A5403

Giving Standardized Estradiol Therapy In Transgender Women to Research Interactions with HIV Therapy: the GET IT RIgHT Study

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

Sponsored by:
National Institute of Allergy and Infectious Diseases

IND #162616

The Antiretroviral Therapy Strategies Transformative Science Group:	**Timothy Wilkin, MD, Chair**
Protocol Chair:	**Jordan E. Lake, MD, MSc**
Protocol Co-Chair:	**Kimberly Scarsi, PharmD, MS**
Protocol Vice Chair:	**Jorge A. Gallardo-Cartagena, MD**
DAIDS Clinical Representative:	**Pablo Belaunzaran Zamudio, MD, DTM&H, MSc**
Clinical Trials Specialist:	**Osarenoma Salawu, MS**

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SIGNATURE PAGE

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 US 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.

Principal Investigator: _____
Print/Type

Signed: _____ Date: _____
Name/Title

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SITES PARTICIPATING IN THE STUDY

A5403 is a multicenter study being conducted at US and non-US clinical research sites.

PROTOCOL TEAM ROSTER

Chair
Jordan E. Lake, MD, MSc
Houston AIDS Research Team CRS
6431 Fannin Street, MSB 2.112
Houston, TX 77030
Phone: 713-500-6759
E-mail: jordan.e.lake@uth.tmc.edu

Co-Chair
Kimberly K. Scarsi, PharmD, MS, FCCP
Northwestern University CRS
University of Nebraska Medical Center
College of Pharmacy
Department of Pharmacy Practice, Room 3021
986145 Nebraska Medical Center
Omaha, NE 68198-6145
Phone: 402-559-9916
E-mail: kim.scarsi@unmc.edu

Vice Chair
Jorge A. Gallardo-Cartagena, MD
Barranco CRS
Av. Almirante Miguel Grau 1010
Lima 15063 PERU
Phone: 51-1-2067800
E-mail: jgallardo@citbm.pe

DAIDS Clinical Representative
Pablo Belaunzaran Zamudio, MD,
DTM&H, MSc
HIV Research Branch
TRP, DAIDS, NIAID, NIH
5601 Fishers Lane, Room 9E40
Rockville, MD 20852
Phone: 240-292-4423
E-mail: belaunzaranzapabf@niaid.nih.gov

Clinical Trials Specialist
Osarenoma Salawu, MS
ACTG Network Coordinating Center
Social & Scientific Systems, Inc.
A DLH Holdings Company
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910-3714
Phone: 301-628-3000
E-mail: osarenoma.salawu@dlhcorp.com

Statisticians
Laura Smeaton, MS
Statistical and Data Analysis Center
Harvard TH Chan School of Public Health
FXB Building, Room 511, 651 Huntington Ave.
Boston, MA 02115-6017
Phone: 617-432-2525
E-mail: smeaton@sdac.harvard.edu

Joseph Puleo, MS
Statistical & Data Analysis Center
651 Huntington Avenue
FXB Building, Room **53**5
Boston, MA 02115-6017
Phone: **617-432-4402**
E-mail: **jpuleo**@sdac.harvard.edu

Data Managers
Lillian Collins, MPH
Frontier Science & Technology Research Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: 716-834-0900, Ext. 7308
E-mail: collins@frontierscience.org

Elizabeth Siciliano, MPH
Frontier Science & Technology Research Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: 716-834-0900, Ext. 7276
E-mail: siciliano@frontierscience.org

DAIDS Pharmacist
Oladapo Alli, PharmD
Pharmaceutical Affairs Branch
DAIDS, NIAID, NIH
5601 Fishers Lane, Room 9E16
MSC #9830
Rockville, MD 20852
Phone: 240-627-3593
E-mail: **oladapo.alli@nih.gov**

Virologist
Sara Gianella, MD
University of California, San Diego AntiViral Research Center CRS
Center For AIDS Research (CFAR)
9500 Gilman Drive
La Jolla, CA 92093-0679
Phone: 858-552-8585 Ext. 7193
E-mail: gianella@ucsd.edu

Pharmacologists
Mackenzie Leigh Cottrell, PharmD, MS
UNC Eshelman School of Pharmacy
120 Mason Farm Road
Chapel Hill, NC 27599
E-mail: mlcottre@email.unc.edu

Kimberly K. Scarsi, PharmD, MS, FCCP
Northwestern University CRS
University of Nebraska Medical Center
College of Pharmacy, Room 3021
986145 Nebraska Medical Center
Omaha, NE 68198-6145
Phone: 402-559-9916
E-mail: kim.scarsi@unmc.edu

Immunologist
Nicholas T. Funderburg, PhD
Ohio State University CRS
School of Health and Rehabilitation Sciences
535A Atwell Hall
453 West Tenth Avenue
Cleveland, OH 43210
Phone: 614-292-7303
E-mail: nicholas.funderburg@osumc.edu

Endocrinologist
Todd Brown, MD, PhD
Johns Hopkins University CRS
Division of Endocrinology and Metabolism
1830 East Monument Street
Baltimore, MD 21287
Phone: 410-502-2327
E-mail: tbrown27@jhmi.edu

Investigators

Carrie Johnston, MD, MS
Weill Cornell Chelsea CRS
1300 York Avenue, A-4
New York, NY 10065
Phone: 518-339-5209
E-mail: cmd9008@med.cornell.edu

Yamikani Mbilizi, MBBS
Blantyre CRS
P.O. Box 1131, Chipatala Avenue
Next to Lions Eye Hospital
Blantyre 265
MALAWI
Phone: 265-1875129
E-mail: ymbilizi@jhu.medcol.mw

Amaya Perez-Brumer, PhD
Dalla Lana School of Public Health
University of Toronto
155 College Street, 5th Floor, Room 554
Toronto, ON, M5T 3M7
CANADA
Phone: 416-978-5178
E-mail: a.perezbrumer@utoronto.ca

Eileen Scully, MD, PhD
Johns Hopkins University CRS
Rangos Building
855 N Wolfe Street, Room 530B
Baltimore, MD 21205
Phone: 410-502-4988
E-mail: escully1@jhmi.edu

Poongulali Selvamuthu, MBBS, MSc, PhD
Chennai Antiviral Research and Treatment (CART) CRS Medical Clinic
Rajiv Gandhi Road
Taramani Chennai 6000113
INDIA
Phone: 91-4426215736
E-mail: poongulali@cartcrs.org

Field Representatives

Joan Gottesman, BSN, RN, CCRP
Vanderbilt Therapeutics (VT) CRS
One Hundred Oaks
719 Thompson Lane, Suite 47183
Nashville, TN 37204
Phone: 615-936-7143
E-mail: joan.gottesman@vumc.org

Morgan Lima, BSN, RN
Vanderbilt Therapeutics (VT) CRS
One Hundred Oaks
719 Thompson Lane, Suite 47183
Nashville, TN 37204
Phone: 615-936-7448
E-mail: morgan.lima@vumc.org

Laboratory Technologist**s**

Lori Caruso, BS
University of Pittsburgh CRS
PTEU/Department of Medicine, ID Division
3550 Terrace Street
Scaife Hall, Room 808
Pittsburgh, PA 15261
Phone: 412-648-9012
E-mail: lcaruso@pitt.edu

Christopher Lane, BS, AAS
University of Rochester
Adult HIV Therapeutic Strategies Network CRS
Infectious Diseases Division
Department of Medicine, Box 689
601 Elmwood Avenue
Rochester, NY 14642
Phone: 585-2789-5574
E-mail: christopher_lane@urmc.rochester.edu

Community Scientific Subcommittee (CSS) Representative

Roslyn Swartz
University of Cape Town Lung Institute (UCTLI) CRS
George Street, Mowbray
Cape Town
SOUTH AFRICA
Phone: 27-603843466
E-mail: yvonieswartz@gmail.com

Community Representatives
Javiera Arnillas
José Santos Chocano 199
Bellavista 07006, Callao
PERU
Phone: +51 902982804
E-mail: a20130319@pucp.pe

B'Yancha Lawson
6431 Fannin Street, MSB 5.159
Houston, TX 77030
Phone: 713-500-5997
E-mail: Byancha.Lawson@uth.tmc.edu

International Site Specialist
Mary Allegra Cermak, MFA
ACTG Network Coordinating Center
Social & Scientific Systems, Inc.
A DLH Holdings Company
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910-3714
Phone: 301-628-3312
E-mail: allegra.cermak@dlhcorp.com

Laboratory Data Manager**s**
Frederic Bone
Frontier Science Foundation
4033 Maple Road
Amherst, NY 14226
Phone: 716-834-0900, Ext 7306
E-mail: bone@frontierscience.org

Sujith Valiyaparambil
Frontier Science Foundation
4033 Maple Road
Amherst, NY 14226
Phone: 716-834-0900, Ext 7291
E-mail: valiyaparambil@frontierscience.org

Laboratory Specialists
Cherisse Heirs, BA
ACTG Laboratory Center at UCLA (ALC-UCLA)
11075 Santa Monica Boulevard, Suite 200
Los Angeles, CA 90025
Phone: 585-397-4730
E-mail: cheirs@milabcentral.org

Laboratory Specialists (Cont'd)
Sara Zabih, MS
ACTG Laboratory Center at UCLA (ALC-UCLA)
University of California, Los Angeles
675 Charles E. Young Dr. South
MRL 4-774
Los Angeles, CA 90095
Phone: 310-794-9084
E-mail: szabih@milabcentral.org

Source Document Specialist
Josie M. Marshall, BS
ACTG Network Coordinating Center
Social & Scientific Systems, Inc.
A DLH Holdings Company
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910
Phone: 303-724-0803
E-mail: josie.marshall@dlhcorp.com

STUDY MANAGEMENT

All general questions concerning this protocol should be sent to actg.teamA5403@fstrf.org via e-mail. The appropriate team member will respond with a "cc" to actg.teamA5403@fstrf.org. A response should generally be received within 24 hours (Monday through Friday).

Protocol E-mail Group
Sites should contact the User Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the actg.protA5403 e-mail group. Include the protocol number in the e-mail subject line. Send an e-mail message to actg.user.support@fstrf.org.

In order to remove site personnel from the actg.protoA5403 e-mail group, contact the User Support Group at the DMC. Include the protocol number in the e-mail subject line.

- **Send an e-mail message to actg.user.support@fstrf.org.**

Clinical Management:
For questions concerning entry criteria, toxicity management, concomitant medications, and co-enrollment, contact the Clinical Management committee (CMC). Send an e-mail message to actg.cmcA5403@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory
For questions specifically related to immunologic, virologic, or pharmacologic laboratory tests, contact the Protocol Immunologist, Virologist, or Pharmacologists. Send an e-mail message to actg.teamA5403@fstrf.org (ATTENTION: Nicholas Funderburg [Immunologist], Sara Gianella [Virologist], or Mackenzie Leigh Cottrell and Kimberly Scarsi [Pharmacologists]).

Participant Registration
For participant registration questions or problems and study identification number SID lists: send an e-mail message to rando.support@fstrf.org or call the DMC Randomization Desk at 716-834-0900, extension 7301.

Data Management

- For nonclinical questions about transfers, inclusion/exclusion criteria, electronic case report forms (eCRFs), randomization/registration, and other data management issues, contact the data manager. Completion guidelines for eCRFs and participant-completed CRFs can be downloaded from the FSTRF website at www.frontierscience.org.
- For transfers, reference the Study Participant Transfer SOP 119, and contact Lillian Collins and Elizabeth Siciliano directly.
- For other questions, send an e-mail message to actg.teamA5403@fstrf.org (ATTENTION: Lillian Collins and Elizabeth Siciliano).
- Include the protocol number, PID, and a detailed question.

DMC Portal and Medidata Rave Problems
Contact DMC User Support. Send an e-mail message to actg.user.support@fstrf.org or call 716-834-0900 x7302.

Protocol Document Questions
For questions concerning the protocol document, contact the Clinical Trials Specialist. Send an e-mail message to actg.teamA5403@fstrf.org (ATTENTION: **Osarenoma Salawu**).

Copies of the Protocol
To request a hard copy of the protocol, send an e-mail message to ACTGNCC@dlhcorp.com. Electronic copies can be downloaded from the ACTG website at https://www.actgnetwork.org.

Product Package Inserts and/or Investigator Brochures
To request copies of product package inserts or investigator brochures, contact the DAIDS Regulatory Support Center (RSC) at RIC@tech-res.com or call 301-897-1708.

Protocol Registration
For protocol registration questions, send an e-mail message to protocol@tech-res.com or call 301-897-1707.

Protocol Activation
For questions related to protocol activation at US sites contact the Clinical Trials Specialist. Send an e-mail message to actg.teamA5403@fstrf.org (ATTENTION: **Osarenoma Salawu**).

For questions related to protocol activation at non-US sites contact the ACTG Site Coordination Group. Send an e-mail message to actgsitecoordination@dlhcorp.com.

Study Product
For questions or problems regarding study product, dose, supplies, records, and returns, call **Oladapo** Alli, Protocol Pharmacist, at 301-496-8213.

Study Drug Orders
Call the Clinical Research Products Management Center (CRPMC) at 301-294-0741.

IND (Investigational New Drug) Number or Questions
The IND number is available on the protocol-specific web page (PSWP). For any questions related to the IND submission, contact the DAIDS RSC at Regulatory@tech-res.com or call 301-897-1706.

Expedited Adverse Event (EAE) Reporting/Questions
Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

Telephone Calls
Sites are responsible for documenting telephone calls made to A5403 team members. Send an e-mail message to actg.teamA5403@fstrf.org.

Protocol-Specific Web Page
Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY OF PROTOCOL-SPECIFIC TERMS

3TC	lamivudine
3TC-TP	lamivudine triphosphate
ART	antiretroviral therapy
ARV	antiretroviral
AUC	area under the concentration-time curve
BIC	bictegravir
BMD	bone mineral density
CBC	complete blood count
CLIA	Clinical Laboratory Improvement Amendments
C-SSRS	**Columbia-Suicide Severity Rating Scale**
CV	coefficient of variation
DAIDS	Division of AIDS
DDI	drug-drug interactions
DHHS	United States Department of Health and Human Services
DRV/c	darunavir-cobicistat
DTG	dolutegravir
DVT	**deep vein thrombosis**
EAE	expedited adverse event
EFV	efavirenz
eGFR	estimated glomerular filtration rate
FDA	Food and Drug Administration
FHT	feminizing hormone therapy
FTC	emtricitabine
FTC-TP	emtricitabine triphosphate
GCLP	Good Clinical Laboratory Practice
GMR	geometric mean ratio
HIV-1	human immunodeficiency virus-1
INSTIs	integrase strand transfer inhibitors
IQA	immunology quality assurance
MACE	**major adverse cardiovascular event**
NIAID	National Institute of Allergy and Infectious Diseases
PD	pharmacodynamics
PI	protease inhibitor
PK	pharmacokinetics

PrEP	pre-exposure prophylaxis
PSWP	protocol-specific web page
PWH	people with HIV
SHBG	sex hormone binding globulin
SOC	standard of care
TAF	tenofovir alafenamide
TBL	**total bilirubin**
TDF	tenofovir disoproxil fumarate
TFV-DP	tenofovir diphosphate
TOST	two-one sided tests
TW	transgender women

SCHEMA

A5403
Giving Standardized Estradiol Therapy In Transgender Women to Research Interactions with HIV Therapy: the GET IT RIgHT Study

DESIGN — A5403 is a phase IIb, 48-week, open-label, non-randomized, three-group trial of adult transgender women (TW) and other individuals identifying as female or transfeminine but with male sex assigned at birth (henceforth referred to in aggregate as TW) living with human immunodeficiency virus-1 (HIV-1). Participants will be on antiretroviral therapy (ART) at entry and receive study-supplied 17-β estradiol for feminizing hormone therapy (FHT) for study weeks 0-48. The primary objectives of the study are to 1) assess whether TW continue to achieve therapeutic concentrations of ART while receiving FHT for 48 weeks, and 2) assess whether serum estradiol concentrations on FHT (across a range of estradiol doses) vary between boosted and unboosted ART regimens.

DURATION — 48 weeks on study.

SAMPLE SIZE — 90 participants (30 per group).

POPULATION — TW with HIV, ≥18 years of age, on ART for ≥24 weeks prior to study entry, on a study-defined ART regimen at screening or willing to switch to one of the below regimens for at least 28 days prior to study entry. Participants must be virologically suppressed at screening (HIV-1 RNA <200 copies/mL or below the lower limit of detection if the local assay lower limit of detection is >200 copies/mL), must have the most recent HIV-1 RNA measurement obtained through routine clinical care between 24 and 96 weeks prior to study entry that is <400 copies/mL, and not be on oral 17-β estradiol for 14 days prior to study entry or injectable 17-β estradiol 30 days prior to entry. Participants must be willing to initiate study provided 17-β estradiol, refrain from any non-study provided estradiol for the study duration, and have no plans for anti-androgen use for weeks 0-24. The trial will aim to enroll at least 50% of participants identifying as non-white or Latine.

REGIMEN — At entry, participants are assigned to analysis groups based on their ART regimen, as below. Participants on other ART regimens at screening, who are willing to switch to one of the below regimens through standard of care, may be enrolled. Participants who switch ART will have to demonstrate continued virologic suppression at a pre-entry visit at least 4 weeks post-switch.

Group 1: Participants taking bictegravir (BIC) + tenofovir alafenamide (TAF) + emtricitabine (FTC)

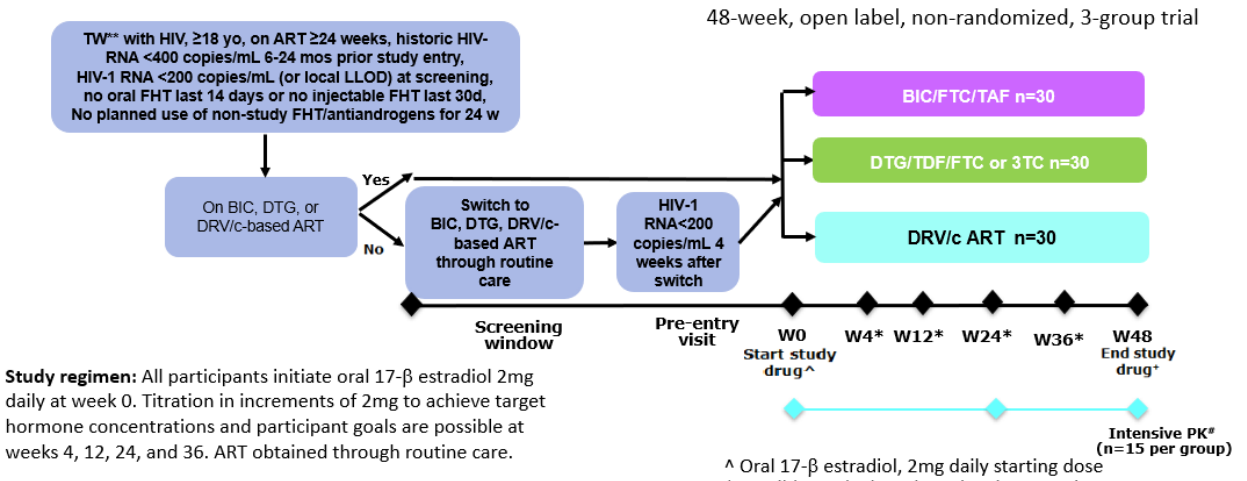
Group 2: Participants taking dolutegravir (DTG) **once daily** + tenofovir disoproxil fumarate (TDF) + (FTC or lamivudine [3TC])

Group 3: Participants taking any regimen containing darunavir plus cobicistat (DRV/c)

Note for all groups: ART will not be provided by the study.

Study treatment provided to all groups: Oral 17-β estradiol 2 mg once daily will be initiated at study entry. At weeks 4, 12, 24, and 36, study clinicians may titrate 17-β estradiol in 2 mg increments to achieve the desired participant goals and target hormone concentrations, as measured locally at each visit.

As phenotypic (physical) changes will lag behind hormone concentrations in many cases, and participant goals will also influence titration decision, guidance on estradiol titration will be provided to sites (see A5403 Manual of Procedures [MOPS]). However, titration will generally occur until hormone concentration targets are achieved. Participants will not be required to titrate to the maximum estradiol concentration, but titration above the recommended therapeutic range will not be permitted for safety reasons.



Study regimen: All participants initiate oral 17-β estradiol 2mg daily at week 0. Titration in increments of 2mg to achieve target hormone concentrations and participant goals are possible at weeks 4, 12, 24, and 36. ART obtained through routine care.

Additional key assessments: Trough samples for sparse ART and 17-β estradiol PK at each visit. FHT acceptability and satisfaction assessed by questionnaire (all) and structured interview (subset of participants) at entry, week 24, and week 48.

^ Oral 17-β estradiol, 2mg daily starting dose
* Possible study drug dose titration, 2mg increments
+ Transition to FHT through routine care
# Plasma ART and serum FHT concentrations pre-dose and 1, 2, 3, 4, 6, and 8 hours post-observed dose

**Identifies as a trans woman or identifies as female or transfeminine but was assigned male sex at birth

Schema Figure 1

1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Primary Hypotheses

1.1.1 Across oral 17-β estradiol doses from 2-10 mg daily, there will not be clinically significant reductions in ART exposure.

