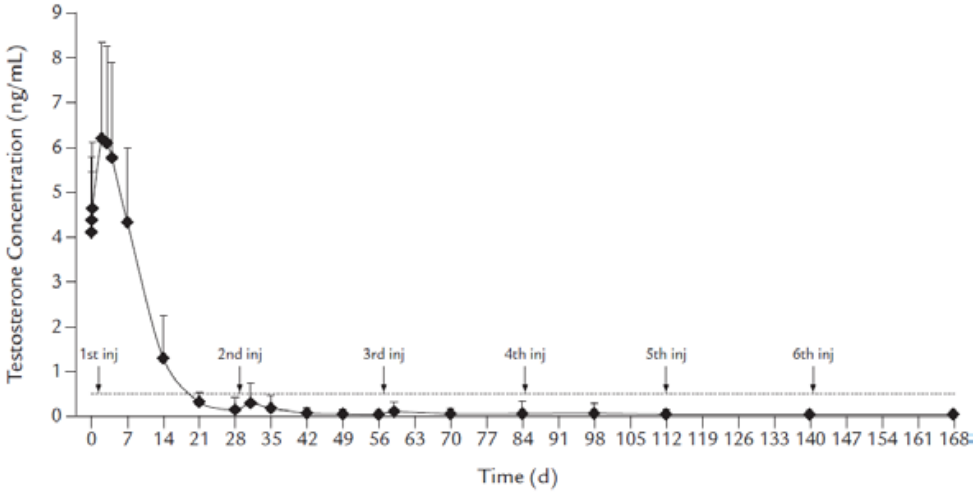


Figure 2. Reduction in testosterone concentrations in cisgender men post-leuprolide acetate administration from a clinical study. Mean plasma testosterone concentrations reached C_{max} of 606 ng/mL 3 days post-injection. By

14 days post-injection, mean concentrations were reduced by 4-fold. By Day 21, 78.7% of study participants reached total testosterone concentrations <0.2 ng/mL. Adapted from Marberger et al.[40].

The use of GnRH agonists, in conjunction with low-dose estrogen, may mitigate some of the side effects associated with high-dose estrogen-containing regimens, and is more potent in its inhibition of testosterone production as compared to anti-androgenic agents (which do not directly inhibit the hypothalamic production of GnRH), resulting in increased demasculinization.

2.3 PrEP Clinical Trials and Pharmacology in TGW

While TGW have been enrolled in HIV clinical trials evaluating PrEP uptake and efficacy, their numbers remain small, understudied, and overall findings may not be applicable to them[5,41,42]. Within the iPrEx trial and the subsequent open label extension cohort, 14% of participants who received PrEP self-identified as TGW. While incidence of seroconversion did not differ between cisgender men who have sex with men (MSM) and TGW, TGW exhibited lower PrEP metabolite concentrations[41]. In PrEP clinical trials, while non-adherence is likely the dominant cause of suboptimal drug concentrations, numerous other factors could modify drug concentrations in addition to adherence. For example, in CGW, vaginal dosing of TFV in the presence of vaginal dysbiosis is associated with lower systemic concentrations of TFV, and is attributed to decreased intravaginal microbiome-mediated TFV metabolism, sufficient enough to reduce the protective efficacy of a TFV gel[43,44]. We and another group have recently observed reduced TFV and FTC concentrations in TGW taking estrogens, possibly reducing the HIV protection effects of PrEP.

TDF has been evaluated for interactions with hormonal contraceptives. Pharmacokinetic (PK) findings demonstrated mean TFV C_{max} and area under the curve (AUC) of 340 ng/mL and 2970 ng-hr/mL, respectively, in CGW taking a norgestimate-ethinyl estradiol oral contraceptive agent; these TFV findings were consistent with historical data in CGW not on oral contraceptives[45]. At the cellular level, however, *in vitro* studies from the female genital tract have demonstrated decreased NRTI uptake in the presence of ethinyl estradiol and etonogestrel in several cell types, including dendritic and CD8+ cells[46]. Further, CD4+ cells from the female genital tract showed decreased TFV-DP concentrations in the presence of progesterone +/- estradiol[47]. It has also been reported that progesterone and testosterone impact NRTI phosphorylation in peripheral blood mononuclear cells (PBMC), indicating another endocrine effect on TFV metabolism[48]. Nicol, et al., reported differential expression of drug transporters between colorectal and vaginal tissue, including a 2.9-fold increase in the *ABCC2* gene in vaginal tissue as compared to colorectal tissue[49]. *ABCC2* encodes the multidrug resistance protein 2, which is involved in estradiol glucuronide clearance[50]. In a recent review, Anderson and colleagues discussed the research gaps in understanding PrEP efficacy in TGW, and highlighted the need for well-controlled clinical trials to more comprehensively describe the impact of hormonal therapies in TGW on PrEP[7].

To begin to address this knowledge gap regarding the potential impact of GAHT on PrEP pharmacology, our group conducted a Center for AIDS Research (CFAR)-funded pilot study to characterize the potential differences in PrEP PK between CGM and TGW[51]. The transgender community, in Baltimore and more broadly, voiced concerns that, despite the high risk of HIV infection in TGW and the promise of PrEP to reduce that risk, there remains substantial concern that PrEP drugs might adversely impact GAHT[5]. This concern, for which there was no evidence to the contrary, creates a reluctance to initiate PrEP among TGW. To begin to address these concerns, we enrolled 8 CGM not on exogenous estrogen and 8 TGW on exogenous estrogen for gender affirmation. Study participants were administered 300 mg TDF/200 mg FTC under direct observation for seven days, enough to establish steady state concentrations based on our prior experience[52]. Unable to fund the

enrollment of larger numbers or to afford the time to recruit estrogen-naïve TGW, we enrolled TGW on a variety of estrogen regimens (**Table 4**). Within the transgender cohort, serum estradiol concentrations needed to exceed 100 pg/mL, which is within the normal reference limits for premenopausal CGW and consistent with the upper limit of normal for postmenopausal CGW on HRT[50]. In addition, 75% of TGW participants also used the anti-androgenic compound spironolactone.

Table 4. GAHT used by TGW enrolled in CFAR pilot[51].

PID	Estrogen	Spironolactone (mg)
1010	Premarin 1.25 mg daily	0
1011	Estradiol 6 mg po daily	200
1012	Premarin 2.5 mg daily	50
1017	Estradiol 0.5 mg IM every 2 wks	50
1018	Estradiol valerate IM 20 mg every 2 wks	200
1019	Estradiol valerate IM 40 mg every 2 wks + Premarin 6.25 mg daily	0
1020	Estradiol 20 mg IM every 2 weeks	100
1021	Estradiol 1.5 mg IM every week	200

On the 8th day of daily observed oral TDF/FTC PrEP dosing, we collected blood for PrEP drug PK testing as well as estradiol, LH, FSH, and total testosterone. Colorectal biopsies were also collected for localized PK and *ex vivo* explant challenge by HIV to estimate HIV susceptibility. Plasma TFV trough concentrations (C_{24}) in TGW were lower by 32% (p = 0.010) when compared to CGM; AUC_{0-24} trended 27% lower (p = 0.065) and clearance (CL_{ss}/F) trended 38% higher (p = 0.065) in TGW compared to CGM. Plasma TFV C_{max} and V/F were not different between gender cohorts. In TGW, plasma FTC trough (C_{24}) and AUC_{0-24} were lower by 32% (p = 0.038) and 24% (p = 0.028) respectively, while CL_{ss}/F was higher by 31% (p = 0.028) when compared to CGM; V/F trended higher in TGW by 26% (p = 0.065) when compared to CGM. FTC C_{max} was not different between cohorts. There were no statistically significant PK differences between TGW and CGM in PBMC or colon tissue for any analytes. However, our pilot was only powered to detect several-fold differences in phosphorylated metabolites. As noted, we did not control for estrogen regimens or use of spironolactone.

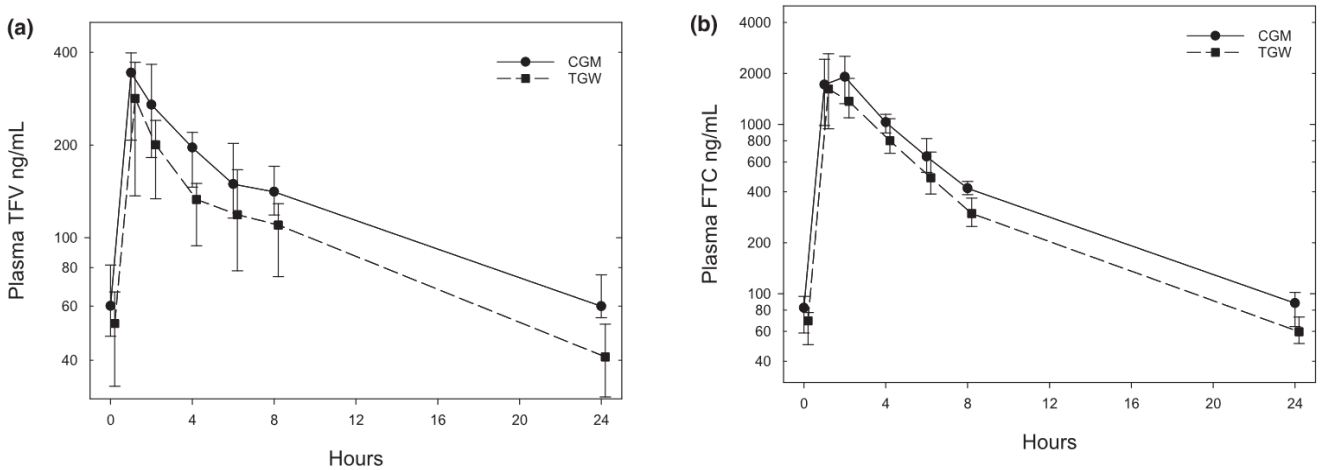


Figure 3. AUC_{0-24} of (a) plasma TFV (left) and (b) FTC (right) in CGM (solid line) and TGW (dashed line).

Despite 100% adherence observed throughout our study, 38% and 13% of TGW in our study appeared to have TFV plasma and PBMC TFV-DP, respectively, which fell below daily adherence

benchmarks from HPTN 066 – used to identify persons with less than daily dosing and potentially reduced PrEP protection.

To further corroborate these findings, the Thai Red Cross reported that estradiol valerate 2 mg and cyproterone acetate 25 mg reduced TFV concentrations by 17% (AUC_{0-24}), while TDF/FTC did not affect plasma estrogen or testosterone concentrations[53]. In contrast to the Hopkins pilot study, the Thai Red Cross study did not assess FTC concentrations or any intracellular concentrations of TFV-DP and FTC-TP; similarly, both studies used estrogen-experienced research participants. In contrast with U.S. practices, the Thai study also used cyproterone for testosterone suppression, which is not licensed for marketing in the U.S., as well as relatively low estrogen doses compared to typical U.S. doses.

There are a number of studies that have aimed to understand the interplay between antiretrovirals and GAHT; however, each differs in their study design and population. A summary of ongoing or completed PrEP/ART trials in TGW are summarized below (**Table 5**):

Table 5. Planned, completed, and ongoing PrEP/ART trials in TGW.

Study	PI	N	GAHT Naïve	Estrogen Regimen	Androgen Regimen	Adherence Measure	Renal Function	Status
Hopkins R01[a]	**Marzinke**	**20**	**yes**	**estradiol (1 mg, 6 mg)**	**leuprolide acetate**	**DOT MEMS**	**mGFR, eGFR cystatin C**	**Planned**
Hopkins CFAR[b]	Shieh	8	no	various	spironolactone	DOT	eGFR	Completed
Thai Red Cross[b]	Hiransuthikul	20	no	estradiol valerate	cyproterone acetate	unclear	-	Completed
Nebraska[c]	Cirrincione	15	no	estradiol (po, sl, td)	spironolactone	unclear	-	Ongoing
UNC Chapel Hill[c]	Cottrell	4	no	varied	unclear	unclear	-	Ongoing
TG Youth & PrEP[c]	Hosek	24	no	estradiol	spironolactone	video DOT	none	Ongoing

2.4 The Contribution of Hormonal Therapies on Renal Function

Recently, two observational studies demonstrated short-term changes in creatinine concentrations in transgender individuals in a GAHT-dependent fashion[54,55]. Decreased creatinine concentrations (implying improved renal clearance) was observed in TGW on a variety of estrogen-based GAHT.
In our pilot, 75% of both CGM and TGW were African American. BMI was higher in TGW (median 30.1) as compared to CGM (22.7), p=0.05. The most striking difference between the two cohorts was in regards to renal function. eGFR using the CKD-EPI equation was estimated as 133 mL/min/BSA in TGW and 111 mL/min/BSA in CGM (p=0.002). This difference was also statistically significant using the eGFR MDRD equation (p=0.005), as well as estimated creatinine clearance using the Cockcroft-Gault equation (p=0.01). These data suggest differential renal function in the presence of exogenous estrogens; however, neither the duration of time on therapy nor direct evaluation of filtration capacity were evaluated in this cohort.

Conversely, exogenous testosterone in transgender men (TGM) resulted in increased creatinine concentrations over time[55]. Previous studies have also demonstrated a similar, short-term impact of hormonal therapies on renal function. Acutely, diabetic CGW with a history of hypertension have shown improved creatinine clearance rates and decreased proteinuria when placed on estrogen-based hormone replacement therapy (HRT) consisting of estradiol and norgestrel[56]. Potential explanations for the impact of exogenous estrogens on improved renal function include hormonal effects on proximal tubule secretion, changes in muscle mass, or changes in renal vasculature.

