Drug-drug interaction study between bictegravir/emtricitabine/tenofovir alafenamide and feminizing hormones in trans women living with HIV

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GCP Statement

This clinical study will be conducted in accordance with applicable Health Canada regulations, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines on current Good Clinical Practice (GCP), and the Declaration of Helsinki.

TABLE OF CONTENTS

1 INVESTIGATOR AGREEMENT

Title: **Drug-drug interaction study between bictegravir/emtricitabine/tenofovir alafenamide and feminizing hormones in trans women living with HIV**

Protocol No.: CTN 331
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This clinical study will be conducted in accordance with applicable Health Canada regulations, ICH guidelines on current GCP, and the Declaration of Helsinki.

I confirm that I have read and understand this protocol and I agree to conduct this clinical study in accordance with the design and specific provisions of the protocol, with the exception of a change intended to eliminate an immediate hazard to participants.

I agree to promptly report to the applicable ethics boards any changes in the research activity and all unanticipated problems involving risks to human participants or others. Additionally, I will not make any changes in the research without prior ethics and sponsor approval, except where necessary to ensure the safety of study participants.

Name	Signature	Date (dd-mmm-yyyy)

2 ABSTRACT

Introduction: Transgender (trans) women have been found to be at higher risk of, and to be disproportionally affected by, HIV for multiple biologic and social reasons. Trans women living with HIV have also been found to have low usage and adherence rates to antiretroviral therapy (ART). Both healthcare providers and trans women, themselves, have expressed concerns of drug-drug interactions (DDIs) between ART drugs and feminizing hormones, which have in turn been shown to contribute to low rates of ART usage amongst trans women living with HIV. The objective of this DDI study is to investigate the pharmacokinetic effects of the common feminizing hormone regimens (oral estradiol [*Estrace® or Lupin-estradiol*] with an anti-androgen (pharmaceutical and/or surgical and/or medical) on the antiretroviral combination bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF;*Biktarvy®*] and vice versa.

Methods and analysis: Participants will be assigned to three groups: group 1 will include 15 trans women living with HIV who are taking feminizing hormones and ART (investigational group); group 2 will include 15 premenopausal cisgender women living with HIV taking ART (ART control group); group 3 will include 15 trans women living without HIV taking feminizing hormones (hormone control group). Women living with HIV will have to be virally suppressed for at least three months and they will have to already be taking B/F/TAF or have their current ART regimen switched to B/F/TAF at baseline. Trans women participants will be required to be on 2 mg oral estradiol or higher and an anti-androgen (pharmaceutical, medical or surgical). Plasma ART drug concentrations will be sampled at the 2-month visit and compared among trans women living with HIV on feminizing hormones and premenopausal cis women living with HIV. Serum estradiol and total testosterone concentrations will be sampled at the baseline and month 2 visits and compared among trans women living with and without HIV. The primary endpoints are B/F/TAF pharmacokinetic parameters (C_{min}, C_{max} and AUC) between trans and cis women living with HIV at month 2 and the estradiol concentrations (C_{min}, $C_{4h,}$ C_{max} and AUC) and the proportions of the month 2 C_{4h} that are within target (200 to 735 pmol/L) between trans women living with and without HIV at month 2. Other endpoints will include the mean estradiol and testosterone concentrations at baseline and the difference between baseline and month 2 estradiol pre-dose and C_{4h}, HIV viral load, adherence, adverse events, patient-reported outcomes (i.e., symptoms), satisfaction with feminizing hormones, satisfaction with ART regimen, and health status. This trial will serve to provide empirical evidence regarding a lack of, or presence of DDIs between B/F/TAF and feminizing hormones.

Dissemination: The findings will be disseminated through publication in peer-reviewed journals as well as presented at national and international conferences and community groups.

Trial registration number: Will be registered once Research Ethics Board (REB) approval is granted.

3 INTRODUCTION

3.1 Issues facing trans women living with HIV

Transgender (trans) women have been found to be at higher risk of, and to be disproportionally affected by, HIV for multiple biologic (e.g., increased risk of biologic transmission via sexual practices) and social reasons (e.g., gender-based stigma and discrimination).[1] Globally, trans women experience disproportionately high rates of HIV compared to cisgender (cis) adults.[1] Canadian archival research estimated that 54% of trans women deaths in Montreal pre-2000 were HIV-related.[2] A study of trans people living in Ontario estimated an HIV prevalence of 2.9%, lower than global estimates, yet ten times higher than the provincial prevalence of 0.23%.[3]

While access to HIV-related healthcare – including initiation of and adherence to antiretroviral treatment (ART) – is essential for the survival and wellbeing of people living with HIV,[4,5] a significant amount of research has described lower ART use and adherence among trans women living with HIV.[6-10] One of the main contributing reasons identified by trans women living with HIV for this discordance in access and adherence is the fear of drug-drug interactions (DDIs) between ART drugs and feminizing hormones.[11] Trans women consider the use of feminizing hormones so vital to their being and lives (e.g., safety) that many trans women living with HIV would rather take feminizing hormones and not ART drugs, if they perceive they have to make a choice.[11] Studies show that many healthcare providers will still withhold feminizing hormones if a trans woman living with HIV is taking ART.[11] Also, trans women living with HIV who believe ART may have negative effects on their feminizing therapy are three times more likely to take higher than prescribed doses of hormones.[12] These concerns can lead to trans women choosing to either not take ART at all, or to take ART and buy their feminizing hormones from the street/black market and be medically unsupervised. Neither of these options are suitable or ideal, and can contribute to potentially severe side effects (e.g., stroke).[13]

The ideal scenario is a shared decision-making medical care model where both the healthcare provider and the trans woman understand the science behind DDIs between ART drugs and feminizing hormones, allowing both important therapies to be prescribed and monitored to meet the needs of the trans woman with medically safe dosing and sustained viral suppression. The objective for this clinical study is just that: to investigate bidirectional DDIs between the most commonly prescribed feminizing hormone regimens (oral estradiol [*Estrace® or Lupin-estradiol*] with an anti-androgen [e.g., spironolactone, cyproterone, finasteride, leuprolide, bicalutamide, dutasteride, and/or orchiectomy, and/or hypogonadism]) and the antiretroviral combination of bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF; *Biktarvy®*).

3.2 Bictegravir (B) with tenofovir alafenamide (TAF) and emtricitabine (F) (B/F/TAF) as a good ART regimen for trans women living with HIV

The current Canadian standard of care for HIV is an ART regimen with two to three active antiretroviral drugs – but more commonly three, typically two from the nucleoside reverse transcriptase inhibitor class along with a third agent.[14] The latest American 2021 DHHS guidelines indicate that the recommended second or third agent for most ART-naïve patients should be an integrase strand transfer inhibitor (INSTI).[15] In today's world, an individual who is newly

diagnosed with HIV will likely be started on a single tablet regimen (STR) to maximize tolerability and adherence. Prior to 2018, there were three commercially available three-drug INSTI-based STRs in Canada: *Stribild, Genvoya and Triumeq*. Each of these STRs, while potent and highly effective, have limitations. Currently in Canada, most patients who were taking *Stribild* have been changed to the newer formulation of *Genvoya,* which contains tenofovir alafenamide (TAF) rather than tenofovir disoproxil fumarate (TDF). TAF has demonstrated significantly lower renal and bone toxicity in clinical trials, which is believed to be related to differences in the disposition of tenofovir (TFV) between the two prodrugs while maintaining high intracellular concentrations at the site of action for HIV. While both are well tolerated, *Genvoya* and *Stribild* have the disadvantage of being formulated with a booster, cobicistat (COBI), and DDIs are a concern with co-administered drugs such as hormone drugs. *Triumeq* is an STR that contains a combination of abacavir (ABC), lamivudine (3TC) and dolutegravir (DTG). *Triumeq* has the disadvantages of requiring an HLA-B*5701 test prior to administration and is associated with life threatening hypersensitivity reactions, which limit its use in HLA-B*5701-positive patients or when an HLA-B*5701test is unavailable*. Triumeq* is also associated with central nervous system side effects, most significantly that of insomnia thought to be related to DTG and perhaps the co-administration with ABC.[16] Furthermore, many studies and guidelines advise about the concern of a potential increase in cardiovascular disease events (CVE) including myocardial infarctions with the use of ABC.[13] The potential increased risk of CVE is of particular importance in trans women taking hormone therapy, as feminizing hormones have also been associated with an increased risk of CVE.[17,18] *Biktarvy®* (B/F/TAF) was approved and has been available in Canada as an STR since 2018 and we believe that it represents a generally well tolerated, convenient, pharmacologically favorable, and highly effective ART strategy for trans women living with HIV. Also, B/F/TAF has been found to be pharmacologically favorable with no DDIs between B/F/TAF and an ethinyl estradiol/norgestimate containing oral contraceptive in healthy adults.[19] However, it is unclear if this is generalizable to feminizing hormones used by trans women, which typically contain higher doses of 17 β -estradiol. **Therefore, for these many reasons, we believe that it is important to assess the DDIs between B/F/TAF and feminizing hormones among trans women living with HIV. Demonstrating a lack of detrimental DDIs between B/F/TAF and common feminizing hormone therapy will provide an evidenced-based approach for the management of HIV among trans women.**

3.3 *DDI findings to date between ART drugs and feminizing hormones amongst trans women*

Recent studies[20-23] with trans women living without HIV who were taking TDF/FTC (*Truvada*) for pre-exposure prophylaxis (PrEP) revealed potential reduced plasma TFV-diphosphate (TFV-DP) and FTC-triphosphate (FTC-TP) concentrations. Two of the studies also showed reduced TFV-TP and FTC concentrations in rectal tissues.[20,23]

The iFACT (Interaction between the use of Feminizing Hormone Therapy and Antiretroviral agents Concomitantly among Transwomen) study[22], done in Thailand, aimed to do a two-way analysis of DDIs to show the effect of PrEP on feminizing hormones and vice versa. This was achieved by giving 20 participants an initial 3-week period of the feminizing hormones, E2

valerate and cyproterone, and then a 2-week period of both the combined feminizing hormone regimen and daily TDF/FTC as PrEP and then a final 3-week of TDF/FTC as PrEP only. Intensive E2 pharmacokinetic (PK) parameters [plasma was collected at 0 (pre-dose), 1, 2, 4, 6, 8, 10, 12 and 24 hours after directly observed medication ingestion] and testosterone concentration at 24 hours (C_{24}) were measured at weeks 3 (without PrEP) and 5 (with PrEP), and intensive TFV PK parameters were measured at weeks 5 (with feminizing hormones) and 8 (without feminizing hormone). The PK parameters revealed a reduction by 12% of TFV area under the curve $[AUC]_{0-24}$ ($p = 0.03$) and 18% of C_{24} of TFV ($p < 0.001$) when participants were on feminizing hormones. There were no significant changes in E2 PK parameters on PrEP. A proposed theory for the reduction involves alteration of drug clearance of TFV by E2.

Similar reductions were noted in another study done by Shieh et al (2018)[20], where plasma TFV and FTC C_{24} (trough) concentrations in 8 trans women on feminizing hormones (on the regimen they were taking) were lower by 32% ($p = 0.010$) and 32% ($p = 0.038$) respectively, when compared to 8 cis men. Both groups of participants were on TDF/FTC as PrEP for 7 days and at the last dose, the PK parameters were measured. Plasma TFV and FTC 24-hr AUC-time curve among the trans women trended toward and were significantly lower by 27% ($p = 0.065$) and 24% ($p = 0.028$), respectively. Estradiol concentrations did not significantly change by TDF/FTC. Rectal concentrations of TFVdp concentrations were 68% lower in trans women when compared to cisgender men, which resonated with another study's finding where rectal TFVdp:dATP, was 7-fold lower in transgender versus cisgender participants ($p< 0.05$) and inversely correlated with female sex hormones ($p< 0.05$)[23]. In this study (2019), patients living with and without HIV who were taking TDF/FTC (as ART or PrEP, respectively) as part of clinical care were enrolled and divided into 3 cohorts: trans women taking feminizing hormones, cis men, and postmenopausal cis women. Blood and rectal tissue were collected at a sampling visit 16–24 hours after the last self-reported TDF/FTC dose. A follow-up visit was conducted within 14 days. Ten participants were taking TDF/FTC as part of ART and 2 were taking PrEP. In blood and rectal tissue, TFVdp/FTCtp and dATP/dCTP concentrations were similar across groups; however, rectal TFVdp:dATP, which is an important effector of PrEP potency, was 7-fold lower in transgender versus cisgender participants ($P < 0.05$) and inversely correlated with female sex hormones ($P < 0.05$) while FTCtp:dCTP did not differ between transgender and cisgender participants.

In January 2020, the first DDI study assessing the impact of feminizing hormones on the drug concentrations of an ART regimen and vice versa was published.[24] This DDI study conducted in Thailand enrolled 20 trans women who were newly diagnosed with HIV. The study was done in a similar fashion as the iFACT study with trans women taking PrEP: the feminizing hormone and ART regimens were tightly controlled during the study and then intensive PK parameters were collected. Participants were given the feminizing hormones, estradiol 2 mg and cyproterone 25 mg once a day only, then the feminizing hormones plus ART (the STR TDF/FTC/efavirenz), then ART only in a chronological order. Intensive E2 PK were measured at weeks 3 (without ART) and 5 (with ART), and intensive TFV and EFV PK were measured at weeks 5 (with feminizing hormones) and 8 (without feminizing hormones). The differences in the mean ratio of E2 AUC, C_{max}, and C_{24h} post-ART vs. pre-ART were 0.72 ($p<0.001$), 0.81 ($p=0.006$), and 0.64 ($p=0.004$),

respectively while the differences in the mean ratio of TFV AUC, C_{24h}, and EFV C_{24h} on vs. off feminizing hormones were 0.86 (p=0.002), 0.83 (p=0.006), and 0.91 (p=0.02), respectively. This implied that E2 PK values were significantly lowered in the presence of an ART regimen consisting of TFD/FTC/efavirenz and vice versa. **As such, since DDIs have been demonstrated between ART drugs and feminizing hormones and since there is a paucity of DDI studies between ART and feminizing hormones amongst trans women, our study is of high importance.**

4 OBJECTIVES AND HYPOTHESES

Primary Objective #1: To compare month 2 PK parameters (C_{min}, C_{max}, AUC) of the ART drugs (B/F/TAF), among trans women living with HIV who are taking B/F/TAF and feminizing hormones (group 1) to pre-menopausal cis women living with HIV who are taking B/F/TAF (group 2); and

Primary Objective #2: To compare the month 2 estradiol concentrations (C_{min}, C_{4h}, C_{max}, AUC) and the proportions of the month 2 C_{4h} that are within target (200 to 735 pmol/L) between trans women living with HIV taking feminizing hormones and B/F/TAF (group 1) and trans women living without HIV taking feminizing hormones and not B/F/TAF (group 3).

Hypotheses: Our first primary hypothesis is that there will be no difference in month 2 B/F/TAF pharmacokinetic (PK) parameters (C_{min}, C_{max}, AUC) between trans women living with HIV and premenopausal cis women living with HIV taking B/F/TAF. Our second primary hypothesis is that there will be no difference in the month 2 estradiol concentrations (C_{min}, C_{4h}, C_{max}, AUC) between trans women living with HIV who are taking B/F/TAF and feminizing hormones and trans women living without HIV who are only taking feminizing hormones.