1.1.2 Over 48 weeks, participants receiving DRV-containing ART will have lower 17-β estradiol exposure than participants receiving DTG- or BIC-containing regimens.

1.2 Secondary Hypotheses

1.2.1 Adverse events related to 17-β estradiol will not differ by ART regimen but may differ by estradiol dose.

1.2.2 Participants receiving DRV-containing ART will require higher doses of 17-β estradiol to achieve similar feminizing hormone therapy (FHT) satisfaction as participants receiving BIC- or DTG-containing ART.

1.2.3 Virologic effectiveness will be maintained in each ART regimen plus FHT over 48 weeks.

1.2.4 Higher 17-β estradiol concentrations will be associated with greater weight gain and greater perturbations of lipids and insulin/glucose.

1.2.5 The relationship between 17-β estradiol dose and serum concentrations will reflect a dose proportional relationship.

1.3 Exploratory Hypotheses

1.3.1 Higher 17-β estradiol concentrations will be associated with decreased cellular HIV transcriptional activity, T cell cycling (Ki67), activation (HLA-DR$^+$CD38$^+$), and PD-1 expression.

1.3.2 Participants in all arms will perceive the intervention acceptability similarly.

1.3.3 17-β estradiol administration will result in changes in sex hormone profiles (e.g., lower total and free testosterone, higher sex hormone binding globulin (SHBG) and free estradiol).

1.3.4 Higher 17-β estradiol concentrations will be associated with greater perturbations of inflammatory biomarker profiles.

1.4 Primary Objectives

1.4.1 To estimate trough concentrations of received ARVs before versus with each dose of oral 17-β estradiol (2-10 mg), both in presence and in absence of concomitant anti-androgen use.

1.4.2 To compare the trough serum estradiol concentrations achieved, at each 17-β estradiol dose, between groups receiving boosted versus non-boosted ART.

1.5 Secondary Objectives

1.5.1 To compare occurrence of FHT-associated adverse effects across ART groups and estradiol doses.

1.5.2 To summarize intervention satisfaction across ART groups.

1.5.3 To compare virologic suppression within ART groups, at each received 17-β estradiol dose over 48 weeks.

1.5.4 To compare the PK parameters (AUC, C_{trough}, C_{max}, T_{max}) of 17-β estradiol over 48 weeks in a subset of TW undergoing intensive PK between groups receiving boosted versus non-boosted ART.

1.5.5 To assess changes in weight, anthropometrics, lipids, glucose, and insulin sensitivity in relationship to estradiol concentrations.

1.5.6 To assess the changes in estradiol concentrations across each 17-β estradiol dosage and ART group.

1.6 Exploratory Objectives

1.6.1 To explore the associations between 17-β estradiol concentrations and cellular HIV transcriptional activity and T cell phenotype (cycling, activation, and exhaustion).

1.6.2 To compare estradiol concentrations measured at a centralized research lab with real-time estradiol concentrations obtained through local labs.

1.6.3 To describe changes in sex hormones following 17-β estradiol administration.

1.6.4 To assess changes in circulating biomarkers of inflammation in relationship to estradiol concentrations.

1.6.5 To explore the relationship between ART and 17-β estradiol PK parameters through population PK modeling and covariate analyses.

1.6.6 To examine the association of 17-β estradiol concentrations and measures of satisfaction with gender affirming therapy using a population pharmacokinetic-pharmacodynamic (PK-PD) model.

1.6.7 To document intervention acceptability across ART groups.

2.0 INTRODUCTION

2.1 Background

Transgender women (TW) are the fastest-growing population of people with HIV (PWH) in the United States [1]. A survey of TW in the US in 2019-2020 found that 42.2% of 1,561 participants were living with HIV [2]. TW also have an extremely high (19%) global prevalence of HIV, and TW of reproductive age have 49-fold higher odds of acquiring HIV compared to a similar age group [3]. Historically, TW have had very few opportunities to participate in clinical research and experience barriers to accessing and engaging in care [4]. As such, there are extremely limited data available on ART optimization for TW with HIV and no evidence-based clinical guidance on the choice of specific agents.

Data suggest access to gender-affirming therapy improves time to initiation and uptake of HIV treatment and decreases treatment interruptions. Therefore, the Centers for Disease Control and Prevention (CDC) describes that access to gender-affirming care is critical to improving HIV outcomes among TW [2]. **Data also suggest that provision of gender-affirming care has been associated with reduced rates of depression, anxiety, and suicidality among transgender persons [5, 6].**

Feminizing Hormone Therapy (FHT): FHT is often an important component of gender-affirming care for TW. FHT typically include exogenous estrogens with or without concomitant anti-androgens [**7**]. However, the doses and regimens used vary widely by provider, geography, and patient preference, and generally involve much higher doses than those used for oral contraception or menopausal transition in cisgender women [**8**] and/or for anti-androgen therapy in cisgender men.

Currently, 17-β estradiol is the preferred agent for FHT due to reduced risk of thromboembolic events compared to ethinyl estradiol. Route of administration varies, and may include pills, intramuscular injections, or transdermal patches. Dosing begins at 1-2 mg daily but can approach 8-10 mg daily or more (in contrast to 0.02-0.045 mg of ethinyl estradiol daily for oral contraceptive use or 1-2 mg daily for post-menopausal replacement therapy). Dosing strategies vary widely by provider and geography, though guidelines such as those of The Endocrine Society and the World Professional Association for Transgender Health can help to guide care [**9**, **10**]. Ultimately, 17-β estradiol dosing is tailored to balance: 1) achieving target serum estradiol and total testosterone concentrations, 2) patient satisfaction with physical and emotional changes (i.e., clinical phenotype (physical), which typically progresses over 2-3 years following

FHT initiation), 3) side effect profiles, and 4) cost. Optimal dose titration of 17-β estradiol is gradual over the first 1-2 years of use [**11**]. Thus, short-term studies cannot adequately assess FHT's efficacy or safely test the doses required by many TW.

Importantly, estradiol exposure (serum concentration) rather than dose is used to define safe thresholds for use and optimize efficacy. Estradiol is known to be a highly variable drug, due to individual differences in drug absorption, distribution, metabolism, and elimination. Therefore, irrespective of the dose administered, the serum concentration is what indicates if an individual is within the desired therapeutic range. More importantly, safety thresholds are established based upon estradiol exposure and not dose. This is a critical point as limited data suggest that nearly one third of trans women in clinical care do not achieve a serum estradiol concentration of >100 pg/mL on even 6 mg or 8 mg of oral estradiol daily [12]. As such, using the protocol-defined titration schematic will allow TW who need or want to maximize safe estradiol exposure to do so.

Exogenous estrogen and antiretroviral drug-drug interactions (DDIs): Though concerns about adverse DDIs between ART and FHT have been documented by both providers and patients, few data exist to direct clinical care [**13**]. Importantly, patient concerns about DDIs may compromise adherence to ART [**13**-**15**]. The United States Department of Health and Human Services (DHHS) guidelines note that DDIs are an important consideration in ART choice for TW and that more data are needed to better guide therapeutic choices [**16**]. Documentation of an absence of significant DDIs with common ART strategies would offer reassurance to both patients and providers.

Studies of low dose ethinyl estradiol used as oral contraception in cisgender females of reproductive age suggest that ethinyl estradiol exposure is influenced by some ART. For example, cobicistat-containing regimens decrease ethinyl estradiol exposure by approximately 30% [**17**, **18**]. DDIs between ART and 17-β estradiol are often extrapolated from ethinyl estradiol data, yet there are known differences in the metabolism of these two estrogens, limiting generalizability. Further, exogenous and endogenous hormones may influence drug metabolizing enzymes or drug transporters [**19**, **20**], and therefore ART disposition and metabolism. In an ongoing study of females in Malawi on efavirenz (EFV)-based ART and hormonal contraception (NCT03153709), higher estradiol concentrations were associated with lower plasma and cervico-vaginal fluid EFV concentrations, a finding hypothesized to be due to induction of P-glycoprotein by estradiol [**21**].

No studies have explicitly been designed to understand the bidirectional relationships of modern ART and estrogen therapies at the doses typically used for FHT in TW [**22**, **23**]. There are few data on the PK and pharmacodynamics of ART or 17-β estradiol in TW using FHT. A recent phase III, randomized, controlled trial of HIV pre-exposure prophylaxis (PrEP) observed lower intracellular concentrations of tenofovir diphosphate (TFV-dp) in dried blood spot specimens from TW compared to cisgender men receiving TDF/FTC. While this finding could reflect a hormone-drug interaction [**24**], the investigators could not exclude adherence differences as a potential explanation [**25**], and standardized FHT was not provided by the study, leaving many open questions. In a study of 20 TW with HIV who

were receiving ART and initiating low-dose FHT (17-β estradiol 2 mg daily), blood tenofovir concentrations were 13% lower after FHT initiation, though still above the concentration believed to be needed to stop viral replication [**26**]. In a separate study of 4 TW and 4 cisgender women, the balance of TFV-dp to its competing substrate (dATP), an important effector of drug potency, was 7-fold lower in rectal biopsies of TW than cisgender women, and exhibited an inverse relationship with serum estradiol concentrations [**19**]. Finally, two additional studies identified lower plasma exposure of tenofovir or FTC in TW, while the intracellular TFV-dp was higher than observed in prior reports [**27**, **28**].

Taken together, these preliminary findings are inconclusive, with limited testing or unknown exposures to estrogen in small groups of participants, yet indicate that FHT may be relevant to the efficacy of ART. There is a clear need for a focused study in larger groups of TW with HIV on commonly used, contemporary ART regimens to inform our understanding of FHT and ART at the estrogen doses commonly used for FHT. Indeed, DHHS guidelines acknowledge that DDI recommendations regarding ART and FHT co-prescription are based on expert opinion, and more data from clinical trials is needed to provide an evidence-based approach to the care of TW with HIV [**16**].

2.2 Rationale

This protocol will address the existing gap in knowledge on optimization of ART and FHT for TW with HIV. FHT is a long-term medication, titrated over 1-2 years to optimal dosing. Thus, short-term studies cannot adequately assess FHT's efficacy or safely test the doses ultimately required by many TW, including testing for dose-dependent DDIs.

Additionally, FHT and ART are both known to contribute to clinically significant metabolic disturbances, including weight gain and lipid and insulin/glucose abnormalities, and anecdotal evidence suggests high rates of new or worsening metabolic disturbances with FHT titration in TW with HIV on ART. However, the severity and sequelae of these changes have not been previously documented in TW with HIV on ART and FHT, yet this knowledge could profoundly affect optimization of care. Therefore, we propose a study duration of 48 weeks to evaluate a range of 17-β estradiol doses. Oral 17-β estradiol was chosen for this evaluation due to ease and range of dose titration options, storage considerations, generic formulation, and international accessibility. All of these factors make oral 17-β estradiol the most practical and widely available option for transition of participants following study closure.

ART in TW with HIV: Integrase strand transfer inhibitors (INSTIs) that do not require PK boosters **(e.g., cobicistat)** are the drugs of choice for most PWH [**16**], and may be an optimal ART base for use with FHT due to the lower potential for DDIs, adverse effects, and food requirements compared to other ART classes. For example, regimens requiring cobicistat may result in the need for additional monitoring and/or FHT dose adjustment. Similarly, as both HIV and FHT are associated with increased cardiovascular disease/thromboembolic risk, abacavir-containing regimens may not be preferred. TW also have high rates of food insecurity and depression, making NNRTI-containing regimens that require dosing with meals or with concomitant neuropsychiatric side

effects less desirable. Given these considerations, a once daily, unboosted INSTI in combination with an abacavir-sparing NRTI backbone may be the preferred ART regimen for TW with HIV using FHT.
However, the potential for excess weight gain with INSTIs [**29**, **30**], which could be enhanced by concomitant FHT-induced anthropometric changes, individualized resistance profiles and other factors may not permit use of INSTIs or unboosted regimens. Therefore, protease inhibitors (PIs) will continue to play a role in ART optimization for some patients for the foreseeable future. Additionally, TW (on average) have lower bone mineral density (BMD) than age-matched controls [**31**], and agents with improved bone health profiles, such as TAF, may be beneficial [**16**]. In summary, evaluating an unboosted INSTI-based ART regimen in addition to a PI-based regimen will more comprehensively inform ART options for TW receiving FHT.

Therefore, we propose investigating the two most commonly used INSTIs in the global ART landscape: BIC/TAF/FTC and DTG/TDF/FTC or 3TC. We selected DRV as a PI-based regimen available domestically and internationally. DRV is a common component of regimens for persons with resistance or virologic failure. We will enroll all participants receiving DRV plus cobicistat, and will allow inclusion of regimens including NRTIs or INSTIs along with DRV. Requiring relative virologic suppression will reduce inclusion of persons with medication adherence issues and reduce confounding of current or recent viremia for key secondary endpoints. In addition, there is a planned grant submission for an HIV reservoir and inflammatory biomarker banked sample substudy.

ART-FHT potential for DDIs: Early data in small studies of individuals receiving FHT and either PrEP or ART indicated some cause for concern regarding the bidirectional DDIs between these two important therapies. Importantly, available data on ART-FHT DDIs in TW have not exceeded 17-β estradiol doses of 2-4 mg daily, which are merely starting doses for many TW. These low doses in prior studies are reflected in low serum estradiol levels, which did not approximate the physiologic concentrations required for most feminization effects, and do not allow for the possibility of a dose-dependent DDI.

Our approach will characterize ART and 17-β estradiol PK and the 17-β estradiol dosing required to achieve target serum estradiol concentrations and participant satisfaction. By addressing both issues, we hope to demonstrate to both TW and their providers that estradiol exposure at the doses used for FHT can be attained without compromise of virologic control or feminizing effects, despite any observed DDIs. This addresses a key research priority for domestic and international communities of TW, as identified in the findings of the ACTG Transgender Working Group.

To determine whether a clinically significant ART-FHT DDI exists, plasma concentrations of BIC, DTG, and DRV will be evaluated in the context of therapeutic thresholds. In the absence of well-defined and widely recognized therapeutic drug monitoring (TDM) targets for all of these ARV, we will use the lower 95% percentile of their trough concentrations from intensive PK studies. However, if new PK/ pharmacodynamic (PD) data become available that improves on this current plan to declare therapeutic exposure, we will adapt our analysis plans.

The existence of a clinically significant DDI for NRTIs will be evaluated on the basis of their active moieties (TFV-DP, FTC-TP, and 3TC-TP) within PBMCs rather than plasma concentrations of the nucleoside analog. The reasoning for this is 2-fold. First, we are enrolling TW taking either TAF or TDF, and these tenofovir prodrugs result in very different plasma PK profiles. Additionally, to date 4 small clinical trials have explored whether an FHT-TFVdp DDI exists with inconsistency in conclusions [**27**, **28**, **32**, **33**]. These trials have all been designed to evaluate TFVdp PK against cisgender controls and some have utilized historical controls. Given the relatively high degree of variability in TFVdp PBMC PK, a DDI study designed to explore between group differences increases risk of a type II error. Our proposed study design offers an improvement in that we will interrogate for individual changes pre- and post-FHT initiation.

Finally, this is the first study that will be able to characterize 17-β estradiol PK and PD measures of FHT effectiveness across a range of 17-β estradiol doses over 48 weeks, given that FHT is titrated to clinical response and safety thresholds, characterizing whether a dose proportional relationship exists for FHT and whether that relationship is altered by DRV**/c** is an important consideration in interrogating whether a clinically significant ART-FHT DDI exists. Previous studies have reported a predictable relationship between dose and concentrations for topically applied estradiol used as hormone replacement therapy in post-menopausal women [**34**, **35**]; however, none have characterized this relationship in the context of FHT for TW or in patients with HIV taking ART.

ART-FHT PK Measurements: To comprehensively address these scientific gaps, we propose serial trough concentrations at each visit, paired with intensive PK sampling in 15 participants per ART group at baseline, and then at two time points (weeks 24 and 48) with varying 17-β estradiol dosing. By combining this sparse and intensive PK approach we have the ability to characterize the PK of both ART and 17-β estradiol after an observed drug dose, which will enrich the sparse sampling after a self-reported dose. PK parameters of both ART and 17-β estradiol can be efficiently compared with non-compartmental methods, target threshold attainment, and combined in a population PK:PD analysis to assess the influence of covariates on PK exposure and PD outcomes.

Rationale for participant intervention and FHT satisfaction surveys: The complexity of dire social realities faced by TW magnify HIV vulnerabilities and limit engagement in routine medical care and research [3, **36**, **37**]. Additionally, in clinical settings, FHT dosing is significantly impacted by individual patient goals and progress towards goals, which we hope to replicate in this study. The inclusion of study satisfaction and acceptability measures are a unique opportunity to not only understand psychosocial impacts of gender-affirming care through this intervention, but also to improve research processes targeting this community. Also, because individual participant goals of FHT vary, and because physical changes will lag behind study dose titrations and timelines for some participants, assessing progress towards individual participant goals over the 48-week study period will be important to understand and contextualize.

Study population: We will enroll only adult TW (individuals currently identifying as trans women, female or transfeminine, but with male sex assigned at birth) with HIV. Because

gender-affirming therapy in adolescents can involve puberty blockers and transition to 17-β estradiol has additional safety considerations during physical development, this study will only enroll adults ≥18 years of age. We will enroll individuals relatively virologically suppressed on the ART regimens described above and who are not receiving FHT at entry to allow for standardized dose titration across groups. The ACTG Transgender Working Group survey of ACTG sites indicate sufficient number of potential participants who fulfill these important criteria already exist in the communities served by the sites. The Working Group's findings also highlighted access to hormones through the study as a major incentive for trial participation due to restricted or difficult access/affordability in many domestic and international locations. Subsequent site surveys have validated the Working Group's findings.

2.2.1 Rationale Summary

To address these open questions, we propose a 48-week, open-label, DDI study among adult TW with HIV on contemporary ART who are initiating or restarting FHT. The proposed ART regimens will allow for inclusion of diverse populations of TW domestically and internationally. Provision of algorithm-based escalation of standardized FHT regimens will allow us to answer the critical scientific questions noted above at the doses of 17-β estradiol commonly used by TW and with a high degree of scientific rigor. As the study design will be modeled after routine clinical care, the study will also be highly clinically relevant. The primary outcome measures will be both 1) ART PK before and after FHT initiation and dose escalation and 2) the serum estradiol concentrations achieved at escalating 17-β estradiol doses between groups receiving boosted versus non-boosted ART. We will assess virologic and safety endpoints as well as participant satisfaction with their FHT goals, and acceptance of our intervention in diverse communities of TW. These data will fill critically important gaps regarding optimization of ART and FHT co-administration for TW for both providers and patients.

3.0 STUDY DESIGN

A5403 is a phase IIb, 48-week, open-label, non-randomized, 3-group trial, of 90 adult (≥18 years) TW living with HIV on suppressive ART and not currently on FHT. All participants will continue on ART and receive study-supplied 17-β estradiol for weeks 0-48. The primary objectives of the study are to determine whether 1) TW achieve therapeutic concentrations of ART while receiving escalating doses of FHT, assessed within each ART group and 2) the trough serum estradiol concentrations achieved at escalating 17-β estradiol doses varies across ART groups, specifically compared between boosted and unboosted ART.

The study consists of three groups, a BIC-treated group (BIC/TAF/FTC; n=30) (Group 1), a DTG-treated group (DTG/TDF/FTC or 3TC; n=30) (Group 2), and a boosted DRV-treated group (DRV/c; n=30) (Group 3), for a total of 90 participants. TW will receive study supplied 17-β estradiol for weeks 0-48. We will target at least 50% enrollment of participants identifying as non-white or Latine.

FHT regimen and monitoring
Oral 17-β estradiol 2 mg once daily will be initiated at study entry. At weeks 4, 12, 24, and 36, study clinicians may titrate 17-β estradiol in 2 mg increments as described in section 5.1.

Pharmacology Study Design
Steady-state antiretroviral and **steady-state** 17-β estradiol PK will be assessed throughout the study using intensive and sparse PK sampling. At entry (week 0), an 8-hour intensive PK visit will occur to collect and store samples to assess ART exposure prior to FHT initiation in 15 participants per ART group (baseline). At week 24, intensive sampling will be repeated in the same 15 participants to assess 17-β estradiol and ART exposure after 24 weeks of continuous FHT. A final intensive PK visit will occur at week 48 **(or premature discontinuation)** to assess 17-β estradiol and ART exposure at the maximal FHT dosing achieved during the study period. In addition, all participants not participating in an intensive PK sampling visit on the same day will have timed, sparse PK sampling collected at each visit to characterize the trough plasma (BIC, DTG, and DRV) and intracellular ART (TFV-DP, FTC-TP, 3TC-TP) concentrations to evaluate the relationship of ART PK exposure across a range of 17-β estradiol doses (see section 6.3.10 for more details).

FHT satisfaction and acceptability
We will measure indicators of intervention satisfaction and acceptability throughout the study period. To measure acceptability, we will ask participants to self-report the degree to which they find the intervention appropriate and useful using Likert-type agreement scales at three study time points: entry, 24 weeks, and 48 weeks. To measure satisfaction, we will use the 12-question Transgender Congruence Scale (TCS), which will assess associations between gender-affirming treatments, perceived gender congruence, and satisfaction at three study time points: entry, 24 weeks, and 48 weeks [**38**]. The TCS has demonstrated high internal consistency (alpha >0.80) and has been utilized in numerous clinical settings [**39**-**41**]. In addition, we will conduct brief, 20-minute, semi-structured interviews with 30 purposively sampled participants across English- or Spanish-speaking sites to provide an opportunity for more in-depth (open-ended) feedback on intervention satisfaction and acceptability at three time points: entry, 24 weeks, and 48 weeks (see section 12.0 for more details).

