Assessment of a new 'boosting' strategy for HIV pre-exposure prophylaxis in healthy volunteers.

Principal Investigator:
Brandon T. Gufford, PharmD, PhD
Assistant Professor of Medicine
Division of Clinical Pharmacology
Indiana University School of Medicine

950 West Walnut Street | Research Institute II (R2), Room 466
Indianapolis, IN 46202
317.274.2847 tel
bgufford@iu.edu

Co-Investigators:
Zeruesenay Desta, PhD
Research Professor of Medicine, Division of Clinical Pharmacology
Adjunct Senior Research Professor, Department of Pharmacology and Toxicology
Indiana University School of MedicineSamir K. Gupta, MD, MS

Vice Chair for Research, Department of Medicine
Associate Professor of Medicine, Division of Infectious Diseases
Director of Indiana Center for AIDS Research
Indiana University School of Medicine

Peter Anderson, PharmD
Professor, Department of Pharmaceutical Sciences
Director of the Pharmaceutical Sciences Graduate Program
University of Colorado Skaggs School of Pharmacy

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1.0 Background

Prevention of HIV transmission is a worldwide imperative. On-demand HIV pre-exposure prophylaxis (PrEP, giving antiretroviral drugs to persons NOT infected with HIV) has been recently reported in the IPERGAY trial results as an effective approach to reduce the risk of HIV transmission among men who have unprotected sex with men.[1] This study utilized a combination of antiretroviral drugs, Tenofovir Disoproxil Fumurate (TDF) and emtricitabine (FTC), taken before and after sexual activity. Although effective, this treatment regimen relies on adherence to intermittent drug administration regimens both before and after sexual activity. Perhaps of more practical importance, sexually active individuals (>2 sexual encounters per week) would require nearly daily drug administration to maintain protection. Poor adherence, especially in the setting of participants not currently in a monogamous relationship, has been suggested as a possible reason why some daily treatments may have failed in preventing HIV infections. As such, methods to improve adherence to pre-exposure prophylaxis regimens with oral antiretrovirals in HIV-negative individuals would be of great value.[2]

TDF, a potent antiretroviral used in combination with FTC for PrEP, rapidly releases tenofovir (TFV) following oral administration, which subsequently is eliminated from the body entirely by the kidney. Part of this elimination process involves active transport of TDF into the urine by organic anion transporter (OAT) 1 and 3 (OAT1 and OAT3). Therefore, drugs inhibiting these transporters would be expected to reduce the renal excretion of TFV and prolong the time that the drug remains active in the body (i.e. prolong the elimination half-life).

Probenecid is known to inhibit human OATs and thereby increases plasma concentrations and prolongs the half-life of many OAT substrates including ampicillin, cefamandole, cefazolin, cefoxitin, cephalothin, nalidixic acid, indomethacin, naproxen, and methotrexate at doses ranging from 500mg to 2g.[3] Probenecid also improves the safety profile of drugs whose nephrotoxicity is mediated via uptake by organic anion transporters (e.g. the antiviral agent cidofovir). Over the past 50 years, probenecid has been combined with antibiotics and antivirals used in the treatment of gonorrhea, influenza, typhoid fever, mycobacterial diseases, and HIV.[3] Peak action (as determined by the effect on penicillin plasma concentrations) after an oral dose of probenecid occurs at about 2 hours and the action lasts for approximately 8 hours with a dose dependent half-life (4 to 12 hours).[4]

It follows that coadministration of TDF with PRO would be expected to reduce the elimination of TFV through the kidney and increase the length of time that TFV remains active in the body. Thus a combination of PRO, a well-tolerated oral drug, with TDF may allow simplified less frequent PrEP dosing, enhance TDF safety, substantially reduce costs, and thus make TDF-based PrEP more available and attractive for the intended population. A boosted single dose strategy of PRO-TDF given before anticipated sexual activity instead of the 4 doses of TDF given before and after currently being implemented would be more practical and may possibly improve adherence. Thus, this strategy should improve PrEP efficacy and compliance. Overall, PRO taken with TDF may drastically improve the likelihood of widespread uptake of pre-exposure prophylaxis in

both resource-replete and resource-limited settings and may greatly reduce the risk of HIV transmission around the world.

In this pivotal proof of concept pharmacokinetic study, we propose to investigate a single dosing strategy for probenecid "boosting" of TDF for on-demand HIV pre-exposure HIV prophylaxis. We estimate that approximately 14 healthy volunteers will need to complete this clinical study to determine if the single boosted PRO-TDF dosing strategy is viable. Data gathered from this study may be used to support future pragmatic trials of PRO-boosted TDF in individuals at high risk for HIV acquisition. Ultimately, the goal of this research is to create a simple and effective on-demand HIV PrEP regimen to reduce the incidence of HIV transmission worldwide.