2.5 Background and Rationale of Planned PrEP-GAHT Interactions in TGW Assessments

Because the interaction between GAHT and PrEP is poorly understood, an investigation on the impact of exogenous hormonal therapies on PrEP effectiveness (and vice versa) is required to optimize dosing regimens for HIV protection, while also not impacting the effects of GAHT on reducing gender dysphoria. Thus, safety, PK, and PD are highly relevant readout in the evaluation of the relationship between GAHT and PrEP.

2.5.1 Safety Assessments

Safety assessments includes history/physical, chemistry/hematology labs at screening and asssement of adverse events, collection of safety labs, review on concomitant medications, and interim history and targeted exams, as needed, during study visits and throughout the study.

2.5.2 Drug Analysis

TFV, FTC, and their metabolites will be measured using validated, liquid chromatographic- mass spectrometric (LC-MS) assays in the CPAL (Dr. Marzinke's laboratory)[52,57]. Post PBMC-lysis and tissue homogenization, drugs will be isolated from specimen sources using either protein precipitation (PrEP in plasma, tissue) or anion exchange chromatography and solid phase extraction (PrEP metabolites in PBMC and tissue) approaches[52,58,59]. Analytes will undergo chromatographic separation and drug-specific ion detection via mass spectrometry. Assay lower limits of quantification are as follows: plasma TFV and FTC: 0.31 ng/mL; tissue TFV and FTC: 0.05 and 0.25 ng/sample, respectively; tissue and PBMC TFV-DP and FTC-TP: 5 fmol/sample and 50 fmol/sample, respectively. All assays are validated in accordance with the FDA, Guidance for Industry: Bioanalytical Method Validation recommendations[60]. The NIH-funded Clinical Pharmacology Quality Assurance (CPQA) Program peer-reviews all bioanalytical methods. Further, the CPAL participates in CPQA proficiency testing for plasma TFV and FTC assays semi-annually[61]. Dr. Marzinke's laboratory is also CLIA-certified and CAP-accredited.

2.5.3 Ex vivo colon tissue explant HIV challenge

Endoscopic colonic biopsies, acquired during PK-intensive visits, can be challenged *ex vivo* with HIV (R5 HIV_{Bal} in our studies) to assess the alterations of susceptibility to HIV infection. With parallel PK testing, this readout can provide evidence of changing protection from HIV over time. *Ex vivo* challenges were previously conducted in our pilot TGW study, and we observed no statistically significant difference in HIV acquisition between TGW and CGM at PrEP steady state. These data suggest that while there may be an impact of hormonal therapies on systemic TFV and FTC concentrations, susceptibility of HIV acquisition in colorectal tissue was not impacted by feminizing therapies. As with phosphorylated analyte concentrations, however, we only had power to detect several-fold changes in explant tissue HIV susceptibility drug regimens. Further, there were a variety of feminizing GAHT used by study participants. The designed PrEP-GAHT Interactions in TGW study will definitively evaluate testosterone ablation or dose-escalated estrogen administration on HIV susceptibility in colonic tissue.

2.5.4 Direct Measurement of Glomerular Filtration

Creatinine clearance and eGFR are indirect estimates of renal filtration. Therefore, GFR will be empirically measured (mGFR) through the administration and evaluation of the exogenous molecule iohexol, which is cleared solely by glomerular filtration without tubular secretion or reabsorption[62-64].

Post-intravenous administration, blood samples will be collected over a 4-hour period and analyzed via by liquid chromatography-UV (LC-UV) at the University of Minnesota.

2.5.5 Hormone Analysis

Safety hormone concentrations, including serum testosterone concentrations, will be measured by the CLIA-certified, CAP-accredited Johns Hopkins Hospital Core Laboratories. To formally evaluate the impact of Truvada® on hormone concentrations, batched analysis of hormone concentrations will be conducted. Serum total and free testosterone concentrations, as well as estradiol measurements, will be conducted via LC-MS analysis. LH and FSH measurements will be analyzed via immunoassay. Samples will be tested at the Brigham and Women's Brigham Research Assay Core, which is a CLIA- certified laboratory accredited by the Joint Commission.

2.5.6 Additional Biomarkers of Renal Function

Additional renal markers will also be measured, including cystatin C, which is a 13 kDa lysosomal cysteine proteinase inhibitor that is freely filtered at the glomerulus, and is completely resorbed and catabolized at the proximal tubules[65]. Cystatin C has also been used as a marker of renal function, and its production and clearance is independent of changes in muscle mass[66,67]. Samples collected for cystatin C will be sent to the Johns Hopkins Hospital Core Laboratories for analysis.

2.5.7 Assessment of Participant Adherence with Study Product/Intervention(s)

To maximize participant safety of and adherence to Truvada® and estrogen-based hormonal therapies, adherence will be monitored using several complementary tools to evaluate Truvada® and estrogen adherence while on study. Adherence will be primarily evaluated via direct observed therapy (DOT). PrEP and hormone doses will be directly observed by study personnel either in person or by live video streaming (e.g., FaceTime, WhatsApp). In the high-dose estrogen phase of the clinical trial, estrogen dosing may be split to a morning and an evening dose; only one dose per day will be monitored using one of the aforementioned strategies. An additional measure to evaluate adherence will be the participant's provision of their pill containers to study visits. IDS will perform a manual pill count. Subjects who demonstrate poor adherence, as determined by study staff, will be required to take the remaining medication under in-person DOT. If the subject misses more than one dose across two of the PK periods, they will be removed from the study. A participant may be replaced at the PI's discretion

2.5.8 Assessment of Gender Dysphoria

Periodic assessments of gender dysphoria will be conducted at baseline and a convenient time during PK visits throughout the study. This will be achieved by asking research participants to rate how they feel about their gender throughout the study.

2.6 Description of Study Products

Tenofovir disoproxil fumarate/emtricitabine (TDF/FTC; Truvada®)

TFV is licensed as the prodrug form, TFV disoproxil fumarate (TDF) which is available in a 300 mg dosage form as well as in fixed dose combination with emtricitabine (200 mg), also an HIV nucleoside reverse transcriptase inhibitor (NRTI), marketed as Truvada® and indicated for prevention of HIV infection in persons at risk of HIV infection as well as for treatment in combination with another NRTI and a more potent antiretroviral drug. TFV is also marketed in other prodrug forms, including TFV alafenamide fumarate (TAF); however, the TDF prodrug form will be evaluated in this phase 1 study.

Tenofovir disoproxil fumarate, an acyclic nucleoside phosphonate diester analog of adenosine monophosphate, is initially converted to tenofovir by diester hydrolysis and further undergoes phosphorylation by cellular enzymes to form tenofovir diphosphate. In turn, tenofovir diphosphate competes with deoxyadenosine 5'-triphosphate, which is then incorporated into DNA resulting in chain termination, thus, preventing the activity of HIV-1 reverse transcriptase. It is also a weak inhibitor of mammalian DNA polymerase alpha, beta, and mitochondrial DNA polymerase gamma.

Emtricitabine is a synthetic nucleoside analog of cytidine. The active metabolite, emtricitabine 5'-triphosphate, inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate, which is then incorporated into nascent viral DNA resulting in chain termination. It is also a weak inhibitor of mammalian DNA polymerase alpha, beta, epsilon and mitochondrial DNA polymerase gamma.

Leuprolide Acetate (Lupron Depot®)

Leuprolide acetate is a synthetic nonapeptide gonadotropin-releasing hormone or luteinizing hormone-releasing hormone (GnRH or LH-RH) analog that exhibits a potent reversible inhibition of gonadotropin secretion, through suppression of testicular and ovarian steroidogenesis. Initial administration of the drug results in an increased luteinizing hormone (LH) and follicle stimulating hormone (FSH) which leads to a transient rise in gonadal steroids, however, its continuous administration results in reduced LH and FSH concentrations.

Estradiol

Estrogens regulate the transcription of a limited number of genes and act through binding to nuclear receptors in estrogen-responsive tissues. Estrogens also modulate the pituitary secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), through a negative feedback mechanism reducing the levels of these hormones seen in postmenopausal women. Estradiol is the principal intracellular human estrogen and is substantially more potent than its metabolites at the receptor level.

Future Application of PrEP-GAHT Interactions in TGW.

Taken together, the PrEP-GAHT Interactions in TGW study will provide criticial information on the two-way impact between gender affirming femininizing therapies and PrEP. Using a traditional drug-drug interaction study design, these five dosing phases will evaluate the influence of PrEP on GAHT pharmacology as well as the roles that testosterone ablation and dose-escalated estrogen have on systemic and localized PrEP concentrations. The findings from this work may result in personalized PrEP dosing regimens for TGW on GAHT to maximize PrEP efficacy.

3 OBJECTIVES AND ENDPOINTS

3.1 Objectives

The global objective of this study is to evaluate the two-way pharmacokinetic-pharmacodynamic (PK-PD) interaction of GAHT (estrogen with testosterone suppression) on TDF/FTC PrEP in TGW through the completion of a five-phase PK-PD study, in which TGW take daily PrEP, followed by a testosterone suppressing agent alone and in conjunction with low- and high-dose estrogen therapy Study objectives including safety, pharmacokinetics (PK) and pharmacodynamics (PD) are essential in understanding the impact of co-administration of PrEP and GAHT on downstream drug and hormone concentrations.

3.1.1 Objectives & Endpoints

Table 2: Study Objectives and Endpoints
*as defined by the *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events,*

Objectives	Endpoints & Data Analysis
• Describe the impact of GAHT on TDF/FTC PrEP PK-PD • Describe the bi-directional impact of TDF/FTC & GAHT combinations	• Non-compartmental analysis of TFV/FTC in plasma and urine • Concentrations of all analytes in blood, PBMC, & colorectal tissue Compare PK1 to PK2, PK3, PK4 for GAHT impact on PrEP • Compare PK5 to PK2-4 for PrEP impact on GAHT
• Describe the impact of GAHT on PrEP suppressed HIV susceptibility	• Describe changes in explant p24 antigen under all PK conditions • Correlation and E_{max} model of drug concentration-p24 relationship
• Explain the mechanism of estrogen impact on PrEP PK	• Mechanistic modeling of PK change • Renal changes with addition of GAHT
• Describe adverse effects associated with PrEP & GAHT	• Grade 2 or higher AEs*
• Assess gender dysphoria will participants are on study product	• Gender dysphoria evaluations at baseline and during PK1 through PK5

Corrected Version 2.1 - July 2017, and Addendum 3 (Rectal Grading Tables for Use in Microbicide Studies)

4 STUDY DESIGN

4.1 Identification of Study Design

This is a Non-IND phase I, open label study of a 5 period drug-drug interaction study to better define the magnitude of PrEP-GAHT drug-drug interactions in order to assure protective dosing in TGW for whom both PrEP and GAHT are indicated and desired.

After completing a screening evaluation, up to 40 individuals will be enrolled to complete 20 evaluable participants at the Johns Hopkins University (JHU). The 20 HIV-negative, self-identifying TGW should be naïve GAHT (including estrogen-based therapies, spironolactone, and progesterone) or willing to abstain from feminizing therapies until serum total testosterone concentrations exceed 200 ng/dL (cisgender male reference interval). Baseline testosterone concentrations will be evaluated at screening; if participants have testosterone concentrations below 200 ng/dL, testosterone levels will be re-evaluated every 2-4 weeks for up to 6 weeks until they exceed 200 ng/dL. If testosterone levels remain <200 ng/dL after 6 weeks, the participant will be considered a screen failure.

Once enrolled and when testosterone concentrations are ≥ 200 ng/dL, eligible participants will receive 300 mg TDF/200 mg FTC (Truvada®, Gilead Sciences), once daily for seven days under aforementioned direct observation (in person observation, live video streaming, or with a time-stamped video) to achieve steady state drug PrEP concentrations. After one week of therapy, participants will undergo intensive PK analysis as well as collection of colorectal biopsies for PD testing (PK1). During the PK-intensive day, iohexol will be administered intravenously for the

empirical determination of renal function and mGFR. While concurrently on PrEP, participants will then be intramuscularly administered depot leuprolide acetate (11.25 mg Lupron®). Two weeks post-injection, when testosterone concentrations are below the lower limit of normal of total testosterone in cisgender men (typically < 200 ng/dL), sampling for PK, PD, and renal function will be performed (PK2). Participants will then immediately begin low-dose oral estrogen therapy (1 mg 17β-estradiol) in conjunction with PrEP for one week, at which time samples will be collected for analyses described above (PK3). While still on PrEP, participants will then transition to high-dose estrogen therapy (6 mg 17β-estradiol) for the remainder of the study. One-week post-high dose estrogen therapy in the presence of PrEP, pharmacological and renal samples will be collected for analysis (PK4). PrEP will then be discontinued, and two weeks later, samples will be collected to assess renal function and hormonal concentrations, and evaluate the presence of any remaining PrEP in plasma, PBMC, or colorectal tissue (though it should be near undetectable levels for most analytes according to our prior data) (PK5). Safety assessments, including history/physical, chemistry/hematology labs at screening and interim history will be performed at each study-intensive visit, as needed. To ensure compliance, participants will have PrEP and hormone dosing monitored via direct observation by study personnel either in person, by live video streaming, or with a timestamped video. In the high-dose estrogen phase of the clinical trial, estrogen dosing may be split to a morning and an evening dose; only one dose per day will be monitored using one of the aforementioned strategies.

Overview of study visits and detailed study events are shown in the Study Schema and outlined in Schedule of Events in **Appendix I**.