Secondary Objectives:

1. To determine the baseline, and the difference between baseline and month 2, estradiol pre-dose and C_{4h} and testosterone concentrations compared between groups 1 and 3 and the proportions within the target;
2. To determine satisfaction with medical gender affirmation for trans women living with HIV (group 1) and living without HIV (group 3) at baseline and month 2, and at the 6 month timepoint for group 1 only;
3. To determine the proportion of participants with an undetectable HIV-RNA (<40 copies/mL) at all follow-up visits (months 1, 2 and 6) for trans and cis women participants living with HIV as a whole cohort and for those who switched to B/F/TAF;
4. To determine adherence for trans and cis women participants living with HIV at baseline and all follow-up visits (months 1, 2 and 6 as a whole cohort and for those who switched to B/F/TAF);
5. To determine the frequency and severity of B/F/TAF-related adverse effects at all follow-up visits (months 1, 2 and 6) for trans and cis women participants living with HIV as a whole cohort and for those who switched to B/F/TAF;

6. To determine the patient-reported outcomes (PROs) at baseline and months 2 and 6for trans and cis women participants living with HIV as a whole cohort and for those who switched to B/F/TAF; as well as those in group 3 at baseline and month 2 as an exploratory analysis;
7. To determine patient satisfaction with ART at baseline and months 2 and 6for trans and cis women participants living with HIV as a whole cohort and for those who switched to B/F/TAF; and
8. To determine participants' health status in all groups at baseline and month 2 and additionally at month 6 for trans and cis women participants living with HIV.

5 METHODS AND ANALYSIS

5.1 Study Design and Setting

This will be a three-armed parallel group, longitudinal, 6-month DDI study. The study will be conducted through Maple Leaf Research (MLR) in Toronto and participants will be recruited through the affiliated clinic and other recruiting clinics. All study visits will be conducted through MLR, either in person or virtually except for the Month 2 visit which must be conducted in person at MLR in Toronto due to the peripheral blood mononuclear cells (PBMC) processing requirement. The study schematic is presented in Appendix A. Unlike the Thai DDI studies[22,24], this study is relatively pragmatic in nature as trans women will be on varying estradiol doses (2 mg or higher) and taking an anti-androgen (pharmaceutical [e.g., spironolactone, cyproterone, finasteride, leuprolide, bicalutamide, dutasteride] and/*or* surgical [orchiectomy] and/*or* medical [hypogonadism]) reason. In the Canadian context, a pragmatic study is the only one possible due to the limited number of trans women in Canada. While this can be seen as a limitation, it is also an advantage as it allows us to capture data on varying scenarios.

The maximum duration of enrolment from screening to the 6 month visit is 9 months (up to 90 days allowed from screening to baseline visit) for cis and trans women living with HIV to complete (group 1, 2). For Group 3 (trans women without HIV) their enrolment will be a maximum duration of 5 months.

An important aspect of this study is the use of community-based research (CBR) principles.[25-30] Research with trans women would not be possible if not done using CBR and working with trans women champions and leaders in the field. This population has a lack of trust for the system including researchers and academic institutions and can often be difficult to enroll into research studies. Our team has been conducting CBR for over a decade and most recently launched the CBR-driven Trans Women HIV Research Initiative (TWIRI) with and for trans women living with HIV. TWIRI is led by Ms. Yasmeen Persad, a trans woman community leader in Toronto and the TWIRI community research coordinator (see www.transwomenHIVresearch.com). Also, the conception of the idea of this study came from the community, and more specifically from the Canadian Institutes of Health Research (CIHR) Canadian HIV Trials Network (CTN) Trans Working Group, made up of mainly trans community members. Also, for this study we will engage the support of a trans woman as a community research participant assistant who will help recruit and who will act as a peer support person on the day of their Baseline and Month 2 study visits

(e.g., by helping participants get to lab for blood draws and help participants fill out surveys (if needed). The community research participant assistant will also ensure that all matters are taken care of, to the best of their ability, to increase trust and ensure a safe environment. Participants may also choose to bring their own support person and this person would receive an honorarium for their time. Furthermore, a TWIRI community advisory board (CAB) has been formed which will review and advise on the study and enrollment.

5.2 Study Population

Participants will be assigned to three groups: group 1 will include 15 trans women living with HIV who are taking feminizing hormones (oral estradiol [2 mg or higher] with an anti-androgen (pharmaceutical [e.g., spironolactone, cyproterone, finasteride, leuprolide, bicalutamide, dutasteride] and/*or* surgical [orchiectomy] and/*or* medical [hypogonadism]) and suppressive ART (investigational group); group 2 will include 15 pre-menopausal cis women living with HIV taking suppressive ART (ART control group); and group 3 will include 15 trans women living without HIV taking feminizing hormones (oral estradiol [2 mg or higher] with an anti-androgen (pharmaceutical [e.g., spironolactone, cyproterone, finasteride, leuprolide, bicalutamide, dutasteride] and/or surgical [orchiectomy] and/or medical [hypogonadism]) (hormone control group). Women living with HIV (groups 1 and 2) will have to be virally suppressed for at least three months on a combination ART regimen with two suppressed viral loads and no clinical suspicion of prior important ART drug resistance. As mentioned above, one unique feature of this study is that a community research participant assistant (a trans woman leader in the Toronto community) will be contracted to assist with recruitment, and visit attendance at Baseline and Month 2 procedures; this will be important as participants will be enrolled from six Toronto sites in addition to Maple Leaf Medical Clinic: 790 Bay Street Clinic in Toronto, Ontario, Canada; Sherbourne Community Health Centre in Toronto, Ontario, Canada; Dr. Raymond Fung's endocrinology clinic in Toronto, Ontario, Canada; Church-Wellesley Clinic in Toronto, Ontario, Canada; St. Michael's Department of Family and Community Medicine at Unity Health in Toronto, Ontario, Canada; and Women's Health in Women's Hands Community Health Centre in Toronto, Ontario, Canada. If recruitment is poor in Toronto, the Montreal site will be added and recruiting primarily from one clinic, Clinique Quartier Latin, which is the clinic that cares for the most trans women living with and without HIV in Montreal. A second clinic in Montreal may also be added to recruit participants, Clinique Médicale Quorum, under the leadership of Dr. Andrew Bui-Nguyen. There is a possibility of adding other sites across Canada, as this study will be advertised through National Trans Networks. Interested participants can also call the sites directly to screen for the trial.

A formative retrospective chart review study has been completed entitled "The Montreal Toronto Trans HIV Study – Characterizing the Trans Women Population living with and without HIV in Montreal and Toronto". This study informed us of the most commonly prescribed feminizing hormones and ART regimens in these populations. Preliminary results reveal that we captured data from the charts of nearly 1500 trans women living with and without HIV cared for in Toronto and Montreal. This high number of trans patients is likely the case because Canada and particularly Toronto are considered trans-friendly urban settings, especially by immigrant

racialized trans people. Of the approximate 1500 patients, 74 in Toronto and 12 in Montreal were identified as living with HIV. Based on this formative study, which involved collaborations with seven clinics serving the majority of trans women accessing feminizing hormones in Toronto and Montreal, we will be ideally positioned for recruitment in the current study. If recruitment is poor in Toronto, the Montreal site will be added and recruiting primarily from one clinic, Clinique Quartier Latin, which is the clinic that cares for the most trans women living with and without HIV in Montreal.

From the preliminary findings from this chart review, we have found that oral estradiol is the most common form of estrogen replacement used, at varying doses. We have also identified that the most common androgen-blocking agents used are spironolactone or cyproterone, split evenly between the two. This has informed the development of the inclusion/exclusion criteria.

6 INCLUSION AND EXCLUSION CRITERIA

6.1 Investigational Population (Group 1)

Inclusion Criteria

1. Is a trans woman or person with transfeminine experience (individual assigned male sex at birth who currently identifies as a woman or person with transfeminine experience and is at any stage of medical, social, or legal transition);
2. Is 18 years of age or older;
3. Is living with HIV;
4. Is currently taking combination ART and has been taking these medications for at least 3 months prior to the screening visit;
5. Is willing to adjust their ART dosing to the morning if they are currently taking it at night for at least 28 days before the baseline visit until the completion of the Month 2 visit;
6. Has had an undetectable viral load for at least 3 months on at least two occasions, where most recent (within 12 months including screening) VL<40 copies/mL and second (within 24 months) VL<200 copies/mL with no suggestion of relevant ART drug resistance prior to screening;
7. Is willing to switch to or stay on B/F/TAF for the duration of the study (i.e., 6 months);
8. Is currently taking oral estradiol (2 mg or higher) and an anti-androgen (pharmaceutical [e.g., spironolactone, cyproterone, finasteride, leuprolide, bicalutamide, dutasteride] and/*or* surgical [orchiectomy] and/*or* medical [hypogonadism]) and has been taking these medications together at the current dose for at least 3 months prior to the screening visit; AND:
    a. Is willing to adjust their oral estradiol dosing to twice a day if they are taking > 2 mg per day (which is currently recommended as best practice) for at least 28 days before the baseline visit until the completion of the Month 2 visit, with the morning dose set at 2 mg (remaining dose can be taken at night), *OR*;
    b. If their estradiol dosing is 2 mg per day and they are currently taking their estradiol at night, they must be willing to switch their dosing to the morning for

at least 28 days before the baseline visit until the completion of the Month 2 visit;

c. Is willing to swallow their oral estradiol if they are currently taking it sublingually or crushing it for at least 28 days before the baseline visit and until the completion of the Month 2 visit;
d. Is willing to keep their anti-androgen (if applicable) medication(s) unchanged until the completion of the Month2 visit;

9. If taking progesterone, is willing to keep the same medication/dose until the completion of the Month 2 visit and must have been taking that dose for at least 3 months prior to the screening visit;
10. Is willing and able to provide full consent for their participation.

Exclusion Criteria

1. Is clinically unable to switch ART to B/F/TAF based on their physician's opinion;
2. Is taking hormonal non-prescription or natural health products in addition to feminizing hormone therapy;
3. Has significant underlying diseases which could worsen due to their participation, or affect their ability to follow the study procedures;
4. Has kidney or liver impairment (ALT > 5X normal; serum creatinine [eGFR] < 30 mL per minute);
5. Is taking medications known to interact with feminizing hormones, B/F/TAF, or its individual components or has taken such medications within the past 28 days prior to baseline (List of prohibited medications in Appendix B1).
6. Has a known hypersensitivity to bictegravir (BIC), emtricitabine (FTC), tenofovir alafenamide (TAF) or to any ingredient in the formulation.

6.2 Comparator Population (Group 2)

Inclusion Criteria

1. Is a cis woman (individual assigned female sex at birth who currently identifies as a woman);
2. Is of reproductive age and 18 years of age or older;
3. Is living with HIV;
4. Has a regular period (every 24 – 35 days), or if period is irregular or absent, it is due to the administration of a progesterone only hormone contraceptive (see Appendix B1); or if uterus removed (hysterectomy), ovarie(s) remain functional (FSH level<27.0 IU/L);
5. Is currently taking combination ART and has been taking these medications for at least 3 months prior to screening;
6. Is willing to adjust their ART dosing to the morning if they are currently taking it at night for at least 28 days before the baseline visit until the completion of the Month 2 visit;
7. Has had an undetectable viral load for at least 3 months on at least two occasions, where most recent (within 12 months including screening) VL<40 copies/mL and second (within 24 months) VL<200 copies/mL with no suggestion of relevant ART drug resistance prior to screening;
8. Is willing to switch to or stay on B/F/TAF for the duration of the study (i.e., 6 months);

9. Is willing and able to provide full consent for their participation.

Exclusion Criteria

1. Is clinically unable to switch B/F/TAF based on their physician's opinion;
2. Is pregnant or is planning on getting pregnant for the duration of the study;
3. Has undergone surgery to remove their ovaries (i.e. oophorectomy) or has a condition in which their ovaries produce little or no estrogen (i.e. primary ovarian insufficiency, hypogonadism) or does not have ovaries naturally (i.e. unilateral ovarian absence); total hysterectomy is allowed as long as ovaries are intact and functional;
4. Is taking an estrogen containing or conjugated estrogens hormonal therapies; IUD and progesterone only contraception are allowed (See Appendix B1/2 for list for both prohibited and allowed contraceptives);
5. Has significant underlying diseases which could worsen due to their participation, or affect their ability to follow the study procedures;
6. Has kidney or liver impairment (ALT > 5X normal; serum creatinine [eGFR] < 30 mL per minute);
7. Has a known hypersensitivity to bictegravir (BIC), emtricitabine (FTC), tenofovir alafenamide (TAF) or to any ingredient in the formulation.
8. Is taking medications known to interact with B/F/TAF, or its individual components or has taken such medications within the past 28 days prior to baseline (List of prohibited medications in Appendix B1/2).

6.3 Comparator Population (Group 3)

Inclusion Criteria

1. Is a trans woman or person with transfeminine experience (defined as individual assigned male sex at birth who currently identifies as a woman or person with transfeminine experience irrespective of medical, social, or legal transition);
2. Is 18 years of age or older;
3. Is HIV negative at screening;
4. Is currently taking oral estradiol (2mg or higher) and an anti-androgen (pharmaceutical [i.e., spironolactone, cyproterone, finasteride, leuprolide, bicalutamide, dutasteride] and/*or* surgical [orchiectomy] and/or medical [hypogonadism]) and has been taking these medications at the current dose for at least 3 months prior to the screening visit, AND:
    a. Is willing to adjust their oral estradiol dosing to twice a day if they are taking > 2 mg per day (which is currently recommended as best practice) for at least 28 days before the baseline visit until the completion of the Month 2 visit, with the morning dose set at 2 mg (remaining dose can be taken at night) OR;
    b. If their estradiol dosing is 2 mg per day and they are currently taking their estradiol at night, they must be willing to switch their dosing to the morning for at least 28 days before the baseline visit until the completion of the Month 2 visit;

c. Is willing to swallow their oral estradiol if they are currently taking it sublingually or crushing it for at least 28 days before the baseline visit until the completion of the Month 2 visit;
d. Is willing to keep their anti-androgen (if applicable) medication(s) unchanged until the completion of the Month2 visit;

5. If taking progesterone, is willing to keep their dose the same until the Month 2 visit and must have been taking that dose for at least 3 months prior to the screening visit;
6. Is willing and able to provide full consent for their participation.

Exclusion Criteria

1. Is taking hormonal non-prescription or natural health products in addition to feminizing hormone therapy;
2. Has taken HIV pre-exposure prophylaxis (PrEP) (i.e, TDF/FTC or TAF/FTC) or HIV post-exposure prophylaxis (PEP) in the prior 28 days from baseline and plans to continue during the study;
3. Has significant underlying diseases which could worsen due to their participation, or affect their ability to follow the study procedures;
4. Has kidney or liver impairment (ALT > 5X normal; serum creatinine [eGFR] < 30 mL per minute);
5. Is taking medications known to interact with feminizing hormones or has taken such medications within the past 28 days prior to baseline (List of prohibited medications in Appendix B1).