2.0 Rationale and Specific Aims

Research Aims

Hypothesis: Probenecid "boosting" can be used to increase TFV concentrations, decrease TDF dosing requirements, and simplify TDF dosing regimens in the setting of on-demand HIV PrEP

Primary Aim: Test whether TFV blood and blood cell concentrations of tenofovir diphosphate (TFV-DP) of a single probenecid-boosted TDF regimen are comparable to those achieved with the current 'on-demand' unboosted TDF dosing regimen of two tablets before and two tablets after sexual activity.

Secondary Aim: Compare the short-term safety and tolerability of the single PRO-TDF regimen compared to the unboosted TDF regimen.

Rationale

Evidence of *probenecid boosting of anti-infective agents:*
Probenecid (1 gram orally) increases amoxicillin systemic exposure, measured by the area under the plasma concentration-time curve (AUC) by 53%[5] and in one small study, reduced dosing requirements by two-thirds.[6] Probenecid also increases acyclovir plasma AUC by 48% following simultaneous administration of 1g probenecid with 1g valacyclovir.[7] Probenecid increased the plasma AUC of cidofovir 24% for male monkeys and 86% for female monkeys administered 30mg/kg probenecid with 2.5mg/kg of cidofovir.[8] One small study compared cidofovir pharmacokinetics in serum using standard probenecid dosing (2g before the cidofovir infusion, then 1g at 2 and 8 hours after the cidofovir infusion) vs. a lower probenecid dose, 2g 1-hour before cidofovir infusion with no subsequent doses, and found the cidofovir pharmacokinetics to be the same.[9] This suggests lower doses of probenecid, such as the 2 gram dose utilized in this study, may offer the same pharmacokinetic "boosting" benefits of higher doses.

3.0 Inclusion/Exclusion Criteria

3.1 Selection of study participants: A total of 16 healthy male volunteers (18 to 55 years old) who meet all inclusion and exclusion criterion listed below will be enrolled to participate in this study. Based on our previous experience, a 15% drop out rate (n=2)

is expected and will be considered to ensure that at least 14 volunteers complete the entire study. Approval to conduct the study will be obtained in advance from the Institutional Review Board (IRB) of the Indiana University. All subjects will sign an IRB approved written informed consent prior to participation in the study Each subject will be provided a copy of the informed consent and allowed sufficient time to read, understand, and formulate questions about any aspect of the study. A member of the study team will carefully review the consent with each subject and answer any questions. Then, potential subjects will undergo a pre-enrollment screening examination (to be accomplished within a maximum of six weeks prior to enrollment into the study) for any medical abnormalities which will include medical histories, vital signs. demographic variables, standard laboratory blood and urine tests, and screening for HIV and HBV infection. The screening will be performed at the Indiana Clinical Research Center (ICRC). During the screening, a blood sample (~15 ml) will be collected from each subject for laboratory tests and urine for urine analyses. An additional ~10 ml will be obtained from each subject to extract genomic DNA for genotyping purposes.

3.2 Inclusion criteria:

1. 18 to 55 years old healthy (as decided from a pre-enrollment screening session described above) male participants within 32% of their ideal body weight.
2. Individuals who agree to refrain from taking any prescriptions medications, over-the-counter medications (including salicylates/aspirin), hormonal agents, and herbal, dietary, and alternative supplements that may interact with the metabolism of those study drugs at least 2 weeks prior to the start of the study and until study completion.
3. Nonsmoker or individuals willing to refrain from smoking or use of tobacco or marijuana for at least one month prior to and until the completion of the study (the entire study lasts for approximately 49 days).

3.3 Exclusion criteria:

Subjects will not be allowed to participate in the study if they at time of study screening:

1. Are underweight (weigh less than 52 kg or 114 lb) or overweight [body mass index (BMI) greater than 32].
2. Females will be excluded to reduce study variability for this first proof of concept study.
3. Have insufficient renal function (estimated Creatinine Clearance ≤ 90 mL/min).
4. Have history of current alcohol or drug abuse (more than 4 alcoholic drinks per day on a regular basis).
5. Have history of intolerance, allergic reactions (e.g. rash) or other forms of hypersensitivities to any of the study medications (tenofovir disoproxil fumurate/ emtricitabine, probenecid).
6. Have taken TDF or FTC as part of PrEP within the past 6 weeks.
7. Any current major illness or chronic illness such as (but not limited to) kidney disease, hepatic disease, diabetes mellitus, gout, hypertension (definition?), coronary artery disease, chronic obstructive pulmonary disease, cancer, chronic active HBV infection, or HIV.
8. History of anemia or any other significant hematologic disorder.

9. Have history or current gastrointestinal disorders such as persistent diarrhea or malabsorption that would interfere with the absorption of orally administered drugs.
10. Have a serious infection within the last week before study enrollment.
11. Have donated blood within the past two months.
12. Have blood results that do not fall in a healthy range (e.g., blood hemoglobin less than 12.0 mg/dl).
13. Are taking on regular basis substances that may interfere with the metabolism (breakdown) of study medications by the body, including prescription medications, over-the-counter, herbal or dietary supplements, alternative medications, or hormonal agents (i.e. oral contraceptives, intra-uterine device with hormones).
14. Have a life style that places subjects at a higher risk for contracting HIV during the study period (e.g. active illicit drug use, excessive alcohol drinking, sexually transmitted infection (including gonorrhea, chlamydia, syphilis, herpes, HPV) within the past one year, or having more than one sexual partner in the past 6 months).
15. Positive HIV antibody test.
16. Positive HBV surface antigen test.
17. Have participation in a research study or use of an investigational drug in the last one month.
18. Are employed or are student under supervision of any of the investigators of this study.
19. Cannot state a good understanding of this study including risks and requirements; are unable to follow the rules of this study.
20. Cannot commit the time requested for this study.