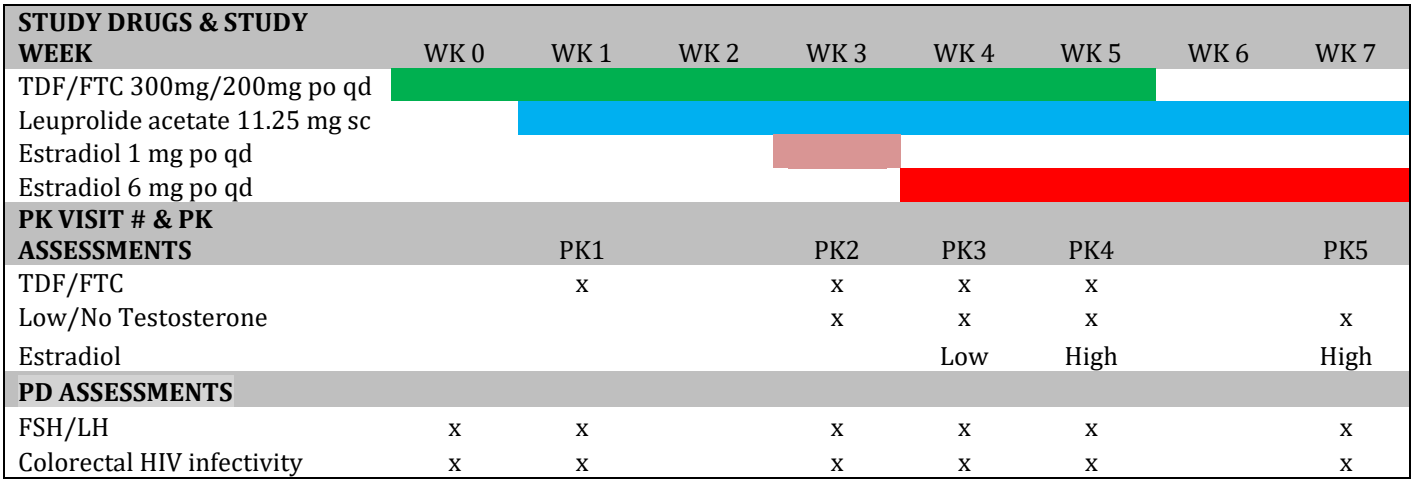


Figure 1. Study Schema for PrEP-GAHT Interactions in TGW Protocol. Dosing and PK sampling phases indicates TDF/FTC PrEP alone (PK1), followed by testosterone suppressing GnRH agonist leuprolide acetate (PK2), then two levels of estradiol (low-dose [PK3] and high dose-estrogen [PK4]), before cessation of PrEP while on high-dose estrogen (PK5). The iterative dosing phases allow assessment of two-way drug-drug interactions: PrEP on GAHT and vice versa. Additionally, the effect of full suppression of testosterone on PrEP, distinct from the estrogen effect, is enabled.

During each PK-intensive day, blood will be collected prior to administration to a PrEP dose, followed by collection at 1, 2, 3, 4, , and 24 hours for TFV ad FTC quantification; blood will also be collected for PBMC isolation at pre-dose and 24 hour time points. Urine will be collected and pooled 0-4 h and 4-24 h post-dose for TFV, FTC and creatinine. Serum will be collected pre-dose on each PK-intensive day for the quantification of estradiol, testosterone, LH, FSH, and cystatin C. For further PK/PD analysis, flexible sigmoidoscopy with rectal tissue biopsies will be collected to measure systemic drug concentrations in colorectal tissue. Biopsies will also be used for *ex vivo* infectivity assays. The sampling schedules and laboratory processing procedures will be described in the Study Specific Procedures (SSP) manual. At each study visit, particpants will have two peripheral intravenous (PIV)

access lines inserted (if applicable and the participant's vascular access allows), one will be used for frequent blood sampling and the second PIV will be used for the administration of iohexol for the direct assessment of renal function. Following iohexol administration, blood GFR sampling will be obtained pre-dose and at 2 and 4 hours hours post-injection. This will occur at baseline and at each PK phase of the study.

Periodic assessments of gender dysphoria will be also be conducted at baseline and at a convenient time during PK visits throughout the study (which coincide with steady-state on each sequential drug regimen). This assessment will involve asking research participants to rate their response to the following statements, based on a 1-5 scale (1 meaning 'completely disagree' and , 5 meaning 'completely agree'.)

- I feel good about my gender today.
- I feel bad about my gender today.

4.2 Description of Study Population

This study will include HIV-negative TGW who meet the criteria outlined in Sections 5.4 and 5.5.

4.3 Time to Complete Accrual

Participant accrual will take approximately 3 years.

4.4 Expected Duration of Participation

Each participant will be on study for approximately 4 months (up to 2 months in screening and 56 days under observationon study drugs). Total study duration is about 4 years.

5 STUDY POPULATION

5.1 Selection of Study Population

The inclusion and exclusion criteria outlined in Sections 5.4 and 5.5 will be utilized to ensure the appropriate selection of study participants.

5.2 Recruitment

Participants will be recruited from a variety of sources, using the following key strategies:

- Listings of prior research participants who have given informed consent to be reached for future studies for which they may be eligible
- Participant referrals (participants refer their friends or partners who may meet eligibility criteria)
- Passive self-referral: interested individuals see an IRB-approved study poster, flyer, brochure, website, or social media posting advertising the study and call the study site directly
- Recruitment services that will use social media-based recruitment strategies involving the posting of IRB-approved recruitment materials

Referrals from other study coordinators and providers, including referrals from care providers of the Chase-Brexton Clinic, and TGW patients under the clinical care of Dr. Adrian Dobs. In order to assist

with medical management of GAHT following study protocol completion, and to ensure appropriate management of any gender dysphoric symptoms that occur while on study, all participants will be required to be under the care of a health care provider for transgender health management. Tha name and contact information of the health care provider will be recorded in the study record.

Study staff will meet as needed to discuss current recruitment status, targets, and strategies. Included in the plans will be measures to specifically target recruitment of TGW, including community outreach to encourage referrals by word of mouth, and use of IRB-approved recruitment materials designed to specifically target recruitment among these populations. Staff also will follow-up with all persons who express an interest in the study to ensure that screening appointments are scheduled and carried out in a timely manner.

5.3 Retention

Once participants enroll in this study, the study site will make every effort to retain them for the duration of follow-up in order to minimize possible bias associated with loss-to-follow-up. Beginning with the informed consent process, components of such procedures include:

- Thorough explanation of the study visit schedule and procedural requirements during the consent process with re-emphasis at each study visit.
- Thorough explanation of the importance and inter-relatedness of all three product doses and sampling phases to the overall success of the study.
- Use of appropriate and timely visit reminder mechanisms (via email and/or telephone/text).
- Immediate and multifaceted follow-up (i.e. phone/text, email, and/or any other communication methods available at the disposal of site's study staff) on missed visits.
- The protocol principal investigator in consultation with the site investigator will determine if research participants who fail to complete all intensive PK visits should be replaced.

5.4 Inclusion Criteria

Individuals who meet the following criteria are eligible for inclusion in this study:

1. 18 years of age or older
2. Self-identifying as a transgender woman
3. Not currently taking any gender affirming hormonal therapy (GAHT) with a total testosterone concentration of ≥ 200 ng/dL, or willing to abstain from feminizing therapies (including estradiol, spironolactone, progesterone, etc.) until total total testosterone concentrations are ≥ 200 ng/dL. Note: Testosterone may be retested every 2-4 weeks during screening to determine eligibility up to 6 weeks.
4. HIV-1 uninfected at screening as documented by Combo Ag/Ab HIV-1/HIV-2 immunoassay
5. Understand and agree to local STI reporting requirements
6. Able and willing to communicate in English
7. Able and willing to provide written informed consent to take part in the study
8. Able and willing to provide adequate information for locator purposes
9. Able and willing to participate in a directly observed study, which may occur in person, using livestreaming or a timestamped video?

10. Availability to return for all study visits, barring unforeseen circumstances
11. Willing to abstain from insertion of anything (drug, enema, penis, or sex toy) in rectum for 72 hours before and 72 hours after each flexible sigmoidoscopy
12. Willing to refrain from aspirin and NSAID use for one week before and after each study biopsy visit
13. Willing and able to use condoms for all RAI for the duration of participation
14. Willing and able to participate in a directly observed study, which may occur in person, using livestreaming or a timestamped video
15. Has an identified healthcare provider for transgender health management
16. Agree not to participate in other research studies involving drugs and/or medical devices for the duration of the study

5.5 Exclusion Criteria

Individuals who meet any of the following criteria at screening will be excluded from the study:

1. Not currently on any PrEP regimen (e.g., Truvada®, Descovy®)
2. History of chronic Hepatitis B infection, as documented by positive HBsAg at screening
3. ≥ Grade 2 laboratory abnormality at baseline as defined by Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 - July 2017, and Addendum 3 (Rectal Grading Tables for Use in Microbicide Studies)
4. Significant colorectal symptom(s) as determined by medical history or by participant self-report (including but not limited to presence of any unresolved injury, infectious or inflammatory condition of the local mucosa, history of inflammatory bowel disease, presence of symptomatic hemorrhoids, and presence of any painful anorectal conditions that would be tender to manipulation)
5. At screening or within the past 2 months: participant-reported symptoms and/or clinical or laboratory diagnosis of active rectal infection requiring treatment per current CDC guidelines or symptomatic urinary tract infection (UTI). Infections requiring treatment include Chlamydia (CT), gonorrhea (GC), syphilis, active HSV lesions, chancroid, genital sores or ulcers, and, if clinically indicated, genital warts. Note that HSV seropositivity with no active genital lesions is not an exclusion criterion. (Note: if an STI apart from HIV is detected, the participant will be referred for treatment and can be retested in 30 days and rescreened once.)
6. History of an underlying clinically significant cardiac arrhythmia or renal disease (including creatinine clearance < 60 mL/min using Cockcroft-Gault equation)
7. Serum phosphate < 2.3 mg/dL
8. History of severe or recent cardiac or pulmonary event
9. History of significant gastrointestinal bleeding
10. Current use of warfarin or heparin or other anticoagulant medications associated with increased risk for bleeding following mucosal biopsy (e.g., daily high dose aspirin [>81 mg], NSAIDs, or Pradaxa®)
11. Use of systemic or anorectal immunomodulatory medications within 4 weeks of enrollment or planned use at any time during study participation

12. Per participant report, use of any rectally administered products containing N-9 (including condoms) or investigational products within 4 weeks of enrollment, or planned use of either at any time during study participation
13. Known allergic reaction to TFV, FTC, leuprolide acetate, or estradiol or other components of the test articles
14. Current known HIV-infected partner(s)
15. Symptoms suggestive of acute HIV seroconversion at screening and enrollment
16. Any other condition or prior therapy that, in the opinion of the investigator, would preclude informed consent, make study participation unsafe, make the individual unsuitable for the study or unable to comply with the study requirements. Such conditions may include, but are not limited to, current or recent history of severe, progressive, or uncontrolled substance abuse, or renal, hepatic, hematological, gastrointestinal, endocrine, pulmonary, neurological, or cerebral disease.

6 STUDY PRODUCTS

6.1 Regimen

After completion of written informed consent and all screening procedures, eligible participants will be asked to come to the DDU research clinic for baseline evaluations, including laboratory assessment of hormonal status and biopsies for baseline HIV explant challenge PD assessment before any study drugs are administered. At this baseline visit, participants will be dispensed one-week supply of 300 mg TDF/200 mg FTC (Truvada®, Gilead Sciences) PrEP. Participants will take one pill daily for seven days under direct observation, starting the day after the baseline study visit, to achieve steady state PrEP drug concentrations.

After 6 days of Truvada® PrEP dosing via DOT, participants will return to the DDU research clinic to undergo the first intensive 24-hour PK sampling visit (Visit # 9, PK1 D1 Visit), including collection of colorectal biopsies for PK and PD testingand administration of intravenous iohexol for the empirical determination of renal function and GFR. At completion of the 24-hour PK sampling visit (Visit #10, PK1 D2 Visit), participants will start another 7 days of Truvada® PrEP via DOT and receive an intramuscular injection of depot leuprolide acetate (11.25 mg Lupron Depot®).

Participants will continue to take Truvada® PrEP via DOT for 5 days, after which, they will return to the DDU research clinic for their 7th dose of Truvada® PrEP via in-person DOT, a 1 week refill of Truvada® PrEP and a safety assessment (Visit #16, Day 14).

After 6 days of Truvada® PrEP dosing via DOT, when testosterone concentrations are projected to be below the lower limit of normal of total testosterone in cisgender men (typically < 200 ng/dL), participants will return to the DDU research clinic to undergo the second intensive 24-hour PK sampling visit (Visit #23, PK2 D1 Visit) including collection of colorectal biopsies for PK and PD testing and administration of intravenous iohexol for the empirical determination of renal function and GFR. At completion of the 24-hour PK sampling (Visit #24, PK2 D2 Visit), participants will start another 7 days of Truvada® PrEP via DOT and begin daily low-dose oral estrogen therapy (1 mg 17β-estradiol), taken concomitantly with Truvada® PrEP via DOT.