Other assessments throughout the study
Anthropometric measurements (including weight, height, minimum waist circumference, and maximum hip circumference), routine labs, HIV-1 RNA, $CD4^+$ and $CD8^+$ T cell counts and percentages, lipids, glucose and insulin, non-estradiol hormone concentrations, stored PBMC, plasma, and serum, **suicidality assessments,** and ART and FHT use and adherence assessments.

Post-trial access
17-β estradiol will not be provided by the study post-trial. If applicable, sites will need to provide options to participants for transitioning to community resources for hormone

acquisition after the study (see MOPS for more detail).

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

4.1.1 Age ≥18 years.

4.1.2 HIV-1, documented by any licensed rapid HIV test or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit at any time prior to study entry and confirmed by a licensed Western blot or a second antibody test by a method other than the initial rapid HIV and/or E/CIA, or by HIV-1 antigen, plasma HIV-1 RNA viral load.

NOTE: The term “licensed” refers to a US FDA-approved kit, which is required for all IND studies, or for sites located in countries other than the United States, a kit that has been certified or licensed by an oversight body within that country and validated internally. Non-US sites are encouraged to use US FDA-approved methods for IND studies.

World Health Organization (WHO) and CDC guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an E/CIA that is based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive), or a Western blot or a plasma HIV-1 RNA viral load.

4.1.3 Assigned male sex at birth and identifies as a TW, female or transfeminine person.

4.1.4 On ART for at least 24 weeks prior to study entry. Regimen changes within the 24 weeks prior to study entry are acceptable, but candidates must have been on a stable regimen for at least 28 days prior to study entry.

4.1.5 On BIC/FTC/TAF, DTG/TDF/FTC or 3TC, or DRV/c-containing ART for at least 28 days prior to study entry (single tablet regimen not required), and with no plans to change ART regimen over the study duration of 48 weeks.

NOTE: Candidates not on the required regimens at screening may switch ART to DRV/**c**-, DTG-, or BIC-based ART through routine clinical care, with the recommendation that TW on PI or NNRTI-based ART switch to DRV/**c**, and TW on other INSTI-based ART switch to BIC or DTG (as locally available). After 28 days, TW who switched ART will have HIV-1 RNA rechecked at the pre-entry visit. Those who remain suppressed may proceed to study entry. TW already on the permitted ART regimens will proceed directly to study entry from screening.

NOTE: DTG dosing is restricted to once daily. Persons on bid dosing are not eligible.

4.1.6 Desire to initiate or restart FHT**, regardless of orchiectomy status**.

4.1.7 HIV-1 RNA <200 copies/mL (or below the assay limit of detection if local assay lower limit of detection is >200 copies/mL) at screening (within 60 days prior to entry) at any US laboratory that has a Clinical Laboratory Improvement Amendments (CLIA) certification or its equivalent, or at any network-approved non-US laboratory that operates in accordance with Good Clinical Laboratory Practice (GCLP) and participates in appropriate external quality assurance programs.

NOTE: For candidates switching ART after screening, an HIV-1 RNA <200 copies/mL (or below the assay limit of detection if local assay lower limit of detection is >200 copies/mL) at pre-entry (at least 4 weeks post-switch) will be required prior to study entry.

4.1.8 HIV-1 RNA <400 copies/mL available through routine clinical care between 24 and 96 weeks prior to study entry and while on ART. The HIV-1 RNA must be the most recent value obtained between **24 and 96 weeks** prior to study entry.

NOTE: Entry will be allowed if the most recent HIV-1 RNA available between **24 and 96 weeks** prior to entry through routine care is a single, unconfirmed plasma HIV-1 RNA >400 but <2000 copies/mL, ***and*** if this single measurement is preceded by an HIV-1 RNA <400 copies/mL and followed by an HIV-1 RNA <400 copies/mL (latter allowable if measured in the **24 weeks** prior to entry and before screening).

4.1.9 The following laboratory values obtained within 60 days prior to study entry by any US laboratory that has a CLIA certification or its equivalent, or at any network-approved non-US laboratory that operates in accordance with GCLP and participates in appropriate external quality assurance programs.

- Hemoglobin ≥9.0 g/dL
- Platelet count ≥75,000/mm^3
- Estimated Glomerular Filtration Rate (eGFR) ≥30 mL/min/1.73m^2 if on or switching to TAF, ≥50 mL/min/1.73m^2 if on or switching to TDF **without cobicistat, or ≥70 mL/min/1.73m^2 if on or switching to TDF in combination with cobicistat**, calculated using the CKD-Epi equation (a calculator is available at: https://qxmd.com/calculate/calculator_251/egfr-using-ckd-epi).
- Aspartate aminotransferase (AST) (SGOT), alanine aminotransferase (ALT) (SGPT), and alkaline phosphatase **are within normal range per local laboratory range**
- Prolactin <**25** ng/dL

4.1.10 Serum estradiol level <75 pg/mL within 60 days prior to study entry.

NOTE: If a participant is on estradiol therapy at screening, the participant may go off estradiol for the required period of time during the screening window and then have their estradiol level assessment performed (not required to be performed at the screening visit). Sites should be aware of the turnaround time for local estradiol levels so that the value can be obtained and the participant enrolled before the screening window ends.

4.1.11 Willingness to avoid the use of prescribed, non-study provided FHT and non-prescribed FHT during the study period, and no planned use of prescribed or non-prescribed anti-androgens for the first 24 weeks of the study.

4.1.12 Ability and willingness of participant to provide informed consent and ability and willingness of participant to undergo study procedures.

4.2 Exclusion Criteria

4.2.1 **K**nown clotting disorders, **active deep vein thrombosis (DVT), pulmonary embolism (PE), or history of these conditions, active arterial thromboembolic disease (e.g., stroke, myocardial infarction), or history of these conditions**.

4.2.2 **Known liver impairment or disease**.

NOTE: **Prior incidental ultrasound, CT, or transient elastography findings of hepatic steatosis ("simple steatosis") without transaminase elevation or known hepatic fibrosis is not exclusionary.**

4.2.3 History of chronic hepatitis B virus (HBV) infection or active HBV infection.

NOTE A: Persons with documented natural (spontaneous clearance) or vaccine-induced HBV immunity (anti-HBsAg >10 UI/mL) are not excluded.

NOTE B: Laboratory results/medical records are required documentation. Participant self-report cannot be used for documentation of viral hepatitis status.

4.2.4 History of current active hepatitis C virus (HCV) infection.

NOTE A: Persons with documented spontaneous clearance of HCV are not excluded.

NOTE B: Persons with documented cure of HCV, defined as negative HCV RNA ≥12 weeks after end of treatment, without known liver

impairment (as per exclusion 4.2.2) are not excluded.

NOTE C: Persons with HCV antibody seropositive within 1 year prior to entry but without standard of care HCV RNA to determine if disease is active will be excluded. If HCV RNA testing is not locally available, then HCV antibody seropositivity alone will be a cause for exclusion.

NOTE D: Laboratory results/medical records are required documentation. Participant self-report cannot be used for documentation of viral hepatitis status.

4.2.5 Prohibited medication use (including drugs with known or expected DDIs with FHT or ART) at time of study entry. See section 5.4.2 for a list of prohibited medications.

4.2.6 Receipt of any estrogen therapy within 14 days prior to study entry for **persons on** oral **FHT**, or within 30 days prior to entry for **persons on** injectable **FH**T.

4.2.7 Known HIV-1 resistance mutations that would preclude remaining on current ART or a switch to a study regimen, in the opinion of the site investigator.

4.2.8 Personal history of breast cancer **or known personal history of breast cancer (BRCA) gene.**

4.2.9 Known or a history of testicular cancer.

4.2.10 Known or a history of gall bladder disease.

4.2.11 Known or suspected pituitary adenoma.

4.2.12 Known allergy/sensitivity or any hypersensitivity to components of study drugs or their formulation.

4.2.13 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.2.14 Suicidal ideation in the past 30 days or suicide attempt in the past 90 days, as reported on the Columbia-Suicide Severity Rating Scale (C-SSRS).

NOTE: Refer to section 8.4 and the MOPS for C-SSRS interpretation and additional management recommendations.

4.2.15 **S**erious illness requiring systemic treatment and/or hospitalization within 30 days prior to entry. Stable (in the opinion of the site investigator) treatments for chronic comorbidities are allowed.

4.2.16 Presence of any other medical condition that would preclude FHT administration for safety reasons, in the opinion of the site investigator.

NOTE: Use of tobacco or other nicotine-containing products is not an absolute contra-indication to FHT. The thromboembolic risk associated with estrogen use in persons who use these products **and options for smoking cessation resources** will be discussed during the informed consent process.

4.3 Study Enrollment Procedures

4.3.1 Prior to implementation of this protocol, and any subsequent full version amendments, each 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, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (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 be reviewed and approved by the DAIDS PRO, and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. 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) **as well as meeting any additional study-specific requirements as determined by the protocol team**, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO, and sites will receive an Amendment Registration Notification from the DAIDS PRO that approves the site-specific ICFs and indicates successful completion of the amendment protocol registration process. A copy of the final Amendment Registration Notification issued by the DAIDS PRO 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.

Once a candidate for study entry has been identified, details will be carefully discussed with the participant. The participant will be asked to read and sign the approved protocol consent form.

Participants from whom a signed informed consent has been obtained may be screened and enrolled, if they otherwise qualify. An ACTG Screening Checklist must be entered through the DMC Study Enrollment System (SES).

4.3.2 Protocol Activation

Prior to enrollment, sites must complete the Protocol Activation Checklist found on the ACTG Member website. This checklist must be approved prior to any screening of participants for enrollment.

4.3.3 Participant Registration

For participants from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, an ACTG Screening Failure Results form must be completed and keyed into the database. Participants who meet the enrollment criteria will be registered to the study according to standard ACTG DMC procedures.

4.4 Co-enrollment Guidelines

- US sites are encouraged to co-enroll participants in A5128, “Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses.” Co-enrollment in A5128 does not require permission from the A5403 protocol chairs.
- Non-US sites are encouraged to co-enroll participants in A5243, “Plan for Obtaining Human Biological Samples at Non-US Clinical Research Sites for Currently Unspecified Genetic Analyses.” Co-enrollment in A5243 does not require permission from the A5403 protocol chairs.
- For specific questions and approval for co-enrollment in other studies, sites should first check the PSWP or contact the protocol team via e-mail as described in the Study Management section.

5.0 STUDY TREATMENT

Study treatment is defined as 17-β estradiol and will be provided through the study.

5.1 Regimens, Administration, and Duration

At entry, participants will be assigned to analysis groups based on their ART regimen, as described below:

Group 1: Participants taking bictegravir (BIC) + tenofovir alafenamide (TAF) + emtricitabine (FTC)

Group 2: Participants taking dolutegravir (DTG) + tenofovir disoproxil fumarate (TDF) + (FTC or lamivudine [3TC])

Group 3: Participants taking any regimen containing darunavir plus cobicistat (DRV/c).

Feminizing hormone therapy regimen, dose escalation, and monitoring
Participants will be taking 17-β estradiol 2 mg by mouth once daily, to be initiated at study entry. At weeks 4, 12, 24, and 36, study clinicians may titrate 17-β estradiol in 2 mg increments. This titration schedule aligns with guidelines that suggest monitoring a minimum of every 3 months during the first year of FHT and to titrate dosage upward over the first 3-6 months and until goals are achieved [**7**, **42**]. An example dose escalation schedule for a participant who chooses to dose escalate at every visit is shown below in Table 5.1-1. Participants who choose not to escalate, or to de-escalate at any given visit, will have a different schedule.

Table 5.1-1: Possible Dose Escalation Schedule

Maximum 17-β Estradiol Dose	Weekly Titration Schedule
2 mg/day (one 2 mg tablet)	Weeks 0-4
4 mg/day (two x 2 mg tablets)	Weeks 4-12
6 mg/day (three x 2 mg tablets)	Weeks 12-24
8 mg/day (four x 2 mg tablets)	Weeks 24-36
10 mg/day (five x 2 mg tablets)	Weeks 36-48

Refer to section 6.3.5 for more detailed clinical and laboratory considerations and to section 8.0 for safety considerations when considering dose escalation. Additional guidance for investigators and study site clinicians is provided in the MOPS.

Participants will remain on study-provided 17-β estradiol for 48 weeks or until study discontinuation procedures are performed. If participants cannot complete their discontinuation assessments within the 48-week time period, additional study drug should be dispensed to ensure participants are on drug at the time of discontinuation assessments.

5.2 Study Product Formulation and Preparation

17-β estradiol will be supplied as 2 mg tablets. Tablets should be stored at controlled room temperature 15-30°C (59-86°F). Dispense in a tight, light resistant container, as defined by the United States Pharmacopeia.

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Acquisition/Distribution

17-β estradiol is manufactured and supplied by Epic Pharma LLC and is available through the NIAID Clinical Research Products Management Center (CRPMC). The site pharmacist should obtain the study product(s) for this protocol by following the instructions in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.*

Any study product not provided by the study must comply with the NIAID (DAIDS) policy that outlines the process for authorizing the use of study products not marketed in the United States in NIAID (DAIDS)-supported and/or -sponsored clinical trials. This policy is available on the NIAID (DAIDS) website at https://www.niaid.nih.gov/sites/default/files/NonFDAapprovedProducts.pdf.

17-β estradiol may be locally sourced by clinical research sites that are unable to import study-provided 17-β estradiol. The A5403 Site Implementation Plan (SIP) must be completed by each site for protocol team notification and authorization of locally sourced 17-β estradiol.

17-β estradiol tablets locally procured by the site and approved for use in A5403 by the protocol team must be stored in accordance with the manufacturer's instructions.

5.3.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC, locally sourced and subsequently dispensed. At US CRSs, all unused study products must be returned to the NIAID CRPMC (or as otherwise directed by the sponsor) after the study is completed or terminated. The procedures to be followed are provided in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.* At non-US CRSs, the site pharmacist must follow the instructions in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks* for the destruction of unused study products.

5.4 Concomitant Medications

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

Additional drug information may be found on the ACTG Precautionary and Prohibited Medications Database located at http://tprc.pharm.buffalo.edu/home/di_search/.

5.4.1 Required Medications

ART in any of the following combinations: BIC/FTC/TAF, DTG **50 mg daily** + TDF and FTC or 3TC, or DRV/c **800/150 mg daily** + other ARVs.

ART will not be provided; participants will obtain it through standard of care (SOC) or co-enrollment in a clinical study (latter if approved by the A5403 study team).

5.4.2 Prohibited Medications

In addition to consulting the lists below, sites are advised to consult the most recent package insert of any medication. Note that topical or short-term use of some otherwise prohibited drugs may be allowed on a case-by-case basis, after discussion with and approval by the A5403 Clinical Management Committee (CMC). Please e-mail the CMC at: actg.cmcA5403@fstrf.org.

5.4.3 Prohibited medications for all participants during the study

5.4.3.1 All other hormones, including but not limited to:

- Delestrogen
- Depotestrogen
- Desogestrel
- Drospirenone
- Estradiol cypionate
- Estradiol Valerate
- Estrogens, Conjugated
- Estrogens, Esterfied
- Ethinyl Estradiol
- Ethynodiol Diacetate
- Etonogestrel via a vaginal ring
- Gestodene
- Hydroxyprogesterone
- Levonorgestrel
- Medroxyprogesterone
- Nandrolone decanoate
- Norelgestromin
- Norethindrone
- Norethisterone enanthate
- Norgestimate
- Norgestrel
- Segesterone
- Progesterone
- Testosterone
- Non-prescribed FHT (“street hormones”)
- Cyproterone acetate
  - Gonadotropin releasing hormone analogues (GnRH) (Goserelin, Leuprolide acetate, Triptorelin)

5.4.3.2 Antiretroviral drugs other than those allowed by the study

5.4.3.3 Moderate-to-strong inducers of CYP3A4, which may include, but not limited to the following:

- St. John’s Wort preparations (Hypericum perforatum)

- Bosentan
- Phenobarbital
- Carbamazepine
- Oxcarbazepine
- Eslicarbazepine
- Phenytoin
- Fosphenytoin
- Apalutamide
- Enzalutamide
- Mitotane
- Rifampin
- Rifapentine
- Rifabutin
- Betametasone
- Dexamethasone

5.4.3.4 Moderate-to-strong inhibitors of CYP3A4 which includes, but are not limited to:

- African potato (hypoxis hemerocallidea)
- Erythromycin
- Clarithromycin
- Fluconazole
- Ketoconazole
- Itraconazole
- Posaconazole
- Voriconazole
- Nefazodone
- Antiarrhythmics (amiodarone, drodenarone, ranolazine)
- Calcium channel inhibitors (Diltiazem, verapamil)
- Dasabuvir plus Paritaprevir/Ombitasvir/Ritonavir (Viekira Pak)
- Ledipasvir/ Sofosbuvir*

*Only applies to participants receiving TDF or TAF.

5.4.4 Precautionary Medications

The following medications are designated as precautionary for use in all participants in the study:

- Thyroid hormone replacement therapy
- Grapefruit juice
- Nirmatrelvir/ritonavir (Paxlovid): When possible, participants receiving Paxlovid therapy should have PK visits at least 3 days after Paxlovid therapy is completed.

The following medications are designated as precautionary medications for all participants receiving bictegravir or dolutegravir containing regimens:

- Antacids or supplements containing polyvalent cations (e.g., aluminum, calcium, copper, iron, magnesium, zinc): Follow product labeling for dose separation

The following medications are designated as precautionary medications for all participants during the first 24 weeks of the study:

- Spironolactone
- Finasteride
- Dutasteride

Anti-androgen therapy will not be offered as part of the study. Participants and providers will be asked to refrain from initiating anti-androgen therapy obtained outside of the study for weeks 0-24, which is within the accepted spectrum of standard of care and will provide additional study rigor [4, **43**]. The aforementioned references highlight the fact that, while concomitant initiation of estradiol and anti-androgen therapy may occur in some clinical settings, it is not the more widely accepted SOC, the effects of different anti-androgens are not equivalent, and concomitant initiation may have disadvantages [**44**, **45**].

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Evaluations

Table 6.1-1: Schedule of Evaluations

Evaluation	Screening	Pre-Entry (if needed)	Entry / Day 0	Post-Entry Evaluations (Weeks)						Premature Study/Treatment Discontinuation Evaluations
				4	**8**	12	24	36	48	
Visit Windows	Within 60 days prior to entry	Minimum 28 days after ART switch		± 7 days		± 14 days				
Documentation of HIV-1	X									
Medical History	X		X							
Medication History	X		X							
Complete Physical Exam	X									
Targeted Physical Exam			X	X		X	X	X	X	X
Nicotine Use			X	X		X	X	X	X	X
Concomitant Medications				X	**X**	X	X	X	X	X
Targeted Medications				X	**X**	X	X	X	X	X
Study Treatment Modifications				X	**X**	X	X	X	X	X
Site Investigator FHT dose assessment			X	X		X	X	X	X	X
Hematology	X		X				X		X	X
Liver Function Tests	X		X	X		X	X	X	X	X

Evaluation	Screening	Pre-Entry (if needed)	Entry / Day 0	Post-Entry Evaluations (Weeks)						Premature Study/Treatment Discontinuation Evaluations
				4	**8**	12	24	36	48	
Visit Windows	Within 60 days prior to entry	Minimum 28 days after ART switch		± 7 days		± 14 days				
Chemistries	X		X	X		X	X	X	X	X
Estradiol concentration	X		X	X		X	X	X	X	X
Prolactin concentration	X									
Total testosterone concentration			X	X		X	X	X	X	X
CD4+/CD8+			X						X	X
Plasma HIV-1 RNA	X	X	X			X	X	X	X	X
HBV surface antigen testing (see section 6.3.8)	**X**									
HCV testing (see section 6.3.8)	**X**									
Fasting lipid panel			X			X	X		X	X
Intensive PK subset (See section 6.3.10)			X				X		X	X
Timed trough PK sampling (See section 6.3.10)			X	X		X	X	X	X	X
Stored Plasma/Serum			X	X		X	X	X	X	X
Stored PBMC			X				X		X	X
C-SSRS questionnaire	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
ART adherence assessment			X	X		X	X	X	X	X

Evaluation	Screening	Pre-Entry (if needed)	Entry / Day 0	Post-Entry Evaluations (Weeks)						Premature Study/Treatment Discontinuation Evaluations
				4	**8**	12	24	36	48	
Visit Windows	Within 60 days prior to entry	Minimum 28 days after ART switch		± 7 days		± 14 days				
FHT adherence assessment**s**				X		X	X	X	X	X
FHT Participant Goals			X						X	X
FHT acceptability and satisfaction surveys			X				X		X	X
Semi-structured interview subset			X				X		X	X
Remote visit					**X**					

6.2 Timing of Evaluations

6.2.1 Screening and Pre-Entry Evaluations

Screening and pre-entry evaluations must occur prior to the participant starting any study medications, treatments, or interventions.