7 DRUGS UNDER INVESTIGATION

In this DDI study two groups of drugs are being assessed:

1. As the HIV ART, the combination of B/F/TAF, known as *Biktarvy®,* which is a widely available STR approved by Health Canada is the drug under investigation. Biktarvy is only available in a single formulation of 50 mg bictegravir (as bictegravir sodium) / 200 mg emtricitabine / 25 mg tenofovir alafenamide (as tenofovir alafenamide hemifumarate). Thus, there is no alternative dosage. Participants in groups 1 and 2 will either have to already be taking B/F/TAF or will be required to switch to it at baseline and they will self-administer B/F/TAF once daily (in the morning) with or without food.
    - For those who switch to B/F/TAF from another ART regimen, there will be no washout period between therapies in order to maintain virologic control. For those participants who switch at baseline to B/F/TAF (switch participants), the drug B/F/TAF (Biktarvy®) will be supplied at baseline at no cost by Gilead Sciences for the duration of the study (i.e., 6 months). Switching ART participants must take B/F/TAF in the morning until at least the Month 2 visit, if they prefer, they can switch from taking their Biktarvy® in the morning to taking it later in the day after the Month 2 visit.
    - For those who are already taking B/F/TAF, they must take B/F/TAF in the morning from the day after the screening until at least the Month 2 visit, if they prefer, they

can switch from taking their Biktarvy® in the morning to taking it later in the day after the Month 2 visit. If required for any reason, B/F/TAF (Biktarvy®) can be supplied at baseline at no cost by Gilead Sciences for the duration of the study (i.e., 6 months).

2. As the feminizing hormone therapy, the drugs under investigation are oral 17 β-estradiol (estradiol) [*Estrace® or Lupin-estradiol®*] as the estrogen and an anti-androgen (pharmaceutical [e.g., spironolactone, cyproterone, finasteride, leuprolide, bicalutamide, dutasteride] and/or surgical [orchiectomy] and/or medical [hypogonadism]). In trans women, the administration of exogenous feminizing hormones is necessary to induce physical changes, and also improves social and emotional adjustment, often in combination with gender affirming surgery.[17,18] While there are different estrogen preparations that are possible, in many parts of Canada oral estradiol is used as it is the only estrogen therapy covered by provincial drug coverage systems. Similarly, while the anti-androgens most commonly used in Canada are spironolactone and cyproterone for the same reason, gender affirming surgeries are becoming more common and thus many trans women may not need an anti-androgen (as they have had an orchiectomy or have a condition such as hypogonadism, for example). Estrogen therapy with 17 β-estradiol is recommended over conjugated estrogens, as it can be easily monitored through laboratory testing and conjugated estrogen is thought to be more thrombogenic. In general, as per the Endocrinology Society Clinical Practice Guideline,[18] estrogen therapy aims to achieve serum estradiol concentrations within the normal range for pre-menopausal women, approximately 370 to 735 pmol/L, but is often variable depending on the assay. Doses of estradiol range from 1 mg to a maximum of 6 mg per day.
   - The University of California, San Francisco (USCF) guidelines recommend splitting the dose to twice a day when the dose is > 2 mg per day. From our formative chart review, we identified that 4 mg once a day (OD) is a common dose and therefore, participants must take 2 mg BID until the Month 2 Visit. Therefore, at the screening visit, trans women participants in both groups 1 and 3 must divide their dose to twice a day (BID) if they are taking > 2 mg per day and take 2 mg in the morning and the rest of the dosing in the evening Participants who are only taking 2mg of estradiol once daily at night must switch their dosing to the morning. This will be beneficial from a PK perspective as then all participants will be taking 2 mg of estradiol in the morning and the heterogeneity of interparticipant estradiol PK will then be reduced.
   - Also, participants who either crush or take the oral estradiol sublingually must switch to swallowing the tablet orally from the day after the screening visit until the Month 2 visit, otherwise the estradiol concentrations will be markedly higher.
   - Participants in groups 1 and 3 will be asked to refrain from the use of estrogenic therapies other than their prescribed feminizing hormone regimen, and that any use of concomitant over-the-counter medications, prescription medications, or natural health products will be reported to study investigators.

The anti-androgen therapies spironolactone and cyproterone both work to prevent the action of testosterone but have vastly different effects on the suppression of testosterone levels. Spironolactone acts mainly as an androgen receptor blocker to directly prevent testosterone from binding and exerting its effects; the suppression of testosterone production is a much weaker effect. Cyproterone also blocks the androgen receptor but also effectively suppresses testosterone production through inhibition of luteinizing hormone secretion.[31,32] Spironolactone is rapidly and extensively metabolized by non-cytochrome p450 mechanisms to numerous active metabolites. These active metabolites have a $t_{1/2}$ of approximately 15 hours and accumulate following multiple doses. Spironolactone and its metabolites are approximately 90% bound to plasma proteins and undergo urinary and biliary elimination.[33] Cyproterone is rapidly and completely absorbed following oral administration with a T_{max} of 3 to 4 hours. The $t_{1/2}$ is approximately 38 hours with biliary (60%) and urinary (33%) excretion of the unchanged drug. Cyproterone is metabolized by CYP3A4 and therefore clinically relevant interactions with CYP3A4 inducers or inhibitors are possible.[33] The effects of antiandrogens cannot be directly evaluated through total testosterone measurement as estrogen supplementation also acts to decrease testosterone, and because these drugs work by multiple mechanisms and do not exclusively reduce total testosterone production. Less common anti-androgen therapies include finasteride, leuprolide, bicalutamide, dutasteride. Finasteride and dutasteride are both 4-aza steroid competitive inhibitors of 5α-reductase (5AR) which is responsible for converting testosterone to dihydrotestosterone (DHT); they are often used by cis men to support hair growth for male pattern baldness and similarly contribute to hair growth for trans women. Bicalutamide is a member of the nonsteroidal antiandrogen (NSAA) group of medications. It works by blocking the androgen receptor, the biologic target of the androgen sex hormones, testosterone and DHT. Like spironolactone, bicalutamide does not lower testosterone levels. Leuprolide is a gonadotropin-releasing hormone (GnRH) analogue acting as an agonist at pituitary GnRH receptors. Agonism of GnRH receptors initially results in the stimulation of LH and FSH by the anterior pituitary initially leading to increased serum estradiol and testosterone levels. However, because propagation of the hypothalamic-pituitary-gonadal (HPG axis) is incumbent upon pulsatile hypothalamic GnRH secretion, pituitary GnRH receptors become desensitised after several weeks of continuous therapy. This protracted downregulation of GnRH receptor activity is the targeted objective of leuprolide therapy and ultimately results in decreased LH and FSH secretion, leading to a dramatic reduction in estradiol and testosterone levels regardless of sex. It is most commonly used by adolescents or trans folks in puberty due to its mechanism of action. Both bicalutamide and leuprolide are used to treat prostate cancer. If participants have a condition in which their testes produce little or no testosterone (i.e. hypogonadism) then the participant will not be required to take an anti-androgen. Finally, orchiectomy is the surgical anti-androgen method that some trans women choose. The testosterone measurement is a secondary outcome and participants are able to enroll regardless of the anti-androgen they are taking. Participants must agree to remain on the same antiandrogen (pharmaceutical/surgical/medical) until after the Month 2 visit.

8 STUDY PROCEDURES

8.1 Engagement with and screening of candidates/recruitment plan

Trans women as well as cis women living with HIV are a sensitive patient population necessitating trauma-informed and participatory approaches. As a research team, we have been practicing CBR in partnership with trans women community leaders for the past 11 years.[34] We have launched TWIRI with a trans woman community leader in Toronto, Yasmeen Persad. Yasmeen is a co-principal investigator of this study and is assisting with its guidance. Yasmeen will also contribute to the study's advertisement through the Toronto community and nationally, which will aid with recruitment. TWIRI has already put together a CAB as well, who have reviewed some of the study documents and heard a presentation about the study; and have shown interest in the study. The final study protocol, documents and the recruitment plan and advertisement will be reviewed with the CAB members, partly facilitating the recruitment. As previously mentioned, a community research participant assistant will be hired to connect with potential candidates, engage them, assist them with the Baseline and Month 2 visits (if preferred by participant, they can also bring their own support person) and to ensure the study is safe for them. This approach will help ensure that research practices are informed, empathetic, and trauma-aware; all of which are necessary for the success of the project.

In addition to the CAB and through Yasmeen and the community research participant assistant's connections, participants will be recruited from the six participating recruitment sites who participated in the aforementioned retrospective Montreal Toronto chart review study. These sites include: Maple Leaf Medical Clinic, Church-Wellesley Clinic, Sherbourne Health Centre, Dr. Raymond Fung's office (the busiest trans endocrinologist in Toronto), and St. Michael's Department of Family and Community Medicine at Unity Health. Since the formative chart review was conducted, we have a sense of the sample of candidates from each site. Site physicians, nurses, and staff have been engaged over the past two years as part of the formative chart review and will be keen to reach out to potential candidates as a member of the circle of care. We will also recruit cis women living with HIV from 790 Bay Street Clinic and Women's Health in Women's Hands Community Health Centre.

If needed, Montreal will have two participating recruitment sites. The central study site, Clinique Quartier Latin, has captured 48 trans women candidates from the chart review. The care providers there will be able to inform potential candidates about the study for potential enrolment. This clinic also provides HIV care and will be able to enroll cis women living with HIV. A second Montreal site has been added, Clinique Médicale Quorum, and one of their physicians has a significant number of trans patients and he is keen to be involved in research. Therefore, recruitment will occur there as well. Recruitment flyers will also be produced and distributed to other clinic centers across Canada for referrals of potential interested candidates.

If recruiting site care providers have interested patients, they will (with verbal consent from their patient) send a referral (for research purposes) to Dr. Mona Loutfy (in Toronto) noting the person's interest, eligibility, and HIV viral loads within the prior 24 months history for those that are living with HIV.

The study will be advertised through the Maple Leaf Medical Clinic website (www.mlmedical.com), Groups of Trans Women, Women Living with HIV and potential study participants can also contact Maple Leaf Research directly to participate. Also, the study has been approved by the CIHR Canadian HIV Trials Network (CTN) and the flyers will be distributed through their networks.

The study procedures to be conducted for each subject are presented in tabular form in Appendix C and described in the text below. Appendix E1/2 also provides additional details about study procedures. For the purpose of this study: one month is equivalent to 28 days.

All the questionnaires to be completed can be found in the DDI Main Study Surveys document.

8.2 Screening Visit

Interested candidates will be screened, consented, and enrolled at MLR in Toronto. All potential candidates will have a screening visit to confirm eligibility. The approved informed consent must be reviewed prior to any study procedures. All participant questions must be answered, and the consent form must be signed before screening study activities can begin. A copy of the signed consent form must be offered / provided to the participant, the original is kept in their study source. This visit can be conducted virtually.

8.3 Screening Visit Procedures

- Written informed consent completed prior to any other assessments (can be completed through eSIGN (e.g ADOBE sign).
- Demographic data (questionnaire), important medical history (including HIV status, gender dysphoria history, surgical history, CIS women- menses history (cycle frequency- see questionnaire), and medications used by the subject to treat or to prevent HIV, feminizing hormones or hormonal contraception, herbal/over-the counter medication, and any other medication used by the subject in the 28 days prior to screening are collected to confirm eligibility. Appendix B1 will be referred to, to confirm eligibility. A doctor visit and/or physical exam is only required if deemed medically necessary by the study personnel and physician; it will be completed by one of the study physicians virtually/ by phone/ or in person (i.e if the participant is experiencing an adverse event or wants to discuss the dosage change for medication).

- <u>Screening Blood Sample Collection</u>:
    - Routine bloodwork: serum creatinine [eGFR] and ALT only for all groups.
    - Hepatitis B Surface antigen testing and Hepatitis C antibody testing for all groups.
    - For groups 1 and 2: a urine protein test will be done
    - For groups 1 and 2: an HIV-1 RNA Viral Load will be drawn to confirm viral suppression.

        - If a HIV-1 RNA Viral Load for groups 1 and 2 is available from their doctors' offices within two weeks prior to the screening visit, these results can be used instead with consent from the participant.
    - For group 3: an HIV-1 RNA Diagnostic Serology will be done to confirm that they are HIV negative.
        - For a positive HIV-1 RNA Diagnostic Serology, the participant will be excluded, and they will be immediately linked to the rapid HIV care programs developed at the Maple Leaf Medical Clinic or their referring physician site.
    - For group 2: serum Beta-HCG test is completed; they will be excluded if positive.
    - For group 2: FSH test will be completed
        - For a FSH level ≥27.0 IU/L, the participant will be excluded and deemed post-menopausal.

- <u>Estradiol Dosing:</u>
    - Trans women taking an oral estradiol dose > 2 mg per day must divide their dose so that they are taking 2 mg in the morning and the rest at night (e.g. 3 mg of estradiol would be divided into 2 mg qAM and 1 mg qPM). There are no known side effects related to this change in dosing.
    - If they need to switch to a divided dose and need a prescription, the participant can meet with a study physician (by phone, video, or in-person) and they will be asked to switch immediately (i.e., the next day).
    - Participants taking an estradiol dose of 2 mg per day at night must switch their dosing to the morning and they will be asked to switch immediately (i.e., the next day).
    - Participants crushing or taking their oral estradiol sublingually must switch to swallowing the tablets and they will be asked to switch immediately (i.e., the next day).
    - Once confirmed or switched to the required oral estradiol dose (as explained above), participants must remain on this same regimen until the completion of the month 2 visit.

- <u>ART Dosing:</u>
    - Women in groups 1 and 2 taking their ART medication at night must switch their dosing to morning and must remain on this same regimen until the completion of the month 2 visit. They will be asked to switch immediately (i.e., the next day). If they are taking Atripla, which is qHS dosing; the participant can switch to morning dosing after baseline with the switch to Biktarvy®.

- <u>Screening window (from the screening to baseline visit):</u>
    - Baseline visit for participants that switch their oral estradiol dosing from the night to the morning <u>or</u> split their dosing <u>or</u> change to swallowing a tablet, must be booked > 28 days *to allow for estradiol concentration stabilization*.

- Baseline visit for participants that switch their ART dosing from the night to the morning, must be booked > 28 days *to allow for ART concentration stabilization.*
- Otherwise, the baseline visit can be scheduled after the HIV-1 RNA Viral Load or HIV-1 RNA Diagnostic Serology results are received around 14 days.
- The maximum screening visit window is 90 days; participants whose baseline visit date will fall past 90 days can be rescreened.

- Questionnaires (to be completed with the coordinator):
  - All groups: Demographic questionnaire
  - Group 2: Menses History

- Participant Instructions:
  - Participant instruction sheet for baseline visit is reviewed and given to participants.
  - Participants will be reminded to avoid drinking grapefruit juice for the duration of the study due to the possibility of interaction with Estrace.

- Follow-Up
  - Study personnel will contact participants who switched their oral estradiol dosing from the night to the morning or split their dosing or changed to swallowing a tablet within a week after their screening visit to confirm that participants have started the new regimen and document the start date.

8.4 Baseline Visit Procedures

- The Baseline procedures may occur as soon as all eligibility criteria are confirmed. The baseline visit must occur within 90 days after the screening visit. This visit can be conducted in person or virtually.
- Important medical history (including HIV status, gender dysphoria history, surgical history, CIS-women menses history (cycle frequency-see questionnaire), and medications used by the subject to treat or to prevent HIV, feminizing hormones or hormonal contraception, herbal/over-the counter medication, and any other medication used by the subject is reviewed to confirm eligibility as per Appendix B1.
- Adverse events will be reviewed and documented in the source document, only AEs related to either estradiol or B/F/TAF determined by the principal investigator will be entered in the CRF.
- Height and weight will be collected for all groups when visit is done in person. If visit is done virtually, it will be done at Month 2.
- A doctor visit and/or physical exam is only required if deemed medically necessary by the study personnel and physician; it will be completed by one of the study physicians virtually/ by phone/ or in person.
- For groups 1 and 2: a urine protein test will be done

- Fasting:
  - All participants are required to fast (no food and drinks [except water]) for 10 hours.