After 5 days of low-dose estradiol in combination with Truvada® PrEP dosing via DOT, participants will return to the DDU research clinic to undergo the third intensive 24-hour PK sampling visit (Visit

#30, PK3 D1 Visit) including collection of colorectal biopsies for PK and PD testing and administration of intravenous iohexol for the empirical determination of renal function and GFR. At completion of the 24-hour PK sampling (Visit #31, PK3 D2 Visit), participants will start another 7 days of Truvada® PrEP via DOT and transition to high-dose estrogen therapy (6 mg 17β-estradiol), which they will continue on for 2 weeks. The high-dose estrogen therapy may be split into two administration periods, one taken in the AM, and one taken in the PM, andonly the estrogen dose taken concomitantly with the Truvada® PrEP dose will be monitored via DOT

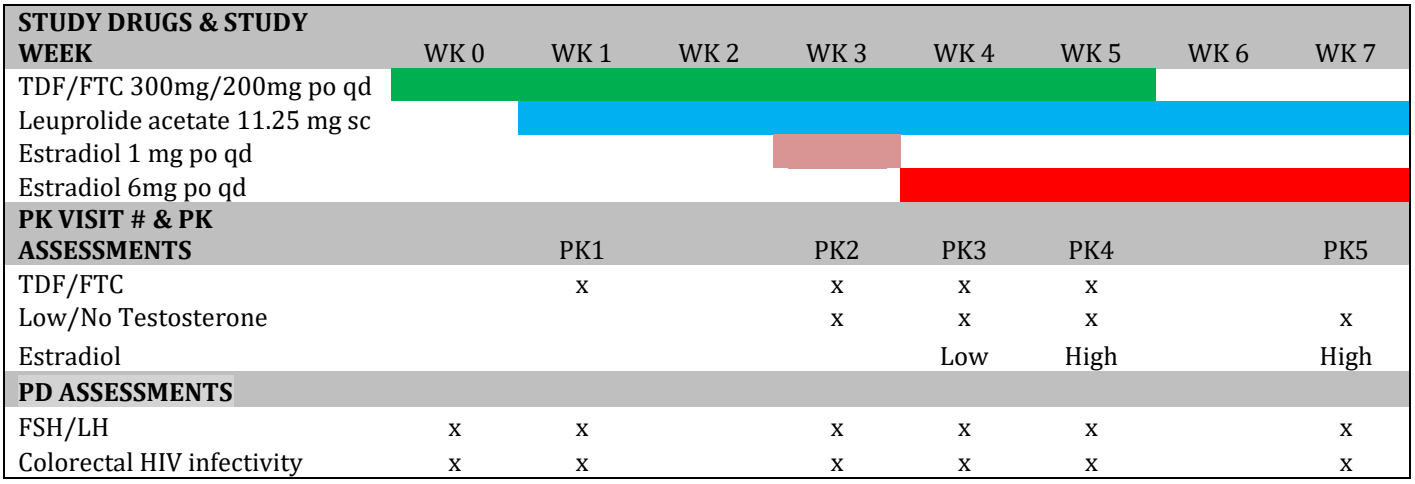
After 5 days of high-dose estradiol in combination with Truvada® PrEP dosing via DOT, participants will return to the DDU research clinic to undergo the fourth intensive 24-hour PK sampling visit (Visit #37, PK4 D1 Visit) including collection of colorectal biopsies for PK and PD testing and administration of intravenous iohexol for the empirical determination of renal function and GFR. Participants will complete Truvada® PrEP dosing at this visit. At completion of the 24-hour PK sampling (Visit #38, PK4 D2 Visit), participants will start another 7 days of high-dose estrodiol via DOT without Truvada® PrEP. Participants will continue to take the high-dose estrodial via DOT without Truvada® PrEP, for 5 days, after which, they will return to the DDU research clinic for their 7th dose of high-dose estrogen therapy via in-person DOT, a one-week refill of high-dose estrodiol and a safety assessment (Visit #44, Day 42).

After 6 days of high-dose estradiol dosing via DOT without Truvada® PrEP, participants will return to the DDU research clinic to undergo the fifth and final intensive 24-hour PK sampling visit (Visit #51, PK5 D1 Visit) including collection of colorectal biopsies for PK and PD testing and administration of intravenous iohexol for the empirical determination of renal function and GFR. Participants will complete the high-dose estradiol at this visit, may elect to stay on estrogen after completion of the study, and this will be coordinated with their primary care providers. Participants will return the next day for the final 24-hour PK sampling visit (Visit #52, PK5 D2 Visit). Participants will complete the high-dose estradiol at this visit, may elect to stay on estrogen after completion of the study, and this will be coordinated with their primary care providers.

After 6 days, participants will return to the DDU research clinic for the final safety visit (Visit # 53).

6.2 Administration

Figure 1. Schema of PK Dosing Phases



6.3 Study Product

Table 6. Study products to be administered during this study.

Trade Name	Study Drug	Formulation	Dose Period	Regimen
Truvada®	tenofovir disoproxil fumarate emtricitabine (fixed dose combination)	300 mg 200 mg	Baseline through Visit 4	1 x 300mg/200mg tablet daily with food
Estrace™	17β-estradiol 17β-estradiol	1 mg 2 mg	Visit 3-4 Visit 4-7	1 x 1 mg tablet daily (1 mg) 3 x 2 mg tablet daily (6 mg)
Lupron Depot®	leuprolide acetate	11.25 mg	Visit 1	prefilled syringe injection

6.3.1 Tenofovir/Emtricitabine

Tenofovir/Emtricitabine: A daily oral dose of TDF/FTC 300 mg/200mg (Truvada®) will be prescribed and dispensed at baseline visit (V 2, day 0) and continued over 5 weeks.
The dose selected is the FDA-recommended oral PrEP dose.

6.3.2 Leuprolide acetate

Lupron Depot® injection 11.25 mg will be administered at Visit #10 (Day 8). The syringe contains sterile lyophilized microspheres, which is leuprolide incorporated in a biodegradable copolymer of lactic and glycolic acids. The product is available in a prefilled dual-chamber syringe containing sterile lyophilized microspheres which, when mixed with diluent, become a suspension. The product is supplied as a kit, containing:

- one prefilled dual-chamber syringe
- one plunger
- alcohol swabs
- a complete prescribing information enclosure

Kits should be stored at 25ºC, with excursions permitted to 15-30 ºC. The suspension will be prepared in the Investigational Drug Service Pharmacy and the injection will be administered by a qualified DDU clinician. The entire contents of the suspension is injected intramuscularly in the ventrogluteal muscle with the needle inserted completely at a 90 degree angle. The syringe should be discarded if not used within 2 hours.

6.3.3 Estrace®

Estradiol: A daily oral low-dose of estradiol (Estrace®) 1mg, will be prescribed and dispensed at Visit #24 (PK2 D2, Day 22) and continued for 7 days. At Visit #31 (PK3 D2, Day 29), the total daily dose of Estrace® will be increased to 6 mg, taken as three 2-mg tablets, daily through Visit #51 (PK5 D1, Day 49). Estrace® tablets will be dispensed by the Johns Hopkins IDS and provided to the participants by the DDU staff. The drug product should be stored at 20-25ºC (USP Controlled Room Temperature).

6.3.4 Iohexol

Iohexol (Omnipaque™ 240; Omnipaque™ 300; Omnipaque™ 350): Iohexol will be prepared and administered in accordance with practices outlined in the Omnipaque™ prescribing information. Specifically, participants will be encouraged to maintain good hydration 24 hours prior to and 24 hours following iohexol administration. A single volume dose of Omnipaque™ will be administered intravenously during the intensive sampling visits so that a total dose of 1500 mg of iohexol is delivered over two minutes. This is the usual adult dosage for measurement of GFR. The concentration of Omnipaque™ used in any individual participant will be kept consistent for all iohexol dose administrations during their participation in the protocol. The iohexol injection will be prepared and dispensed by the Johns Hopkins IDS Pharmacy and administered by a qualified clinician in the

Johns Hopkins University Institute for Clinical Research (ICTR) unit. Omnipaque™ should be stored at controlled room temperature, 20°-25°C (68°- 77°F); excursions permitted to 15°-30°C (59°-86°F) [see USP Controlled Room Temperature]. Samples will be analyzed at the University of Minneosta.

6.4 Study Product Supply and Accountability

The Hopkins Investigational Drug Service (IDS) Pharmacy will be used to support the study, which includes labeling, drug storage, dispensing and obtaining drug supplies. Inventory management such as recordkeeping requirements, tracking of drug supply, and accounting for all doses dispensed will also be done through IDS.

6.5 Accountability

All study products will be available to the study staff through the designated site pharmacy or compounding pharmacy, once they have been labeled appropriately by the PoR. The PoR is required to maintain complete records of all study products received and dispensed.

6.6 Dispensing

Study products will be dispensed from the pharmacy to the designated study staff for an enrolled participant, upon receipt of a written prescription from an authorized prescriber.

6.7 Retrieval of Unused Product

Any dispensed, unused study product will be returned to the investigational drug pharmacy.

6.8 Concomitant Medications and Procedures

With the exception of medications listed as prohibited, enrolled study participants may use concomitant medications during study participation. All concomitant medications, over-the-counter preparations, vitamins and nutritional supplements, recreational drugs, and herbal preparations reported throughout the course of the study will be recorded in the source documents and on case report forms designated for that purpose.

6.9 Prohibited Medications and Procedures

Participants will be advised to refrain from RAI or any practices which include rectal insertion of any product, including those used during sexual intercourse (drug/medication, penis, other objects, sex toy, or douche) for 72 hours before and after each flexible sigmoidoscopy with biopsy collection.

Study participants will be prohibited from using the following medications throughout the study period: heparin (including Lovenox®), warfarin, Plavix® (clopidogrel bisulfate), immunomodulatory medications, rectally administered products containing N-9 (including over-the-counter products), or investigational products. Other drugs, such as aspirin or NSAIDS that are associated with increased likelihood of bleeding following mucosal biopsy, should be restricted for use prior to flexible sigmoidoscopy as required by clinical guidelines.

Based on the Truvada® U.S. prescribing information, concomitant use of TFV with medications that decrease renal function or compete for active tubular secretion may increase concentrations of TFV or other renally eliminated medications.

If participants report using any of these medications or products while on study or within the specified time frame noted, their study product administration will be put on hold, pending discussions with study site investigator and/or DAIDS MO. Study staff will actively review medication use with each research participant during screening and throughout study participation. Participants will also be queried as to their adherence regarding insertion of rectal products 72 hours before and after each biopsy collection visit.

6.5 Recommended Procedures

Condoms will be provided for use by all participants enrolled in this study. Using condoms will be recommended for all sexual encounters where the participant will be engaging in receptive anal intercourse (RAI), for the duration of study participation. In addition to instructions regarding abstinence prior to and following flexible sigmoidoscopy with biopsy, participant education about risk reduction will be provided. These will be included in instructions provided as Pre-Post visit reminders and STI risk reduction. In the event that a participant needs additional supply of condoms between visits, she may request these from study staff at any time.

7 STUDY PROCEDURES

7.1 Procedure overview

This section includes information on visit-specific study procedures. An overview of the study visits and evaluations is available in protocol Appendix I: Schedule of Events. Unless otherwise specified, the laboratory procedures listed in this section are performed at the local study site laboratories.

7.2 Visit 1 - Screening Visit

Prescreening via a phone call or email will take place with potential participants. Participants interested in the study can request that the informed consent documents be mailed out or emailed to them prior to the Screening Visit.

Written informed consent will be obtained at the screening before any procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined. Participants who do not meet the eligibility criteria may be rescreened at a later date, if appropriate, as in the case of a concomitant medication washout period. In such cases, the length of the washout period will be determined by the PI based on safety considerations and the elimination half-life of the concomitant medication. Screening labs may be repeated once to determine eligibility.

The following activities will take place at the screening visit:

Table 10: Visit #1 - Screening Visit

Component	Procedure/Analysis

Administrative	• Obtain written informed consent
	• Assign participant ID (PTID)
	• Assess eligibility
	• Obtain demographic and locator information
	• Provide counseling:
	  ○ HIV/STI risk reduction counseling
	  ○ HIV pre/post test counseling
	  ○ Participant education: flexible sigmoidoscopy procedure with rectal tissue biopsy

Clinical	• Obtain complete medical history, documenting pre-existing conditions • Review and record concomitant medications • Perform general physical exam • Obtain vital signs – blood pressure (BP), heart rate (HR), temperature (temp), height (inches) and weight (kg)

Safety and Screening Labs*	• Collect blood specimens for:
	  • Complete Blood Count (CBC) with differential
	  • Comprehensive Metabolic Panel (CMP)
	  • Prothrombin Time (PT)/Partial Thromboplastin Time (PTT)
	  • PO_4
	  • Lipid Panel
	  • Estradiol
	  • Testosterone, total only
	  • HBsAg
	  • HIV testing
	  • Syphilis testing (confirmatory test as needed)
	• Collect rectal swab for:
	  • Rectal NAAT for GC/CT

* *Screening laboratory values are valid up to 60 days*. Additional tests can be ordered for abnormal POCT urine dipstick results.

7.3 Visit #2 – Baseline Visit (Day 0)

The Baseline visit serves to establish pre-dose conditions for safety assessments, hormone levels, kidney function, and PD assessment of HIV challenge of colon explant tissue.

If deemed eligible based on the results from the screening visit, participants will be contacted to schedule a baseline visit and asked to refrain from RAI and the insertion of anything in their anorectum for 72 hours prior to the visit.

The Baseline Visit must take place ≤60 days after the Screening Visit. If >60 days pass before the Baseline Visit, participants will need to be re-screened and re-consented.