Screening
Screening evaluations to determine eligibility must be completed within 60 days prior to study entry unless otherwise specified. The estradiol assessment can occur any time after screening has been initiated that is appropriate based upon timing of the participant's last dose of estradiol. Confirmation of serum estradiol <75 pg/mL must be received prior to study entry.

In addition to data being collected on participants who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Results form and entered into the ACTG database.

Pre-Entry
The pre-entry visit is only required for participants who switched ART at screening. HIV-1 RNA testing must be completed at least 28 days after ART switch. HIV-1 RNA results must be received prior to study entry (refer to inclusion criterion 4.1.7).

6.2.2 Entry Evaluations

Entry evaluations must occur within 60 days following screening evaluations. Participants must begin 17-β estradiol within 3 days after study registration. Participants must arrive fasting (refer to section 6.3 for a definition of fasting). If a participant is not fasting, they must return in the fasting state for entry blood draw prior to starting 17-β estradiol.

Site Investigator FHT dose assessment at week 0 will ensure the participant is still willing and able to safely initiate FHT at the starting dose of 2 mg daily.

Intensive PK sampling will occur on a subset of participants at entry prior to initiation of 17-β estradiol. Sparse, timed, trough PK sampling will occur for all participants not participating in the intensive PK visit on the same day as entry. Because intensive PK sampling will capture the trough, those participants do not need a sparse sample collected at entry.

Semi-structured interviews will occur on a subset of participants at entry prior to initiation of 17-β estradiol.

6.2.3 Post-Entry Evaluations

For all post-entry evaluations, if a participant is unable to attend a visit within the

specified window, every attempt should be made to bring the participant in for an out-of-window visit rather than skip the visit, unless the next visit window has opened.

On-Treatment Evaluations
A study visit will occur at week**s** 4 **and 8**, with a window of ±7 days. **Note that week 8, is a remote (phone, text, video chat, etc.) visit that will include the evaluations indicated in the SOE.** Study visits will occur at weeks 12, 24, 36, and 48, with a window ±14 days. Participants must arrive fasting for all **in-person** post-entry visits (see definition of fasting in section 6.3). If a participant is not fasting, they must return in the fasting state within the study window for blood draw and prior to any 17-β estradiol dose escalation. Note that the participant should be on study-provided 17-β estradiol at the time of the week 48 blood draw. Additional medication should be dispensed, if necessary, to ensure this.

Timed, trough PK sampling will occur for all participants at all **in-person** visits except those who are undergoing intensive PK sampling at the same visit. Intensive PK sampling on a subset of participants will occur at weeks 24 and 48 visits.

Semi-structured interviews will occur on a subset of participants at weeks 24 and 48. See section 12.4 for additional details.

Site Investigator FHT dose assessment at weeks 4, 12, 24, and 36 will include a determination of whether dose escalations, reductions or interruptions are warranted. Additionally, determination of whether any interventions/referrals for sequelae of FHT (e.g., lipid or glucose elevations, worsening depression) are warranted. At week 48 or premature study discontinuation, the site investigator assessment will include plans for transitioning FHT and associated care provision to local providers.

NOTE: Lab results (fasting chemistries, liver function tests, local estradiol and total testosterone concentration) must be available prior to any changes in 17-β estradiol dose (unless changed to accommodate an adverse event).

Event-Driven Evaluations
Detectable viremia (as defined in section 8.**8**) and site investigator suspects failure with drug resistance requiring a change in ART will lead to premature treatment and study discontinuation evaluations. If the site investigator suspects non-adherence and would not otherwise change ART, the participant may remain on study.

Participant report of suicidal ideation or attempt, worsening depression, or other acute psychiatric condition will lead to permanent discontinuation of study-provided 17-β estradiol, and the participant should be immediately referred to behavioral health and substance use counseling services for further evaluation and treatment. The A5403 CMC should be notified within

72 hours. When the participant is stable to do so, the premature study discontinuation evaluations must be completed, and the participant will be taken off study.

NOTE: Suicidal ideation or attempt will be defined by answers provided on the C-SSRS. See the MOPS for scoring and interpretation instructions, and for additional considerations.

NOTE: Though suicidal ideation or attempt, worsening depression, or other acute psychiatric condition necessitates discontinuation of study product, if changes in mental health are documented to be transient and due to another cause such as acute intoxication, rechallenge may be considered if the participant has returned to their baseline mental status and if approved by the participant, study investigator, protocol team and CMC.

6.2.4 Discontinuation Evaluations

Evaluations for Registered Participants Who Do Not Start Study Treatment
All eCRFs must be keyed for the period up to and including the entry visit.

Discontinuation Evaluations
Participants should discontinue study once they complete all week 48 or premature discontinuation evaluations. Refer to the Discontinuation Log in the A5403 eCRF completion guide. Participants who require additional monitoring for follow-up of adverse events, etc., should be managed as per section 8.0.

Premature Treatment Discontinuation Evaluations
Participants who prematurely permanently discontinue study treatment will have discontinuation evaluations performed per the schedule of evaluations (SOE) prior to being taken off the study and preferably prior to discontinuing 17-β estradiol (unless the reason for discontinuation is one that requires immediate cessation of study product for safety reasons).

Premature Study Discontinuation Evaluations
Participants who prematurely discontinue from the study before week 48 will have the study discontinuation evaluations performed per the SOE prior to being taken off the study and preferably prior to discontinuing 17-β estradiol (unless the reason for discontinuation is one that requires immediate cessation of study product for safety reasons).

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS website for information about what must be included in the source document:

https://www.niaid.nih.gov/sites/default/files/score-source-documentation-requirements.pdf.

Each study site and laboratory involved in this study must comply with the DAIDS policy on Requirements for DAIDS Funded and/or Sponsored Laboratories in Clinical Trials Policy, which is available at https://www.niaid.nih.gov/sites/default/files/laboratorypolicy1.pdf.

All stated evaluations are to be recorded on the eCRF unless otherwise specified. Refer to section 7.0 for information on the DAIDS AE Grading Table and AE reporting of AE requirements.

The protocol team and/or study monitoring entity (e.g., SMC) may determine that additional source data associated with procedures or evaluations performed per protocol should be entered into eCRFs so that the data can be used for analysis or to otherwise assist with interpretation of study findings. In such cases, sites will be officially instructed to enter the additional data into eCRFs from available source documentation.

Fasting Instructions
Fasting is required at entry, all post-entry visits, and the premature discontinuation visit. Fasting is defined as nothing to eat or drink, except for prescription medications or water, for at least 8 hours before a blood draw.

6.3.1 Documentation of HIV-1

Section 4.1.1 specifies assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on the eCRF.

6.3.2 Medical History

The medical history must include all signs and symptoms regardless of grade and all diagnoses identified by the ACTG criteria for clinical events and other diagnoses regardless of grade within the past 30 days. In addition, the following diagnoses should be recorded regardless of when the diagnosis was made:

- AIDS-defining conditions
- Bone fractures (verbal history accepted)
- Coronary heart disease, cardiovascular disease, myocardial infarction
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes or pre-diabetes
- Blood clots or clotting disorders **(exclusionary)**
- Cerebrovascular accident
- Cholecystitis
- Gallstones
- **Orchiectomy or chemical castration**
- **Chronic active HBV (exclusionary)**
- **HCV status, including history of spontaneous clearance or cure (exclusionary)**

Any allergies to any medications and their formulations must also be documented.

6.3.3 Medication History

A medication history must be present, including start and stop dates. The table below lists the medications that must be included in the history.

Table 6.3.3-1: Medication History

Medication Category	Complete History or Timeframe
Antiretroviral therapy1	24 weeks prior to study entry
Immune-based therapy	60 days prior to study entry
Blinded study treatment	60 days prior to study entry
HIV-1-related vaccines	60 days prior to study entry
Prescription drugs for treatment of opportunistic infections	60 days prior to study entry
Prescription drugs for prophylaxis of opportunistic infections	60 days prior to study entry
Prescription drugs (other)	60 days prior to study entry
Alternative therapies	60 days prior to study entry
Dietary supplements	60 days prior to study entry
Sex-hormone medications or sex-hormone analogues or antagonists*	Most recent hormone use and type and estimate of total years on FHT[2]

[1] Record the start date of the current ART.
[2] Refer to hormone eCRF.
* Includes: estrogens, progesterones, or testosterone therapy; leuprolide or other synthetic gonadotropin-releasing hormone; tamoxifen, raloxifene, aromatase inhibitors or any other androgen, anti-androgen, estrogen, or progesterone analogue or antagonist therapy.

6.3.4 Clinical Assessments

<u>Complete Physical Examination</u>
A complete physical examination will be performed per the SOE and is to include, at a minimum:

- Examination of the skin, head, mouth, and neck
- Auscultation of the chest
- Cardiac exam
- Abdominal exam
- Examination of the lower extremities for edema
- Signs and symptoms
- Diagnoses
- Height (in cm)
- Weight (in kg); calculated body mass index
- Vital signs (temperature, pulse, respiration rate, and blood pressure)

Targeted Physical Examination
A targeted physical examination will be performed per the SOE and is to be driven by any previously identified or new signs or symptoms including diagnoses or adverse events that the participant has experienced since the last visit.

The exam must include:
- Vital signs (temperature, pulse, respiration rate, and blood pressure)
- Anthropometric measurements (weight, minimum waist circumference and maximum hip circumference). Refer to the A5403 MOPS.
- Signs and symptoms assessment
- **Recording of interim orchiectomy per participant report, if applicable**

Refer to section 7.2 for AE collection requirements.

Nicotine Use
History of nicotine use is required to be recorded on the eCRF per the SOE. Post-entry, record nicotine use per the SOE.

Concomitant Medications
Per the SOE, any new and discontinued concomitant medications, including vaccines, must be recorded on the eCRFs.

Targeted Medications
Per the SOE, any new, discontinued or modified ART or non-study provided hormones or anti-androgens (and other non-study FHT) must be recorded on the eCRFs. Participants who pursue anti-androgen therapy outside the study within the first 24 weeks will not be discontinued from the study. The use of anti-androgen therapy and other outside FHT use will be addressed during data analysis.

Study Treatment Modifications
Per the SOE, record all study drug modifications, including initial doses, participant-initiated and/or protocol-mandated modifications, and inadvertent and deliberate interruptions since the last visit. All interruptions of study-supplied drug, regardless of duration, must be recorded. Record any permanent discontinuation of treatment.

6.3.5 Site Investigator FHT Dose Assessment

Site investigator FHT dose assessment will be performed per the SOE, and dose will be modified per the investigator's discretion based on discussion with the participant and review of clinical information. Participants will be reminded about potential side effects by study staff at each visit and be encouraged to call the site should they experience a side effect. Detailed guidance to investigators

regarding clinical considerations is provided in the MOPS. Date of participant contact to communicate dosing decisions will be reported on the eCRF. Reasons for no dose escalation at a visit, or dose decrease will also be reported on the eCRF.

At weeks 4, 12, 24, and 36, study investigators may titrate 17-β estradiol in 2 mg increments. At these visits, the decision to escalate the 17-β estradiol will be individualized, after a discussion between the investigator and the participant, taking into consideration: a) the targeted serum hormone concentrations (as locally measured), b) achievement of desired phenotypic changes, and c) safety (refer to section 8.0 for safety considerations) and tolerability (see MOPS) considerations. At week 48, the investigator assessment will primarily focus on assessing tolerability of the final dose and transitioning of participant care to community-based settings, where available.

Participants will not be required to titrate to the maximum estradiol concentration, but also will not be allowed to titrate above the recommended therapeutic range for safety reasons. Dose titration will generally occur until hormone concentration targets are achieved, as phenotypic changes will lag behind target hormone concentrations in many cases. Participant goals and safety assessments will also influence titration decisions (see MOPS for guidance on estradiol titration). Targets for hormone concentrations are based on the Endocrine Society guidelines [**7**, **11**] and, for estradiol, from personal communication with Dr. Vin Tangpricha of the World Professional Association of Transgender Health (WPATH). Target hormone concentrations are defined as both:

1) A serum estradiol concentration of ≤200 pg/mL; and
2) A serum total testosterone concentration of <50 ng/dL*

* There are some clinical scenarios where complete testosterone suppression is not in line with participant goals. In these cases (see MOPS), only serum estradiol concentrations will guide titration decisions.

6.3.6 Laboratory Evaluations

At screening, pre-entry, and entry all laboratory values must be recorded per the SOE.

Blood draws for lab evaluations at screening and pre-entry will not be in fasted state. Blood draws for lab evaluations will be in fasted state starting at study entry and at all post-entry visits.

For post-entry assessments, record all laboratory values for serum creatinine, glucose, AST, ALT, total cholesterol, LDL cholesterol, triglycerides, and HDL cholesterol regardless of grade; record abnormal laboratory findings as per section 7.2.

Hematology
Complete blood count (CBC) with differential to include: hemoglobin, hematocrit, white blood cell count [WBC], differential WBC, absolute neutrophil count (ANC), platelets.

Liver Function Tests
Total bilirubin, AST [SGOT], ALT [SGPT], alkaline phosphatase, indirect bilirubin.

Chemistries
Serum glucose, potassium, creatinine, total protein, albumin.

Estradiol Concentrations
Estradiol hormone concentrations will be done per the SOE. Screening and entry values will be random levels. Post-entry, concentrations should be timed to be a trough concentration based on time of participant's last dose (20-28 hours post-dose).

Non-estradiol Hormone Concentrations
Prolactin and total testosterone will be done per the SOE.

6.3.7 Immunologic Studies

CD4+/CD8+
Obtain absolute CD4+/CD8+ T-cell count and percentage per the SOE. All laboratories must possess a CLIA certification or equivalent (US sites) or IQA certification (non-US sites).

6.3.8 Virologic Studies

Plasma HIV-1 RNA
Screening HIV-1 RNA must be performed within 60 days prior to study entry at a local laboratory that possesses a CLIA certification or equivalent (US sites) or appropriate External Quality Assurance (EQA) Program (non-US sites). Eligibility will be determined based on the screening value. Pre-entry HIV-1 RNA will be performed at the local laboratory only for participants who switched ART (per section 4.1.5). For entry and post-entry evaluations, the laboratory must be certified by an appropriate EQA Program.

Viral hepatitis testing
Testing for viral hepatitis status should be documented from medical records, if available. If HBV status prior to starting ART or HCV status within the last year (with the exception of documentation of prior spontaneous HCV clearance or cure) is not available, testing at screening should occur. HBV testing will be defined as HBV surface antigen testing. HCV testing will be defined as HCV antibody testing, followed by HCV RNA testing when HCV antibody testing is positive.

NOTE: In persons with positive HCV antibodies with no available HCV RNA testing, HCV antibody seropositivity alone will be a cause for exclusion, as per section 4.2.4.

6.3.9 Metabolic Studies

Fasting lipid panel
Triglycerides, total cholesterol, direct LDL cholesterol and HDL cholesterol will be performed as a safety lab per the SOE.

6.3.10 Pharmacokinetic Sampling

For additional details regarding PK, please see section 11.0, the MOPS, and the laboratory processing chart (LPC) found on the A5403 PSWP.

Intensive PK subset (n=15 per group)
An 8-hr intensive PK visit will occur per the SOE for a subset of 15 participants per group identified at enrollment. Whole blood samples will be collected pre-dose (within 30 min prior to ART and 17-β estradiol dosing), and then 1, 2, 3, 4, 6, and 8 hours after dosing. Non-viable PBMC samples will be processed from blood collected at the pre-ART, 3- hour post, and 8-hour post time points. Note the pre-dose collection reflects the trough concentration.

If the intensive PK visit is occurring on the same day as the study visit, draw fasting labs and then provide breakfast or allow the participant to eat food they brought to the visit. There are no fat or calorie content requirements. ART and 17-β estradiol should be administered with food (during meal or at the conclusion of the meal) and should be given at the same time, or within 15 minutes of each other.

Dates, times and dose amounts of the three doses of ART and 17-β estradiol prior to the intensive PK sampling period should be documented on the eCRF. **The PK visit should be rescheduled if the participant reports missing any of the prior three doses of ART and 17-β estradiol.** At entry, there is no expected 17-β estradiol doses prior to the PK sampling **and 17-β estradiol should not be started until after the entry 8-hour intensive PK ART sampling is completed.** Any ART or 17-β estradiol dose administered during the intensive PK sampling time period should be documented.

Timed Trough PK samples
Plasma, serum, and non-viable PBMCs will be collected before daily ART and 17-β estradiol administration in all participants to assess trough PK of ART and 17-β estradiol. For participants who are completing an intensive PK visit on the same day as the study visit, an additional trough sample is not required. All samples will be collected at each visit per the SOE, except non-viable PBMCs will not be collected at week 4.

Concentrations will be assessed from plasma and non-viable PBMC samples collected within 22-26 hours after the last dose of ART and 17-β estradiol. If necessary, participants should bring ART and 17-β estradiol to the clinic visit and may administer the doses after blood collection. Timing of the participant's medication schedule should be reviewed at entry and at each visit to ensure sample collection will occur within the specified window.

NOTE: Collection of NON-viable PBMCs for PK studies do not require IQA oversight.

6.3.11 Stored Plasma/PBMC/Serum

Per the SOE, plasma, viable PBMCs, and serum samples will be stored for inflammatory (hs-IL-6, EN-RAGE, oxLDL, d dimer), immunologic and virologic assessments, for metabolic testing (high molecular weight adiponectin and insulin), and for hormone testing, such as sex hormone binding globulin (SHBG), estradiol, total and free testosterone.

NOTE: Only sites certified and in good standing by the IQA Cryopreservation PT Program at the Duke Human Vaccine Institute are required to collect VIABLE PBMCs.

6.3.12 Columbia-Suicide Severity Rating Scale (C-SSRS) Questionnaire

The C-SSRS will be administered per the SOE. The C-SSRS eCRF is posted on the DMC Portal in the Forms Management Utility.

NOTE: Refer to section 8.4 and the MOPS for C-SSRS interpretation and additional management recommendations.

6.3.13 ART Adherence Assessment

ART adherence assessment eCRF will be administered per the SOE. The adherence eCRF is posted on the DMC Portal in the Forms Management Utility.

NOTE: Site staff or investigators should review the results of the adherence assessment during the visit, and counsel participants on adherence if any concerns become apparent.

6.3.14 FHT Adherence Assessment**s**

FHT adherence assessment eCRF will be administered**, and the pill count eCRF will be completed per the SOE.** The adherence eCRF **and the pill count eCRF are** posted on the DMC Portal in the Forms Management Utility.

NOTE: Site staff or investigators should review the results of the adherence assessments during the visit, and counsel participants on adherence if any

concerns become apparent.

6.3.15 FHT Participant Goals

FHT participant goals will be collected per the SOE. The checklist eCRF is posted on the DMC Portal in the Forms Management Utility.

6.3.16 FHT Acceptability and Satisfaction Surveys

FHT acceptability and satisfaction surveys will be administered per the SOE. The survey eCRF is posted on the DMC Portal in the Forms Management Utility.

6.3.17 Semi-structured Interview Subset

For a subset of English- and Spanish-speaking participants, a semi-structured interview regarding FHT acceptability and satisfaction will be conducted by Dr. Amaya Perez-Brumer of the A5403 protocol team or her delegate per the SOE. Refer to section 12.0 for more information. Note that participants must consent to participating in the optional interviews (refer to Informed Consent for Interviews) to be considered for participation. Results from this are not required to be reported on an eCRF.

6.3.18 Remote Visit

A remote visit will be conducted by site staff, per the SOE.

7.0 ADVERSE EVENTS AND STUDY MONITORING

7.1 Definition of Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or diagnosis that occurs in a study participant during the conduct of the study REGARDLESS of the attribution (i.e., relationship of event to medical treatment/study product/device or procedure/intervention). This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition.

7.2 Adverse Event Collection Requirements for This Protocol

All AEs must be recorded on the eCRFs if any of the following criteria have been met.

- All Grade ≥3 AEs
- All AEs that lead to 17-β estradiol interruption or dose reduction, regardless of grade.
- All AEs meeting **serious adverse event (**SAE**)** definition or **Expedited AE** (EAE) reporting requirement (see section 7.3.2)

- The following AEs will be recorded at Grade ≥1 if:
  - Lipid abnormalities, if values were normal at baseline (if abnormal at baseline, worsening by at least 1 grade should be reported)
  - Glucose abnormalities, if values were normal at baseline (if abnormal at baseline, worsening by at least 1 grade should be reported)
- The following AEs will be recorded at Grade ≥2:
  - Cholecystitis
  - Elevated liver enzymes
  - Hypertension
- The following will be recorded regardless of grade:
  - Coronary heart disease or other cardiovascular disease
  - Cancer (exclusive of basal/squamous cell skin cancer)
  - Diabetes mellitus or pre-diabetes
  - Any vascular events, including arterial events (strokes and myocardial infarctions) or deep venous thrombotic events (venous thromboembolism, deep venous thrombosis, retinal vein thrombosis, and pulmonary embolism)

NOTE: SAEs or events meeting EAE reporting requirements should also be entered into the DAIDS Adverse Experience Reporting System (DAERS), an Internet-based reporting system.

All AEs that are reported must have their severity graded. To grade AEs, sites must refer to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, which can be found on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

SAEs

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above).

