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

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Statistical Design and Power Plan

Specific Aim 1. Evaluation of the two-way PK-PD interaction of gender affirming exogenous estrogen (with testosterone suppression) on TDF/FTC PrEP in transgender women (TGW).

Study Design and Endpoints

Study Goal: The quantitative description of the two-way drug-drug interaction of gender-affirming hormone therapies (GAHT; testosterone suppressing GnRH antagonist leuprolide acetate alone and in combination with estradiol at two dose levels) and oral PrEP (TDF/FTC) in a study of 5 PK sampling periods.

Aim	Objectives	Endpoints & Data Analysis
1.a	• Describe the impact of GAHT on TDF/FTC PrEP PK-PD • Describe the bi-directional impact of TDF/FTC & GAHT combinations	• Non-compartmental analysis of TFV/FTC in plasma and urine • Concentrations of all analytes in blood, PBMC, & colorectal tissue Compare PK1 to PK2, PK3, PK4 for GAHT impact on PrEP • Compare PK5 to PK2-4 for PrEP impact on GAHT
1.b	• Describe the impact of GAHT on PrEP suppressed HIV susceptibility	• Describe changes in explant p24 antigen under all PK conditions • Correlation and Emax model of drug concentration-p24 relationship
1.c	• Explain the mechanism of estrogen impact on PrEP PK	• Mechanistic modeling of PK change • Renal changes with addition of GAHT
1.d	• Describe adverse effects associated with PrEP & GAHT	• Grade 2 or higher AEs (according to DAIDS Table for Grading Adverse Events, Corrected Version 2.1 - July 2017) by PK period

Study Design: Eligible participants will receive 300 mg TDF/200 mg FTC (Truvada®, Gilead Sciences), once daily for seven days under direct observation to achieve steady state drug PrEP concentrations. After one week of therapy, participants will undergo intensive PK analysis as well as collection of colorectal biopsies for PD testing (PK1). During the PK-intensive day, iohexol will be administered intravenously for the empirical determination of renal function and glomerular filtration rate (GFR). While concurrently on PrEP, participants will then be intramuscularly administered depot leuprolide acetate (3.75 mg Lupron™). Two weeks post-injection, sampling for PK, PD, and renal function will be performed (PK2). Participants will then immediately begin low-dose oral estrogen therapy (1 mg 17β-estradiol) in conjunction with PrEP for one week, at which time samples will be collected for the analyses described above (PK3). While on PrEP, participants will then transition to high-dose estrogen therapy (6 mg 17β-estradiol) for the remainder of the study. One week post-high dose estrogen therapy in the presence of PrEP, pharmacological and renal samples will be collected for analysis (PK4). A second injection of Lupron™ will commence at this time; PrEP will then be discontinued, and two weeks later, samples will be collected to assess renal function and hormonal concentrations (PK5). The presence of any remaining PrEP in plasma, PBMC or colorectal tissue will also be evaluated. All PrEP drug dosing will be administered under direct observation (DOT). Safety assessments include history/physical, chemistry/hematology labs at screening and interim history before each phase of the study.

Study Participants: 20 HIV-negative, self-identifying TGW naïve to GAHT, or abstinence from affirming therapies until testosterone concentrations reach the cisgender male range.

Sample analysis: The Marzinke CPAL will assay various specimens collected throughout the study (analytical methods are described in the **primary grant proposal**). Hormone testing will be performed via immunoassay or mass spectrometric methodologies at the Brigham and Women's Hospital (Boston, MA, USA). Iohexol sample analysis will be performed at the University of Minnesota to enable determination of measured GFR (mGFR).

Data Analysis:

Pharmacokinetic parameters in all matrices will be estimated using non-compartmental analysis, including the maximum concentration (C_{max}), time to maximum concentration (T_{max}), and area under the concentration versus time curve (from 0-24 hours, $AUC_{0\text{-}24h}$) for plasma TFV and FTC. These will be summarized using descriptive statistics by PK period. Colorectal tissue concentrations of TFV, FTC, TFV-DP, and FTC-TP will be summarized by time interval and PK period using descriptive statistics. Compartmental modeling using population PK methods are described in **Specific Aim 2** data analysis below. ***Sample size is conditioned primarily on***

plasma TFV and FTC as well as PBMC TFV-DP and FTC-TP. Dr. Chaturvedula will perform the PK analysis with Drs. Marzinke and Hendrix.