The following activities will take place at the baseline visit:

Table 11: Visit #2 – Baseline Visit (Day 0)

Component	Procedure/Analysis

Administrative	• Confirm participant understood and signed informed consent
	• Review/update locator information
	• Confirm eligibility
	• Ensure participant complied with pre-visit instructions
	• Provide counseling and education:
	  ○ HIV/STI risk reduction counseling
	  ○ Pre/Post visit reminders
	  ○ Flexible sigmoidoscopy with rectal tissue biopsy information sheet

Clinical	• Review/update medical history • Review/update concomitant medications • Review any available test results • Conduct gender dysphoria assessment • Perform symptom-directed physical exam, as needed • Obtain vital signs (HR, BP, temp, weight) • Insert PIV x2 (if applicable)

Safety Labs:	• Collect blood specimens for:
	  • Complete Blood Count (CBC) with differential
	  • Comprehensive Metabolic Panel (CMP)
	  • PO_4
	  • Estradiol
	  • Testosterone, total only
	  • HIV testing

Study Product	• Adminster Iohexol • Dispense 7 day supply of TDF/FTC

Study Labs:	• Collect blood specimens for:
	  • Estradiol
	  • Testosterone, free and total
	  • LH/FSH
	  • Cystatin C

Study Labs: GFR	• Collect blood specimens for GFR calculations:
	  • Pre-Dose
	  • 2hrs
	  • 4hrs

Cleansing Douche	• Cleansing douche will be administered at least one hour prior to flexible sigmoidoscopy
Rectal Specimens	• Perform flexible sigmoidoscopy with rectal tissue biopsy collection (5)

7.4 Directly Observed Therapy (DOT) Visits (Conducted prior to Intensive PK Sampling Visits

The following activities will take place at the DOT visits:

Table 12: DOT Visits

Component	Procedure/Analysis
Clinical	• Assess/Update AEs • Review/update medical history • Review/update concomitant medications • Perform symptom-directed physical exam as needed
Study Product	• Participant will take TDF/FTC or estradiol dose under direct observation by study personnel.

7.5 Visits # #23, #30, #37 and #51 - Intensive PK Sampling Visits Day 1 (PK D1 Visits)

The following activities will take place at the PK Day 1 Visits:

Table 13: Intensive PK Sampling Visits Day 1 (PK D1 Visits)

Component	Procedure/Analysis
Administrative	• Review/update locator information Ensure participant complied with pre-visit instructions • Adherence monitoring
Clinical	• Assess/Update AEs • Review/update medical history • Review/update concomitant medications • Review any available test results • Conduct gender dysphoria assessment • Perform symptom-directed physical exam, as needed • Collect vital signs (HR, BP, temp, weight) • Insert PIV x2 (if applicable)
Safety Labs	• Collect blood specimens for: • Serum Cr • Testosterone, only total (Visit #23, PK2 D1 only) • HIV Ag/Ab
Study Labs	• Collect blood specimens for: • Estradiol • Testosterone, free and total • LH/FSH • Cystatin C
Study Labs: GFR	• Collect blood specimens for GFR calculations: Pre-Dose • 2hrs • 4hrs
Study Labs: PK and PBMC	• Collect blood specimens for PK: • Pre-Dose • 1hr • 2hrs • 3hrs4hr • Collect blood specimens for PBMC • Pre-Dose
Study Labs: Urine	• Collect urine specimens for TFV/FTC and Creatinine • 0-4hrs • 4-24 hrs (collected at home by participant)

7.6 Visits #10, #24, #31, #38, #52 - Intensive PK Sampling Visits Day 2 (PK D2 Visits)

The following activities will take place at the PK Day 2 Visits:

Table 14: Intensive PK Sampling Visits Day 2 (PK 24hr Visits)

Component	Procedure/Analysis
Administrative	• Provide counseling and education: o HIV/STI risk reduction counseling o Pre/Post visit reminders Flexible sigmoidoscopy with rectal tissue biopsy information sheet
Clinical	• Assess/Update AEs • Review/update medical history • Review/update concomitant medications • Review any available test results • Perform symptom-directed physical exam, as needed • Collect vital signs (HR, BP, temp)
Study Labs: PK and PBMC	• Collect blood specimens for PK: • 24hrs • Collect blood specimens for PBMC: • 24 hrs • Collect 4-24hrs Urine
Cleansing Douche	• Cleansing douche will be administered at least one hour prior to flexible sigmoidoscopy
Rectal Specimens	• Perform flexible sigmoidoscopy with rectal tissue biopsy collection (5)
Study Product	• Dispense TDF/FTC (Visits #10, 24, 31) • Inject Lupron Depot (Visit #10) • Dispense Estradiol, 1 mg (Visit #24) • Dispense Estradiol, 6 mg (Visits #31, 38)

7.7 Visits #16, #44 and #53 - Safety Visits

The following activities will take place at the Safety Visits:

Table 15: Safety Visits

Component	Procedure/Analysis

Administrative	• Review/update locator information
	• Provide counseling and education:
	  ○ HIV/STI risk reduction counseling
	  ○ Pre/Post visit reminders

Clinical	• Assess/update AEs • Review/update medical history • Review/update concomitant medications • Review any available test results • Perform symptom-directed physical exam, as needed • Collect vital signs (HR, BP, temp)

Safety Labs	• Collect blood specimens for:
	  • CBC with differential[1]
	  • CMP[1]
	  • PO_4[1]
	  • Lipid Panel[2]
	  • Testosterone[2], total only
	  • Syphilis[2]
	  • HIV testing[1]
	• Collect rectal swab for:
	  • Rectal NAAT for GC/CT[2]

Study Product	• Dispense TDF/FTC (Visit #16) • Dispense Estradiol 6 mg (Visit #44)

[1] Safety labs collected at Visit #16 and #53

[2] Safety labs collected only at Visit #53

7.8 Exit Follow-up Phone Call

After the final study visit, follow-up call or early termination visit, a final contact may be required to provide laboratory test results and post-test counseling. All final contacts will be documented in participant study records.

7.9 Interim Visits

Interim visits may be performed at any time during the study. All interim contacts and visits will be documented in the source documentation and on applicable case report forms.

Some Interim Visits may occur for administrative reasons. For example, the participant may have questions for study staff. Other interim visits may occur for clinical purpsoes, such as to measure testosterone levels or in response to AEs experienced by study participants. When interim visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care, if clinically indicated.

The following activities will take place at Interim Visits:

Table 16: Interim Visit

Component	Procedure/Analysis
Administrative	• Review/update locator information •
Clinical	• Assess/update AEs • Review/update medical history • Review/update concomitant medications • Review any available test results • Perform symptom-directed physical exam, as needed • Obtain vital signs (HR, BP, Temp), as needed
Specimens	• Collect specimens as appropriate/indicated

7.5 Participants Who Withdraw or Are Withdrawn from the Study

If the participant withdraws or is withdrawn from the study after receiving study product, an Early Termination Visit will be conducted, if possible.

The following activities will take place at the Early Termination Visit:

Table 17: Early Termination Visit

Component	Procedure/Analysis
Administrative	• Review/update demographic and locator information • Review STI risk reduction counseling and provide condoms
Clinical	• Assess/update AEs • Review/update medical history • Review/update concomitant medications • Review any available test results • Perform symptom-directed physical exam, as needed • Obtain vital signs (HR, BP, Temp)

Safety Labs and STI Screening	• Collect blood specimens for:
	  • CBC with differential
	  • CMP
	  • Lipid Panel
	  • Estradiol
	  • Testosterone, total only
	  • HIV Testing
	  • Syphilis
	• Collect rectal swab for:
	  • Rectal NAAT for GC/CT

7.6 Participants Who Become Infected with HIV

During the study protocol, subjects will be regularly tested for HIV exposure and queried for high-risk behaviors. If there is a concern of possible HIV exposure, HIV and STI screening will be offered at that time. If a participant self-reports seroconversion while on study, or if one of the HIV tests performed during the study protocol is confirmed positive, study staff will provide counseling and direct the participant to the appropriate HIV care.

Study staff will also request documentation of lab results or, failing availability of test results, will perform HIV Testing, with confirmatory testing as needed. These results will be kept in the subject's study chart and recorded on study case report forms (CRFs).

Participants will be withdrawn from the study if they test positive for HIV antibody or RNA. Any participant who tests positive for HIV while enrolled in the study will be immediately removed from the study and referred to the appropriate care provider for additional counseling and treatment as necessary. HIV 4th generation EIA and RNA test results will be available prior to any subsequent study intervention. No additional doses of study product will be dispensed. Participants will be compensated for their study participation up until the time of removal.

7.10 Clinical Evaluations and Procedures

The following history and physical exam components will be conducted at select visits.

Medical History:

- Each participant will have a complete review of their medical history at screening and will be asked about any changes to their medical history at each visit.
- Concomitant medications will be reviewed and recorded at screening and updated as needed at every study visit.

Physical Exam:

- A complete physical examination will be completed at screening and a targeted symptom-directed physical exam will be done during subsequent study visits as needed.
- Height (at screening only) and Weight (at screening and PK iohexol visits only)
- Vital signs
    - Heart Rate/Pulse
    - Blood Pressure
    - Temperature

Rectal Exam, Cleansing Douche, and Rectal Specimen Collection:

The participant will be positioned in the left lateral decubitus position for the following procedures:

- Rectal exam: The examiner will conduct a visual examination of the anus and surrounding area and note any abnormality. The examiner will then insert a lubricated (Good Clean Love, Good Clean Love, Inc. Eugene, OR) gloved finger into the anal canal and sweep around the internal anal circumference.
- Rectal specimen collection:
    - Swabs for GC/CT will be inserted into the rectum and placed in contact with the rectal wall, turned 360 degrees and then removed.
- A pre-procedural cleansing douche will be self-administered by the participant prior to all flexible sigmoidoscopies.

- Rectal tissue biopsy collection via flexible sigmoidoscopy: Up to 5 biopsies will be taken via a flexible sigmoidoscope at approximately 15-20 cm using large-cup biopsy forceps.

7.11 Laboratory Evaluations

7.11.1 Clinical Laboratory Testing

The local clinical laboratory or designee will run the following, as indicated:

- Blood specimens:
  - CBC with differential
  - CMP, which includes blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase test (ALP), sodium (Na), potassium (K), calcium (Ca), carbon dioxide (CO2), chloride (Cl), glucose, albumin, total bilirubin, total protein, and calculated eCRF (calculated by the JH laboratories using CKD-EPI)
  - Phosphate (PO_4)
  - HBsAg
  - HIV Testing
  - Syphilis Testing
  - Testosterone, total only
  - Estradiol
- Rectal swabs:
  - Rectal GC/CT (NAAT)

7.11.2 Research Laboratory Testing

Johns Hopkins University:

- Plasma and PBMC cell lysate for PK (Marzinke)
- Colonic tissue homogenate and extracted cell lysates for PK/PD (Marzinke)
- Urine for PK and creatinine
- Cystatin-C

University of Minnesota:

- Iohexol

Brigham and Women's Hospital:

- Testosterone, total and free
- Estradiol
- LH
- FSH

7.12 Specimen Collection, Handling, and Processing

Study sites will adhere to the standards of good clinical laboratory practice, DAIDS Laboratory Requirements and site standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens at the local laboratories.
(http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/gclp.pdf)

Specimen collection, testing, and storage at the site laboratories will be documented per standard site practice. In cases where laboratory results are not available due to administrative or laboratory error, sites are permitted to re-draw specimens that are intended for use in the screening as well as ongoing safety assessments process. Specimens will be handled in accordance with Requirements for DAIDS Sponsored and/or Funded Laboratories in Clinical Trials.

7.13 Storage of Specimens for Future Use

Study staff will store all specimens collected according to site SOPs. Specimens will not be labeled with any personal identifiers. Storage of all study samples will follow local standard operating procedure to ensure the anonymity and confidentiality of the trial research participants. Specimens remaining at the end of the study will be transferred to a designated central bio-repository after all protocol-required and quality assurance testing has been completed. Study participants' permission to do so will have been documented by signing informed consent which will include appropriate IRB-approved language. All screening samples for individuals who do not eventually enroll in the study will be destroyed.

7.14 Biohazard Containment

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

8 ASSESSMENT OF SAFETY

8.1 Safety Monitoring

The study site investigator is responsible for continuous close safety monitoring of all study participants and for promptly alerting the PI and the protocol team if unexpected concerns arise. A sub-group of the protocol team, including the grant PI, co-PI, and study site investigatorswill serve as the Protocol Safety Review Team (PSRT). Study status reports will be submitted to the PSRT every month for review.

These reports will include all adverse events (AEs) reported for the study, regardless of their relationship to the study products.

The study team will meet, as needed, throughout the period of study implementation to review the safety data, discuss product use management, and address any potential safety concerns.

8.2 Clinical Data Safety Review

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are the first layer and are responsible for the initial evaluation and reporting of safety information at the participant level, and for promptly alerting the PSRT if unexpected concerns arise. In the event of any study participant experiencing an AE ≥ Grade 3, the study site investigator will review and discuss the appropriate study management with the PSRT.

During the trial, the PSRT will review status reports described above and conduct calls to clarify the data as appropriate. The content, format and frequency of the safety reports will be agreed upon in advance of study implementation. If necessary, external experts representing expertise in the fields of HIV drug pharmacology, biostatistics, and medical ethics may be invited to review the events.

8.3 Adverse Events Definitions and Reporting Requirements

8.3.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a study product whether or not considered related to the product. The term “investigational product” for this study refers to TDF/FTC, leuprolide acetate, and estradiol.

Study participants will be instructed to contact the study site staff to report any AEs they may experience at any time between enrollment and completion of their participation. In the case of a life-threatening event, they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. Study site will obtain written permission from the participant to obtain and use records from non-study medical providers to complete any missing data element on a CRF related to an adverse event. All participants reporting an untoward medical occurrence will be followed clinically, until the occurrence resolves (returns to baseline) or stabilizes.

The study site investigators will determine AE resolution or stabilization in their best clinical judgment, but may seek medical consultation regarding follow-up or additional evaluations of an AE from the PSRT. Study site staff will document in source documents and CRFs all AEs reported by or observed in enrolled study participants, regardless of severity and presumed relationship to study products. AEs will be graded by the standard Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 - July 2017.