7.3 EAE Reporting to DAIDS

7.3.1 Expedited Reporting of Adverse Events to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

The DAERS, an internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs may be submitted using the DAIDS EAE Form. This form is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting.

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at (DAIDSRSCSafetyOffice@tech-res.com).

7.3.2 Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.
- The study agent for which expedited reporting is required is 17-β estradiol.
- In addition to the SAE Reporting Category identified above, other AEs that must be reported in an expedited manner are*:*
  - Any vascular events, including arterial events (strokes and myocardial infarctions) or deep venous thrombotic events (venous thromboembolism, deep venous thrombosis, retinal vein thrombosis, and pulmonary embolism)
  - Severe headache of new onset (Grade ≥3) or worsening of headache leading to Grade ≥3 headache

7.3.3 Grading Severity of Events

The DAIDS AE Grading Table, corrected Version 2.1, July 2017, must be used and is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

7.3.4 Expedited AE Reporting Period

- The expedited AE reporting period for this study is no later than 3 days as per the EAE manual.
- After the protocol-defined EAE reporting period, unless otherwise noted, only suspected, unexpected serious adverse reactions (SUSARs), as defined in Version 2.0 of the DAIDS EAE Manual, will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

7.4 Study Monitoring

The protocol team will monitor the conduct and safety of the study via regularly scheduled summaries of accrual, study discontinuation, study treatment disposition, AEs, and data and specimen completeness, as appropriate, as per the Study Progress, Data, and Safety Monitoring Plan (SPDSMP).

During the study, the safety and tolerability of the study medication will be monitored by toxicity reports presenting laboratory and clinical data. The subset of the team receiving these reports (as per the specifications in the SPDSMP) will discuss these reports on regularly scheduled conference calls or by e-mail. Any concerns will be presented to the DAIDS Clinical Representative.

The DAIDS Clinical Representative will review and assess select EAE reports for potential impact on the study participant safety and protocol conduct as per DAIDS policies, guidance documents, and SOPs as applicable.

This trial will be formally monitored at least annually by an ACTG-appointed Study Monitoring Committee (SMC) (as per appointment via the ARTS TSG). The study will undergo interim feasibility and safety review by the SMC. The first interim review will occur no more than one year after enrollment of the first participant or 25 participants complete 24 weeks of follow-up (in order to have conduct and safety data on at least two possible estradiol dose titrations), whichever occurs earlier. An additional SMC review may be triggered if accrual at 18 months after first enrollment is less than 50% of the total expected accrual (e.g., fewer than 45 of anticipated 90 participants across all groups). An interim review by the SMC will also be triggered if, at any time the following occurs: if greater than 25% of currently accrued participants (if at least 30 participants have been enrolled) are lost or withdrawn from study follow-up. **Any of the following will trigger an expedited safety review for potential trial modification by the SMC:**

- **Any death that is not clearly attributed to a cause other than the study product (e.g., accident, trauma)**
- **2 or more Grade 3 or higher, treatment-related adverse events**
- **2 or more Grade 2 or higher allergic/hypersensitivity reactions related to study treatment**
- **2 or more of the same Grade 3 or higher, unexpected, treatment-related adverse events (and screening and accrual will be held until the SMC expedited safety review has occurred).**

An interim review may also be convened if a concern is identified by the DAIDS clinical representative, the study chairs, or study statistician in consultation with the team, based on safety reports distributed to the team at frequency specified in the SPDSMP or as requested by any member of the CMC. See section 10.5.1 for statistical and other considerations related to interim monitoring.

Detailed plans for study monitoring are outlined in a Study Monitoring Plan developed by the Statistical and Data Management Center (SDMC) prior to enrollment of the first participant.

8.0 CLINICAL MANAGEMENT ISSUES

8.1 Toxicity

8.1.1 General instructions for management of Grades 1-4 AEs **are** provided in this section. Refer to section 8.2 and further for information on specific toxicities.

8.1.2 Grade 1 or 2

Participants who develop a Grade 1 or 2 AE or toxicity thought to be related to study-supplied 17-β estradiol may continue 17-β estradiol at the discretion of the site investigator, and will be followed carefully **until resolution or stabilization**.

If the site investigator or participant chooses to discontinue any study-supplied 17-β estradiol, the site should notify the A5403 CMC within 72 hours. The premature treatment/study discontinuation evaluations must be completed, and then the participant will be taken off study.

8.1.3 Grade 3 or 4

For Grade 3 or 4 AE or toxicity that is related to study-supplied 17-β estradiol, 17-β estradiol must be held, participants must be followed carefully, and the A5403 CMC should be notified within 72 hours.

The participant should be followed until the AE has decreased to Grade ≤2. After the AE has decreased to Grade ≤2, the A5403 CMC should be consulted to see whether study medication rechallenge at a current or reduced dose is permissible. If it is not, the premature study discontinuation evaluations must be completed, and then the participant will be taken off study.

8.1.4 Serious Adverse Event

For Grade 3 or 4 SAE (as defined in section 7.2) that is related to study-supplied 17-β estradiol, 17-β estradiol must be permanently discontinued and the A5403 CMC should be notified within 72 hours. The participant should be followed until the AE has decreased to Grade ≤2. The premature study discontinuation evaluations must be completed, and then the participant will be taken off study.

8.2 Deep Vein Thrombosis**, Other Thromboembolic Event, or Major Adverse Cardiovascular Event (MACE)**

If DVT, **thromboembolic event, or MACE** is suspected **or diagnosed**, 17-β estradiol should be **discontinued immediately**, and the A5403 CMC should be notified within 72 hours. **If an alternate diagnosis is confirmed,** rechallenge at the current or a reduced dose may be permitted after consultation with the A5403 CMC and local providers.

The sudden onset of partial or complete loss of vision, or a sudden onset of proptosis, diplopia**, or migraine** should lead to discontin**uation of treatment,** pending examination. If examination reveals papilledema or retinal vascular lesions, estrogens should be permanently discontinued.

8.3 Elevated Liver Tests

If any of the following criteria are met, the study-provided 17-β estradiol must be permanently discontinued and the A5403 CMC should be notified within 72 hours. The participant should be followed until the AE has decreased to Grade ≤2. The premature study discontinuation evaluations must be completed, and then the participant will be taken off study.

Specific criteria:

- **ALT or AST >8 times (x) upper limit of normal (ULN)**
- **ALT or AST >5 x ULN for more than 2 weeks**
- **ALT or AST >3 x ULN and total bilirubin (TBL) >2 x ULN or international normalized ratio (INR) >1.5**
- **ALT or AST >3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).**

NOTE: If a participant experiences elevated ALT or AST >3 times ULN, liver tests (ALT, AST, bilirubin, alkaline phosphatase) will be repeated within 3 to 5 days.

8.4 Suicidal Ideation or Attempt, Worsening Depression, or Other Acute Psychiatric Condition

If a participant reports suicidal ideation or attempt, worsening depression, or other acute psychiatric condition, the study-provided 17-β estradiol must be permanently discontinued and the participant should be immediately referred to behavioral health and substance use counseling services for further evaluation and treatment. The A5403 CMC should be notified within 72 hours. When the participant is stable to do so, the premature study discontinuation evaluations must be completed, and the participant will be taken off study.

NOTE: Suicidal ideation or attempt will be defined by answers provided on the C-SSRS. See the MOPS for scoring and interpretation instructions, and for additional considerations.

NOTE: Though suicidal ideation or attempt, worsening depression, or other acute psychiatric condition necessitates discontinuation of study product, if changes in mental health are documented to be transient and due to another cause such as acute intoxication, rechallenge may be considered if the participant has returned to their baseline mental status and if approved by the participant, study investigator,

protocol team and CMC.

8.5 New Diagnosis of Breast Cancer

The premature treatment/study discontinuation evaluations must be completed, and then the participant will be taken off study.

8.6 New Cancer Diagnosis Other than Breast, Basal, and Squamous Cell Skin Cancer

17-β estradiol should be held. Rechallenge at the current or a reduced dose may be permitted after consultation with local oncologist and the A5403 CMC. If it is not, the premature study discontinuation evaluations must be completed, and then the participant will be taken off study.

8.7 Sustained Hypertension

Sustained hypertension is defined as new hypertension or significant increase in blood pressure in a participant with hypertension while on 17-β estradiol and that persists at Grade ≥2 despite antihypertensive therapy. 17-β estradiol should be held, the A5403 CMC should be notified within 72 hours, and attributability should be assessed by the site investigator. Rechallenge at the current or a reduced dose may be permitted. If it is not, the premature study discontinuation evaluations must be completed, and then the participant will be taken off study.

8.8 Detectable Viremia

If a participant has a detectable viral load at >200 copies/mL, the HIV-1 RNA should be repeated at least 7 days later and the participant should have a review of recent medication adherence. If detectable viremia is confirmed, the investigator should assess whether this is likely to be (a) failure due to drug resistance, or (b) failure due to treatment interruptions or incomplete adherence. If, in the opinion of the site investigator, the detectable viremia is likely due to (b) and reinitiation of the same regimen with enhanced adherence would achieve resuppression, then the participant may continue on study on the same regimen. If the site investigator suspects failure with drug resistance and/or considers that the participant should change treatment regimen, then the participant will have the premature study discontinuation evaluations completed, and then the participant will be taken off study.

9.0 CRITERIA FOR DISCONTINUATION

9.1 Permanent and Premature Treatment Discontinuation

- Drug-related toxicity (see section 8.1).
- Request by participant to terminate treatment.
- Clinical reasons believed life-threatening by the physician, even if not addressed in section 8.0 of the protocol.

- Requirement for prohibited concomitant medications.
- At the discretion of the ACTG, IRB/EC, FDA, NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, or investigator

9.2 Premature Study Discontinuation

- Failure by the participant to attend 3 consecutive clinic visits.
- Detectable viremia and site investigator suspects failure with drug resistance (as defined in section 8.**8**) requiring change of ART.
- ART change or discontinuation.
- 17-β estradiol discontinuation.
- Participant repeatedly does not adhere to study medications as prescribed, per the site investigator's discretion.
- Request by the participant to withdraw.
- Request of the primary care provider if she or he thinks the study is no longer in the best interest of the participant.
- At the discretion of the ACTG, IRB/EC, FDA, NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, or investigator.

10.0 STATISTICAL CONSIDERATIONS

10.1 General Design Issues

This is an open label, non-randomized, multicenter trial to evaluate both ART and estradiol exposure (PK) in TW living with HIV who are suppressed on one of **three** targeted ART regimens and are starting or restarting FHT with oral 17-β estradiol.

While the ultimate goals of FHT are internal/external gender congruence, how this is defined varies greatly across individuals (i.e., high heterogeneity within the sample/population), and the time horizon for goal attainment is longer (1 to 3 years), than is feasible for a one-year trial. Therefore, this trial will use mixed methods approach on participant satisfaction/progress towards transgender congruence, as outlined in the behavioral analysis (section 12.0).

Also, the current trial focuses on two primary PK-related questions related to the interaction between estradiol FHT and ART: Does titration to estradiol doses needed for long-term use to attain FHT goals of gender congruence cause changes in ART PK that could impact ART efficacy? Are there differences observed between ART regimens in serum estradiol exposure across the FHT dosing titration scheme? Since most of the PK studies of FHT in this key population have been short in duration and have tested fixed and low doses of estradiol, this trial is designed to fill important knowledge gaps.

All participants will start oral 17-β estradiol at 2 mg/day, and will be evaluated at weeks 4, 12, 24, and 36 for the purposes of titrating the estradiol dose in 2 mg increments based on safety, tolerability and participant preference, using locally tested, real-time total serum estradiol and total testosterone. The titration algorithm, as described in the

MOPS, does not permit escalation of FHT that exceeds hormone target ranges. Potential adjustment of doses of oral 17-β estradiol over the 48-week period of the trial following the trial visits include to 4, 6, 8 and 10 mg/day. De-escalation for tolerability or toxicity will be allowed.
The PK analysis approaches for the primary objectives utilizes both a multi-period, fixed sequence (participants as their own control) design for the primary objective for ART PK, and a parallel group design (where groups are defined and compared based on current ART regimen) for the primary objective for estradiol PK. Each of these primary objectives utilizes sparse, but timed (i.e., trough) PK sampling. At entry, participants are assigned to one of 3 cohorts based on their current ART regimen (ART = required concomitant medication) containing BIC, DTG or DRV/**c**. Other required ART are as defined in the study schema.

Parallel group comparisons (i.e., between ART groups for the estradiol primary PK outcome) are subject to two sources of bias: 1) imbalances between non-randomized ART groups on known or unknown factors associated with estradiol PK outcomes; and 2) informative missingness due to the titration scheme whereby estradiol dose adjustment is partially based upon the outcome being analyzed (i.e., confounding by indication). Therefore, caution is warranted for inference at the higher estradiol doses. These issues are described in more detail in the **A**nalys**e**s section below (section 10.6).

The total sample size accrual goal is 90; or 30 per ART cohort. As analyses are specified at each dose of oral 17-β estradiol, the effective sample size based on observed titration of estradiol over time, is anticipated to be smaller than the enrolled sample. Refer to the sample size section (section 10.4) and **A**nalyses section (section 10.6) below for more details.

A subgroup of participants (who opt-in via informed consent) will undergo intensive PK sampling at a subset of study visits (weeks 0, 24, and 48) for the purposes of estimating the following 17-β estradiol and ART PK parameters which cannot be reliably estimated from sparse trough-timed sampling: Area under the concentration-time curve (AUC), C_{max}, and T_{max}. (Note: additional PK parameters may be modeled and so this is not an exhaustive list.) The sample size for this intensive PK subgroup is 15 per ARV group, and is derived in the sample size section below (section 10.4).

This trial is open label at all levels (participants, clinic research staff, and trial team).

All trial outcomes are defined for the 48-week period following oral 17-β estradiol initiation. The primary completion date (PCD) for the study is when the final participant enrolled is evaluated at week 48 for the primary outcomes.

10.2 Outcome Measures

Primary and secondary outcome measures listed below will be addressed in the study's primary Statistical Analysis Plan (SAP), which will define the content of the Primary Analysis Report. This report will form the basis for the primary study manuscript(s) and results reporting to ClinicalTrials.gov. Outcome measures related to other objectives

intended for subsequent publications are listed under Other Outcome Measures.

10.2.1 Primary Outcome Measures

10.2.1.1 Trough concentration of the analytes BIC, DTG, and DRV in plasma at each received dose of oral 17-β estradiol (0 (pre-FHT or baseline), 2, 4, 6, 8, 10 mg) summarized at the participant level as the following two outcomes:

a. Intra-person geometric ratios of trough ART concentration at each received estradiol dose relative to pre-FHT (i.e., baseline).
b. Indicator of concentration being above drug-specific externally defined threshold.

10.2.1.2 Trough serum total estradiol assessed at each received dose of oral 17-β estradiol (i.e., 2 mg, 4 mg, 6 mg, 8 mg, 10 mg), as quantified via batch testing at central lab.

10.2.2 Secondary Outcome Measures

10.2.2.1 Trough concentration of the analytes TFV-DP, FTC-TP, and 3TC-TP in non-viable PBMCs at each received dose of oral 17-β estradiol (0 (pre-FHT or baseline), 2, 4, 6, 8, 10 mg) summarized at the participant level as the following two outcomes:

1. Intra-person geometric ratios of trough ART concentration at each received estradiol dose relative to pre-FHT (i.e., baseline).
2. Indicator of concentration being above drug-specific externally defined threshold.

10.2.2.2 Occurrence of any reported adverse event deemed related to 17-β estradiol over 48 weeks.

10.2.2.3 Occurrence of SAEs and new events of CAD/CVD, cancer, DM/pre-DM, and vascular event, defined in section 7.2, over 48 weeks.

10.2.2.4 Indicators of testosterone suppression at each received dose of oral 17-β estradiol, defined as any serum total testosterone <50 ng/dL.

10.2.2.5 Indicators of virologic suppression of HIV, defined as plasma HIV-1 viral load <50 copies/mL, measured over 48 weeks.

10.2.2.6 Changes in overall transgender congruence score from baseline, measured over 48 weeks.

10.2.2.7 Area under the curve over 8 hours (AUC 0-8h) of estradiol based on intensive sampling among a subset of participants.

10.2.2.8 Absolute and percent changes in weight and BMI from baseline, measured over 48 weeks.

10.2.2.9 Absolute and percent changes from baseline in minimum waist and maximum hip circumference and waist-hip ratio measured over 48 weeks.

10.2.2.10 Absolute changes in fasting lipids from baseline (e.g., direct LDL, total cholesterol, triglycerides, HDL), measured over 48 weeks.

10.2.2.11 Absolute changes in glucose and insulin sensitivity (HOMA-IR) from baseline, measured over 48 weeks.

10.2.3 Other Outcome Measures

10.2.3.1 Changes in overall intervention acceptability (per participant self-report via survey and interview) between baseline and weeks 24 and 48.

10.2.3.2 Changes in inflammation from baseline to weeks 4, 12, 24, 36, and 48 post initiation of oral 17-β estradiol, as measured by select biomarkers of inflammation (see section 6.3).

10.2.3.3 Serum total estradiol at 4, 12, 24, 36, and 48 weeks post initiation of oral 17-β estradiol, as measured in real time at local labs for the purposes of real-time estradiol titration.

10.2.3.4 Cellular HIV transcriptional activity.

10.2.3.5 PK parameters (e.g. Cmax, Tmax) of estradiol based on intensive sampling, among a subset of participants.

10.2.3.6 T-cell cycling and activation, as measured by Ki67, HLA-DR^{+}CD38^{+} and PD-1 expression.

10.2.3.7 Absolute changes from baseline, measured at 4, 12, 24, 36, and 48 weeks post initiation of oral 17-β estradiol in sex hormones.

10.2.3.8 PK parameters (e.g., AUC, C_{max}, T_{max}) of ART based on intensive sampling at 24 and 48 weeks, among a subset of participants.

10.3 Randomization and Stratification

There is no randomization to study groups or stratification in this trial. During the enrollment process, participants will be assigned to cohorts/groups for the purposes of analysis, based upon their current ART regimen. Further partitioning or other adjustments for analyses will be based on post-enrollment events including adherence to or changes to ART and study treatment of 17-β estradiol, use of anti-androgens (e.g.,

spironolactone) after week 24, and observed oral 17-β estradiol dosing based on titration over 36 weeks. The subgroup for intensive PK will be self-selected by endorsement via informed consent. See section 10.6 for more details.

10.4 Sample Size and Accrual

10.4.1 Sample Size for ART Primary PK Objective

For the primary objective/outcome of trough ART concentration using fixed sequence design, the sample size is derived using assumptions on the standard deviation of intra-person differences in natural log-transformed trough concentrations (i.e., transformed geometric ratios). Since direct estimates of these standard deviations are rarely reported, even in fixed-sequence designs, we used published estimates of between-person coefficient of variation of trough concentrations of the respective ARTs. Combining these various assumptions of correlations of log-transformed trough concentrations within a person (e.g., 0 = no correlation, or independent observations, 0.25 and 0.5), and the following relationship between coefficient of variation (CV) and standard deviation of the log-transformed trough concentration under the assumption of log-normally distributed troughs {s.d. = square root [ln(CV^2+1)]}, we calculated the standard deviation of the difference (on the log scale, which corresponds to the geometric ratio), using the formula : var(x1 – x2) = var(x1) + var(x2) – [2* correlation(X1,X2) * stdev(X1)*stdev(X2)].

The published inter-person coefficient of variation of C_{trough} of BIC = 0.352 from PK fact sheet from University of Liverpool PK Research Labs [**46**]; of C24 of DTG = 0.47 (using largest estimated from dosing with high fat meal, other CVs range from .34 to 0.44) [**47**]. Max intrasubject variability on log C_{max} of components among DRV/**c**, FTC, and TAF was 0.36 and thus the range of standard deviations below covers DRV as well [**47**, **48**].

Table 10.4.1-1: Variation in C_{trough} for BIC and DTG

Reference for Between Person CV in C_{trough}	Hypothesized Correlation Between C_{trough} Concentrations in Same Participant	Standard Deviation of Intra-Person Difference in Log $C_{troughs}$
BIC = 0.352	0.0	0.48
	0.25	0.42
	0.50	0.34
DTG = 0.47	0.0	0.63
	0.25	0.55
	0.5	0.45

Once the standard deviation on the log difference is assumed, then normal assumptions on the natural log-scaled trough concentrations, may be applied. The hypothesis for the ART primary PK objective is an equivalence hypothesis (i.e., not clinically significant reductions). Therefore, two-one sided tests (TOST) using the t-test and pre-specified no effect bounds for the geometric mean ratio (i.e., the mean of the intra-person geometric ratios) can be used to calculate statistical power for

the equivalence hypothesis.

Additional assumptions include what effective sample sizes might be expected at higher than 2 mg doses of estradiol based on the realization of the titration scheme. Since participants might titrate up at different rates, the assumptions in the immediate table below use the simplifying assumption to equate number of "late titraters" with numbers lost to follow-up (i.e., these cancel out). In primary analyses, only one observation per estradiol dose will contribute to calculation of outcomes. (See **A**nalys**e**s section (section 10.6).for more details.)