- Dosing Times:
  - Participants that split their oral estradiol into AM & PM dosing need to document the time of their evening doses (the day prior to and the evening of the baseline visit).
  - Participants need to document the time of oral estradiol and ART (if applicable) the day before and the day of the visit.

- Breakfast:
  - A Breakfast of 350-500 calories must be consumed within 30 minutes of starting. The start time must be documented.

- Blood Sample Collection:
  - Blood Draw Group 1
    - Pre-dose serum estradiol and testosterone samples are collected 5-25 minutes after breakfast start time. Time must be recorded.
    - Routine bloodwork (CBC, electrolytes [sodium, chloride, potassium], serum creatinine [eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids).
    - HIV-1 RNA Viral Load & Lymphocyte Subsets (CD4 and CD8).
      - If there is an HIV-1 RNA viral load blip (i.e., > 40 and < 500 copies/mL), the participant needs to repeat the HIV-1 RNA Viral Load in 2-3 weeks; if the repeat viral load is still > 200 copies/mL, they will be withdrawn from the study.
      - If the baseline HIV-1 RNA viral load is detectable at > 500 copies/mL, they will be withdrawn from the study; and replaced with another participant.
    - Feminizing hormones and current ART are taken 30 minutes (±5 mins) after the start of breakfast. Time must be documented.
    - One (1) serum estradiol sample is collected 4 hours (±10 mins) after feminizing hormones are taken. Time must be documented. If the visit is conducted virtually, this sample will be optional.

  - Blood Draw Group 2:
    - Serum estradiol and testosterone samples are collected 5-25 minutes after breakfast start time. Time must be recorded.
    - Routine bloodwork (CBC, electrolytes [sodium, chloride, potassium], serum creatinine [eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids) and serum Beta-HCG.
    - HIV-1 RNA Viral Load & Lymphocyte Subsets (CD4 and CD8).

- If there is an HIV-1 RNA viral load blip (i.e., > 40 and < 500 copies/mL), the participant needs to repeat the HIV-1 RNA Viral Load in 2-3 weeks; if the repeat viral load is still > 200 copies/mL, they will be withdrawn from the study.
- If the baseline HIV-1 RNA viral load is detectable at > 500 copies/mL, they will be withdrawn from the study; and replaced with another participant.

▪ The participants in group 2 <u>do not</u> have a C4h estradiol sample collected.
▪ The pregnancy test must be negative, if positive, the participant will be withdrawn and considered a screen failure.

o <u>Blood Draw Group 3:</u>
▪ Pre-dose serum estradiol and testosterone samples are collected 5-25 minutes after breakfast start time. Time must be recorded.
▪ Routine bloodwork (CBC, electrolytes [sodium, chloride, potassium], serum creatinine [eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids).
▪ HIV-1 RNA Diagnostic Serology
- If an HIV-1 RNA Diagnostic Serology returns positive, the participant will be immediately linked to the rapid HIV care programs developed at the Maple Leaf Medical Clinic or their referring physician site.

▪ <u>Feminizing hormones are taken 30 minutes</u> (±5 mins) after the start of breakfast. Time must be documented.
▪ One (1) serum estradiol sample is collected 4 hours (±10 mins) after feminizing hormones are taken. Time must be documented. If the visit is done virtually, this sample is optional.

- <u>B/F/TAF Dosing</u>:
  - Participants in Groups 1 and 2 not already taking B/F/TAF as their ART regimen, must switch to B/F/TAF at baseline visit (they will take their first dose of B/F/TAF the next day after the baseline visit). They speak with a study physician (virtually/ by phone/ or in person) to discuss possible side effects and adherence; the study physician is to enter the B/F/TAF prescription in the chart. Only Participants that switch their ART regimen to B/F/TAF at baseline are booked for a virtual month 1 standard of care visit.
  - Document start date of B/F/TAF.
  - Switch participants in group 1 and 2 are dispensed 2 bottles of B/F/TAF at baseline as part of the study. If participants taking B/F/TAF at start of study need assistance with medication, please contact the study team for further instructions.

- <u>Questionnaires</u>:
  - Groups 1: FEM-SQ, Adherence, HSIDM PRO, HIVTSQs, SF-36.
  - Groups 2: Adherence, HSIDM PRO, HIVTSQs, SF-36, Menses History (to be completed with a coordinator).

- Groups 3: FEM-SQ, HSIDM PRO, SF-36.

- Participant Instructions:
  - Participant Instruction Sheet for month 2 visit is given & reviewed for day before and day of visit instructions.

- Follow-Up
  - Study personnel will contact group 1 and 2 participants who switched to B/F/TAF within a week after their baseline visit to confirm that participants have started the new regimen and document the start date and any side effects (if any).

8.5 Month 1 Visit Procedures (Group 1 and 2 who switched to B/F/TAF only)

- The Month 1 standard of care visit can be conducted virtually / by phone with the study physician (as per clinical procedures).
- This study visit is scheduled relative to the day that participants started B/F/TAF (i.e., Start Date + 28 days [± 5 days]).
- Review and document any changes in medical history (including, gender dysphoria history, surgical history, and medications used by the subject to treat HIV, feminizing hormones or hormonal contraception, herbal/over-the counter medication, and any other medication used by the subject. This will be reviewed to confirm eligibility as per Appendix B1.
- Adverse events will be reviewed and documented in the source document, only AEs related to either estradiol or B/F/TAF determined by the principal investigator will be entered in the CRF.

8.6 Month 2 Visit Procedures

- Participants must come to Maple Leaf Research in Toronto to complete the Month 2, due to the PBMC processing requirements. Additional travel support will be provided for participants travel and the stay in Toronto (see #22 Ethics & Dissemination for more details).
- Month 2 visit is scheduled 8 weeks [± 5 days] after the baseline visit, with exception to participants who switched to B/F/TAF, month 2 visit is scheduled 8 weeks after the B/F/TAF start date [± 5 days].
- Review and document any changes in medical history (including HIV status, gender dysphoria history, surgical history, CIS-women menses history (questionnaire) and medications used by the subject to treat or to prevent HIV, feminizing hormones or hormonal contraception, herbal/over-the counter medication, and any other medication used by the subject. This will be reviewed to confirm eligibility as per Appendix B1.
- Adverse events will be reviewed and documented in the source document, only AEs related to either estradiol or B/F/TAF determined by the principal investigator will be entered in the CRF.
- For participants that started the study already taking B/F/TAF - A doctor visit and/or physical exam is only required if deemed medically necessary by the study personnel and

physician; it will be completed by one of the study physicians virtually/ by phone/ or in person.

- For participants that switched to B/F/TAF at baseline- A standard of care visit is required, it can be conducted virtually / by phone or in person with the study physician (as per clinical procedures) to review and discuss possible side effects and adherence.

- Fasting
    - All participants are required to fast (no food and drinks (except water) for 10 hours.

- Dosing Times:
    - Participants that split their oral estradiol into AM & PM dosing need to document the time of their evening doses (the day prior to and the evening of the Month 2 visit).
    - Participants need to document the time of oral estradiol and ART (if applicable) the day before and the day of the visit.

- Breakfast
    - A Breakfast of 350-500 calories is given upon arrival to all participants.
    - The start time for breakfast must be documented.
- Weight will be collected for all groups.

- Blood Sample Collection:
    - A catheter can be inserted for the day to perform the required laboratory work otherwise a regular butterfly needle or multi-sample blood collection needle can be used (decision is up to participant and lab tech based on comfort and safety).

    - Blood Draw Group 1:
        - Pre-dose samples:
            - serum estradiol and testosterone and pre-dose whole blood B/F/TAF PK samples are collected 5-25 minutes after breakfast start time (see lab manual for processing instructions). Time must be recorded.
            - Routine bloodwork (CBC, electrolytes [sodium, chloride, potassium], serum creatinine [eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids)
            - HIV-1 RNA Viral Load
                - If there is an HIV-1 RNA viral load blip <200, participant needs to repeat in 2-3 weeks.
        - Feminizing hormones and B/F/TAF are taken 30 minutes (±5 mins) after the start of breakfast. Time must be documented.
        - Post-dose samples:
            - Seven (7) post-dose serum estradiol samples are collected 1H, 2H, 3H, 4H, 6H, 8H, 24H (±10 mins) after feminizing hormones are

taken on morning of month 2 visit (see lab manual for processing instructions). All times must be documented.
- Nine (9) post-dose whole blood B/F/TAF PK plasma samples are collected 0.5H, 1H, 1.5H, 2H, 3H, 4H, 6H, 8H, 24H (±10 mins) after B/F/TAF is taken on morning of month 2 visit (see lab manual for processing instructions). All times must be documented.
- If any timepoints for estradiol and B/F/TAF PK sample collection fall out of the window, blood sample will be drawn regardless, and time must be documented.

- DBS whole blood sample is collected 2H after B/F/TAF is taken (±10 mins) (see lab manual for processing instructions).
- PBMC whole blood samples are collected 2H after B/F/TAF is taken (±10 mins). The processing of samples must start within 30 minutes at the Dr. Rupert Kaul's University of Toronto lab. (see lab manual for processing instructions).
- Participants can take their AM oral estradiol and B/F/TAF after the 24H samples are collected.

- Blood Draw Group 2
  - A urine pregnancy test must be negative, if positive, the participant must be withdrawn.
  - Predose samples:
    - Serum estradiol and testosterone samples and B/F/TAF pre-dose whole blood samples are collected 5-25 minutes after breakfast start time (see lab manual for processing instructions). Time must be recorded.
    - Routine bloodwork (CBC, electrolytes [sodium, chloride, potassium], serum creatinine [eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids) and serum Beta-HCG.
    - HIV-1 RNA Viral Load,
      - If there is an HIV-1 RNA viral load Blip <200, participant needs to repeat in 2-3 weeks.
  - B/F/TAF medication is taken 30 minutes (±5 mins) after the start of breakfast. Time must be documented.
  - Post-dose samples:
    - Nine (9) post-dose whole blood B/F/TAF PK plasma samples are collected 0.5H, 1H, 1.5H, 2H, 3H, 4H, 6H, 8H, 24H (±10 mins) after B/F/TAF is taken on morning of month 2 visit (see lab manual for processing instructions). All times must be documented.
    - If any timepoints for B/F/TAF PK sample collection falls out of the window, blood sample will be drawn regardless, and time will be documented
  - DBS whole blood sample is collected 2H after B/F/TAF is taken (±10 mins) (see lab manual for processing instructions).

- PBMC whole blood samples are collected 2H after B/F/TAF is taken (±10 mins). The processing of samples must start within 30 minutes at the Rupert Kaul lab. (see lab manual for processing instructions).
- Participants can take their AM B/F/TAF after the 24H samples are collected.

- Blood Draw Group 3
  - Pre-dose samples:
    - Pre-dose serum estradiol and testosterone samples are collected 5-25 minutes after breakfast start time. Time must be recorded.
    - HIV-1 RNA Diagnostic Serology
      - If an HIV-1 RNA Diagnostic Serology returns positive, they will be immediately linked to the rapid HIV care programs developed at the Maple Leaf Medical or their referring physician site. The participant will be withdrawn.
    - Routine bloodwork (CBC, electrolytes [sodium, chloride, potassium], serum creatinine[eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids).
  - Feminizing hormones are taken 30 minutes (±5 mins) after the start of breakfast. Time must be documented.
  - Post-dose samples:
    - Seven (7) serum estradiol samples are collected 1H, 2H, 3H, 4H, 6H, 8H, 24H (±10 mins) after feminizing hormones are taken on morning of month 2 visit (see lab manual for processing instructions). All times must be documented.
    - If any timepoints for estradiol PK sample collection falls out of the window, blood samples will be drawn regardless, and time will be documented
  - Participants can take their AM oral estradiol after the 24H samples are collected.

- If the participant does not complete all of the required estradiol and/or PK time points or any estradiol values are abnormal the principal investigator needs to be consulted to determine if Month 2 procedures should be repeated.

- Questionnaires:
  - Groups 1: FEM-SQ, Adherence, HSIDM PRO, HIVTSQs, SF-36.
  - Groups 2: Adherence, HSIDM PRO, HIVTSQs, SF-36, Menses History (to be completed with a coordinator).
  - Groups 3: FEM-SQ, HSIDM PRO, SF-36.

- B/F/TAF Dosing:
  - Dispense 4 bottles of B/F/TAF to group 1 and 2 participants (If required).

- If B/F/TAF was switched to the morning, it can be switched back to the evening after this visit.
- Participants are not required to keep empty bottles.

- Next steps:
  - The Month 2 visit will be the last study visit for Group 3 participants.
  - After completion of this visit participants can change back to their original estradiol dosing if they prefer. If needed, a new prescription can be requested from the study physician.
    There will a Month 6 visit for participants in Group 1 and 2. Remind participants to return any leftover study medication at month 6, empty bottles are not returned.

8.7 Month 6 Visit Procedures (Group 1 & 2 only)

- Month 6 is scheduled 24 weeks [± 5 days] after the baseline visit. This visit can be conducted virtually.
- Important medical history (including HIV status, gender dysphoria history, surgical history, CIS-women menses history) and medications used by the subject to treat HIV, feminizing hormones or hormonal contraception, herbal/over-the counter medication, and any other medication used by the subject are reviewed and updated as required. This will be reviewed to confirm eligibility as per Appendix B1.
- Adverse events will be reviewed and documented in the source document, only AEs related to either estradiol or B/F/TAF determined by the principal investigator will be entered in the CRF.

- For participants that started the study on B/F/TAF- A doctor visit and/or physical exam is only required if deemed medically necessary by the study personnel and physician; it will be completed by one of the study physicians virtually/ by phone/ or in person.
- For participants that switched to B/F/TAF at baseline- A standard of care visit is required, it can be conducted virtually / by phone or in person with the study physician (as per clinical procedures) to review and discuss possible side effects and adherence.
- Blood Sample Collection:
  - Routine bloodwork (CBC, electrolytes [sodium, chloride, potassium], serum creatinine [eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids(non-fasting).
  - HIV-1 RNA Viral Load
    - If there is an HIV-1 RNA viral load blip or viremia (i.e., > 40 copies/mL), the participant needs to repeat the HIV-1 RNA Viral Load in 2-3 weeks.
  - Lymphocyte Subsets (CD4 and CD8).

B/F/TAF Dosing:

- Participants that switched to B/F/TAF as their ART regimen, can either switch back to their old regimen or continue taking B/F/TAF after this visit. The study physician can provide them with a prescription.

- Document the last dose of B/F/TAF in the study. Participants need to return any leftover study medication. Participants are not required to keep empty bottles.

- Questionnaires:
  - Group 1:
    - FEM-SQ, Adherence, HSIDM PRO, HIVTSQs, HIVTSQc, SF-36.
  - Group 2:
    - Adherence, HSIDM PRO, HIVTSQs, HIVTSQc, SF-36, Menses History (to be completed with a coordinator).

9 SCREEN FAILURES

- A screen failure is an individual who consents to participate in this study but does not complete the baseline visit because they do not meet inclusion / exclusion criteria.
- Trans women who test positive for HIV at screening are considered screen failures.
- Cis women who have a positive pregnancy test at screening are considered screen failures.
- Any screen failure must be replaced with another participant until the group number is met.