Renal Function will be assessed by several methods including estimation of iohexol clearance for estimation of mGFR, estimation of eGFR by use of the CKD-EPI equation, and assessment of cystatin C to asses GFR as well. Renal function analysis will be led by our nephrologist, Dr. Grams.

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

Safety data will be included for any participant who receives one dose of study drug. Adverse event frequency, grade, duration, and resolution will be captured and summarized using descriptive statistics. Sample size is not conditioned by this objective. Our biostatistician, Dr. Rosenblum, will perform these analyses.

Statistical analysis. All PK, PD, and safety endpoints will be summarized using descriptive statistics. Each of these classes of readout will be evaluated in a paired statistical test, selected based on the distribution of the data, to test the effect of (1) PrEP on hormones and (2) hormones on PrEP. Contrast (1) will be evaluated by paired comparison of the PrEP alone condition (PK#1) with each of the sequentially additive hormone conditions (leuprolide testosterone ablation [PK#2], plus low dose estrogen [PK#3], and finally, plus high dose estrogen [PK#4]). Contrast (2) will be tested by paired comparison of hormones alone (PK #5) with hormones plus PrEP (PK #4).

Sample size analysis: For paired analyses of change within participants between any of the 5 pharmacologic conditions, ***20 research participants provide 80% power to detect a 37% relative difference in PBMC TDF-DP concentration.*** This is with 2-sided 5% Type I error and assuming a standard deviation of 0.3 (in terms of $\log_{10}$ concentration). This is the observed within subject standard deviation of PBMC TFV-DP measurements over 5 weeks in the HPTN 066 trial data set. In a more recent ongoing study (also a sequential hormone-PrEP interaction study), we have smaller PBMC TFV-DP variation and estimate the ***ability to detect a 24% change***. Detection of the more conservative 37% change in active TFV-DP drug concentrations in PBMC is of ***roughly similar magnitude as we saw with plasma TFV in the CFAR Pilot study.*** **A 37% difference is also a smaller difference than the difference between daily dosing and 4 times per week dosing** (43%), which is a key contrast in the **Aim 2** clinical trial simulation. We are keenly interested to determine if the fall in TFV and FTC active moieties is as large as the PK difference in daily dosing used in iPrEx as compared to on demand 4/week (on average) dosing in IPERGAY. Given that the richest PK/PD relationships in oral PrEP studies are established in PBMCs, this basis of our study sample size decisions. The variation in colorectal tissue TFV-DP and FTC-TP is relatively much larger and we anticipate sufficient power only to detect differences as large as 73%. Differences of 20% will be detectable in plasma TFV and FTC.

Due to the multiple comparisons being conducted, we will control the familywise Type I error rate for each analyte across the different experimental conditions by using the graphical multiple testing procedures of Bretz and colleagues[1]. The advantage of these procedures is that they provide improved power compared to simpler procedures such as Bonferroni or Holm's step-down method.

Regarding assessments of percent above specific target concentrations (for example 4 or 7 doses per week equivalent), the maximum width of the 95% confidence interval (CI) for the fraction with plasma TFV or PBMC TFV-DP above the protective level is approximately 0.44, which occurs when the point estimate is 0.5 and the corresponding 95% CI is (0.28, 0.72). These estimates indicate that half of TGW participants would fall below the desired target concentrations, indicating adjustment of dosing recommendations. If the point estimate is 0.9, the 95% confidence interval is approximately (0.77, 1.00). Having 90% of TGW above the target concentrations is our target. If we fall below that target, a recommendation for dose adjustment would be strongly considered. This outcome would also be highly comparable to the HPTN 066 target concentration threshold values which were based on 90% sensitivity (100% specificity) in identifying daily dosing of TDF/FTC in a directly observed dosing setting. These examples of dichotomous outcome assessments are presented in order to give a rough idea of the level of uncertainty that can be expected for estimating these population proportions using the data generated by this trial.

For the safety assessment, the following table describes the probability of missing a specified adverse event for a range of true adverse event rates given a sample size of 20 research participants.

	Probability of Missing an Event
Event Rate	**N=20**
0.01	0.818
0.02	0.668
0.03	0.543
0.04	0.442
0.05	0.358
0.06	0.290
0.07	0.234
0.08	0.189
0.09	0.151
0.10	0.122
0.11	0.097
0.12	0.078
0.13	0.062
0.14	0.049
0.15	0.039
0.16	0.031
0.17	0.024
0.18	0.019
0.19	0.014
0.20	0.011

Specific Aim 2: Comparison of the likelihood of HIV protective concentrations during daily dosing and the IPERGAY (4 dose per week) regimen, with and without GAHT, using population PK modeling and simulation of Aim 1 data.