The following hypothesized effective sample sizes (Table 10.4.1-2) representing the assumptions above are used for statistical power calculations below in Table 10.4.1-3. These estimates are purposely conservative.

Table 10.4.1-2: Projected Sample Sizes Across Doses of 17-Beta EstradiolDose of Oral 17-β Estradiol

Dosing	Hypothesized Effective Group Sample Size per ART	Basis of Effective Sample Size
2 mg/day	30	100% of 30
4 mg/day	28	95% of 30
6 mg/day	25	90% of 28
8 mg/day	20	80% of 25
10 mg/day	15	75% of 20

Assumptions in Table 10.4.1-3 below include true (alternative) geometric mean ratio (GMR) of 1.0 (i.e., equivalence), and alpha (significance level) of 0.05 (which is equivalent to a 90% CI due to two-one sided tests at 0.05).

Table 10.4.1-3: Statistical Power for ART Primary Objective

Standard deviation of intra-person differences in log C_{trough} of ARV	Effective Sample size per 17-β estradiol dose and ART group	No Effect Bounds	Statistical power
0.35	28 (or higher)	(0.80, 1.25)	0.90 (or higher)
	25		0.85
	15	(0.75, 1.33)	0.83
0.45	25	(0.75, 1.33)	0.85
	20	(0.70, 1.43)	0.92
	15		0.80
0.50	30	(0.75, 1.35)	0.84
	28		0.81
	20	(0.70, 1.43)	0.85

Standard deviation of intra-person differences in log C_{trough} of ARV	Effective Sample size per 17-β estradiol dose and ART group	No Effect Bounds	Statistical power
0.55	28	(0.70, 1.43)	0.91
	25		0.87
	20		0.75
0.65	30	(0.70, 1.43)	0.80
	28		0.77
	25		0.69

Thus, from the table above, for an effective sample size of 20 within an ART analysis group, standard deviation of intra-person differences in log C_{trough} (ARV) of 0.45, there is 92% power to declare equivalence under no-effect bounds of (0.70, 1.43).

While various NEB are given above to demonstrate statistical power under various scenarios, the reference interval used in analysis will be (0.70,1.43) due to the higher variability of the C_{trough} parameter [**49**].

10.4.2 Sample Size for Estradiol Primary Objective

For the primary objective/outcome of comparing serum estradiol between groups defined by ART regimen at each of the doses of oral 17-β estradiol, a parallel groups PK design is utilized so that the geometric mean ratio is formed by difference in log-transformed means between ART groups. To size for this design, a key assumption is the between participants CV in the PK of serum estradiol. The CV of C_{trough} of serum estradiol is extracted from the iFACT trial, which tested for the drug-drug-interaction of another form of oral estradiol (estradiol valerate) given at 2 mg/day with ART regimen EFV/FTC/TDF in ART naïve transgender women in Thailand [**26**]. From this trial, the CV on the C_{24} of estradiol was 0.60 (i.e., highly variable). For this objective, testing framework is superiority (as opposed to equivalence in the previous objective), comparing the DRV/**c** ART group against the other 2 ART groups.

Under the following alternative GMRs, power is calculated for various hypothesized effective sample sizes (see Table 10.4.1-2 above for estimates on effective sample sizes at various doses of oral 17-β estradiol) (other assumptions include 0.05 significance level using TOST):

Table 10.4.2-1: Statistical Power for Estradiol Primary Objective

Alternative GMR for Serum Estradiol (DRV/other ART)	Sample Size in DRV Group	Sample Sizes in Other ART Groups Combined	Statistical Power
0.70	30 (e.g., 2 mg dose)	60 (e.g., 2 mg dose)	0.81
	28 (e.g., 4 mg dose)	56 (e.g., 4 mg dose)	0.78
0.65	25 (e.g., 6 mg dose)	50 (e.g., 6 mg dose)	0.88
0.63	20	40	0.85
0.60	15	30	0.81

By Table 10.4.2-1 above, as the effective sample sizes decrease over increasing estradiol doses (since not all participants are anticipated to dose-escalate to the highest levels), the alternative GMR **that** can be detected with adequate **power** (~80% or higher) decreases from 0.70 to 0.60. Note, the sample sizes above make the simplifying assumption that the effective sample sizes in ART group will be similar across the increasing oral 17-β estradiol doses by observed titration.

10.4.3 Sample Size for Intensive PK Subsample

The approach for calculation is similar to the primary objective for PK, but utilizes intensive sampling for estimation of estradiol PK parameters of C_{max} (and T_{max}), and AUC. From the iFACT trial [**26**] the maximum CV between AUC(24hrs) and C_{max} of estradiol was 0.27. Using this assumed CV, the estimated standard deviation of the log transformed parameters is 0.265. Under normality assumptions for log transformed parameters, a sample size of 15 has 80% power (conditional probability) that a 2-sided, 95% CI on the estradiol PK parameter has a half-width of 0.168. Exponentiating provides example CIs of (422.7, 591.5) around a hypothetical mean of 500 (e.g., AUC), and (42.3, 59.1) around a hypothetical mean of 50 (e.g., C_{max}).

10.4.4 Accrual

Complete accrual to all study groups is anticipated to take 12-18 months. However, it is not assumed for this accrual to be uniform over this time period, or by ART group. Since there are not any previous network trials accruing exclusively from this key population, other trials do not inform benchmarks for the rate of accrual. The survey to potential participating network sites suggests that sufficient numbers of potential participants exist in the communities already targeted by those sites to allow enrollment within this time frame. Due to differences in regulatory approvals in different locations, it is anticipated that accrual during the first 6 months of the study may be primarily from US sites. But it is anticipated that accrual will be rapid following the approval of the study at non-US CRSs.

There is a target to enroll at least 50% participants who identify as non-white or Latine.

10.5 Data and Safety Monitoring

10.5.1 Interim Monitoring Guidelines

It is recommended that the trial be stopped in the case of any death that is caused by study treatment, and that trial modification (including stopping) should be considered in the case of unexpected serious illness (i.e., SAE) caused by the study treatment. Section 7.4 also lists AE triggers for expedited safety review, which may also result in trial modification following SMC review.

Any/all SMC reviews will include administrative/trial conduct data (e.g., accrual, adherence to ART and study treatment, ART changes, realization of titration scheme, including timing of estradiol dose changes relative to study visits, drop-out [including withdrawals and losses to follow-up]); as well as safety information (reported AEs and SAEs) including safety-related outcomes (e.g., protocol defined virologic failure and targeted events). Because there are no plans to initiate batched centralized PK testing prior to study completion, there are no plans for inclusion of early information on either of the primary outcomes (ART or estradiol). Locally measured estradiol concentrations will be available to sites for clinical management of FHT, but will not be summarized for SMC review due to inter-assay variability between laboratories. The realization of titration will provide information on how these local hormone concentrations were used in trial conduct.

Between SMC review, routine monitoring reports are distributed to the team and are detailed in the Study Progress, Data, and Safety Monitoring Plan (SPDSMP): screening, accrual, data delinquency and availability, study and treatment status, ART changes, collected specimen availability with attention to samples for batched PK testing (both sparse and intensive), and safety. These routine reports are distributed as per the SPDSMP to the team (exact distribution as per SPDSMP), which includes the DAIDS clinical representative.

Detailed plans for study monitoring will be outlined in a SPDSMP developed by the SDMC prior to enrollment of the first participant.

10.5.2 Analysis Plan

See **section 10.5.1** for **monitoring guidelines**.

10.6 Analyses

For both of the primary PK analyses, inclusion in the analysis is based upon no evidence of non-adherence or misuse of either ART regimen or oral 17-β estradiol, from any source (e.g., including but not limited to self-report and objectives measures of adherence, such as undetectable drug levels, or estradiol levels indicative of extra-trial dosing such as PK results) around the timing of samples drawn for PK that would violate steady state assumptions for the drugs in question (i.e., estradiol and targeted ART). Because participant preference is part of the titration process, analyses will be as-treated, based on the received dose of estradiol, rather than the prescribed dose. This is because the study goal is to understand the biologic associations between oral 17-β estradiol and ART drug concentrations. Analyses will also be as-treated with respect to changes in ART regimen.

All analyses will use log-transformed concentrations of drug or hormones or PK parameters calculated from those concentrations of drug or hormones in order to utilize normality assumptions in the analysis.

Analyses will be informed and adjusted by the following (and **details on subgroup** approaches will be described in the **primary** SAP [**publicly posted]):** Subset of trial sample restarting FHT versus naïve to FHT (with particular relevance to transgender congruence scale and FHT acceptability outcomes), and use of anti-androgen during study follow-up. **Specifically, supplementary analyses will be within subgroups by naïve versus restarting FHT, and separately, by use versus non-use of anti-androgens.**

While the plan is to conduct the primary (final) analysis once the last participant has met the primary completion date (week 48 evaluations), and the routine process for final analysis preparation of the database has completed, the following is an alternate plan under the contingency that one (or more) ART cohort completes accrual and follow-up far in advance of the others. In this case, objectives which do not require inclusion of the ART cohort still under accrual or in follow-up (e.g., objectives which include analyses within ART cohort) may undergo final analysis and public presentation once the data to support those analyses have completed the quality control and assurances process and are considered final. In this contingency, the final analysis will occur in 2 stages.

10.6.1 Primary Analysis

For the primary ART PK analysis, participants serve as their own control; thus no bias is introduced by absence of randomization.

10.6.1.1 The primary objective will be approached with 2 primary analyses. The first is to calculate the GMR of the C_{trough} of each ARV, using the fixed sequence design, and at each dose of oral 17-β estradiol. The reference period for each GMR will be the pre-FHT (baseline) period when C_{trough} of the ARV is measured in the absence of oral 17-β estradiol. To handle cases of multiple observations at the same estradiol dose; the first sampled among qualifying C_{trough} concentrations at steady-state will be used in the primary analysis. (Note: other approaches [e.g., geometric mean or observed minimum, or repeated measures modeling to include all concentrations] may be employed as supplementary analyses on this objective and will be detailed in the SAP.)

The geometric means and CVs of $C_{troughs}$ of each ARV analyte within each estradiol dose will be calculated. Other descriptive statistics at each estradiol dose include key percentiles (e.g., 25th, 50th, 75th).

The 90% confidence interval about each GMR will be calculated (and compared to a reference interval of [0.7,1.43]), and will not be adjusted for multiplicity due to evaluation at 2, 4, 6, 8 and 10 mg/day doses of oral 17-β estradiol. The location and coverage of the confidence interval will be the focus of inference, rather than the p-value from a hypothesis test.

A reference interval wider than the conservative (0.8,1.25) bioequivalence interval was chosen due to the higher variability of C_{trough} parameter (versus other parameters like AUC that combine multiple measurements). This approach has been used in other trials when the PK parameter has exhibited high variability [**49**, **50**].

The second component of the ARV primary analysis is to address the hypothesis that ART exposure in the presence of potentially increasing doses of oral 17-β estradiol remains above externally defined thresholds. This analysis will calculate the absolute number and proportion of participants whose C_{trough} of ARV is below a specified reference level at a particular dose of oral 17-β estradiol. Thresholds will be defined from the lower 95% percentile of their trough concentrations from intensive PK studies (exact values will be provided in the SAP). See the trial rationale section 2.2 above for more details on the rationale of these thresholds. Exact (based on Wilson-score) 95% confidence intervals about the observed proportions will be calculated.

10.6.1.2 Primary analysis of estradiol PK objective

For this primary objective, participants are compared between ART groups (boosted versus not), and thus utilizes a parallel groups PK design. At each oral 17-β estradiol dose, the GMR of C_{trough} of serum total estradiol between groups will be calculated along with the respective 90% CI. Cases of multiple observations within a participant at the same estradiol dose will be handled by utilizing the earliest (in calendar time) for the primary analysis. [Note: additional methods are being explored for supplementary analyses including geometric mean.] Both unadjusted and adjusted GMRs will be calculated, where adjusted GMRs will be based on pre-specific prognostic factors, and may rely on prioritizing factors included for adjustment based on significance tests for imbalance on these factors between analysis groups. See discussion below on bias for the rationale for adjustment of GMRs.

The geometric means and CVs within each analysis group will also be calculated. Other descriptive statistics at the analysis group level include key percentiles (e.g., 25th, 50th, 75th) of the serum total estradiol measured as well as Wilcoxon-Rank Sum tests between analysis groups.

If ART regimen changes occur that would represent switch to the other comparison group, then subsequent estradiol concentrations will be excluded from analysis.

Unlike the ART PK objective where a fixed sequence design was possible, comparisons here are subject to bias based on confounding by prognostic

factors for serum total estradiol which may be imbalanced between analysis groups not allocated via randomization in this trial, as well as confounding by indication via the estradiol titration scheme as described above (10.1). While the former source of bias may be mitigated via calculating adjusted GMR for known and measured prognostics factors which have large imbalances between analysis groups (as proposed above), the later bias is more difficult to tackle. Importantly, confounding by indication is not anticipated to occur or impact the analysis and interpretation at the same rate throughout the trial. If confounding by indication is present, it is expected to be greater at higher FHT dose levels. For instance, because everyone is started at the 2 mg dose and evaluated at week 4 at this dose before implementing the titration scheme, confounding by indication does not apply for this estradiol dose. It is anticipated that this bias may be minimal at the lower doses of estradiol, which represent the earlier phases of the titration scheme (e.g., 4 mg/day and potentially 6 mg/day). Therefore, this issue is of more concern at the higher doses of estradiol (6-10 mg/day, where it could substantively impact the results. Therefore, the interpretation of comparisons at higher doses, as well as interpretation of results at lower versus higher doses, will require caution and acknowledgment of this issue.

10.6.2 Secondary Analyses

Each of the safety outcomes will be summarized by ART group and also by 17-β estradiol dose at time of outcome. Two degree of freedom tests will explore if the number of participants with qualifying safety outcomes differs across the three ART groups, with pairwise comparisons informed by the results of the omnibus testing. Association of 17-β estradiol dose with safety outcomes will utilize estimation of each safety outcome incidence in order to adjust for time of exposure to various estradiol doses. For laboratory outcomes where results are reported regardless of grade, changes from pre-FHT and from lower estradiol doses will be summarized.

Analyses of 17-β estradiol PK parameters from intensive sampling on a subset of participants will use parallel methods to the primary methods described above, where the difference may be the reference interval for no interaction.
Proportion of participants with VL <50 copies/mL over time by ART group, and by 17-β estradiol dose at time of measurement will be reported. Missing viral loads will be excluded from this analysis.

Proportion of participants with suppressed testosterone (among those not taking anti-androgens **and who have not undergone orchiectomy**, missing results excluded) will be summarized by ART group and by 17-β estradiol dose at time of measurement.

Overall transgender congruence scores and changes from baseline to weeks 24 and 48 will be summarized by ART group and by 17-β estradiol dose at time of

measurement. Mean Scores will be modeled by both ART group and 17-β estradiol dose to test secondary hypothesis.

Anthropometric (weight, BMI, waist, hip, and waist-to-hip ratio) outcomes, and both absolute and percent changes will be summarized by ART group and by 17-β estradiol dose at time of measurement. The hypothesized association of 17-β estradiol dose with increased changes will be assessed by comparing changes within each person adjusting for time and 17-β estradiol dose (e.g., change in outcome per month at a given dose). The reference for each person will be the pre-FHT measurement (i.e., baseline), resulting in a ratio at each dose.

A comprehensive SAP will be prepared to further address analyses of all outcomes to address all objectives.

11.0 PHARMACOLOGY PLAN

11.1 Pharmacology Objectives

See section 1.0 for study objectives. If changes or unexpected results in ART trough concentrations are observed, additional analysis of intensive PK samples may be conducted to better describe those changes. If that occurs, the following objectives will be explored:

11.1.1 To describe the PK parameters (area under the concentration-time curve [AUC], C_{trough}, C_{max}, T_{max}) of ART in a subset of TW undergoing intensive PK in the context of historical data from cisgender individuals.

11.1.2 To determine if intra-individual ART PK parameters (AUC, C_{trough}, C_{max}, T_{max}) change with increasing 17-β estradiol dosage over 48 weeks in a subset of TW undergoing intensive PK.

11.2 Pharmacology Study Design

This is a pharmacology study, thus, the pharmacology study design is specified in the overall study design described in section 3.0.

A combined intensive and sparse sampling strategy will be used to characterize the **steady-state** ART and **steady-state** 17-β estradiol PK. Intensive sampling will occur, in a subset of participants, at baseline prior to 17-β estradiol exposure in this population of TW, and weeks 24 and 48 at escalating 17-β estradiol doses. Sparse sampling will enrich these data and contribute to a population PK model that can investigate covariates on drug exposure and to assess the relationship between drug exposure and study-related outcomes.

Intensive PK samples will be analyzed in batch for 17-β estradiol at defined time point(s)

(see LPC). Samples of plasma and PBMC from each time point will be stored and evaluated for ART concentrations, as needed, to explain pharmacokinetic results related to the sparse trough concentrations.

11.3 Primary and Secondary Data, Modeling, and Data Analysis

Intensive PK analysis

The plasma PK parameters for 17-β estradiol include: maximum concentration (C_{max}), time to maximum concentration (T_{max}), minimum concentration (C_{min}), time to minimum concentration (T_{min}), oral clearance (CL/F), volume of distribution (Vd), elimination half-life (T1/2), C_{trough}, time of C_{trough}, and area under the concentration-time curve over 8 hours ($AUC_{0\text{-}8h}$). Standard noncompartmental techniques will be used to determine these PK parameters using the software package Phoenix WinNonLin (Certara®). The AUC will use the linear up/log down version of the trapezoidal rule. This version of the trapezoidal rule uses linear interpolation between untransformed data up to C_{max}, and between log-transformed data from C_{max} through C_{last}. C_{max} will be taken as the maximum observed concentration. T_{max} is the time at which C_{max} occurs. C_{trough} will be taken as the pre-dose measurement. Apparent oral clearance will be calculated as CL/F. The T1/2 will be determined using regression analysis when possible along with other estimates of exposure as deemed appropriate based on review of the data. This noncompartmental analysis may be supplemented with a compartmental analysis and or a nonlinear mixed effects population PK analysis.

If intensive ART samples are evaluated, the same methods will be used to evaluate these intensive samples. TFV-DP and FTC-TP or 3TC-TP concentrations in PBMCs will be collected on a limited basis therefore no PK parameters will be calculated. Descriptive statistics will be used to summarize the concentrations as follows: C_{3h}, C_{8h}, C_{trough}, $C_{average}$.

Trough and intracellular concentrations

ARV will be measured from pre-dose samples according to section 6.3.9 to derive C_{trough}. BIC, DTG, DRV, TFV, FTC, and 17-β estradiol will be measured from plasma and TFV-DP and FTC-TP or 3TC-TP will be measured from PBMCs. The concentrations will be at a single time point; therefore, no PK parameters will be calculated.

See section 10.6 for statistical analyses on all PK outcomes (PK parameters or timed concentrations).

11.4 Population Pharmacokinetic/Pharmacodynamic Analyses

The objective and intended outcome of this analysis is to investigate relationships among the concentrations of ART and 17-β estradiol with any participant characteristics that influence the PK of the medications and relationships with treatment outcomes. Conventional measures of exposure will be obtained from the pharmacokinetic analyses. PD models may be in the form of a linear or a sigmoid E_{max} relationship.

For this analysis, we will determine the population PK characteristics of ART and 17-β estradiol using all PK data collected across multiple observations in order to inform between- and within-individual variability. This will be approached using appropriate modeling software with a goal to identify participant characteristics (covariates) that may influence these parameters, including the influence of concomitant medications. An error model will be identified and selected using a stepwise process and based on improvements in standard diagnostic plots and estimate precision. If necessary, poorly identified structural parameters, such as the absorption rate constant, may be fixed to usual adult values. Covariates will include age, weight, body mass index, race, estimated glomerular filtration rate, and concomitant medications. The influence of each covariate on the PK characteristics of the drug will be tested sequentially. At the end of the analysis, all covariates that show an influence on the parameters will be evaluated again by comparison of the full model (with all factors included) with a model from which each of the factors is deleted sequentially.

After the model that best describes the plasma PK characteristics of ART and 17-β estradiol is identified, we will next develop a linked PK and PD model to investigate relationships among the PK parameters of the drug, and measure effectiveness.

11.5 Anticipated Outcomes

These PK studies will provide information on ART and 17-β estradiol when combined in TW living with HIV. The PK data will fill gaps in our knowledge about effective co-prescribing of ART and hormones for gender-affirming therapy.

12.0 BEHAVIORAL SCIENCE

12.1 Rationale

The complexity of dire social realities faced by TW can magnify HIV vulnerabilities and limit engagement in the HIV continuum of care and research [3, **36**, **37**]. The inclusion of study satisfaction and acceptability measures are a unique opportunity within A5403 to not only understand psychosocial impacts of gender-affirming care through this intervention but also improve research processes targeting this community.

Importantly, because not all TW will enter this study FHT-naïve, and because individual goals of FHT initiation vary widely between participants, additional qualitative work in a subset of participants will be critical to interpreting the quantitative data obtained.

12.2 Design

The embedded behavioral science study is a two-pronged, concurrent, design. The quantitative survey component will be administered to all A5403 participants. Semi-structured, in-depth interviews will be conducted at the same time points on a smaller sample of TW purposively recruited from English- or Spanish-speaking sites (due to interviewer language restrictions and the need for qualitative interviews to have accurate

interpretation/not be biased by 3rd party translation variation). Participants will be selected from the subset of persons providing written informed consent to be considered for this optional component.