10 MANAGING POSITIVE HIV DIAGNOSTIC SEROLOGY

- If an HIV-1 RNA Diagnostic Serology returns positive, the participants will be immediately linked to the rapid HIV care programs developed at the Maple Leaf Medical or their referring physician site. The participant will be withdrawn.

11 MANAGING HIV-1 RNA BLIPS

- If there is an HIV-1 RNA viral load blip (i.e., > 40 and < 500 copies/mL), the participant needs to repeat HIV-1 RNA Viral Load in 2-3 weeks; if the repeat viral load is still > 200 copies/mL, at baseline or month 2, they will be withdrawn from the study and replaced with another participant from the same group.
- If the baseline or month 2 HIV viral load is detectable at > 500 copies/mL, they will be withdrawn from the study; and replaced with another participant.

12 STUDY WITHDRAWAL

If a participant cannot or does not complete the study for any reason or is lost to FU they need to be replaced with a participant from the same group. Please contact the study principal investigator to discuss.

- If a participant has a high-risk HIV exposure and HIV post-exposure prophylaxis (PEP) is required for a month-participants, needs to be withdrawn but can be rescreened after 30 days.

- For a positive HIV-1 RNA Diagnostic Serology, the participant will be excluded/withdrawn, and they will be immediately linked to the rapid HIV care programs developed at the Maple Leaf Medical Clinic or their referring physician site.
- If there is an HIV-1 RNA viral load blip (i.e., > 40 and < 500 copies/mL), the participant needs to repeat HIV-1 RNA Viral Load in 2-3 weeks; if the repeat viral load is still > 200 copies/mL, at baseline or month 2, they will be withdrawn from the study and replaced with another participant from the same group.
- If the baseline or month 2 HIV viral load is detectable at > 500 copies/mL, they will be withdrawn from the study; and replaced with another participant.
- If CIS woman becomes pregnant between screening and month 2 they will be withdrawn and replaced with a participant.
- Participants are free to withdraw from participation in the study at any time upon request.
- The participant will be withdrawn if any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- The participant will be withdrawn if they meet an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

13 EVALUATION, RECORDING, AND REPORTING OF ADVERSE EVENTS

13.1 Definitions

13.1.1 Adverse Event (AE)

In this study, an adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation participant, administered a study medication/intervention, which has a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational intervention.

During each visit with the participant, information on AEs will be gathered and documented accordingly. AEs will be graded as mild, moderate, severe, or life-threatening and assessed by causality as related to the study intervention.

In this study, any other new conditions that are not related to study drugs will be captured in the Medical History and do not need to be graded. Stable chronic conditions which are present prior to clinical trial entry and any worsening of these pre-existing conditions will also be accounted for in the participant's medical history.

13.1.2 Serious Adverse Events (SAE)

In this study, an SAE is defined as an AE meeting one of the following criteria at any dose:

- Results in death during the period of protocol-defined surveillance

- Is a life-threatening event (defined as a participant at immediate risk of death at the time of the event)
- Results in in-patient hospitalization or prolongation of existing hospitalization during the protocol-defined surveillance
- Results in persistent or significant disability or incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions)
- Is a congenital anomaly or birth defect

Any other important medical event that may not result in one of the above outcomes, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

Participants will be monitored during the study period for SAEs. If an SAE is ongoing at the time a participant discontinues/completes the study, the SAE will be followed until the Investigator agrees that the event is satisfactorily resolved, becomes chronic, or that no further follow-up is required.

13.2 AE Description: Relationship to Study Treatment

For all collected AEs (including SAEs), the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below:

Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to Investigational Product administration and cannot be explained by concurrent disease or other products or chemicals. The response to withdrawal of the product (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.

Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the Investigational Product, is unlikely to be attributed to concurrent disease or other products or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.

Possibly Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the participant's clinical

condition, other concomitant events). Although an adverse drug event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to Investigational Product administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other products or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not related: The AE is completely independent of Investigational Product administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

13.2 Follow-Up for Adverse Events

Any AE that occurs between the time that a study participant commences the study and the time that the participant departs the study at the end of the final study visit (or at the time of early discontinuation of the participant from the study for any reason) will be captured and recorded. At each contact with the participant, the investigator (or designate) must seek information on AEs by specific questioning and, as appropriate, by examination.

AEs that had previously been reported by the study participant will also be reassessed for duration, intensity and possible reoccurrence. Assessment of safety will include clinical observation and monitoring of study related lab results.

All AEs (including SAEs) will be followed until resolution or until the investigator and the clinical/medical monitor are in agreement that the AE has resolved, stabilized or become chronic and no further follow-up is required.

13.3 SAE Reporting

All SAEs which occur during the course of the study must be reported to the Sponsor-Investigator within 24 hours of the site becoming aware of the event.

SAEs will be reported to:

Dr. Mona Loufty

Phone:	416-725-9566
Fax:	416-465-0502
E-mail:	mona.loufty@wchospital.ca

13.4 Mandatory Reporting to Health Canada

Per section C.05.014 of the Canadian Food and Drug Regulations, SAEs meeting Health Canada expedited reporting criteria (serious and unexpected adverse drug reactions) will be submitted to Health Canada. Expedited reporting of reactions that are serious but expected is not required. Expedited reporting is not required for serious events from clinical investigations that are considered unrelated to the study treatment, whether or not the event is expected.

During the trial, the Sponsor-investigator will inform Health Canada in an expedited manner of any serious, unexpected adverse drug reaction in response to the use of the study treatment that has occurred during the study, as follows:

a) Where it is neither fatal nor life-threatening, within 15 days after becoming aware of the information;
b) Where it is fatal or life-threatening, within 7 days after becoming aware of the information. Within 8 days after having initially informed Health Canada of the fatal or life-threatening SAE, submit as complete a report as possible. Follow-up reports of fatal or life-threatening reactions must include an assessment of the importance and implication of the findings, including relevant previous experience with the same or similar drugs.

The Investigator is responsible for informing the local Research Ethics Board (REB) of SAEs that occur at the site in compliance with local requirements.

14 POTENTIAL RISK TO HUMAN PARTICIPANTS TAKING BIKTARVY®

A few risks are associated with taking Biktarvy® for people living with HIV-1. The only absolute contraindication to the drug is a known hypersensitivity to the drug itself. Severe acute exacerbations of hepatitis B have been reported in patients co-infected with HIV-1 and HBV, and it may occur with discontinuation of Biktarvy®. Therefore, patients co-infected with HIV-1 and HBV who discontinue Biktarvy® should be closely monitored with clinical and laboratory follow-up for at least several months after stopping treatment.

15 CLINICAL TRIAL DATA ON ADVERSE REACTIONS TO BIKTARVY®

The adverse event profile of Biktarvy® is very favorable. The primary safety assessment of Biktarvy® was based on Weeks 48, 96, and 144 pooled data from 1274 patients in two randomized, double-blind, active-controlled trials (Study 1489 and Study 1490). The most common adverse reactions (all grades) reported in at least 5% of patients in the Biktarvy® group in study 1489 were diarrhea, nausea, and headache. No adverse events in at least 5% of patients in the Biktarvy® group were reported in Study 1490. The safety profile of Biktarvy® was consistent through week 144 in both studies.

Additional adverse reactions (all grades) occurring in less than 2% of patients in Study 1489 and Study 1490 included:

- Gastrointestinal disorder: abdominal pain, dyspepsia, flatulence, vomiting.
- Psychiatric Disorders: depression.
- Skin and subcutaneous tissue disorders: rash.
- Suicidal ideation or suicide attempt (in patients with a pre-existing history of depression or psychiatric illness)

The majority of adverse reactions were Grade 1. Only 0.9% of patients in both the 1489 Study and the 1490 Study randomized to Biktarvy® discontinued due to an adverse event, compared to the 1.6% and 1.8% in the abacavir [ABC]/DTG/lamivudine [3TC] and DTG + FTC/TAF arm respectively.

16 RISKS OF ESTRADIOL THERAPY

A few risks are associated with taking estrogen therapy for transwomen. These risks include venous thrombosis/thromboembolism, increased risk of cardiovascular disease, weight gain, decreased libido, hypertriglyceridemia, elevated blood pressure, decreased glucose tolerance, gallbladder disease, benign pituitary prolactinoma, mental health effects and infertility. Since participants in this study will be already taking estradiol prior to entering the study, these risks have been being monitored by their primary prescribing physician and the ongoing risk in the study are considered low.

17 ADVERSE REACTIONS TO ESTRADIOL

The adverse event profile of estradiol is very favorable. Women rarely have severe side effects from taking estradiol. The most common adverse reactions are abdominal pain, nausea and vomiting. Other possible adverse reactions are depression, nervousness, and/or irritability, allergic reaction and rash, increased blood sugar levels, change in blood pressure, acne, change in cholesterol and/or triglyceride levels, change in weight. As study participants will be already taking estradiol prior to entering the study and are to be on a stable dose, side effects are likely to have already occurred and be resolved and managed by their primary prescribing physician. Any adverse event that occurs related to estradiol and any lab results obtained from this study will be sent to the primary physician, with the permission of the participant to help monitor the risks stated above.

18 HANDLING, STORAGE, AND ACCOUNTABILITY OF B/F/TAF

B/F/TAF must be stored in a secured place within the temperature range specified on the label. The storage temperature should be continuously monitored and recorded with a calibrated (if

not validated) temperature monitoring device(s). Only authorized study personnel should be allowed access to the study interventions. Storage conditions will be assessed during pre-study activities. B/F/TAF will be dispensed in their original marketed packaging containing 30 tablets in each bottle. Empty bottles are not required to be returned to study sites; however, participants should return any leftover study medication at the end of study, or a note written in chart explaining otherwise.

19 RISK MANAGEMENT

Risk minimization, management, and assessment procedures have been implemented in the study to minimize and assess potential risks to participants who participate in this clinical study with Estrace® or Lupin-estradiol and Biktarvy®. Components include: (1) specific study entry and exclusion criteria to ensure that participants who have underlying characteristics that potentially increase their risk for an adverse outcome are excluded; (2) protocol-specific procedures for minimizing and managing certain AEs, such as diarrhea, nausea, headache, etc. related to Biktarvy® and Estrace® or Lupin-estradiol; (3) ongoing follow-up at Month 1 for participants who switched to Biktarvy® at baseline and at Month 6 for participants living with HIV.

Any participants switched to Biktarvy® who do not tolerate the medication will be returned (rescued) to their prior antiretroviral therapy (ART) regimen and withdrawn from the study.

20 PK SAMPLE COLLECTION AND ANALYSIS

20.1 Antiretroviral PK Samples

Whole blood samples for storage of ART plasma PK concentrations will be collected from the participants as mentioned above. Similarly, PBMC samples will be collected to assess TFV-DP and FTC-TP concentrations at one time point (2h) during the study visit. These samples will be processed at Dr. Rupert Kaul's University of Toronto's research laboratory.

In addition, dry blood spots (DBS) will be collected to assess TFV-DP and FTC-TP. While PBMC is the preferred sample for intracellular drug concentration testing, there is an increasing body of literature supporting the use of DBS which has shown correlation with the PBMC values and adherence.[35] Due to the ease of taking this sample, a C_{2h} sample will be collected at Month 2 visit. The advantage of doing DBS testing is the ease of collection and processing.

Bioanalysis of all samples (minus the plasma TAF concentrations) will be performed under the direction of Dr. Kim Scarsi at the Antiviral Pharmacology Laboratory at the University of Nebraska Medical Center, Omaha, Nebraska, United Stated of America (USA). Dr. Scarsi is one of the top PK and DDI researchers in the USA and has particular expertise in studying DDIs between ART drugs and hormonal contraceptive medications. She has carried out such work for the US AIDS Clinical Trial Group studies over the years; all assays in her lab are validated according to Federal

Drug Association (FDA) guidance and evaluated in the NIH DAIDS Clinical Pharmacology Quality Assurance Program.
The assay ranges are as follows:

- BIC plasma 20-10,000 ng/mL
- FTC plasma 10-1,500 ng/mL
- TFV-DP PBMC and DBS 50-10,000 fmol/sample
- FTC-TP PBMC and DBS 1,000-200,000 fmol/sample

Plasma TAF concentration analyses and reporting will be performed at QPS LLC, Delaware Technology Park, 3 Innovation Way, Suite 240, Newark, Delaware, 19711, USA.
The TAF plasma assay is:

- TAF method QPS 60-1578 with assay range of 1-1000 ng/mL.

Concentrations of antiretrovirals will be determined in plasma samples using the currently approved bioanalytical methodology; no human DNA analysis will be performed on these samples.

20.2 Estradiol Level Samples

Serum estradiol samples will be collected from the participants as mentioned above. Estradiol bioanalysis will be performed under the control of Life Labs.

21 DATA MANAGEMENT

Visit data collection forms (eCRFs) are available electronically using a secure electronic system and will be created and managed in REDCap through the CIHR Canadian HIV Trials Network (CTN) in Vancouver, BC; data will be kept on their institutional secure server. Research site study personnel can access the electronic eCRFs in real-timeParticipant questionnaires are available through the electronic system REDCap; they will be self-administered by participants electronically in real-time at each visit. If the participant prefers, a research coordinator can assist them completing the questionnaires. Paper questionnaires are available as backup. Data entry verification will include algorithms that automatically check completed forms for missing, out-of-range, or inconsistent values. Aberrant entries will be checked according to the data management agreement and reported promptly to the original interviewer for immediate reconciliation. An audit trail will be created for all data changes. The database will be maintained on a secure server and in a password-protected partitioned drive and backed up daily.

22 ADHERENCE TO PROTOCOL

22.1 Protocol Amendments

All protocol amendments will be reviewed and approved and if applicable submitted to the applicable regulatory agencies for prior approval or notification. The Investigator must sign and date the amendment prior to implementation. All protocol amendments must also be submitted to the ethics committee.

22.2 Protocol Deviations

No deviations from this protocol will be permitted without the prior written approval of the Sponsor-Investigator, except when the modification is needed to eliminate an immediate hazard or hazards to participants. Any deviations that may affect a participant's treatment or informed consent, especially those increasing potential risks, must receive prior approval from the REB unless performed to remove an immediate safety risk to the participants. In this case it will be reported to the REB and the Sponsor immediately thereafter. Any departures from the protocol must be documented.

23 ENDPOINTS AND ADDITIONAL VARIABLES OF INTEREST

23.1 Primary endpoints

***Primary endpoints #1*:** relates to the comparison of the PK parameters of interest, AUC, maximum (C_{max}) and minimum (C_{min}) plasma concentration of BIC, FTC, and TAF, and PBMC and DBS TFV-DP and FTC-TP concentrations will be compared between trans women living with HIV (group 1) and premenopausal cis women living with HIV (group 2).

These are the standard PK endpoints for DDI studies.[36] The FDA Guidance document on topic indicates that AUC and C_{max} are the standard change in drug exposure parameters to be measured.[37] We will also be reporting C_{min} which is standard in the field. The FDA indicates that the PK results of DDI studies should be presented as the geometric mean ratio of the observed PK exposure measures with and without the perpetrator drug and include the associated 90% confidence interval (CI). It is also recommended to report on the measures of the observed variability of the interaction.

Primary endpoints #2: relates to the hormone concentrations routinely tested when trans women are taking feminizing hormones, namely estradiol concentrations. The primary endpoints for estradiol concentrations will be C_{min}, C_{4h}, C_{max}, and AUC and the proportions of the month 2 C_{4h} that are within target (200 to 735 pmol/L) and will be compared between groups 1 and 3. Testosterone concentration will not be assessed as a primary endpoint due to its high variability.