8.3.2 Serious Adverse Events

Serious adverse events (SAEs) will be defined as AEs occurring at any dose that:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongations of existing hospitalization

Note: Per ICH SAE definition, hospitalization itself is not an adverse event, but is an outcome of the event. Thus, hospitalization in the absence of an adverse event is not regarded as an AE, and is not subject to expedited reporting. The following are examples of hospitalization that are not considered to be AEs:

- Protocol-specified admission (e.g. for procedure required by study protocol)
- Admission for treatment of target disease of the study, or for pre-existing condition (unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator)
- Diagnostic admission (e.g. for a work-up of an existing condition such as persistent pretreatment lab abnormality)
- Administrative admission (e.g. for annual physical)
- Social admission (e.g. placement for lack of place to sleep)
- Elective admission (e.g. for elective surgery)

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the research subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.3.3 Adverse Event Relationship to Study Product

The relationship of all AEs to study product will be assessed by the study investigators. The relationship categories that will be used for this study are:

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

8.4 Expedited Adverse Event (EAE) Definitions and Reporting Requirements

Expedited Adverse Event Reporting

Events that meet SAE criteria and are unexpected (not previously reported to the agency or included in the IB) must be reported in an expedited manner. The study Principal Investigator will assume responsibility for the reporting of Serious Adverse Events to the Johns Hopkins Medicine Institutional Review Board (JHM IRB). Copies of this final report will be forwarded to the DAIDS PO for this protocol.

Unexpectedly Fatal or Life-threatening Event

Unexpectedly fatal or life-threatening events, will be reported to the JHM IRBvia telephone and fax as soon as possible, but no more than seven calendar days after receipt of the information. Any additional information received will be sent to the IRB as soon as possible, but no more than 15 days after receipt of the information.

Serious and Unexpected Event

Serious and unexpected events must be reported to the JHM IRB within 15 days. Also required to be reported to the agency within 15 days are any laboratory findings suggesting significant risk for human subjects including:

- Carcinogenicity
- Mutagenicity

- Teratogenicity

Follow-Up Information
Additional relevant information received about an event must be reported as soon as possible to the JHM IRB with a copy to the DAIDS PO, in no more than 15 days.

Report Format
Safety reports will be drafted by the study team, in consultation with the Principal Investigator and DAIDS PO using the JHM IRB Protocol Event Reporting format.

For all EAEs submitted, the study site must file an update to the DAIDS PO with the final or stable outcome unless the initial EAE submitted had a final or stable outcome noted already.

Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 dated July 2017 and Addendum 3 (Rectal Grading Tables for Use in Microbicide Studies) will be the primary tools for grading adverse events for this protocol. In cases where an AE is covered in multiple tables, Addendum 3 (Rectal Grading Table for Use in Microbicide Studies) will be the grading scale utilized.

The DAIDS AE Grading Tables are available on the RSC website at
https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables

EAE Reporting Period

- The expedited AE reporting period for this study is defined as the entire study duration for an individual participant (from enrollment until the participant's final study contact).
- After the protocol-defined AE reporting period, unless otherwise noted, only suspected unexpected serious adverse reactions will be reported to the DAIDS PO if the study staff becomes aware of the events on a passive basis (from publicly available information).

8.5 Reporting of Adverse Reactions to the Responsible IRBs

The study site investigator will report adverse reactions to the responsible IRBs in accordance with respective IRB policies and procedures.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the study site investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting, the study site investigator will report it as soon as possible in accordance with respective IRB policies and procedures.

8.6 Social Harms Reporting

Although study sites make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-infected or at "high risk" for HIV

infection). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities and/or employer.

Social harms that are judged by the study site investigator to be serious or unexpected will be reported to the DAIDS Program Officer and the responsible site IRB (JHU) at least annually or according to individual IRB requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed.

8.7 Withdrawal of Subjects Due to Adverse Events

Clinical Management Section 9 below includes guidelines for withdrawal of research participants from this study.

9 CLINICAL MANAGEMENT

Guidelines for clinical management and product hold/discontinuation are outlined in this section. In general, the study site investigator has the discretion to hold study product at any time if he or she feels that continued product use would be harmful to the participant, or interfere with participant's acute medical condition (such as e.g. viral gastroenteritis), or with treatment deemed clinically necessary according to the judgment of the investigator.

9.1 Grading System

The primary grading system for this study is *Rectal Grading Table for Use in Microbicide Studies,* which is Addendum 3 to the *DAIDS AE Grading Table Version 2.1, dated July 2017*; it can be found at Regulatory Support Center (DAIDS RSC) website:

https://rsc.niaid.nih.gov/sites/default/files/rectal-tox-table-updates-05-11-2012.pdf

9.2 Dose Modification

This study involves five different test conditions composed of five different drug combinations. If any of the study products are not well tolerated according to either the research participant who requests withdrawal, the clinical judgement of a study clinician, or if a participant experiences a Grade 3 AE attributable to the study product, then the subject will be discontinued form the study, followed until AE resolution or stabilization, and replaced. The participant will be included in the safety analysis. Only research participants who successfully complete all five test conditions and PK assessments will be considered evaluable for PK and PD endpoints.

All Grade 3 or greater AEs will be reviewed with the PSRT, or designees, as soon as they occur. A Grade 4 AE also requires immediate PSRT, or designee, involvement, an automatic pause in further dosing, and review by PSRT. Study participants who experience the following will be permanently discontinued from product use, but may continue with study sampling if deemed medically appropriate by the PSRT:

- Any Grade 3 event deemed related to study products
- Any Grade 4 event

The PSRT will consider replacement of any subject who fails to complete all five intensive PK sampling visits.

9.3 Discontinuation of Study Product(s) in the Presence of Toxicity

Study participants will be permanently discontinued from product use and discontinued from the study following appropriate safety evaluation by the study site investigator or a designated sub-investigator in the event of the following:

- HIV seroconversion
- Any Grade 3 event deemed related to study products
- Any Grade 4 event
- Clinical reasons determined by the investigator or PSRT

9.4 General Criteria for Discontinuation of Study Product(s)

Study participants will be permanently discontinued from product use by the study site investigator or a designated sub-investigator in the event of the following:

- HIV seroconversion
- Any Grade 3 event deemed related to study products
- Any Grade 4 event
- Clinical reasons determined by the investigator or PSRT

9.5 Criteria for Progression to the Next Dosing Phase

In the event of a Grade 2 event deemed related to study product, the PSRT will consider participant safety in determining if the participant may remain on protocol with close follow-up.

9.6 Criteria for Early Termination of Study Participation

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

9.7 Study Participant Replacement

Individuals that miss or incorrectly complete a PK intensive visit may be replaced at the discretion of the protocol chair. Accordingly, the protocol chair will determine if research participants who fail to complete all biopsy collection sessions should be replaced. Replacement subjects would be assigned the same sampling schedule as the subject they are replacing.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and Summary of Design

Eligible participants will receive 300 mg TDF/200 mg FTC (Truvada®, Gilead Sciences), once daily for seven days under direct observation to achieve steady state drug PrEP concentrations. After one week of therapy, participants will undergo intensive PK analysis as well as collection of colorectal biopsies for PD testing (PK1). During the PK-intensive day, iohexol will be administered intravenously for the empirical determination of renal function and glomerular filtration rate (GFR). While concurrently on PrEP, participants will then be intramuscularly administered depot leuprolide acetate (11.25 mg Lupron^{TM}). Two weeks post-injection, sampling for PK, PD, and renal function will be performed (PK2). Participants will then immediately begin low-dose oral estrogen therapy (1 mg 17β-estradiol) in conjunction with PrEP for one week, at which time samples will be collected for the analyses described above (PK3). While on PrEP, participants will then transition to high-dose estrogen therapy (6 mg 17β-estradiol) for the remainder of the study. One week post-high dose estrogen therapy in the presence of PrEP, pharmacological and renal samples will be collected for analysis (PK4). PrEP will then be discontinued, and two weeks later, samples will be collected to assess renal function and hormonal concentrations (PK5). The presence of any remaining PrEP in plasma, PBMC or colorectal tissue will also be evaluated. Truvada® PrEP or hormone drug dosing will be administered under direct observation (DOT). Safety assessments include history/physical, chemistry/hematology labs at screening and interim history before each phase of the study.

10.2 Study Endpoints

Study endpoints to be assessed are each paired with specific objectives as listed in Section 3, Objectives & Endpoints.

10.3 Study Hypotheses

This protocol and derived data will provide information to test the following study hypotheses:

- GAHT decreases TDF/FTC PrEP concentrations in an estradiol dosage-dependent manner; but there is no effect of TDF/FTC PrEP on feminizing GAHT (as assessed by testosterone measurements).

10.4 Accrual and Sample Size

Up to a total of 40 individuals will be enrolled at at JHU, in order to complete 20 evaluable participants. Participants will be HIV-1 seronegative, self-identify as a TGW, generally healthy, and at least 18 years of age. Healthy participants must also not be on any hormonal therapies, or be willing to abstain from estrogen or anti-androgenic therapies, until testosterone concentrations reach > 2 ng/mL.

Based on prior studies with similar eligibility requirements, is the expected enrollment rate would be 1 participant per month. Therefore, accrual is expected to take approximately 20 months.

For paired analyses of change within participants between the 5 pharmacologic conditions, 20 research participants provides 80% power to detect a 37% relative difference in PBMC TDF-DP concentration, assuming a standard deviation of 0.3 (in terms of log10 concentration), which is the observed standard deviation of PBMC TFV-DP measurements in the HPTN 066 trial data set. This

will provide sufficient power to detect a similar change in active drug concentrations in PBMC as we saw with plasma TFV in the TGW Pilot study.

10.5 Randomization and Blinding

Enrolled participants will not be randomized, as this is by necessity an open-label study. This will be an unblinded study. As described in Section 9, in the event that a study site investigator is concerned that a participant might be put at undue risk by continuing product use, the product use may be discontinued.

10.6 Data Analysis

10.6.1 Descriptive Statistics and Graphics

Descriptive statistics and graphics will be used to summarize the characteristics of endpoints among the five dosing phases. Continuous measures will be summarized by means and standard deviations or median and interquartile range depending on data distribution. For binomial endpoints, 95% exact confidence intervals will be calculated. Given the small sample size and possible non-normality of the data, non-parametric tests will be used to test for differences among (Kruskal-Wallis) and between (Wilcoxon rank sum test) test formulations.

10.6.2 Safety

For the safety analysis, the number and the frequency of all adverse events (AEs) will be tabulated for each of the five dosing phases. To be able to determine whether excessive AEs are occurring, the proportion of subjects that experience an AE will be calculated for each phase. A single summary outcome of this type (yes/no) can be reasonable assumed to follow a Bernoulli distribution.

Table shows for selected true underlying rates between .01 and .20 the probability of one or more participants experiencing AE's in a sample of 20. From this table we can see that we have at least a 80% chance of one or more participants experiencing an AE when the true rate is at least 8% and a probability of approximately 18% when the true rate is no more than 1%. Unless the true rate is at least 2%, the probability of two or more subjects experiencing AEs will be quite small – 5%.

Table 18: Probability of Events for Selected True Rates of AEs

Event Rate	Probability of missing toxicity (1-event rate)^n	Power to detect one or more events 1-(1-p)^n	Power to detect two or more events	Power to detect three or more events
0.01	0.818	0.182	0.017	0.001
0.02	0.668	0.332	0.060	0.007
0.03	0.544	0.456	0.120	0.021
0.04	0.442	0.558	0.190	0.044
0.05	0.358	0.642	0.264	0.075
0.06	0.290	0.710	0.340	0.115
0.07	0.234	0.766	0.413	0.161
0.08	0.189	0.811	0.483	0.212
0.09	0.152	0.848	0.548	0.267

0.10	0.122	0.878	0.608	0.323
0.11	0.097	0.903	0.662	0.380
0.12	0.078	0.922	0.711	0.437
0.13	0.062	0.938	0.754	0.492
0.14	0.049	0.951	0.792	0.545
0.15	0.039	0.961	0.824	0.595
0.16	0.031	0.969	0.853	0.642
0.17	0.024	0.976	0.877	0.685
0.18	0.019	0.981	0.898	0.725
0.19	0.015	0.985	0.916	0.761
0.20	0.012	0.988	0.931	0.794

Additional safety analyses will also tabulate the number and type of AEs observed overall, and by severity, site, and study product. AEs that lead to discontinuation of study participation will be tabulated separately.

10.6.3 Pharmacokinetics

Pharmacokinetic parameters in all matrices will be estimated using non-compartmental analysis, including the maximum concentration (Cmax), time to maximum concentration (Tmax), and area under the concentration versus time curve (from 0-24 hours, AUC0-24h) for plasma TFV and FTC. These will be summarized using descriptive statistics by PK period. Colorectal tissue concentrations of TFV, FTC, TFV-DP, and FTC-TP will be summarized by time interval and PK period using descriptive statistics. Sample size is conditioned primarily on plasma TFV and FTC as well as PBMC TFV-DP and FTC-TP.

10.6.4 Explant Studies

Pharmacodynamic antiviral effects will be explored by assessing HIV-1 p24 antigen production after ex vivo HIV challenge of colorectal biopsy explants from participants sampled at each PK phase of the study. Paired PK-PD data pairs will be pooled across all study participants within each condition and fit to variations of the traditional Emax model to establish the concentration-response relationship for each condition. Comparisons across conditions will also be compared. Sample size is not conditioned on this readout.

10.6.5 Missing Data

All reasonable efforts will be made to obtain complete data for all participants; however, missing observations will occur due to missed visits, participants lost to follow-up, or noncompliance. Research participants who fail to complete dosing and evaluations relevant to all study product doses for any reason may be replaced at discretion of the study site investigator after consultation with the Sponsor. Data for PK, distribution, and permeability will be required of all periods of the study or the research participant will be replaced.