12.3 Measures of Satisfaction and Acceptability

We will measure/track indicators of intervention satisfaction and acceptability at study weeks 0, 24, and 48 (or premature treatment or study discontinuation). To measure acceptability, we will ask participants to self-report the degree to which they find the intervention appropriate and useful using Likert-type agreement scales at weeks 0, 24, and 48 (or premature discontinuation). To measure satisfaction, we will use the 12-question Transgender Congruence Scale (TCS), which will assess associations between FHT, perceived gender congruence, and satisfaction at weeks 0, 24, and 48 (or premature discontinuation) [**38**]. The TCS has demonstrated high internal consistency (alpha >0.80) and has been utilized in numerous clinical settings [**39**, **40**, **41**].

In addition, Dr. Perez-Brumer and her team (as opposed to site staff) will conduct brief, 20-minute, semi-structured, web-based interviews with 30 purposively sampled English- and Spanish-speaking participants by group: BIC-treated group (n=10) (Group 1); a DTG-treated group (n=10) (Group 2) ; and a DRV/**c**-treated group (n=10) (Group 3). This design will provide an opportunity for more in-depth (open-ended) feedback on intervention satisfaction and acceptability across groups and over time. The semi-structured interview guide in English and Spanish is posted on the A5403 PSWP.

12.4 Procedures and Analysis

For the survey components assessing acceptability and satisfaction, participants will complete these at weeks 0, 24, and 48 (or premature discontinuation). Participants may be assisted by a study staff member, if necessary. Survey may be conducted via paper. Study staff will provide real-time support to participants and receive training to ensure confidentiality and standardized data collection at weeks 0, 24, and 48 (or premature discontinuation).

For qualitative interviews assessing satisfaction, all planned interviews are expected to last around 20 minutes and will be conducted online via an online video conferencing platform (i.e., Microsoft Teams, Skype, Zoom, WhatsApp). Participants will be purposively recruited from survey completers based on clinical site, and by group (i.e., BIC-treated group, a DTG-treated group, and a DRV/**c**-treated group (see MOPS for recruitment guidelines) [**51**, **52**]. Interviews will be conducted in English and Spanish by a bilingual medical sociologist (Dr. Amaya Perez-Brumer) with extensive experience conducting qualitative data collection with transgender communities and/or a University of Toronto transgender doctoral trainee who is a licensed social worker and has experience in qualitative research methods. All interviews will be audio-recorded, and transcribed by an outside company. Once the interview is transcribed, the audio recording will be erased from the digital recorder. Audio recordings are considered source documents, and a copy will be kept on a secure password protected server for 5 years at the University of Toronto. Dr. Amaya Perez-

Brumer will have access to personal information and information provided in the interviews. Data will be de-identified. Audio recordings and other information will be kept strictly confidential. For more detail about confidentiality, refer to Informed Consent for Interviews.

For qualitative data, our sample size is driven by the goal of saturation [**53**]. Guided by immersion crystallization, we will analyze transcripts based on inductive and deductive approaches to identify themes and relations between themes [**54**, **55**]. Anticipated main analysis will assess lived experience of taking FHT and ART and participating in the research study, and both quantitative and qualitative assessments will collect information in the following key domains: to assess goals (body modification and study participation), perceived impacts of ART and FHT, and experience with clinic and staff. These qualitative assessments will provide invaluable, supplemental, contextual support to the quantitative data obtained through the participant survey, informing the current data interpretation and future interventions.

13.0 DATA COLLECTION AND MONITORING

13.1 Records to Be Kept

Electronic case report form (eCRF) screens will be made available to sites for data entry. Participants must not be identified by name on any data submitted to the DMC. Participants will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon registration.

13.2 Role of Data Management

13.2.1 Instructions concerning entering study data on eCRFs will be provided by the ACTG DMC. Each CRS is responsible for keying the data in a timely fashion.

13.2.2 It is the responsibility of the ACTG DMC to assure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

13.3 Clinical Site Monitoring and Record Availability

Monitoring visits may be conducted on-site or remotely. Remote visits may include remote source document verification using methods specified for this purpose by NIAID. Remote monitoring visits may be performed in place of, or in addition to, onsite visits to ensure the safety of study participants and data integrity [**56**]. The site **must** make available study documents for site monitors to review utilizing a secure platform that is HIPAA and 21 CFR Part 11 compliant. The Data Management Center will configure Medidata Remo**te** Source Review (RSR) and make it available to all sites. We encourage sites to use the DMC provided Medidata RSR platform but other potential platform options include: Veeva SiteVault, site-controlled SharePoint or cloud-based portal, and direct access to Electronic Medical Record (EMR). Other secure platforms that are 21 CFR Part 11 compliant may be utilized, as allowed by the DAIDS Office of Clinical Site Oversight (OCSO).

13.4 Reporting Protocol Deviations

This protocol follows the requirements to report protocol deviations per SOP ACTG-153. The site principal investigator and personnel are responsible for identifying and reporting deviations. Once protocol deviations are identified, corrective actions are to be developed by the site and implemented promptly. Protocol deviations must be sent to the IRB/EC per their guidelines.

Protocol deviations must be recorded on the study protocol deviation eCRF.

14.0 PARTICIPANTS

14.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document (Informed Consent and Authorization to use and Disclose Protected Health Information) and any subsequent modifications will be reviewed and approved by the IRB or EC responsible for oversight of the study. A signed consent form will be obtained from the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant and this fact will be documented in the participant's record.

14.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the ACTG, IRB/EC, FDA, NIAID, OHRP, other local, US, or and international regulatory entities as part of their duties.

14.3 Study Discontinuation

The study may be discontinued at any time by the ACTG, IRB/EC, FDA, NIAID, OHRP, or other country-specific government agencies as part of their duties to ensure that research participants are protected.

15.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by ACTG policies.

16.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other 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 CDC and the National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

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INFORMED CONSENT AND AUTHORIZATION TO USE AND DISCLOSE PROTECTED HEALTH INFORMATION

Sponsor / Study Title:	National Institute of Allergy and Infectious Diseases (NIAID) / "Giving Standardized Estradiol Therapy In Transgender Women to Research Interactions with HIV Therapy: the GET IT RIgHT Study"
Protocol Number:	A5403
Principal Investigator: (Study Doctor)	«PiFullName»
Telephone:	«IcfPhoneNumber»
Address:	«PiLocations»

SUMMARY

PURPOSE
This is a research study and your participation in this study is voluntary. The reasons this study is being conducted are:

- To see if human immunodeficiency virus (HIV) antiretroviral medicines that you are taking continue to work well in transgender women and other individuals identifying as female or transfeminine but with male sex assigned at birth (referred to as transgender women throughout this form) when taken with feminizing hormone therapy (FHT), also known in this study as estradiol; and
- To see if estradiol levels in blood vary between boosted and un-boosted HIV medicines when transgender women are taking different doses of FHT.

STUDY TREATMENT
The study drug in this trial is 17-β estradiol. Throughout this consent form, the study drug will be referred to as estradiol. At study entry, you will start oral estradiol 2 mg tablet once daily. At weeks 4, 12, 24, and 36, the study doctor may change the estradiol dose by 1 tablet to achieve any goals you may have and depending upon your blood hormone levels.

NUMBER OF PARTICIPANTS
There will be 90 participants: 30 in three different groups. The groups are based on what HIV medicines individuals are taking.

LENGTH OF STUDY
The study will last for 48 weeks, or about 12 months.

REQUIRED ACTIVITIES
At all visits, some blood will be collected from a vein in your arm. At all visits, you will arrive fasting. This means that you will not eat or drink anything other than water and your prescribed medications for

at least 8 hours before the visit. You will also complete surveys, and you may participate in interviews.

RISKS

The following serious side effects have been reported with the study drug, estradiol:

- Numbness or weakness on one side of your body, or in your arm or leg, sudden or severe headache, problems with speech or walking **(stroke). It is possible that this risk may be increased if you are using tobacco or nicotine products. Options for resources to help you quit smoking will be provided to you if you are using these products and if you desire to learn about these resources.**
- Swelling in your hands, ankles, or feet
- Fever, diarrhea, muscle pain, dizziness, fainting
- Sudden and severe stomach pain, nausea, vomiting, **gallbladder disease**
- **Changes in thyroid function**
- Vision changes
- Allergic reaction: Itching or hives, swelling in your face or hands, swelling or tingling in your mouth or throat, chest tightness, trouble breathing
- Breast tenderness **and breast enlargement**
- Chest pain that may spread, coughing up blood, trouble breathing/shortness of breath **(blood clot in lungs)**
- Blistering, peeling, red skin rash
- Lumps in breast
- Pain in your leg (calf) **that may be a blood clot**
- Changes in hair growth **or hair loss**
- Stomach cramps, bloated feeling
- Weight gain
- **Headaches**
- Increase in cholesterol **(a type of fat)**
- **Increase in triglycerides (a type of fat)**
- Blood sugar increases
- **Heart attack**
- **High blood pressure**
- **Breast cancer**
- **Liver problems**
- **Mood changes**

It is not fully known how well HIV is controlled in the body when HIV medications and estradiol are taken together.

Taking blood may cause some discomfort, bleeding, bruising, and/or swelling where the needle enters the body, lightheadedness, and in rare cases, fainting, or infection.

There is the possible risk that taking estradiol could lead to your HIV medications not controlling your HIV as well, which could increase the chance of passing HIV to other people. You should discuss your choices with your health care provider to choose the best way for you to prevent the spread of HIV to your partner(s).

BENEFITS
As part of this study, you will receive estradiol as FHT. FHT is used by some transgender women as part of their gender-affirming care. However, it is possible FHT may not benefit you.

OTHER CHOICES
You may choose to not participate in the study. Instead of being in this study, you have the option of FHT available to you or starting FHT under the care of your regular doctor or other health care provider. Please talk to your regular doctor about these and other choices available to you.

INTRODUCTION

You are being asked to take part in this research study because you take medicines for HIV, and you are a transgender woman or a female or transfeminine person but with male sex assigned at birth. We will say transgender woman throughout this form.

This study is sponsored by the National Institutes of Health (NIH). The study doctor is in charge of this study at this site. Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

This research study is being done to help us understand if the feminizing hormone, estradiol, affects commonly used HIV medication drug levels in blood. Estradiol is used for gender-affirming therapy by transgender women. Its use in this study is considered investigational, which means it is not approved by the Food and Drug Administration (FDA) for gender-affirming therapy by transgender women. It is FDA approved for other uses. The reason this is important to understand is because if estradiol does affect HIV medication drug levels in blood, this may mean that the HIV medication may not work as well in transgender women taking hormones as it should. We will also try to understand whether the feminizing effects of estradiol in transgender women vary depending upon what HIV regimen it is combined with. This study will help us understand whether HIV medications and estradiol have an effect on one another when taken together. This study will fill important gaps in knowledge about HIV medications and estradiol co-administration for transgender women living with HIV and clinical providers.

The study has three (3) groups:
1. Group 1: Participants taking the HIV medications Bictegravir (BIC) + tenofovir alafenamide (TAF) + emtricitabine (FTC)
2. Group 2: Participants taking the HIV medications Dolutegravir (DTG) + tenofovir disoproxil fumarate (TDF) + FTC or lamivudine (3TC)

3. Group 3: Participants taking any HIV medications containing darunavir plus cobicistat (DRV/c).

Which group you are in is determined by the HIV medication you are taking.

You will continue taking your existing HIV medications while on the study or switch to one of the above regimens prior to study entry. Your HIV medications will not be provided by the study.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

Information Collected at Screening

There is some information that we collect on everyone who is screened for an AIDS Clinical Trials Group (ACTG) study. As part of your screening visit, some demographic (for example, age, gender, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, viral load) information will be collected from you. We also collect information on whether you use (or have used) intravenous (IV – into a vein) drugs.

We will collect this information even if you do not enroll in this study. This information is collected so that ACTG researchers may help determine whether there are patterns or common reasons why people do not join a study.

If you agree to join this study, you will be asked to sign this consent form. After you have signed the form, the research staff will determine if you are eligible to join the study. If you are eligible, you may also be able to participate in three 20-minute interviews for this study. If you are interested, you will indicate your interest on Attachment A at the end of this form, and you will sign a separate consent form.

Estradiol

The study drug in this trial is estradiol. Starting at study entry, you will start taking estradiol by mouth 2 mg once daily. At weeks 4, 12, 24, and 36, the study doctor may change the estradiol dose by 1 tablet to achieve any goals you may have. The study doctor will talk to you about what hormone levels they are trying to achieve, depending upon your personal goals. They will monitor hormone levels in your blood to make sure you are on target towards those goals but also that your hormone levels are staying within safe ranges.

Blood Draw

Throughout the study you will have blood drawn. You will be told the results for routine laboratory tests for safety and local hormone level testing.

Screening

If you would like to be in this study, after you have read and signed this consent form, you will come to the clinic for a screening visit to make sure you meet the requirements for joining the study. This will take about an hour. At this visit:

- You will have a physical exam and answer questions about your medical history and any medications you are taking or have taken in the past.
- **You will complete a questionnaire related to suicidal thoughts or attempts.**

- You will have blood drawn. This blood will be used for the following tests:
  - For routine lab tests for safety.
  - To measure the amount of HIV in your blood.
  - To measure the level of prolactin in your blood. Prolactin is a protein best known in helping with milk production.
  - Blood estradiol level if you report that you have not recently taken estradiol. If you have recently taken estradiol, you will be asked to come back later to have your estradiol level checked (but before you enter the study).
  - **You may need to be tested for hepatitis B virus and hepatitis C virus if you don't have documentation for this testing.**

Pre-Entry
If you change your HIV medication to one of the regimens allowed in the study, you will need to take that medication for at least 28 days (about 4 weeks) before you can enter the study. You will have a Pre-Entry visit at least 4 weeks after you have changed your HIV medication to measure the amount of HIV in your blood. This visit will take about 20 minutes. You will have this visit before you officially enter the study.

If you enter the study
If the tests at screening show that you are eligible for the study, you will be assigned to one of three groups, depending on the HIV medicines you are taking.

Entry / Day 0
After your screening visit (and pre-entry visit, if applicable), you will come in for an entry visit. You will arrive fasting for this visit. This means that you will not eat or drink anything other than water and your prescribed medications for at least 8 hours before the visit. Do not take your HIV medications before your study visit. If you usually take them in the morning, bring them with you to the study visit. The study staff will remind you when you need to fast. If you are not fasting for this visit, the visit will be rescheduled, and you will need to return to the clinic on a different day.

At this visit:
- You will have a shortened physical exam and answer questions about your medical history and any medications you are taking or have taken in the past.
- **You will be asked about nicotine use.**
- You will have blood drawn that will be used for the following tests:
  - For routine lab tests for safety.
  - To measure the amount of HIV and CD4+/CD8+ (strength of your immune system, the system that helps your body fight infections) in your blood.
  - Total testosterone (a hormone that stimulates development of male secondary sexual characteristics) and estradiol concentration.
  - Some of the blood will be used for study-required testing during or at the end of the study. You will not receive the results of this testing.
  - Timed trough sampling: Blood will be collected before daily antiretroviral therapy (ART) and estradiol administration, except participants completing an intensive pharmacokinetic (PK) visit.

- About 15 participants in each group will have blood drawn for PK testing over 8 hours after you take your HIV medication. PK testing is testing to see how the levels of the study drugs rise and fall in your blood over time. Please refer to Attachment A to indicate your interest in participating in PK testing.
  - For PK testing, the blood collection time points will be at hour 0 (before you take your HIV medicine), and at 1, 2, 3, 4, 6, 8 hours after taking the HIV medicine.
- You will be asked about your goals for estradiol use.
- You will start taking oral estradiol 2 mg (1 tablet) once daily. **If you are participating in PK testing, you will start taking estradiol after the 8-hour PK testing is complete.**
- You will complete a few surveys about satisfaction and acceptability of estradiol and the trial.
- **You will complete a questionnaire related to suicidal thoughts or attempts.**

Post-Entry Evaluations

You will have five more **in-person** visits after your entry visit (total of six visits). These visits will be at weeks 4, 12, 24, 36, and 48. You will arrive fasting for these visits. Do not take your HIV medications before your study visit. If you usually take them in the morning, bring them with you to the study visit. The study staff will remind you when you need to fast. If you are not fasting for these visits, these visits will be rescheduled, and you will need to return to the clinic on a different day.

At these visits:

- You will have a shortened physical exam.
- You will be asked about nicotine use.
- You will be asked about your HIV medication and study drug use and adherence **(including study staff performing pill counts)**.
- You will have blood drawn that will be used for the following tests:
  - For routine lab tests for safety.
  - To measure the amount of HIV and CD4+/CD8+ (strength of your immune system, the system that helps your body fight infections) in your blood.
  - Total testosterone and estradiol concentration.
  - Some of the blood will be used for study-required testing during or at the end of the study. You will not receive the results of this testing.
  - Timed trough sampling: Blood will be collected before daily ART and estradiol administration, except participants completing an intensive PK visit.
- At weeks 24 and 48 **(or premature discontinuation),** the same 15 participants in each group who had blood drawn for intensive PK at entry will have blood drawn for PK testing over 8 hours as described above. Blood will be drawn before ART and estradiol dosing.
- At weeks 4, 12, 24, 36, and 48 oral estradiol dose may be increased, decreased, or stay the same, depending upon your goals, how you are feeling on the study drug, and the results of your blood tests.
- At weeks 24 and 48, you will complete a few surveys about satisfaction and acceptability of estradiol and the trial.
- **You will complete a questionnaire related to suicidal thoughts or attempts.**

If you are having suicidal thoughts or feel in crisis, call the study doctor at the telephone number listed on the first page of this form. You can also call or text the National Suicide & Crisis Lifeline at 9-8-8 or 1-800-273-TALK (8255). The Lifeline numbers are answered 24 hours a day every day of the year by a skilled, trained counselor. You can also present to a healthcare provider, your local emergency room, or call 9-1-1 to be connected to local emergency services.

- At week 48, you will be asked again about your goals for estradiol use.
- At week 48, you will be referred to community resources for continuing estradiol after the study is over.

You will have a remote visit at week 8. At this visit:

- **You will be asked about medications you're taking and any modifications to study treatment.**
- **You will complete a questionnaire related to suicidal thoughts or attempts.**

CAN I CHOOSE THE TYPES OF RESEARCH THAT MY SAMPLES AND INFORMATION ARE USED FOR?

You cannot choose the types of research that your samples and information are used for because some of your blood will be stored and used for study-required PK, virologic [viruses], immunologic [blood cells] and metabolic or inflammatory [the physical and chemical changes that take place in the body] testing. You will not be told the results from these study-required tests.

The labels on your samples, including those that are stored for later testing, will be coded and will not include any private information that has been collected about you. This means that no one looking at the labels or at other information will be able to know that the samples or information came from you.

The tests described above are required by this study. If you do not agree to the storage or testing that has been described above, you should not join this study. If you stop the study early, investigators may keep and analyze already-collected data.

Please refer to Attachment B to consent for use of your samples in other studies.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 90 participants will take part in this study: 30 in each of the three groups.

HOW LONG WILL I BE IN THIS STUDY?

You will be in the study for 48 weeks, or about 1 year.

WHY WOULD THE STUDY DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early if:
- You do not attend three consecutive study visits.
- The study doctor suspects HIV medication drug resistance and needs you to change your HIV meds.
- You are not able to take the study drug as prescribed.
- **The study doctor discontinues the study drug based on how you answer questions about and or report suicidal ideation or attempts, or other changes in your mental health, or worsening depression.**
- You are not taking estradiol as prescribed by the study (such as taking higher or lower doses than what you agreed with your study doctor).
- You change your HIV medications for some other reason.
- You request to be taken off the study.
- Your primary care doctor decides the study is no longer in your best interest or that it is unlikely that you will be able to comply with the study requirements.
- The study is stopped or cancelled.

The study doctor may also need to take you off estradiol without your permission if:
- The study doctor or your primary care doctor identifies a reason why continuing the estradiol may be harmful to you.
- You need a treatment that you may not take while taking estradiol.
- You have adverse events (side effects) related to estradiol.

If you must permanently stop taking 17-β estradiol due to suicidal ideation or attempt, worsening depression, or other acute psychiatric conditions, you will be immediately referred to behavioral health and substance use counseling services for further evaluation and treatment.

If you have to stop taking estradiol early or you are taken off the study early, you will have a Premature Study Treatment or Study Discontinuation visit and have several evaluations.

IF I HAVE TO PERMANENTLY STOP TAKING STUDY-PROVIDED DRUGS OR ONCE I LEAVE THE STUDY, HOW WOULD DRUGS BE PROVIDED?

If you must permanently stop taking estradiol before your study participation is over, you may check with your regular doctor or local clinic for options. The study staff will discuss other options that may be of benefit to you.

<u>After the study</u>
After you have completed your study participation, the study will not be able to continue to provide you with estradiol you received on the study. If continuing to take these or similar drugs/agents would be of benefit to you, the study staff will discuss how you may be able to obtain them.

WHAT ARE THE RISKS OF THE STUDY?