In general, estrogen therapy aims to achieve serum estradiol concentrations within the normal range for pre-menopausal women; approximately 200 to 735 pmol/L.[18,31] Oral micronized 17 β -estradiol is rapidly absorbed and is metabolized in both the gastrointestinal (GI) tract and liver to estrone (E1), which is further metabolized to estrone sulfate (E1S) and other conjugates.[32,38] The oral bioavailability of 17 β -estradiol is approximately 5% due to a large first pass effect[39] and the half-life ($t_{1/2}$) of oral estradiol is often reported to be 0.5-1h.[40] Conversely, when measured in PK studies, the elimination $t_{1/2}$ has been found to range from approximately 12-20 hours.[32] This extended $t_{1/2}$ is likely the result of enterohepatic recirculation and continuous interconversion between estradiol, E1, and E1S.[32,39] At 2 mg dosages of 17 β -estradiol administered once daily acutely, the C_{max} of estradiol was 489.6 pmol/L (at 8 hours), which reduced to a 24h C_{min} of 146.8-220.2 pmol/L/mL. As can be seen, the C_{min} is below the desired estradiol concentration target in these studies and therefore, is the reason that most clinicians do not use the estradiol C_{min} as the

concentration used to monitor estradiol dosing clinically. In another study, C_{max} was C_{4h}. It is predicted that the C_{max} will be at 4 hours; however, this is variable and not well-established. Therefore, a full series of estradiol concentration samples will be taken from 1 hour to 8 hours after administration. Clinicians indicate to their trans patients taking estradiol to take the pill at night and go to the laboratory in the morning to capture the estradiol level at 8-12 hours post-dose. We have chosen 8 hours post dosing as our final peak sample as 12 hours will not be feasible with laboratory and research facility hours. Since the timing of the estradiol concentration is tricky, we are currently undertaking a supplemental pilot study to draw the full series of estradiol concentrations from 8 trans women living with HIV on ART and 8 trans women living without HIV who meet our inclusion and exclusion criteria to inform the final endpoints for this study.

23.2 Secondary Endpoints

***Secondary endpoints #1*:** are the baseline estradiol pre-dose and C_{4h} and total testosterone concentrations, and these will be compared between groups 1 and 3 and assessed to see the proportion that meets target. The baseline estradiol pre-dose and C_{4h} and total testosterone concentrations will be taken on variable ART regimens for the trans women living with HIV; the month 2 estradiol pre-dose and C_{4h} and total testosterone concentrations will be taken from the trans women living with HIV who are taking B/F/TAF; and the change in estradiol pre-dose and C_{4h} and total testosterone concentrations from baseline to month 2 in those who switched to B/F/TAF can be compared to those who did not and to those in group 3. Group 3 values also provide information on the degree of variability of estradiol concentrations. The desired concentrations of estradiol are between 200-735 pmol/L and the desired concentration for testosterone is < 2 nmol/L. The frequency and proportion of those within the target of 200-735 pmol/L for estradiol concentration and < 2 nmol/L for testosterone concentrations will be reported as well as the frequency and proportion that are below and above target.

Testosterone concentrations are routinely measured in the care of trans women after initial start of feminizing hormones and are easier to test than estradiol as the concentration is less impacted by the timing of its sampling. For ease, the testosterone concentration in our study will be measured at the same time as the estradiol. Free testosterone represents the portion of testosterone unbound to serum proteins and depends on levels of sex hormone binding globulin (SHBG).[40] While free testosterone can be measured, assays are unreliable. Consensus is lacking on the role of free vs. total testosterone concentrations. Total testosterone concentrations are reliable and readily available; however, they do not describe the actual bioavailable testosterone level. Bioavailable testosterone is free testosterone plus testosterone weakly bound to albumin. SHBG is elevated in the presence of estrogen, and in particular with exogenous estrogen supplementation, more so with oral estrogen than with parenteral routes due to first pass hepatic activity.[18] The Endocrine Society recommends monitoring of the total testosterone level, with a target range of < 2 nmol/L and for that reason, we will be measuring total testosterone concentration.

Secondary endpoint #2: Total score on the FEM-SQ, a questionnaire for measuring satisfaction with medical gender affirmation, will be compared between groups 1 and 3 throughout the study period. The FEM-SQ is a new questionnaire developed by our team through mixed-methods survey development including expert consultation and focus groups with 23 trans women (version 1), semi-structured interviews with over 10 trans women (version 2), and quantitative data collection (survey administration) with 300 trans women (version 3). While hormone concentration measurement is numerically easier to capture, understanding how satisfaction with gender affirmation differs between trans women taking feminizing hormones and ART and those only taking feminizing hormones without ART is of critical importance for understanding and reducing barriers to ART uptake.

Secondary endpoint #3: Proportion of patients with an undetectable HIV-RNA (<40 copies/mL) will be compared between groups 1 and 2 throughout the study period.

As all participants living with HIV will have an undetectable HIV-1 RNA viral load at baseline, the number and proportion of those maintaining virologic suppression at month 1 (for those who switched to B/F/TAF at baseline), month 2 and month 6 will be reported for those in group 1 and group 2 as a whole cohort and for those who switched to B/F/TAF.

Secondary endpoint #4: ART adherence will be assessed at each visit for groups 1 and 2 using a validated visual analogue scale (VAS) of ART use over the prior month at each visit.[41] This VAS is known as the Medication Adherence Self-Reported Inventory (MASRI) and was developed by Walsh, Mandalia and Gazzard and validated and published in *AIDS* in 2002. It starts with a permissive statement prefaced in order to encourage accurate responses. This reads as `We understand that many people on anti-HIV medication find it very difficult to take it regularly and often miss doses. We won't be surprised if you have missed lots of doses as well. We need to know how many doses you have missed'. The MASRI VAS is for the proportion of doses taken in the preceding month. The instructions for the VAS read: `put a cross on the line below at the point showing your best guess about how much medication you have taken in the last month. We would be surprised if this was 100% for most people, e.g., 0% means you have taken no medication; 50% means you have taken half your medication; 100% means you have taken every single dose of medication'. The VAS ranges from 0 to 100% in 10% intervals. For validation, the VAS was compared to the gold standards of MEMS Caps (MC) and pill count (PC). The mean self-reported adherence by VAS over the preceding month was 93.3% (SE, 1.2%) and was strongly associated with both MC (r. 0.63; P, 0.001) and PC (r. 0.75; P, 0.001). In multivariable analysis, the strongest association between a MASRI item and MC was for the VAS. Also, the VAS items were inversely associated with viral load (p=0.01) confirming clinical importance.

Secondary endpoint #5: Frequency and severity of B/F/TAF related adverse effect for participants in groups 1 and 2 who have changed to B/F/TAF at each follow-up visit.

Adverse effects will be recorded using the NIAID standardized toxicity grading system (Appendix D) and will be reported as a frequency and proportion of with any adverse effect, any grade 2 or higher and any requiring stopping the treatment.

Secondary endpoint #6: PROs will be assessed for groups 1 and 2 at all study visits using the HIV Symptom Index Distress Module (HSIDM) at baseline, months 2 and 6. However, since the scale does not mention HIV and asks about symptoms solely, group 3 participants will be asked to complete it at baseline and month 2.

The HSIDM, also referred to as the HIV Symptom Index (HSI), is a validated patient-reported outcome (PRO) instrument that assesses the burden of 20 common symptoms associated with HIV treatment or disease. The instrument has been validated repeatedly and is available in French and English. It is considered to be the gold standard in HIV symptom research. Participants are asked about their experience with each of 20 symptoms during the past 4 weeks using a 5-point, Likert-type scale. Response options and scores are as follows: (0) "I don't have this symptom;" (1) "I have this symptom and it doesn't bother me;" (2) "I have this symptom and it bothers me a little;" (3) "I have this symptom and it bothers me;" (4) "I have this symptom and it bothers me a lot." The 20 symptoms comprise of fatigue/loss of energy, difficulty sleeping, nervous/anxious, diarrhea/loose stools, changes in body composition, feeling sad/down/depressed, bloating/pain/gas in stomach, muscle aches/joint pain, problems with sex, trouble remembering, headaches, pain/numbness/tingling in hands/feet, skin problems/rash/itching, cough/trouble breathing, fever/chills/sweats, dizzy/light-headedness, weight loss/wasting, nausea/vomiting, hair loss/changes, and loss of appetite/food taste. The score is most commonly dichotomized symptoms being not bothersome (scores of 0 or 1) or bothersome (scores of 2, 3 and 4). The overall bothersome symptom counts at baseline and at months 2 and 6 are generated by counting the number of individual symptoms scored as bothersome per participant and can therefore be reported as a frequency and proportion of those with no or any bothersome symptoms and a median (IQR) of the number of bothersome symptoms.

Secondary endpoint #7: Since taking ART regimens is a big part of this study, we will be asking about satisfaction with ART regimen using the HIV Treatment Satisfaction Questionnaire (HIVTSQ). There are two versions of the HIVTSQ that have been developed. At the baseline, month 2 and month 6 visits, the 10-item HIVTSQ status version (HIVTSQs) will be used. The developers, Woodcock and Bradley, found that nine of the 10 items in the HIVTSQ performed well, but one of the items, the one on "demands" did not.[42] So, they developed a revised questionnaire called the HIVTSQ new change version (HIVTSQc).[43] The HIVTSQc will be used in addition to the HIVTSQs at month 6. The HIVTSQs was validated in 2001 with 150 people living with HIV with principal components analyses suggesting that participant ratings of nine items could be summed to compute the total satisfaction scale (Cronbach's alpha 0.82), and/or divided into the subscales of 1) general satisfaction/clinical (alpha 0.80) and 2) lifestyle/ease (alpha 0.74). The HIVTSQs also showed construct validity: with negative correlation to viral load (Spearman's r - 0.33 $p < 0.01$). The HIVTSQc was validated with 97 participants and confirmatory factor analysis supported the two subscales: 1) general satisfaction/clinical change with an alpha 0.85 and 2)

lifestyle/ease change with an alpha 0.88) and the 10-item *treatment satisfaction change* scale with an alpha 0.92 which also met construct validity. Since these studies, the HIVTSQ has been expanded to include 12 items and is superior and we have purchased the license for the use of the 12-item HIVTSQs and HIVTSQc in both French and English.

The questionnaires are both scored out of 66. The HIVTSQs score is calculated by summing the answers to questions 1 to 11 which have a range from 0 to 6 making a score that has a range from 0 to 66 with a higher score meaning that one is more satisfied with their treatment. Item number 12 is not included in the composite score, it is stand alone. The assessed change or an improvement in medication satisfaction, the total HIVTSQs score from the baseline is subtracted from the follow up score. For the HIVTSQc, which is the same as the HIVTSQs except for minor word alterations in question 9, answers from questions 1 to 11 are added up but this time the answers range from -3 to + 3 to assess deterioration and improvement in satisfaction compared to before. The total score ranges from -33 to +33.

Secondary endpoint #8: Participant health status in all groups will be assessed using the SF-36 at baseline and month 2, which allows us to convert to a utility and conduct cost-effectiveness analyses in the future, if desired. The SF-36 will be repeated for Groups 1 and 2 at the 6-month visit.

The SF-36 is a 36-item, patient-reported survey of patient's health status, or quality of life, and is one of the most commonly used scales to measure health status as it can be converted to a utility and used in health economics.[44] The SF-36 consists of eight scale scores (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health). These eight scale scores are the weighted sums of the questions in their section and then each scale is directly transformed into a 0-100 scale on the assumption that each question carries equal weight. SF-36 is interpreted in ascending fashion with lower scores equating to more disability and higher scores meaning less disability. The individual scale scores and overall scale score are most often reported as a mean and standard deviation (SD). We are choosing the SF-36 as the health status measure as it is the most robust, it is available in French and English, and it is free of charge.

Additional variables: Basic sociodemographic variables will be collected such as age [to be reported as a median with interquartile range (IQR)] and race/ethnicity [White, African, Caribbean Black (ACB), Indigenous or Other (N/%)]. Clinical variables will include weight (median/IQR), height (median/IQR), body mass index (median/IQR), and hepatitis B or C infection (N/%). For the trans women participants, dose of estradiol will be recorded (N/% and median/IQR), which anti-androgen/orchiectomy/medical is used (N/%) and its dose (N/%), and whether gender affirming surgery has taken place, more specifically orchiectomy which can impact testosterone concentrations. For participants living with HIV, duration of HIV infection (median/IQR), duration of ART (median/IQR), which ART regimen they are currently taking, baseline VL (median/IQR), baseline CD4 count (median/IQR) will be collected. For cis women, a menstrual history will be recorded including if their cycles are regular (N/%).

24 STATICAL ANALYSES AND SAMPLE SIZE CALCULATION

24.1 Statistical analyses

Baseline sociodemographic and clinical variables (including age, city of care, race/ethnicity, baseline ART drugs, CD4 count, etc.) will be summarized using medians and IQRs and means and SDs for continuous variable, when appropriate, and frequencies and proportions for categorical variables and presented in table format.

For the ART drugs' PK parameters, we will be determining the 2-month PK data of plasma BIC, plasma FTC, plasma TAF, and PBMC and DBS TFV-DP and FTC-TP for trans and cis women living with HIV. For each woman, plasma AUC, C_{max} and C_{min} will be reported. Analyses will take place on logarithmically transformed AUC, C_{max} and C_{min} data. The geometric mean ratio (GMR) will be calculated by comparing the trans women (group 1) to cis women (group 2) and the associated 90% CIs will be reported. The two groups will be considered similar if the observed GMR is not less than 0.7 when comparing group 1 to group 2. A 30% lower exposure was chosen as an acceptable difference for this secondary objective, as 30% is a conventional reduction in exposure that is often considered acceptable and does not require consideration of a dose adjustment. To establish bioequivalence between groups would require a significantly higher sample size, which is not likely to be necessary given what we know about the metabolism/elimination of the proposed ART and feminizing hormones.

Since a full series of estradiol concentrations will be done over time at month 2 for groups 1 and 3, for each woman, serum AUC, C_{max}, C_{4h}, and C_{min} for estradiol will be reported. As above, analyses will take place on logarithmically transformed AUC, C_{max}, $C_{4h,}$ and C_{min} data. The geometric mean ratio (GMR) will be calculated by comparing the trans women living with HIV (group 1) to trans women living without HIV (group 3) and the associated 90% Cis will be reported. The two groups will be considered similar if the observed GMR is not less than 0.7 when comparing group 1 to group 3. A 30% lower exposure was chosen as an acceptable difference for this secondary objective, as 30% is a conventional reduction in exposure that is often considered acceptable and does not require consideration of a dose adjustment.

Another estradiol endpoint is determining the proportion of estradiol C_{4h} concentrations that are within range or out of range at month 2. The target range for estradiol is 370 to 735 pmol/L for the Endocrine Society and 200 to 500 pmol/L for Sherbourne Health and therefore our target range will be from 200 to 735 pmol/L. These proportions will be compared between groups 1 and 3 using the Chi-square test. The proportions of below, within and above target at baseline and month 2 as a bar graph with 3 colours, one for each target (within, below, above) and the change in categories compared from month 2 to baseline will also be presented using chi-square testing.

The secondary hormone endpoints to be assessed are the mean estradiol C_{min} and C_{4h} and total testosterone concentrations at baseline and month 2 in groups 1 and 3. The hormone concentrations will be reported using means/SD and compared using the independent t-test.