Study Goal: Estimate the relative level of protection afforded by the standard IPERGAY regimen compared to

Aim	Objectives	Endpoints & Data Analysis
2	• Assess HIV protective effect of 2 PrEP regimens with & without GAHT	• Update existing population PK PrEP models for impact of GAHT • Simulate PrEP RCT with daily dosing and Ipergay dosing, both with and without GAHT (6 regimens in total)

the on demand IPERGAY (4 dose) regimen in TGW on GAHT.

While once daily PrEP is currently recommended for optimal prevention of HIV acquisition in heterosexual cisgender women (CGW) at high risk of HIV infection, the IPERGAY PrEP dosing strategy (two doses before sex followed by a single dose for 2 days after sex), led to an 86% relative risk reduction in men who have sex with men (MSM), the highest level of protection in the modified intent-to-treat analysis for any of the primary PrEP randomized clinical trials[2]. This success is attributed to high rates of adherence to the prescribed regimen in the wake of proven efficacy in the iPrEx trial of daily TDF/FTC in MSM and TGW. iPrEx had lower levels of adherence. Consequently, there is a growing interest in this dosing strategy for prevention, which circumvents the requirement for daily oral PrEP.

In both iPrEx and IPERGAY, there were TGW on GAHT, but the numbers were relatively much fewer than the CGM who were not hormones. Therefore, there is a low level of confidence in the protective efficacy of oral TDF/FTC by either the daily or the on demand 4 dose IPERGAY regimen in TGW on GAHT. Thus, the impact of GAHT on PrEP remains an untested and critically important clinical management question. To indirectly address this question in the absence of a randomized clinical efficacy trial in TGW, we propose a clinical trial simulation

of 6 drug regimens: standard daily and 4 dose IPERGAY PrEP regimens, both with and without gender affirming hormone manipulation (testosterone ablation with high and low dose estrogen).

One fundamental assumption inherent in the clinical trial simulation is that TGW and CGM both have the same level of protection if the concentration of active drugs (TFV-DP and FTC-TP) are the same in PBMC and colorectal tissue, and that there is no change in susceptibility of the colorectal tissue itself mediated by hormonal manipulation. While there is no empiric evidence that there are differences in susceptibility of tissue based on hormonal levels, the differences that do exist in levels of protection afforded by PrEP between MSM (whose primary HIV risk is receptive anal sex) and CGW (whose primary risk is receptive vaginal sex) has been largely explained by PK and native deoxynucleotide triphosphate differences between colorectal and vaginal tissue. In addition, the colorectal tissue PK between men and women seem consistently the same, so the estrogen difference between CGM and CGW does not appear to modify local colorectal PK. However, the estrogen difference between CGM and CGW is much smaller than between TGW on high-dose estrogen therapies and CGM. For the purposes of the clinical trial simulation, we are assuming the high-dose estrogen in our TGW is not increasing the susceptibility of colorectal tissue for drug uptake. There simply is insufficient evidence to address this in a quantitative manner. Empiric data from clinical efficacy trials is inadequate as sexual histories do not provide enough granular data to know what percentage of sexual exposures in women are vaginal or anal. While these data would be helpful to inform our current simulation issue, this information is unavailable. Accordingly, our simulation will use the same target PBMC and colorectal tissue concentrations of active PrEP drugs as those that correlate with high levels of HIV protection in prior randomized controlled trials of MSM.

The benefit of performing a formal clinical trial simulation based on population PK models updated to account for low testosterone and elevated estrogen levels is that the simulation will enable us to introduce sources of heterogeneity inherent in any study population. Sources that will be included in the model will focus on variation in the estrogen effects on TFV and FTC PK, and evaluate that variation on potential efficacy outcomes in the simulation. Predicting the impact by simply reducing the active drug concentrations by the fraction observed in our small pilot does not adequately account for the many sources of variation across a study population. The clinical trial simulation will account for all covariates known to affect adherence, PK, and PD, based prior modeling work by our group and others. This approach will provide more robust estimates of protection, comparable to a clinical trial. The future utility of such an exercise provides better information than available at present when considering varied dosing regimens. In addition, the clinical trial simulation allows comparison of different trial designs to judge which are most efficient in finding differences – in either PrEP dosing regimens, concomitant hormonal regimens, or both – should they truly exist.