The study will assess interactions between estradiol and your HIV medicines. It is important for you to be aware of the side effects of estradiol, so you and the study team can manage the risks. The list below is what is known from studies of estradiol involving women who are peri- or post-menopausal.

Estradiol

The following serious side effects have been reported with the study drug, estradiol:

- Numbness or weakness on one side of your body, or in your arm or leg, sudden or severe headache, problems with speech or walking (stroke). It is possible that this risk may be increased if you are using tobacco or nicotine products. **Options for resources to help you quit smoking will be provided to you if you are using these products and if you desire to learn about these resources.**
- Swelling in your hands, ankles, or feet
- Fever, diarrhea, muscle pain, dizziness, fainting
- **Sudden and severe** stomach pain, nausea, vomiting, gallbladder disease
- Changes in thyroid function
- Vision changes
- Allergic reaction: Itching or hives, swelling in your face or hands, swelling or tingling in your mouth or throat, chest tightness, trouble breathing
- Breast tenderness and breast enlargement
- Chest pain that may spread, coughing up blood, trouble breathing/shortness of breath (blood clots in lungs)
- Blistering, peeling, red skin rash
- Lumps in breast
- Pain in your leg (calf) **that may be a blood clot**
- Changes in hair growth **or hair loss**
- **Stomach cramps, bloated feeling**
- Weight gain
- Headaches
- Increase in cholesterol (a type of fat)
- Increase in triglycerides (a type of fat)
- Blood sugar increases
- Heart attack
- High blood pressure
- **Breast cancer**
- **Liver problems**
- **Mood changes**

It is unknown how well HIV is controlled in the body when HIV medications and estradiol are taken together.

Risks of Blood Draws
Taking blood may cause some discomfort, bleeding, bruising, and/or swelling where the needle enters the body, lightheadedness, and in rare cases, fainting, or infection.

Passing HIV to Other People
There is the possible risk that taking estradiol could lead to your HIV medications not controlling your HIV as well, which could increase the chance of passing HIV to other people. You should discuss your choices with your health care provider to choose the best way for you to prevent the spread of HIV to your partner(s).

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

As part of this study, you will receive estradiol for 48 weeks. FHT is used by transgender women to enhance their gender-affirming care and well-being. For example, the estradiol you receive could help in development of female physical characteristics, such as breast growth, weight gain in the hips and buttocks, reduced or softer facial hair growth, etc. However, it is possible FHT may not benefit you. Information learned from this study may help others who are transgender women and who have HIV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Enrollment in the study is voluntary. You may choose to not participate in the study. Instead of being in this study, you have the option of starting FHT under the care of your regular doctor or other health care provider.

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless you have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (see below); if you have consented to the disclosure, including for your medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research participants.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or

program evaluation by the agency which is funding this project or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study. The Certificate of Confidentiality will not be used to prevent disclosure for any purpose you have consented to in this informed consent document.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

Your records may be reviewed by the US Food and Drug Administration (FDA), the ACTG, the US Office for Human Research Protections (OHRP), or other local, US, and international regulatory entities as part of their duties, Advarra Institutional Review Board (IRB) (a committee that protects the rights and safety of participants in research), National Institutes of Health (NIH), study staff, study monitors, and their designees.

Also, any publication of this study will not use your name or identify you personally.

A description of this clinical trial will be available on https://ClinicalTrials.gov as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT ARE THE COSTS TO ME?

The study will pay for the study drug (estradiol), research-related tests and assessments. Taking part in this study may lead to added costs to you and your insurance company. In some cases it is possible that your insurance company will not pay for these costs because you are taking part in a research study. Costs may include but not limited to transportation costs, time away from work or home.

WILL I RECEIVE ANY PAYMENT?

«Compensation»

You will be paid up to a total of $xx.xx if you complete this study. You will be paid for the visits you complete according to the following schedule:

- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.

If you do not complete the study, for any reason, you will be paid for each study visit you do complete.

You will be paid _____ *["following each completed visit", "monthly", "quarterly", "at the end of your participation in the research study", "following each completed visit or at the end of your participation in the research study, whichever you prefer"]*.

If you have any questions regarding your compensation for participation, please contact the study staff.
[OR]
You will not receive any monetary compensation for your participation in this study.

[If applicable:] We will reimburse you for the cost of *[describe: e.g., traveling to your study visits]*. You will be reimbursed approximately *[e.g., 2 weeks, 1 month, etc.]* after you submit your travel receipts to the study staff.

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of this study, you will be given immediate treatment for your injuries. The cost for this treatment could be charged to you or your insurance company.

There is no program for compensation through the NIH.
You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. Your site will share a summary of the results when they are ready to be presented. Your study staff can answer any questions you may have.

WHOM TO CONTACT ABOUT THIS STUDY

During the study, if you experience any medical problems, suffer a research-related injury, or have questions, concerns or complaints about the study such as:

- Whom to contact in the case of a research-related injury or illness;
- Payment or compensation for being in the study, if any;
- Your responsibilities as a research participant;

- Eligibility to participate in the study;
- The study doctor's or study site's decision to withdraw you from participation;
- Results of tests and/or procedures;

Please contact the study doctor at the telephone number listed on the first page of this consent document.

If you seek emergency care, or hospitalization is required, alert the treating physician that you are participating in this research study.

An institutional review board (IRB) is an independent committee established to help protect the rights of research participants. If you have any questions about your rights as a research participant, contact:

- By mail:
  Study Subject Adviser
  Advarra IRB
  6100 Merriweather Dr., Suite 600
  Columbia, MD 21044
- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: Pro00066234.

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date

AUTHORIZATION TO USE AND DISCLOSE PROTECTED HEALTH INFORMATION

If you decide to be in this study, the study doctor and study staff will use and share health data about you to conduct the study. Health data may include:

- Your name.
- Address.
- Phone number.
- Date of birth.
- Medical history.
- Information from your study visits, including all test results.

Health data may come from your study records or from existing records kept by your doctor or other health care workers.

For this study, the study staff may share health data about you with authorized users. Authorized users may include:

- Representatives of University of California, Los Angeles (UCLA) and National Institute of Allergy and Infectious Diseases (NIAID).
- Representatives of ACTG.
- Representatives of Advarra IRB (an Institutional Review Board that reviews this study).
- The Food and Drug Administration (FDA) and other US federal and state agencies.
- Government agencies to whom certain diseases (like HIV, hepatitis, and STDs) must be reported.
- Governmental agencies of other countries.
- Outside individuals and companies, such as laboratories and data storage companies, that work with the researchers and sponsor and need to access your information to conduct this study.
- Other research doctors and medical centers participating in this study, if applicable.
- A data safety monitoring board which oversees this study, if applicable.

Your health data will be used to conduct and oversee the research, including for instance:

- To see if the study drug works and is safe.
- To compare the study drug to other drugs.
- For other research activities related to the study drug.

Once your health data has been shared with authorized users, it may no longer be protected by federal privacy law and could possibly be used or disclosed in ways other than those listed here.

Your permission to use and share health data about you will end in 50 years unless you revoke it (take it back) sooner.

You may revoke (take back) your permission to use and share health data about you at any time by writing to the study doctor at the address listed on the first page of this form. If you do this, you will not be able to stay in this study. No new health data that identifies you will be gathered after your written request is received. However, health data about you that has already been gathered may still be used and given to others as described in this form.

Your right to access your health data in the study records will be suspended during the study to keep from changing the study results. When the study is over, you can access your study health data.

If you decide not to sign and date this form, you will not be able to take part in the study.

STATEMENT OF AUTHORIZATION
I have read this form and its contents were explained. My questions have been answered. I voluntarily agree to allow study staff to collect, use and share my health data as specified in this form. I will receive a signed and dated copy of this form for my records. I am not giving up any of my legal rights by signing and dating this form.

_____	_____
Participant's Name (print)	Signature and Date
_____	_____
Witness's Name (print)	Signature and Date

ATTACHMENT A: OPTIONAL PROCEDURES

There are two different optional procedures (as described below) that will advance the scientific goals of this study. Not all participants can do these optional procedures, but we would like to know if you would be interested in doing these optional procedures. You may indicate your interest in both procedures, if you wish. If you are not interested in the optional procedures, it will not affect your ability to take part in the main study.

1. Blood drawn for pharmacokinetic (PK) testing

About 15 participants in each group will have blood drawn for PK testing. You will be asked to remain at the clinic all day (about 10 hours) for an intensive PK blood sampling. For this intensive PK sampling, you will have blood drawn several times over a period of 8 hours to measure the amount of HIV medicine and estradiol in your blood. **Except the entry visit, t**he sample collection time points will be at hour 0 (before you take your HIV medicine and estradiol), and at 1, 2, 3, 4, 6, and 8 hours after taking the HIV medicine and estradiol doses. **At the entry visit, the sample collection time points will be at hour 0 (before you take your HIV medicine), and at 1, 2, 3, 4, 6, and 8 hours after taking the HIV medicine. At the entry visit, you will start taking estradiol after the 8-hour PK testing is complete.** To avoid many needle sticks, a small tube (intravenous [IV] device) may be placed into a vein in your arm and left in place during your stay. You will be able to move around and should not have any significant pain or discomfort once the tube is in place. The site will provide food for you, or you may bring food, if you prefer.

Blood drawn for PK testing will occur at entry and weeks 24 and 48 **and at the Premature Study Treatment or Study Discontinuation visit, if you discontinue the study or study treatment early.**

Please indicate below if you are interested in this optional procedure. No matter what you decide, it will not affect your participation in the main study.

_____ (initials) YES, I am interested _____ (initials) NO, I am not interested

2. Interviews

Interviews will be conducted among 30 participants who speak English or Spanish. The interviews are expected to last around 20 minutes and will be conducted online via an online video conferencing platform. All interviews will be audio-recorded. Interviews will occur at weeks 0, 24, and 48.

Please indicate below if you are interested in participating in the interviews. No matter what you decide, it will not affect your participation in the main study. If you indicate you are interested, you will be asked to review and sign a separate consent form.

_____ (initials) YES, I am interested _____ (initials) NO, I am not interested

ATTACHMENT B: CONSENT FOR USE OF SAMPLES IN OTHER STUDIES

When samples are no longer needed for this study, the ACTG may want to use them in other studies and share them with other researchers. These samples are called “extra samples”. The ACTG will only allow your extra samples to be used in other studies if you agree to this. If you have any questions, please ask.

Identifiers will be removed from your samples and from any private information that has been collected about you. This means that no one looking at the labels or at other information will know that the samples or information came from you.

Extra samples are stored in a secure central place called a repository. Your samples will be stored in the ACTG repository located in the United States.

There is no limit on how long your extra samples will be stored.

When a researcher wants to use your samples and information, their research plan must be approved by the ACTG. Also, the researcher’s institutional review board (IRB) or ethics committee (EC) will review their plan.

IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the ACTG will send your samples to the researcher’s location. This means that researchers who are not part of the study team may use your samples without asking you again for your consent.

You will not be paid for your samples. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you.

You may withdraw your consent for research on your extra samples at any time, and the specimens will be discarded.

Please choose the response that matches what you want by putting your initials in the space provided. Please ask the staff any questions that you have before you indicate your selection.

<u>Research Without Human Genetic Testing</u>
If you agree, some of your blood that is left over after all required study testing is done may be stored (with usual protection of your identity) and used for ACTG-approved HIV-related research that does not include human genetic testing.

____ (initials) I understand and I agree to this storage and possible use of my blood.

OR

____ (initials) I understand but I do not agree to this storage or possible use of my blood.

Research with Human Genetic Testing

The ACTG has two different studies that collect samples for genetic testing.

If you live in the US, this study is called ACTG A5128, Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses.

If you live outside of the US, this study is called ACTG A5243, Plan for Obtaining Human Biological Samples at Non-US Clinical Research Sites for Currently Unspecified Genetic Analyses.

Your site might ask you if you would like to participate in one of these studies if it is being done where you live. If you would like to participate, you will sign a separate consent form.

Your extra samples will not be used for human genetic testing unless you sign and date a consent form for A5128 or A5243.

INFORMED CONSENT FOR INTERVIEWS

Sponsor / Study Title:	National Institute of Allergy and Infectious Diseases (NIAID) / "Giving Standardized Estradiol Therapy In Transgender Women to Research Interactions with HIV Therapy: the GET IT RIgHT Study"
Protocol Number:	A5403
Principal Investigator: (Study Investigator)	«PiFullName»
Telephone:	«IcfPhoneNumber»
Address:	«PiLocations»

SUMMARY

PURPOSE	To help the research team understand how satisfied you are with the study and your experience with gender-affirming care and human immunodeficiency virus (HIV) services more broadly.
STUDY ACTIVITY	Interviews will be conducted throughout the study.
NUMBER OF PARTICIPANTS	30 participants.
LENGTH OF ACTIVITY	Approximately 20 minutes per interview.
REQUIRED ACTIVITY	Participate in three audio-recorded 20 minute virtual interviews.
RISKS	You may feel uncomfortable discussing some topics. The interviews will be audio-recorded, so there is a risk that someone may recognize your voice. However, there are procedures in place to reduce this risk, which are described later in this form.
BENEFITS	These discussions will help the research team understand how satisfied you are with the study and your experience with gender-affirming care and HIV services more broadly. You may also learn more about your experiences and motivations for study participation through discussion during your interview.
OTHER CHOICES	You do not have to take part in these interviews.

INTRODUCTION

Extreme stigma faced by many transgender women can increase vulnerability to HIV and, at times, limit access to needed healthcare. By participating in interviews (brief conversations) about your experience participating in the GET IT RIgHT Study, you will help the research team understand how satisfied you are with the study and your experience with gender-affirming care and HIV services more broadly. Everything in the main study consent form you signed before still applies to your participation in this study unless otherwise noted in this form.

This is a consent form. It gives you more information about the interviews. The study staff will talk with you about this information. You are free to ask questions about these interviews at any time. You can contact the study investigator using the information on the first page of this form with any interview-related questions. Please make sure that each of your questions have been answered before providing consent. If you agree to participate in the interviews, you will be asked to sign this consent form. You will get a copy to keep.

WHAT DO I HAVE TO DO?

You will be asked to participate in a 20-minute interview via an online video conferencing platform (for example, Microsoft Teams, Skype, Zoom, WhatsApp) at three different times during the study. Interviews will occur at entry and weeks 24 and 48 (or if you stop estradiol or the study early). Interviews will be conducted with 30 participants. These interviews will be completely confidential and will not impact your participation in the GET IT RIgHT Study. Your participation is completely voluntary and you can stop at any moment.

Before beginning the interview, the interviewer will ask you if your understanding of the interview's aims and the content of the questions is clear. During the interview, you will be asked about your demographics (ethnicity, race, current living situation, school/employment, social support, and health background). You will also be asked questions about your participation and satisfaction regarding this study, prior experiences accessing care, and your plans in accessing future care.

Interviews will be conducted in English or Spanish by a bilingual medical sociologist with extensive experience conducting interviews with transgender communities and/or by a University of Toronto transgender doctoral trainee who is a licensed social worker and has experience with interviews.

All interviews will be audio recorded. Once your interview is transcribed (put into written form) word-for-word, the audio-recording will be erased from the digital recorder. A copy of your recording will be kept on a secure password protected server for 5 years.

Individuals will be recruited based on clinical site and by group. In order to schedule the interview, study staff at your site will collect your contact information and send it to a GET IT

RIgHT study investigator, and the study investigator may contact you, if you are selected, to schedule the interviews.

WHAT ARE THE RISKS RELATED TO THE STUDY ACTIVITY?

A potential risk of participating in this study is that a question may make you feel uncomfortable, during and/or after your participation, if it touches upon a personal or sensitive topic. If, while participating in an interview, you experience any feelings of discomfort or otherwise do not wish to continue, you may skip a question at any time, stop the interview, and/or withdraw from the interview. Your withdrawal from the interview will not have any negative impacts on your participation with the GET IT RIgHT study.

You will have audio recording of your voice so there is the risk that someone may recognize your voice. However, once the audio recordings are collected, each audio file will be coded. The recordings will be transcribed by the study staff and no names will appear in the written transcripts.

ARE THERE BENEFITS TO THE STUDY ACTIVITY?

By participating in interviews (brief conversations) about your experience participating in the GET IT RIgHT Study, you will help the research team understand how satisfied you are with the study and your experience with gender-affirming care and HIV services more broadly. You may also benefit from these interviews by learning more about your experiences and motivations for study participation through discussion during your interview. Information learned from the interviews may help other people in the future.

WHAT OTHER CHOICES DO I HAVE BESIDES THESE INTERVIEWS?

You do not have to participate in these interviews. This will not affect your study participation in GET IT RIgHT study nor your routine healthcare.

WHAT ABOUT CONFIDENTIALITY?

The study investigator will have access to personal information and to information that you provide during the interview. Your interview data, including direct quotes, may appear in academic journals, university seminar presentations, in research conference presentations, and other relevant work where academic research is shared; however, your name and other personally identifying information will never appear or be communicated publicly when the study findings are shared. Demographic information, such as age range, ethnic or cultural background, sexual orientation, gender identity, and school/employment status may be used to describe the study participants. The study investigator will make every effort possible to ensure that the data cannot clearly identify you. It is important to consider, however, that unless you specifically request that certain information (such as things that you’ve said) not be shared, any comments

that you make during the interview will be available to the research team to integrate into their communication of the study findings.

Data Collection
Your interview will be audio-recorded. The study investigator will save these recordings on a password-protected computer and ensure that the recordings, as well as all other information, are kept strictly confidential. Any personal identifying information will be removed (de-identified) from the audio-recordings, and de-identified audio recordings will be transcribed at an outside company that will sign a non-disclosure agreement to ensure the utmost confidentiality. Please avoid mentioning specific names (such as first and last names) during your interview, including those of partners, friends, family members, or healthcare providers that you may reference during your interview. You may instead choose to use a pseudonym (a fake name) or nickname. A random nickname will be chosen to represent you as a participant by the research team during data analysis.

Study information that does not identify you
Any personal identifying information will be removed (de-identified). The research team will remove information that you share with us that can identify you and will replace it with a random and anonymous study number (for example, Participant 01). The information that you provide that is identifiable, such as demographic information, will be stored separately from your interview recording and transcript. This will further safeguard the link between your personal identifying information and the comments that you share with us in your interview. All information that you share with us, including personal health information, will be kept confidential and will not be shared with anyone outside the study team unless required by law. Your identifying information will not be shared in any publications, reports, or presentations that come from this study. During and after your interview, you reserve the right to explicitly request that information that you have shared not be mentioned in publications, presentations, or other methods of sharing the study findings. You may request to withdraw your interview from the study at any time, and without any negative impact on your participation in the GET IT RIgHT study.

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, this research is covered by a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless you have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (see below); if you have consented to the disclosure, including for your medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research participants.

The Certificate cannot be used to refuse a request for information from personnel of the United

States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the agency which is funding this project or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

The Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

Your records may be reviewed by the US Food and Drug Administration (FDA), the AIDS Clinical Trials Group (ACTG), the US Office for Human Research Protections (OHRP), or other local, US, and international regulatory entities as part of their duties, Advarra Institutional Review Board (IRB) (a committee that protects the rights and safety of participants in research), National Institutes of Health (NIH), study staff, study monitors, drug companies supporting this study, and their designees.
Also, any publication of this study will not use your name or identify you personally.

A description of this clinical trial will be available on https://ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT ARE THE COSTS TO ME?

Costs are described in the study consent you already signed.

WILL I RECEIVE ANY PAYMENT?

You will be paid up to a total of $xx.xx if you complete this study. You will be paid for the visits you complete according to the following schedule:

- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.

If you do not complete the study, for any reason, you will be paid for each study visit you do complete.

You will be paid _____ *[“following each completed visit”, “monthly”, “quarterly”, “at the end of your participation in the research study”, “following each completed visit or at the end of your participation in the research study, whichever you prefer”]*.

If you have any questions regarding your compensation for participation, please contact the study staff.
[OR]
You will not receive any monetary compensation for your participation in this study.
[If applicable:] We will reimburse you for the cost of *[describe: e.g., traveling to your study visits]*. You will be reimbursed approximately *[e.g., 2 weeks, 1 month, etc.]* after you submit your travel receipts to the study staff.

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of this study, you will be given immediate treatment for your injuries. The cost for this treatment could be charged to you or your insurance company.

There is no program for compensation through the NIH.

You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

These interviews are completely voluntary. You may choose not to continue in this study or you may leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. Your site will share a summary of the results when they are ready to be presented. Your study staff can answer any questions you may have.

WHOM TO CONTACT ABOUT THIS STUDY

During the study, if you have questions, concerns or complaints about the study such as:

- Payment or compensation for being in the study, if any;
- Your responsibilities as a research participant;
- Eligibility to participate in the study;
- The study investigator’s or study site’s decision to withdraw you from participation;

<u>Please contact the study investigator at the telephone number listed on the first page of this consent document</u>.

If you seek emergency care, or hospitalization is required, alert the treating physician that you

are participating in this research study.

An institutional review board (IRB) is an independent committee established to help protect the rights of research participants. If you have any questions about your rights as a research participant, contact:

- By mail:
  Study Subject Adviser
  Advarra IRB
  6100 Merriweather Dr., Suite 600
  Columbia, MD 21044
- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: Pro00066234.

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered, and you agree to continue with this study activity, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date