Furthermore, for trans women living with HIV who were on a different former ART regimen prior to baseline and were switched to B/F/TAF, the mean change in estradiol C_{4h} and total testosterone concentration from baseline to month 2 can be assessed using the paired t-test. Similarly, the hormone concentrations (estradiol and total testosterone) for group 2 will also be assessed and can be compared in a similar fashion as an exploratory analysis.

Other secondary outcomes that will be reported include medical gender affirming satisfaction using a scale which will be summarized using a mean and SD for each visit and using a repeated measures diagram and compared between groups using the independent t-test. Viral response which will be reported using frequency and proportion of participants who maintain viral suppression at months 1, 2 and 6. Adherence will be reported using the VAS,[41] which is a Likert scale and will be summarized using a mean and SD for each visit and using a repeated measures diagram. Adverse event rate to B/F/TAF will be reported for groups 1 and 2 using frequencies and proportions. The PRO scale used is most commonly dichotomized and each symptom will be reported as frequencies and proportions for the various symptoms and compared from baseline to month 2 and 6 for both groups 1 and 2. An exploratory analysis will be conducted to compare to group 3. Finally, drug regimen satisfaction will be reported as a means (SDs) and compared between groups 1 and 2 at baseline and month 2 and 6 using a t-test. Overall, SF-36 scores will be reported as means (SDs) compared between groups at baseline and month 2 for all groups and additionally at month 6 for groups 1 and 2.

24.2 Sample size Justification

The sample size is being determined primarily for the primary hypothesis of no difference between ART plasma AUC, if we assume coefficient of variation (CV) [AUC] for each ART drug of 0.33 (based on Gallant, 2017 Lancet for BIC; AUC=96 mcg*h/mL, SD=32), the proposed sample size of 15 participants in group 1 and 2 provides 80% statistical power to detect 30% lower exposure during feminizing hormone therapy use based on an alpha of 0.1, consistent with the FDA guidance to report GMR + 90% confidence intervals between each group.

25 ETHICS AND DISSEMINATION

25.1 Ethical considerations

This study will be conducted in accordance with the ethical principles for human research outlined in the Declaration of Helsinki, the Tri-council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2 (2018), and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)'s Good Clinical Practice Standards which are all followed in Canada.

25.2 Informed consent

All participants will be given detailed oral and written information about the study. Consent forms describing in detail the study medication/intervention(s) study procedures and risks will be given to each participant and written documentation of informed consent is required prior to starting any study procedures. Participants must sign an informed consent document that has

been approved by a participating centre's REB prior to any procedures being done specifically for the trial. Each participant should have sufficient opportunity to discuss the study, have all of their questions addressed and consider the information in the consent process prior to agreeing to participate. Participants may withdraw consent at any time during the course of the study without prejudice. The informed consent form will be signed and dated by the participant and the investigator or delegate. The original signed informed consent form will be retained in the participant's study files and a copy will be provided to the participant.

The informed consent process must be conducted, and form signed before the participant undergoes any screening procedures that are performed solely for the purpose of determining eligibility for the study.

The informed consent will be available in English and revisions to the consent form will be made according to requirements of the REB. Annual REB renewals will be made to the REB and all required amendments will be made in a timely fashion.

25.3 Compensation

All participants are provided the following to account for food, travel, childcare, time, and other expenses:

	Screening	**Baseline**	**Month 1**	**Month 2 (Day 1)**	**Month 2 (Day 2)**	**Month 6**
Group 1	$40	$80	$20	$160	$40	$40
Group 2	$40	$50	$20	$160	$40	$40
Group 3	$40	$80		$160	$40	

For participants that need to travel from outside of the Greater Toronto Area for the study visits, study related travel expenses will be covered on a case-to-case basis. Expenses must be pre-approved with study team. Meal allowance will be calculated based on current CIHR standards for meals not provided during study visits (Breakfast $19.10, Lunch $18.90, Dinner $47.35).

25.4 Confidentiality

All participant-related information including CRFs, laboratory specimens, evaluation forms, reports, etc. will be kept strictly confidential. All records will be kept securely at study sites behind 2 locked doors and will be only accessible to research staff. Participants will be identified only by means of a coded number specific to each participant. All computerized databases will identify participants by numeric codes only and will be password protected. All digital files will be restricted to study personnel and will be kept on secure servers at Maple Leaf Medical Clinic.

Due to questionnaires being sent to the participant's email when visits are conducted virtually, email addresses for each participant will be collected in the eCRF. However, during data export this identifying information will be removed to maintain confidentiality.

Upon request, and in the presence of the investigator or their representative, participant records will be made available to the study sponsor, monitoring groups representative of the study sponsor, representatives of funding groups, and applicable regulatory agencies for the purpose of verification of clinical trial procedures and/or data, as is permissible by local regulations.

All study materials will be kept for 15 years before destruction as per Health Canada Guidelines.

25.5 Research Ethics Board (REB)

Research ethics approval for the conductance of this study will be obtained from Veritas IRB. Ethics reviews will also be sought at Unity Health Toronto. Informed consent will be obtained prior to study activities at the screening visit.

The REB will review all appropriate study documentation to safeguard the rights, safety, and well-being of the participants. The study will be conducted only at sites where ethics approval has been obtained. A copy of the protocol (including protocol amendments), all versions of informed consent forms, other information to be completed by participants such as survey instruments or questionnaires, and any proposed advertising/recruitment materials must be reviewed and approved by the REB prior to implementation of the trial. The investigator will be responsible for obtaining REB approval of the annual Continuing Review throughout the duration of the study. The investigator will notify the REB of serious adverse events as applicable. The investigator will seek prior ethics approval for any protocol deviations except when the change is intended to eliminate an immediate hazard to participants. In this case, the protocol deviation will be promptly reported.

25.6 Dissemination plan

Data analyses and then dissemination will begin when all study participants have completed the month 2 study visit and the data up to that point has been collected and verified. The results will be published and presented at national and international conferences, on the Maple Leaf Medical Clinic website, and to community groups as appropriate. Also, other knowledge translation tools useful for community members such as infographics, fact sheets and community reports will be produced in consultation with the CAB aimed to support trans women living with HIV to understand the findings and issues with DDIs between ART drugs and feminizing hormones. Also, results in the form of infographics will be provided to all recruitment sites and study participants if desired.

26 CONCLUSION AND IMPACT

This study is of global importance for trans women living with HIV, an underserved population, and their care providers. If our hypotheses are correct (i.e., that there are no clinically significant

DDIs and impact of feminizing hormones on B/F/TAF and vice versa), trans women living with HIV that live in jurisdictions where care providers are still restricting feminizing hormones due to potential DDIs with ART can be reassured and provided the therapies that they need. Subsequently, trans women who are not taking ART due to fear from such restrictive care from care providers and are purchasing hormones illegally could be reassured and access the care they need in an informed manner. Furthermore, the results of this study are important in light of the reduced TDV and FTC concentrations found amongst trans women taking TDF/FTC as PrEP and feminizing hormones. As we will be reporting on plasma TAF and FTC and intracellular PBMC and DBS TDV-DP and FTC-TP levels, this is likely crucial information.

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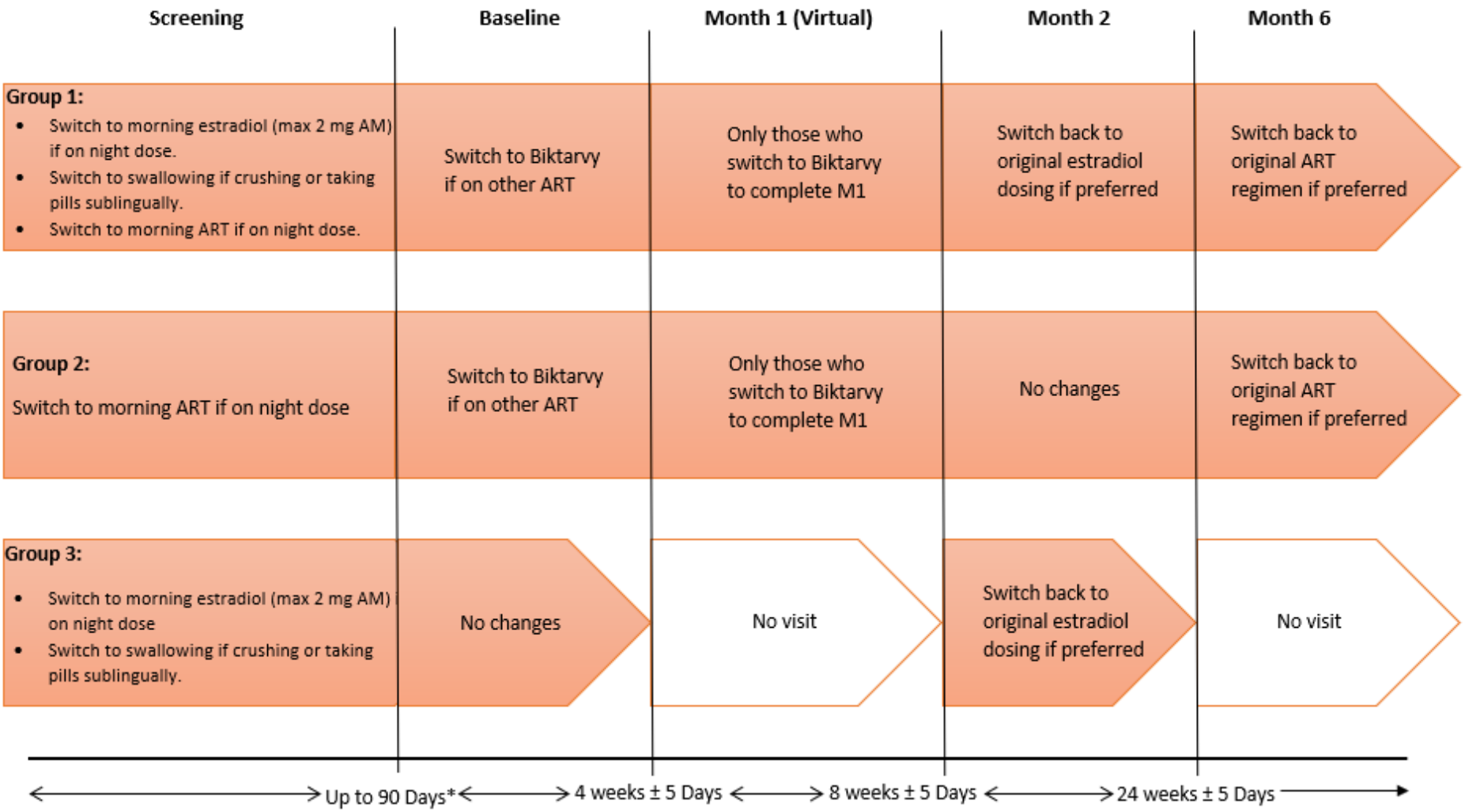
APPENDIX A – Study Schematic



*Participants can be re-screened if the screening process takes more than 90 days.

Group 1: 15 trans women living with HIV

Group 2: 15 cis women living with HIV

Group 3: 15 trans women living without HIV

APPENDIX B1 – List of Prohibited Medications

Additional Hormonal Therapy:	
Estrogen containing products and conjugated estrogens	**Combined Oral Contraceptives:** Ethinyl estradiol/levonogestrel, ethinyl estradiol/desogestrel, ethinyl estradiol/norethindrone, ethinyl estradiol/norethindrone/ethinyl estradiol, ethinyl estradiol/norgestimate, ethinyl estradiol/drosperinone, ethinyl estradiol/norgestrel **Patch:** norelgestromin/ethinyl estradiol **Ring:** etonogestrel/ethinyl estradiol **Conjugated estrogens**
Allowed Contraceptives	Copper IUD Levonorgestrel IUDs (e.g. Mirena) Progesterone- only contraception incl. Depo-Provera

Prohibited medications with Biktarvy (bictegravir, emtricitabine, tenofovir alafenamide): *Refer to table in Appendix C for DDIs which can be managed	
Anticonvulsants	Carbamazepine Fosphenytoin- Phenytoin Oxcarbazepine Primidone
Anti-HBV	Adefovir
Anti-infectives	Clarithromycin Erythromycin Rifabutin Rifampin
Antiarrhythmics	Dofetilide Verapamil
Antidepressants	Nefazodone St. John's Wort
Antifungals	Fluconazole Itraconazole Ketoconazole Posaconazole Voriconazole
Antihypertensive	Bosentan
Antineoplastic agents	Apalutamide Enzalutamide

Antiprogesterone	Mifepristone
Barbiturate	Phenobarbital
CNS Stimulant	Modafinil

Prohibited medications with Estradiol:	
Anti-infectives	Clarithromycin Erythromycin Rifabutin Rifampin
Antiarrhythmic	Verapamil
Anticonvulsants	Carbamazepine Fosphenytoin- Phenytoin Oxcarbazepine
Antidepressants	Nefazodone St. John's Wort
Antifungals	Fluconazole Itraconazole Ketoconazole Posaconazole Voriconazole
Antihyperlipidemic	Gemfibrozil
Antihypertensive	Bosentan
Antineoplastic Agents	Apalutamide Enzalutamide Tamoxifen
Antiprogesterone	Mifepristone
Barbiturate	Phenobarbital
CNS Stimulant	Modafinil
Immunosuppressant	Cyclosporine (systemic)

Prohibited medications with Cyproterone:	
Anti-infectives	Clarithromycin Erythromycin Rifabutin Rifampin
Antiarrhythmic	Verapamil
Anticonvulsants	Carbamazepine Fosphenytoin- Phenytoin Oxcarbazepine
Antidepressant	Nefazodone St. John's Wort

Prohibited medications with Cyproterone:	
Antifungals	Fluconazole Itraconazole Ketoconazole Posaconazole Voriconazole
Antihypertensive	Bosentan
Antineoplastic Agents	Apalutamide Enzalutamide
Antiprogesterone	Mifepristone
Barbiturate	Phenobarbital
CNS Stimulant	Modafinil

Prohibited medications with Spironolactone:	
Antihypertensive	Eplerenone
Diuretics	Amiloride Triamterene
Electrolyte Supplements	Potassium salts
Immunosuppressants	Cyclosporine (systemic) Tacrolimus (systemic)

Prohibited medications with Estradiol Testing Assay:	
Antineoplastics Aromatase Inhibitor	exemestane (Aromasin)
Estrogen receptor antagonists	fulvestrant (Faslodex)

APPENDIX B2 – Drug Interactions and recommendations on management

Medications interacting with Biktarvy (bictegravir, emtricitabine, tenofovir alafenamide):		
Agents	**Mechanism of DDI**	**Management**
Polyvalent cation containing products (e.g. Mg, Al, Ca, Fe) Calcium or iron supplements, cation containing antacids or laxatives, sucralfate, buffered medications	May decrease the concentration of Bictegravir.	Administer Biktarvy 2 hours before or 2 hours after taking medications or oral supplements containing polyvalent cations. Biktarvy and medications or oral supplements containing polyvalent cations can be taken together with food.