Study Design: We propose modeling and clinical trial simulation building by drawing upon PK and PD data from three sources: **Aim 1**, prior studies of ours with rich PK data, large randomized clinical trials with sparse PK data, and HIV protective efficacy data.

Aim 1 (detailed above) will provide the most important PK data for performing the definitive clinical trial simulation. There is no prior study of PrEP pharmacology in TGW that has a rich a sampling scheme (both in terms of sampling times after a dose as well as breadth in biological matrices evaluated), which is needed for robust model building and subsequent trial simulation.

We will begin preliminary model building with data already available from our CFAR pilot study (which included a similar sampling schemes, but a smaller number of participants) as well as hundreds of TGW in iPrEx. Our group has already been working on PK models from iPrEx PK data (as well as PK and efficacy data from several other randomized clinical trials), but we have not had the data on concomitant medications to know which TGW in iPrEx were on GAHT. Dr. Glidden (biostatistician at UCSF), who has been our collaborator in the pooled randomized clinical trial PrEP studies (including iPrEx) modeling project to date, has committed to serve as consultant in our current proposal and will provide the necessary data on hormonal drug use in iPrEx to enable model revisions immediately. The data files will all be de-identified and fully compliant with HIPAA regulations and local IRB policy for data transfer. We have followed these regulations and IRB policy in that earlier ongoing project form its inception.

The modeling work begins as revision, not *de novo*, because we have already published a series of population PK models, largely in smaller non-efficacy trials that are richly sampled like MTN-001 (Chaturvedula, Hendrix). We have also been building upon those models by incorporating essential efficacy outcomes (Rosenblum, Hendrix, Savic) using individual-level data for TFV and FTC analytes and clinical HIV seroconversion outcomes

(Partners PrEP, TDF 2, IAVI, iPrEx). This preliminary model revision can occur in the early grant years while the **Aim 1** trial is ongoing. After conclusion of the **Aim 1** clinical trial, these tentative models will be revised with the addition of much richer Aim 1 data.

These final models will then be used to simulate six dosing regimens, daily dosing and IPERGAY regimen (2+1+1 dosing), each with and without gender affirming therapy at low and high exogenous doses, to evaluate the frequency of achieving protective levels of TFV-DP and FTC-TP in PBMC (against previously established HIV protection benchmarks).

Table 1. Comparison of Study Arm dosing regimens in planned clinical trial simulation

Study Arm	TDF/FTC PrEP Regimen	Hormonal Regimen
1	daily	none
2	daily	leuprolide + low estrogen
3	daily	leuprolide + high estrogen
4	on demand (2+1+1)	none
5	on demand (2+1+1)	leuprolide + low estrogen
6	on demand (2+1+1)	leuprolide + high estrogen

Data and Statistical Analysis

Modeling and Simulation: Using pharmacological parameters acquired during the **Aim 1** clinical trial as well as the additional data sources described immediately above, we will build population PK models considering biological covariates including age, sex, weight, mGFR and eGFR, hepatic status, concomitant medications (hormone concentrations). Clinical trial simulation will link (1) the revised population PK models, (2) prior iPrEx-related PD models of HIV protection, and (3) adherence models based on our prior Markov chain models of adherence with informative demographic covariates. The trial simulations will be performed to extrapolate the impact of dose-escalated estrogen therapy on systemic and localized TFV and FTC concentrations and their subsequent influence on HIV seroconversion when dosed according to the standard daily dosing and IPERGAY regimen (see table above).

Sample Size: As with traditional multiple regression analysis, the number of covariates is limited by the number of observations. In prior model building, typical covariates that are included in the final model include only weight, creatinine clearance, and age. The proposed clinical study (**Aim 1**) will generate richer data (more PK samples in a dosing interval and more biological matrices) under more dose-regimen conditions (5 proposed) than all of the prior studies. Therefore, the smaller sample size (20, when compared to the other studies is well compensated by the richness of the data. In addition, we believe that pooling of data from various other PrEP studies provide higher power to the entire analysis.

References

1. Bretz, F. *et al.* Graphical approaches for multiple comparison procedures using weighted Bonferroni, Simes, or parametric tests. *Biometrical J.* **53,** 894–913 (2011). PMCID: PMC3427907.
2. Molina, J.-M. *et al.* On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. *N. Engl. J. Med.* **373,** 2237–2246 (2015). PMID: 26624850.