Plasma and PBMC samples obtained outside the specified window due to schedule conflicts with anoscopy and biopsy collection will not substantially affect our ability to capture data needed for study endpoints and will not adversely affect achieving related study objectives.

As previously noted, all available data will be used for safety purposes. All available data will also be used for descriptive purposes. Formal statistical comparisons between study phases, however, must necessarily be limited to subjects who complete use of all dosing phases.

The familywise error rate corrections will be used to adjust for multiple comparisons.

Missing data adjustment methods will be investigated using multiple imputation and sensitivity analyses in the event of possible informative missingness.

11 DATA HANDLING AND RECORDKEEPING

11.1 Data Management Responsibilities

Study staff will provide case report forms and site Standard Operating Procedures as part of the study activation process. All case report forms that will be used as source documents will be identified.

Close coordination between protocol team members will be necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, adherence, and follow-up will be monitored closely by the team.

11.2 Source Documents and Access to Source Data/Documents

All study sites will maintain source documents and data in accordance with *Requirements for Source Documentation:*

https://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf

Each investigator will maintain and securely store complete, accurate, and current study records throughout the study. This described study is a non-IND trial and there are no investigational products involved in the study. There will be no marketing application based on the data or findings from this work.

Study records must be maintained on site for the entire period of study implementation. Thereafter, instructions for record storage will adhere to the DAIDS Storage and Retention of Clinical Research Records Policy. No study records may be moved to an off-site location or destroyed prior to receiving approval from both DAIDS and Sponsor.

11.3 Quality Control and Quality Assurance

Quality control and quality assurance procedures will be performed by all study sites as specified in *Requirements for Clinical Quality Management Plans:*

https://www.niaid.nih.gov/sites/default/files/qmppolicy.pdf

11.4 Study Coordination

Study implementation will also be guided by a common study-specific procedures manual that provides further instructions and operational guidance on conducting study visits; data and forms processing; specimen collection, processing, and shipping; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations.

Close coordination between protocol team members is necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, retention, follow-up, and AE incidence will be monitored closely by the team.

12 CLINICAL SITE MONITORING

Non-network study monitoring will be carried out with prior approval of the study monitoring plan by DAIDS. Site monitoring visits will be conducted to assess overall study compliance, as required per *Requirements for On-Site Monitoring*:

https://www.niaid.nih.gov/sites/default/files/onsitemonitor_reqs.pdf

Study monitors will visit the site to complete the following:

- Assess compliance with the study protocol, Good Clinical Practices (GCP) guidelines, and applicable regulatory requirements, including US CFR Title 45 Part 46 and Title 21 Parts 50, 56, and 312
- Review informed consent forms, procedures, and documentation
- Perform source document verification to ensure the accuracy and completeness of study data
- Verify proper collection and storage of biological specimens
- Verify proper storage, dispensing, and accountability of investigational study products
- Assess implementation and documentation of internal site quality management procedures
- Assess site staff training needs

Johns Hopkins University Clinical Pharmacology will provide site study monitoring. Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, CRFs), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of DAIDS and U.S. regulatory authorities. A site visit log will be maintained at the study site to document all visits. The outcomes of the monitoring visits and the subsequent reports of resolutions of any identified problems will be provided to DAIDS.

13 HUMAN SUBJECTS PROTECTIONS

The investigators will make efforts to minimize risks to participants. Volunteers and study staff members will take part in a thorough informed consent process. Before beginning the study, the investigators will have obtained IRB approval, and the protocol will have been submitted to the appropriate regulatory agencies involved in this trial, including amendment of IND# 126136. The investigators will permit audits by the NIH, Sponsor, and Office for Human Research Protections (OHRP), the FDA or any of their appointed agents.

13.1 Institutional Review Boards

The participating study site is responsible for assuring that this protocol, the associated site-specific informed consent documents, and study-related materials are reviewed by an IRB responsible for oversight of research conducted at the study sites. Any amendments to the protocol, informed consent documents, and other study-related materials must be approved by the study site IRB prior to implementation.

Subsequent to the initial review and approval, the study site IRB must review the study at least annually. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others.

13.2 Protocol Registration

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

Site-specific informed consent forms (ICFs) *WILL NOT* be reviewed or approved by the DAIDS PRO, and sites will receive an Initial Registration Notification when the DAIDS PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. The site will not receive any additional notifications from the DAIDS PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

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

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the *DAIDS Protocol Registration Manual*, available at
https://www.niaid.nih.gov/sites/default/files/prmanual.pdf

13.3 Risk-Benefit Statement

13.3.1 Risks

General

Disclosure of STI status may cause sadness or depression in volunteers. Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of

questions. Participants may experience slight embarrassment from receiving a douche administered by the clinical staff. Although all efforts will be made to protect participant privacy and confidentiality, it is possible that participant's involvement in the study could become known to others, and that social harms may result (i.e. become known as HIV-infected or at "high risk" for HIV infection). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families, employer, and/or communities.

Participants in sites requiring partner notification in response to diagnosed STIs or HIV infection could have problems in their relationships with their sexual partners. Participants also could have problems in their partner relationships associated with use or attempted use of study products.

Risk of blood draws and PIVs

Phlebotomy and insertion and use of peripheral intravenous catheters (PIVs) are generally safe procedures, but there are some risks. Common risks include discomfort, site pain, tenderness, bleeding, bruising, redness, or swelling due to vein irritation from the needle or catheter. Less common risks include local site infection, infiltration or the leakage of fluid into the surrounding area/tissue, or clotting of the vein. In rare cases, it may result in fainting. Very rarely PIVs can be associated with a venous air embolism where air enters the vein and can potentially travel to your heart, brain, or lungs and cause damage. Study staff will adhere to universal precautions and infection control guidelines to reduce risks.

Rectal Swabs

There is the risk of mild discomfort from this procedure, in addition to a slight risk of bleeding.

General Risks of Cleansing Douche

The main risk from having a douche is temporary discomfort. The douche bottle nozzle consists of hollow plastic tube, tapered slightly toward the opening, and coated with paraffin as lubricant. It is about the thickness of a pencil and will be passed through the anal canal to administer approximately 120-125 mL of fluid into the rectum. This may cause a “bloated” or “cramping” feeling. Some air may be pumped into the rectum as well, causing flatulence. The tube is small, but it might cause some anal or rectal discomfort if the subject has any hemorrhoids or other painful conditions. There is a remote possibility that the douche tip could cause perforation.

TDF/FTC PrEP

In PrEP trials of TDF/FTC, more than 2% of participants receiving TDF/FTC reported adverse reactions of headache, abdominal pain and decreased weight more frequently than those receiving placebo. Other side effects associated with TDF/FTC are less frequent. Side effects associated with use of TDF or FTC are listed below:

The following side effects have been associated with the use of TDF:
- Upset stomach, vomiting, gas, loose or watery stools
- Generalized weakness
- Dizziness
- Depression
- Headache
- Abdominal pain
- Worsening or new kidney damage or failure
- Inflammation or swelling and possible damage to the pancreas and liver
- Shortness of breath

• Rash
• Allergic reaction: symptoms may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, a general feeling of illness or a potentially serious swelling of the face, lips, and/or tongue
• Bone pain and bone changes such as thinning and softening which may increase the risk of breakage
• Muscle pain and muscle weakness

The following side effects have been associated with the use of FTC:
• Headache
• Dizziness
• Tiredness
• Inability to sleep, unusual dreams
• Loose or watery stools
• Upset stomach (nausea) or vomiting
• Abdominal pain
• Rash, itching, which sometimes can be a sign of an allergic reaction
• Skin darkening of the palms and/or soles
• Increased cough
• Runny nose
• Abnormal liver function tests, which could mean liver damage
• Increases in pancreatic enzyme
• Increased triglycerides
• Increased creatine phosphokinase, which could mean muscle damage

Leuprolide acetate

Commonly reported side effects of leuprolide include: arthropathy, asthenia, decreased testicular size, dermatological reaction, edema, gastrointestinal disease, headache, hot flash, increased lactate dehydrogenase, pain, signs and symptoms of injection site, urination disorder, and diaphoresis.
Other side effects include: respiratory system disorder, abnormal hepatic function tests, dehydration, dizziness, dyspnea, impotence, increased serum alkaline phosphatase, increased serum aspartate aminotransferase, insomnia, myalgia, nausea and vomiting, neuromuscular disease, paresthesia, prolonged partial thromboplastin time, sleep disorder, and vertigo.

17β-estradiol

Commonly reported side effects of estradiol include: cerebrovascular accident, infection, malignant neoplasm of breast (in women, though theoretical in TGW), headache, and mastalgia.
Other side effects include: abdominal pain, limb pain, pruritus, sinusitis, nausea, and skin rash. Venous thromboembolism risk associated with oral estradiol varies in the literature ranging from no additional risk to 4-times greater than background risk, which is itself low, ranging from 1 in 1,000 to 1 in 10,000 population. Several cases of prolactin elevations and growth of pituitary prolactinoma have been reported.

Iohexol Infusions

Iohexol is approved for use as an intravenous contrast agent, but is very commonly used in clinical research settings for direct GFR measurements in healthy research participants. Side effects of iohexol associated with intravenous use are generally either (1) chemotoxic reactions resulting from the physicochemical properties of the contrast media, as well as the dose, speed of injection, and hemodynamic disturbances and/or injuries to organs or vessels perfused by the contrast medium; or,

(2) idiosyncratic reactions including all other reactions, which occur more frequently in patients 20 to 40 years old. Idiosyncratic reactions may or may not be dependent on the amount of dose injected, the speed of injection, and the radiographic procedure. Idiosyncratic reactions are subdivided into minor, intermediate, and severe. The minor reactions are self-limited and of short duration; the severe reactions are life-threatening and treatment is urgent and mandatory. As in our prior use of iohexol (JHMIRB Protocol #IRB00033945), we will obtain an IND exemption for an FDA approved drug being used in a non-approved population. This is regularly approved under 21 CFR 312.2(b) given that there is no intent to modify the label, no intent for commercial purposes, performed under IRB approval, and the dose, route, frequency, and intended population does not pose an increased risk with iohexol use.

Flexible Sigmoidoscopy with Rectal Tissue Biopsies

Flexible sigmoidoscopy is a commonly practiced medical procedure and the endoscopic procedures done in this trial will not involve any unusual risks or discomforts. The risks associated with these procedures include mild discomfort and the feeling of having a “bloated stomach”. Endoscopic biopsies are painless and heal quickly, usually within 3 days. On extremely rare occasions, the endoscopic procedure or biopsies may lead to pain, infection (sepsis), bleeding or perforation of the gastrointestinal tract. Perforation occurs approximately once out of every 1 in 1,000 to 10,000 procedures. If this extremely rare complication occurs, antibiotics and surgery to repair the tear may be necessary.

Prep instructions for sigmoidoscopy will include no solid food eight hours prior to the procedure. A cleansing douche will be administered as enema prep for all flexible sigmoidoscopy procedures

13.3.2 Benefits

Participants in this study will experience no direct benefit. Participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of safe and effective interventions to prevent HIV transmission. Participants also may appreciate the benefit of earlier diagnosis of STIs; additionally, participants will be referred for treatment for any incidental findings detected during screening and other study-related examinations.

13.4 Informed Consent Process

It is the responsibility of the study site investigator to ensure that the Elements of Informed Consent (21 CFR 50.25 and ICH GCP 4.8.10) and Health Insurance Portability Accountability Act (HIPAA) guidelines are followed and documented in the source document file. The process for obtaining informed consent from potential research participants should be clearly documented and appropriately filed within the site’s standard operating procedures.

Written informed consent will be obtained from each study participant prior to initiation of any study procedures. Written informed consent also will be obtained for long-term specimen storage and possible future testing. In obtaining and documenting informed consent, the investigators and their sub-investigators will comply with applicable local and US regulatory requirements and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with the Requirements for Source Documentation at:

https://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf

Participants will be provided with copies of the informed consent forms. The study site is responsible for developing study informed consent forms for local use, based on the templates in the Appendices that describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations.

The informed consent process will cover all elements of informed consent required by research regulations. In addition, the process will specifically address the following topics of importance to this study:

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

The informed consent process will include an assessment of each potential participant's understanding of concepts identified by the protocol team as essential to the informed consent decision. Study sites will follow the IRB guidance regarding this assessment.

If during the trial, a consent revision is being presented, including new information that might affect the research participant's willingness to participate, participants will be informed of the revisions. If a research participant terminates the study and consent form revision occurs after their participation has ended, they do not need to sign the revised consent form.

13.5 Participant Confidentiality

All study procedures will be conducted in private and every effort will be made to protect participant privacy and confidentiality to the extent possible. The study site will implement confidentiality protections that reflect the local study implementation plan and the input of study staff and community representatives to identify potential confidentiality issues and strategies to address them. In addition to local considerations, the protections described below will be implemented at all sites.

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens and other reports will be identified by a coded number only, to maintain participant confidentiality. Forms, lists, logbooks, appointment books and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All local databases will be secured with password-protected access systems. Participants' study information will not be released without their written permission, except as necessary for review, monitoring, and/or auditing.

The site investigator will make study documents (e.g., consent forms, drug distribution forms, eCRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the FDA, the NIAID, the OHRP, JHU legal representatives (attorneys that may review records in the case where there is concern about a possible research-related injury or violation of confidentiality) and other JHU staff and other local, US, or international regulatory entities for confirmation of the study data.

13.6 Special Populations

This section outlines considerations made for the inclusion or exclusion of special populations in this study.