*taken from Biktarvy drug monograph

APPENDIX C – Schedule of Events

Protocol Activity	Screening	Baseline	Month 1*	Month 2	Month 6	Notes
Informed Consent	W					
Inclusion & Exclusion	W					
Enrollment & Information	W					
Demographic Data (questionnaire – Screening Only), Medical History, Concurrent Medication History, Menses Questionnaire	W	W	X	W	X	Demographics (e.g. age, race, education, etc.(questionnaire) screening only; medical history (e.g. FHT history, HIV history, menses history questionnaire, etc.) and medication history (e.g. gender affirming surgery, chronic ongoing conditions, psychiatric, major medical conditions) ; feminizing hormones, hormonal contraception, medications used to treat or prevent HIV, herbal/over-the counter medication, and any other medication used in the 30 days prior to screening). The medical and medication history is updated at each visit as required.
Height and weight		W		W		Height will only be collected at Baseline.
Doctor Visit & Physical Exam	O	O	X	O	O	A doctor visit and/or physical exam is only required if deemed medically necessary by the study personnel and physician. If a participant switches to B/F/TAF, they will be seen by a physician at each visit to allow for follow-up of side effects and adherence.
AE related to B/F/TAF or oral estradiol		X	X	X	X	
BLOOD WORK						
Routine blood work	W	W		W	X	Routine blood work includes CBC, electrolytes [sodium, potassium, chloride], serum creatinine[eGFR], ALT, total bilirubin, glucose, HbA1C, and lipids. For screening serum ALT & serum creatinine[eGFR] only.

Protocol Activity	Screening	Baseline	Month 1*	Month 2	Month 6	Notes
Hepatitis B Surface antigen & Hepatitis C antibody	W					
FSH Test	C					
Urine Pregnancy and/or Serum Beta-HCG	C	C		C	C	Urine pregnancy test is only required at month 2.
Urine Protein Test	X	X				
HIV-1 RNA Diagnostic Serology	Z	Z		Z		
HIV-1 RNA viral load	X	X		X	X	
Lymphocyte Subsets CD4 + CD8		X			X	
Serum testosterone and estradiol levels		W/T		W		All participants: pre-dose estradiol and testosterone concentrations are collected at baseline & month 2. Trans women have an additional estradiol C_{4H} level at baseline (optional if visit is virtual).
Seven (7) post-dose serum estradiol levels (1h, 2h, 3h, 4h, 6h, 8h, 24h)				T		
Ten (10) ART PK plasma samples (pre-dose, 0.5h, 1h, 1.5h, 2h, 3h, 4h, 6h, 8h, 24h)				X		
PBMC (2h) & DBS (2H) for TFV-DP and FTC-TP				X		
QUESTIONNAIRES						
FEM-SQ		T		T	T	
Adherence		X	X	X	X	
HSIDM PRO		W		W	X	
HIVTSQs		X		X	X	
HIVTSQc					X	
SF-36		W		W	X	

W: All to complete (Group 1, 2, 3)
X: Cis and trans women living with HIV to complete (group 1, 2)
O: If deemed necessary
T: Trans women to complete (Group 1, 3)
Z: Women living without HIV to complete (group 3)
C: Cis women living with HIV to complete (group 2)[1]

Abbreviations: BIC, bictegravir; F or FTC, emtricitabine; TAF, tenofovir alafenomide; PK, pharmacokinetics; DBS, dried blood spot; TFV-DP, tenofovir diphosphate; FTC-TP, emtricitabine triphosphate; FEM-SQ, Feminizing Medical Gender Affirmation Satisfaction Scale; HSIDM, HIV Symptom Index Distress Module; PRO, patient-reported outcomes; HIVTSQ, HIV Treatment Satisfaction Questionnaire; SF-36, Short Form 36.

Study visits:

- The baseline visit for participants that do not need to change their ART and/or estradiol dose/timing can be booked as soon as screening results are available (approximately 14 days due to HIV-1 RNA Diagnostic Serology and HIV-1 RNA viral load results).
- The baseline visit for participants required to change their estradiol dosing and/or ART dose/timing must be scheduled at least 28 days after the changed dose.
- Participants requiring a switch to B/F/TAF will be switched at baseline, preferably as soon as screening results are received but they will be allowed to complete their current ART prescriptions (which could take as long as 90 days) before switching to B/F/TAF.
- The maximum screening visit window is 90 days; participants whose baseline visit date will fall past 90 days can be rescreened.

* Month 1 is required for participants who change their ART regimen to B/F/TAF; it must be scheduled at least 28 days after the B/F/TAF start date . Standard of Care visit, can be done virtually.

- Month 2 is scheduled 8 weeks after baseline, except for the participants who change their ART regimen to B/F/TAF, month 2 is scheduled 8 weeks after the date B/F/TAF was started.
- Month 6 is scheduled 24 weeks after baseline except for the participants who change their ART regimen to B/F/TAF, month 6 is scheduled 24 weeks after the date B/F/TAF was started.
- Study visits will have a buffer window of ± 5 business days.

APPENDIX D – NIAID standardized toxicity grading system

Item	Grade 1 Toxicity	Grade 2 Toxicity	Grade 3 Toxicity	Grade 4 Toxicity
1.2 Hematology				
Hemoglobin (g/dL)	9.5 - 10.5	8.0 - 9.4	6.5 - 7.9	< 6.5
Absolute Neutrophil Count (x 109/L	1.0 - 1.5	0.750 - 0.999	0.500 - 0.749	<0.500
Platelets (x 109/L)	75 - 99	50 - 74.9	20 - 49.9	<20 or diffuse petechiae
WBC (cells/L)	2 x 10^9 – 2.499 x 10^9	50 x 10^9 – 100 x 10^9	1 x 10^9 - 1.499 x 10^9	< 1 x 10^9
1.3 Enzymes				
AST (SGOT)	1.25 - 2.5 x upper normal limit	2.6 - 5 x upper normal limit	5.1 - 10 x upper normal limit	> 10 x upper normal limit
Alkaline phosphatase	1.25 - 2.5 x upper normal limit	2.6 - 5 x upper normal limit	5.1 - 10 x upper normal limit	> 10 x upper normal limit
Amylase	1.1 - 1.5 x upper normal limit	1.6 - 2.0 x upper normal limit	2.1 - 5.0 x upper normal limit	> 10 x upper normal limit
1.4 Chemistries				
ALT	1.25 – 2.5 x upper normal limit	2.5 - 5 x upper normal limit	5 - 10 x upper normal limit	> 10 x upper normal limit
Cholesterol (mmol/L)	5.18 – 6.19	6.19 – 7.77	> 7.77	NA
Glucose (mmol/L) Fasting	6.11 – 6.95	6.95 – 13.89	13.89 – 27.75	> 27.75
Glucose (mmol/L) Nonfasting	6.44 – 8.89	8.89 – 13.89	13.89 – 27.75	> 27.75
Hyponatremia (mmol/L)	130 - 135	123 - 129	116 - 122	< 116 or mental status changes or seizures
Hypernatremia (mmol/L)	146-150	151-157	158-165	> 165 or mental status changes or seizures
Hypokalemia (mmol/L)	3.0 - 3.4	2.5 - 2.9 or replacement Rx required	2.0 - 2.4 or intensive replacement Rx req. or hospitalization req.	< 2.0 or paresis or ileus or life-threatening arrhythmia
Hyperkalemia (mmol/L)	5.6 - 6.0	6.1 - 6.5	6.6 - 7.0	> 7.0 or life threatening arrhythmias
Hypoglycemia (mmol/L)	3.0 - 3.5	2.2 - 2.9	1.7 - 2.1	< 1.7 or mental status changes or coma
Hyperglycemia (mmol/L) (note if fasting)	6.4 - 8.9	9.0 - 13.9	14.0 - 28.0	> 28 or ketoacidosis
Hyperbilirubinemia (µmol/L)	24 - 33	34 - 55	56 - 110	> 110
LDL (mmol/L)	3.37 – 4.12	4.12 – 4.90	> 4.90	NA
Potassium, High (mmol/L)	5.6 – 6.0	6.0 – 6.5	6.5 – 7.0	> 7.0
Potassium, Low (mmol/L)	3.0 – 3.4	2.5 – 3.0	2.0 – 2.5	< 2.0
Total Bilirubin	1.1 – 1.6 x upper normal limit	1.6 – 2.6 x upper normal limit	2.6 – 5.0 x upper normal limit	> 5.0 x upper normal limit
Sodium, High (mmol/L)	146 – 150	150 – 154	154 – 160	>160
Sodium, Low (mmol/L)	130 - 135	125 - 130	121 - 125	> 160
Triglycerides (mmol/L)	1.71 – 3.42	3.42 – 5.7	5.7 – 11.4	> 11.4
Urea	1.25 - 2.5 x upper normal limit	2.6 - 5 x upper normal limit	5.1 - 10 x upper normal limit	> 10 x upper normal limit
Creatinine (µmol/L)	130 - 180	181 - 360	361 - 720	> 720 or dialysis required
1.5 Lymphocyte Subsets CD4				
Absolute CD4+ Count (cells/L)	300 - 400	200 - 300	100 - 200	> 100

APPENDIX E1 – PROTOCOL REQUIRED LAB ASSESSMENTS

<table>
<tr><th>Laboratory Assessments</th><th colspan="4">Parameters</th></tr>
<tr><td>Hematology</td><td colspan="4">Complete blood count (CBC)
- White blood cell count (WBC)
- Hemoglobin (Hb)
- Mean corpuscular volume (MCV)
- Platelet Count</td></tr>
<tr><td rowspan="3">Chemistry</td><td colspan="2">Total Bilirubin</td><td colspan="2" rowspan="2">Lipids
- Cholesterol
- Triglycerides (TGs)
- High-density lipoprotein (HDL – C)
- Low – density lipoprotein (LDL-C)
- Non-HDL – C
- Total Cholesterol (TC)/HDL – C</td></tr>
<tr><td colspan="2">Electrolytes
- Sodium
- Chloride
- Potassium</td></tr>
<tr><td>Alanine Aminotransferase (ALT)</td><td>HbA1c</td><td>Glucose</td></tr>
<tr><td>Hepatitis screening (PHL)</td><td colspan="2">Hepatitis B virus surface antigen (HBsAg)</td><td colspan="2">Hepatitis C antibody (if positive perform plasma hepatitis C virus quantitative test)</td></tr>
<tr><td>Hormone</td><td>Estradiol – 17 Beta</td><td>Total Testosterone</td><td colspan="2">Follitropin (FSH)</td></tr>
<tr><td>Lymphocyte Subsets CD4 + CD8</td><td colspan="4">CD4+ Percent
CD4+ Absolute Count
CD8+ Percent
CD8+ Absolute Count
CD4/CD8 ratio</td></tr>
<tr><td>Pregnancy testing</td><td colspan="2">Serum β human chorionic gonadotropin (β – hCG) pregnancy test</td><td colspan="2">Urine β – hCG pregnancy test</td></tr>
<tr><td>Urine</td><td colspan="4">Protein test</td></tr>
<tr><td>Human immune deficiency virus-1 (HIV – 1) serology (PHL)</td><td colspan="4">HIV Ab/Ag CMIA Screen
Geenius™ Confirmation Assay
p24 Ag ELFA</td></tr>
<tr><td>HIV – 1 RNA Viral Load (PHL)</td><td colspan="4">Roche cobas® HIV-1 assay or current Public Health Ontario testing platform</td></tr>
<tr><td rowspan="3">Pharmacokinetics (PK)</td><td colspan="4">Investigational antiretroviral therapy (ART) PK samples will be collected for both Group #1 and Group #2</td></tr>
<tr><td colspan="4">Dried blood spot (DBS) for Group #1 and Group #2</td></tr>
<tr><td colspan="4">Human peripheral blood mononuclear cells (PBMC) for Group #1 and Group #2</td></tr>
</table>

APPENDIX E2 – REQUIRED BLOOD VOLUMES

Group 1:

	GROUP 1	ML of Blood	Screen	Base	Month 2	Month 6
HIV-1 RNA Viral Load	1- Pearl	6	6	6	6	6
Lymphacyte Subset (CD4 + CD8)	1- EDTA	4	0	4	0	4
Standard Blood#1	1-SST	6	0	6	6	6
Standard Blood#2	1-EDTA	4	0	4	4	4
ALT & eGFR	1- SST	6	6	0	0	0
HBV/HVC serology	1- SST or Red	6	6	0	0	0
Testosterone pre-dose			0			0
estradiol pre-dose	1-SST	6	0	6	6	0
estradiol C1H	1-SST	6	0	0	6	0
estradiol C2H	1-SST	6	0	0	6	0
estradiol C3H	1-SST	6	0	0	6	0
estradiol C4H	1-SST	6	0	6	6	0
estradiol C8H	1-SST	6	0	0	6	0
estradiol C24H	1-SST	6	0	0	6	0
ART PK pre-dose	1- EDTA	6	0	0	6	0
ART PK C0.5H	1- EDTA	6	0	0	6	0
ART PK C1H	1- EDTA	6	0	0	6	0
ART PK C1.5H	1- EDTA	6	0	0	6	0
ART PK C2H	1- EDTA	6	0	0	6	0
ART PK C3H	1- EDTA	6	0	0	6	0
ART PK C4H	1- EDTA	6	0	0	6	0
ART PK C6H	1- EDTA	6	0	0	6	0
ART PK C8H	1- EDTA	6	0	0	6	0
ART PK C24H	1- EDTA	6	0	0	6	0
PBMC (18ML)	3- CPT	20	0	0	20	0
DBS C2h	1- EDTA	4	0	0	4	0
		TOTAL	18	32	142	20

Group 2:

	GROUP 2	ML of Blood	Screen	Base	Month 2	Month 6
HIV-1 RNA Viral Load	1- Pearl	6	6	6	6	6
Lymphacyte Subset (CD4 + CD8)	1- EDTA	4	0	4	0	4
Standard Blood#1	1-SST	6	0	6	6	6
Standard Blood#2	1-EDTA	4	0	4	4	4
ALT & eGFR	1- SST	6	6	0	0	0
HBV/HVC serology	SST or Red	6	6	0	0	0
Serum Pregnancy	1- SST	6	6	6	6	6
Testosterone pre-dose			0			0
estradiol pre-dose	1-SST	6	0	6	6	0
ART PK pre-dose	1- EDTA	6	0	0	6	0
ART PK C0.5H	1- EDTA	6	0	0	6	0
ART PK C1H	1- EDTA	6	0	0	6	0
ART PK C1.5H	1- EDTA	6	0	0	6	0
ART PK C2H	1- EDTA	6	0	0	6	0
ART PK C3H	1- EDTA	6	0	0	6	0
ART PK C4H	1- EDTA	6	0	0	6	0
ART PK C6H	1- EDTA	6	0	0	6	0
ART PK C8H	1- EDTA	6	0	0	6	0
ART PK C24H	1- EDTA	6	0	0	6	0
PBMC (18ML)	3- CPT	20	0	0	20	0
DBS Cmin	1- EDTA	4	0	0	4	0
		TOTAL	24	32	112	26

Group 3:

	GROUP 3	ML of Blood	Screen	Base	Month 2
HIV-1 RNA Diagnostic Serology	**1- red**	6	6	6	6
Standard Blood#1	**1-SST**	6	0	6	6
Standard Blood#2	**1-EDTA**	4	0	4	4
ALT & eGFR	**1- SST**	6	6	0	0
HBV/HVC serology	**SST or Red**	6	6	0	0
Testosterone pre-dose			0		
estradiol pre-dose	**1-SST**	6	0	6	6
estradiol C1H	**1-SST**	6	0	0	6
estradiol C2H	**1-SST**	6	0	0	6
estradiol C3H	**1-SST**	6	0	0	6
estradiol C4H	**1-SST**	6	0	6	6
estradiol C8H	**1-SST**	6	0	0	6
estradiol C24H	**1-SST**	6	0	0	6
		TOTAL	18	28	58