13.6.1 Women

All minorities are eligible for the proposed studies, and no individual will be excluded on the basis of racial or ethnic background. We expect the racial and ethnic composition of our studies to be similar to the demographics of the community served by the Johns Hopkins Hospital, Chase-Brexton Health Services and Whitman-Walker Health (referral bases). Approximately 70% of the transgender population are racial and ethnic minorities at both our primary patient referral partner, Chase-Brexton Clinic, as well as in our past transgender women (TGW) clinical studies in our Drug Development Unit (DDU) at The Johns Hopkins Hospital. Focused ongoing efforts to encourage clinical trial participation by minorities within the Johns Hopkins program have resulted in enhanced participation. This high percentage of racial and ethnic minorities also reflects the demographics of the larger HIV epidemic in Baltimore and Washington, DC.

This is a phase I pharmacokinetic study targeted to self-identifying TGW. We will exclude cisgender men and cisgender women from participation in the study based on study design. Inclusion criteria include self-identifying TGW who are naïve to gender affirming hormonal therapy or are willing to abstain from feminizing therapies until total testosterone concentrations reach a cisgender male reference interval. As the proposed study is aimed to evaluate drug-drug interactions between PrEP and exogenous gender affirming estrogen-based hormonal therapies, we are therefore limiting enrollment to self-identifying TGW to increase the efficiency of successful recruiting based on behavioral grounds.

13.6.2 Children

Pediatric TGW aged 18-21 are eligible to participate and provide clinical materials for laboratory studies. Children less than 18 years of age will be excluded.

13.7 Compensation

Pending IRB approval, participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits, child care, and time away from work according to site's practice. Reimbursement amounts will be specified in the site's informed consent form.

13.8 Communicable Disease Reporting

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

13.9 Access to HIV-related Care

13.9.1 HIV Counseling

HIV test-related counseling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study. Testing will be performed in accordance with the algorithm in Appendix II. Counseling will be provided in accordance with standard HIV counseling policies and methods at the participating study site and additionally will emphasize the unknown efficacy of the study products in preventing HIV infection. In accordance with NIH policies, participants must receive their HIV test results to take part in this study. Referral for additional counseling related to testing or diagnosis will occur if needed or requested by the participant.

13.9.2 Care for Participants Identified as HIV-infected

Participants will be provided with their HIV test results in the context of post-test counseling. Per site SOPs, participants found to be HIV-infected will be referred to available sources of medical and psychosocial care and support, and local research studies for HIV-infected adults.

13.10 Study Discontinuation

This study may be discontinued at any time by DAIDS/NIAID, Sponsor, , the OHRP, or site IRB.

14 LIST OF APPENDICES

APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS
APPENDIX II: HIV TESTING ALGORITHM
APPENDIX III: RECTAL TISSUE BIOPSY ALLOCATION
APPENDIX IV: TOXICITY TABLES
APPENDIX V: SAMPLE INFORMED CONSENT

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APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

STUDY VISIT		Screen	Baseline	DOT	PK SERIES 1 D1	PK SERIES 1 D2	DOT	Safety	DOT	PK SERIES 2 D1	PK SERIES 2 D2	DOT	PK SERIES 3 D1	PK SERIES 3 D2	DOT	PK SERIES 4 D1	PK SERIES 4 D2	DOT	Safety	DOT	PK SERIES 5 D1	PK SERIES 5 D2	Safety (+ 2 weeks)
Visit Number		1	2	3-8	9	10	11-15	16	17-22	23	24	25-29	30	31	32-36	37	38	39-43	44	45-50	51	52	53
Study Day		-60	0	1-6	7	8	9-13	14	15-20	21	22	23-27	28	29	30-34	35	36	37-41	42	43-48	49	50	56
ADMINISTRATIVE																							
Informed Consent		x																					
Eligibility assessment		x	x																				
Obtain/Update Locator Information		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Obtain/Update Medical History/Concomitant Meds		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Assess/Update AEs		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Adherence Monitoring			x		x					x			x			x					x		
Gender Dysphoria Assessment			x		x					x			x			x					x		
CLINICAL																							
Physical Exam[1]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	x	x
Vital Signs (BP HR, Temp, Ht^2, Wt^2)		x	x		x	x		x		x	x		x	x		x	x		x		x	x	x
Insert PIV x 2 (if applicable)			x		x					x			x			x					x		
Hormone Testing	Estradiol	x	x																				x
	Testosterone (total only)	x	x							x													x
Safety Labs	CBC w/ Diff	x	x					x															x
	CMP (eGFR)	x	x					x															x
	PO_4	x	x					x															x
	PT/PTT	x																					
	Serum Cr (eGFR)				x					x			x			x					x		
	Lipid panel[5]	x																					x
STD Testing: Blood	HBsAg	x																					
	Syphilis	x																					x
	HIV	x	x		x			x		x			x			x					x		x

STUDY VISIT		Screen	Baseline	DOT	PK SERIES 1 D1	PK SERIES 1 D2	DOT	Safety	DOT	PK SERIES 2 D1	PK SERIES 2 D2	DOT	PK SERIES 3 D1	PK SERIES 3 D2	DOT	PK SERIES 4 D1	PK SERIES 4 D2	DOT	Safety	DOT	PK SERIES 5 D1	PK SERIES 5 D2	Safety (+ 2 weeks)
Visit Number		1	2	3-8	9	10	11-15	16	17-22	23	24	25-29	30	31	32-36	37	38	39-43	44	45-50	51	52	53
Study Day		-60	0	1-6	7	8	9-13	14	15-20	21	22	23-27	28	29	30-34	35	36	37-41	42	43-48	49	50	56
CLINICAL CONTINUED																							
STD Testing: Rectal Swab	GC/CT NAAT	x																					x
Cleansing Douche			x			x					x			x			x					x	
DOSING/STUDY PRODUCT																							
Iohexol Administration			x		x					x			x			x					x		
Dispense TDF/FTC			x			x		x			x			x									
DOT Dose of TDF/FTC				x	x	x	x	x	x	x	x	x	x	x	x	x								
Lupron Injection®						x																		
Dispense Estradiol 1 mg											x													
DOT Dose of Estradiol 1mg											x	x	x											
Dispense Estradiol 6 mg														x			x		x					
DOT Dose of Estradiol 6mg														x	x	x	x	x	x	x	x			
STUDY SPECIMEN COLLECTION																							
Serum Estradiol			x		x					x			x			x					x		
Serum Testosterone (free and total)			x		x					x			x			x					x		
Serum LH, FSH			x		x					x			x			x					x		
Serum Cystatin			x		x					x			x			x					x		
Plasma PK TFV, FTC	Pre-Dose				x					x			x			x					x		
	1hr																						
	2hr																						
	3hr																						
	4hr																						
	24hr					x					x			x			x					x	
PBMC TFV-DP, FTC-TP	Pre-Dose				x					x			x			x					x		
	24hr					x					x			x			x					x		

STUDY VISIT		**Screen**	**Base line**	**DOT**	**PK SERIES 1 D1**	**D2**	**DOT**	**Safety**	**DOT**	**PK SERIES 2 D1**	**D2**	**DOT**	**PK SERIES 3 D1**	**D2**	**DOT**	**PK SERIES 4 D1**	**D2**	**DOT**	**Safety**	**DOT**	**PK SERIES 5 D1**	**D2**	**Safety (+ 2 weeks)**
Visit Number		1	2	3-8	9	10	11-15	16	17-22	23	24	25-29	30	31	32-36	37	38	39-43	44	45-50	51	52	53
Study Day		-60	0	1-6	7	8	9-13	14	15-20	21	22	23-27	28	29	30-34	35	36	37-41	42	43-48	49	50	56
STUDY SPECIMEN COLLECTION CONTINUED																							
Iohexol GFR	Pre-Dose		x		x					x			x			x					x		
	2hrs																						
	4hrs																						
Urine TFV, FTC, Cr	0-4hr				x					x			x			x					x		
	4-24hr					**x**					**x**			**x**			**x**					**x**	
Colon Biopsies TFV, FTC, TFV-DP, FTC-TP, Explant HIV Challenge[6]			x			x					x			x			x					x	

[1] Full physical exam at screening only, targeted PE at all other visits as needed

[2] Height at screening only, weight at screening and iohexol visits only

[3] Additional test may be ordered for abnormal results

[4] DOT may consist of in person observation, live video streaming, or with use of a time-stamped video

[5] Fasting, total cholesterol, triglycerides, HDL, LDL

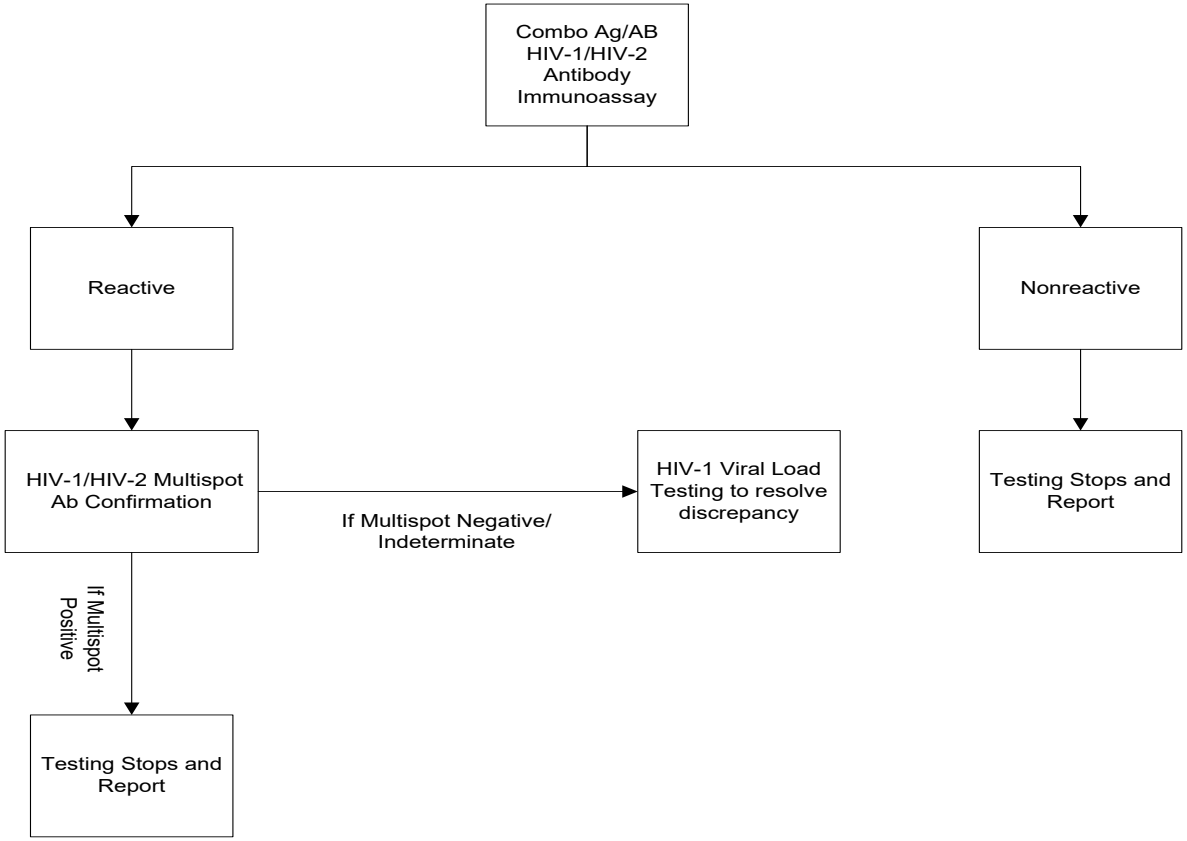
[6] Colonic tissue collection for PK/PD - 5 total biopsies collected, 2 for PD (explant), 3 for PK studies.

[7]If a participant has difficult vein access and is not a candidate for 2 PIVs, a single PIV is acceptable; a saline flush will be performed after iohexol administration, and a waste tube will be collected prior to collection of samples for iohexol measurement of drug quantification.

APPENDIX II: HIV TESTING ALGORITHM

In accordance with CDC guidelines for HIV serology testing, The Johns Hopkins Hospital HIV Laboratory has moved to a 4th generation assay which is a more sensitive and specific assay. The 4th generation assay is a combined antigen and antibody assay for HIV1 and HIV2 serological detection. All reactive samples will be confirmed with a rapid multi-spot assay for HIV-1 and HIV-2. The Western blot confirmatory assay is no longer performed. Any discrepant results will go on to HIV RNA viral load testing. As with the previous HIV serology, no reactive results will be released prior to confirmatory testing.

JHH HIV Lab HIV Screening Testing Algorithm Overview



APPENDIX III: Rectal Tissue Biopsy Allocation

Table 19. Number of Samples Taken at Each Biopsy Visit & Their Allocation for Testing

	Baseline	24 hr Post-PK Intensive Visits
PK (tissue homogenates)	3	3
Explant	2	2

Of note, the overall total number of biopsies for the entire study, given one baseline visit and the sampling visits, will be 30 biopsies per participant.

APPENDIX IV: TOXICITY TABLES

The *Division of AIDS Rectal Grading Table for Use in Microbicide Studies (DAIDS AE Grading Table Addendum 3, Clarification dated May 2012)* will be the primary tool for grading adverse events for this protocol.

Adverse events not included in the *Rectal Grading Table* will be graded by the most current *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. (July 2017).* In cases where an AE is covered in both tables, *DAIDS AE Grading Table Addendum 3* will be the grading scale utilized.

All Division of AIDS grading tables are available online at: http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx

APPENDIX V: SAMPLE INFORMED CONSENT FORM

This is a sample consent form document only. Each participating site is responsible for developing its own informed consent documents. Institutions may require specific informed consent language (e.g., HIPAA language), have different policies on compensation, financial obligation, costs and injury, etc.