4.0 Enrollment/Randomization

4.1 Enrollment:

Subjects will be recruited from 1) IRB approved campus fliers affixed to community bulletin boards; 2) verbal script using the attached template; or 3) via email of the pre-recruitment script. No subject will be excluded from the study on the basis of ethnicity or race. We will include all minorities. We expect that our recruitment of healthy volunteers will reflect the ethnic and racial demographics of the Indianapolis metropolitan based on current local clinic and hospital visits, state census and previous studies at the Indiana University School of Medicine. Having completed multiple similar trials in the past, we have developed experience in recruiting subjects and in managing the complex logistics of clinical trials.

4.2 Randomization:

Subjects will be randomly allocated to enter the study in either the control or treatment arm using a blocked design to ensure equal numbers of subjects in each sequence group (n=8, control to treatment; n=8, treatment to control). Following a washout period of at least 6 weeks, each individual will crossover to the next study phase. The randomization table will be constructed prior to subject enrollment by the Principal Investigator using the Statistical Analysis Software (SAS®) randomization procedure. In the event that an individual is withdrawn from the study, an alternate participant will replace that individual using an identical study sequence to maintain the block design. A total of 16 subjects will be initially enrolled in an effort to ensure that at least 14 subjects complete the entire study. If needed, additional subjects will be screened and enrolled until the target enrollment of n=14 subjects have completed the entire study.

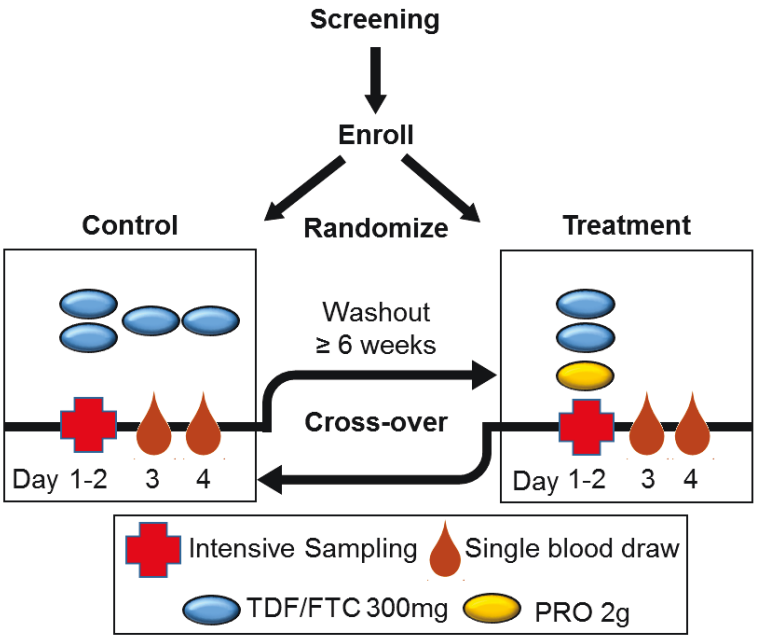
5.0 Study Procedures

5.1 Study Design:

The study will be a randomized, open-label, cross-over clinical pharmacokinetic trial to investigate a strategy for probenecid "boosting" in the setting of tenofovir DF/emtricitabine for HIV PrEP. The study will be conducted at the Indiana University Clinical Research Center. All samples will be processed and the amount of tenofovir/FTC in plasma, blood, and urine, and tenofovir diphosphate and emtricitabine in peripheral blood mononuclear cells will be determined using validated analytical methods developed by the investigators at the University of Colorado. Probenecid plasma and urine concentrations will also be measured using an in-house assay. Following completion of the study, the secondary aim will be accomplished via analysis of selected samples collected at baseline and following treatment. Those selected samples will be assessed for urinary markers of proximal tubulopathy (urine total protein, albumin, creatinine, phosphorus, retinol binding protein, and beta-2-microglobulin) and serum alkaline phosphatase, osteocalcin, procollagen type 1 N propeptide, cystatin C, and creatinine to determine if the probenecid boosting strategy does indeed lead to less potential renal and bone toxicity.

5.2: Study Procedures:

Overview: The study will be a randomized, open-label, cross-over clinical pharmacokinetic trial to investigate a strategy for probenecid "boosting" in the setting of tenofovir DF/emtricitabine for HIV PrEP in healthy volunteers. Potential subjects will undergo a screening appointment with those deemed eligible being enrolled and randomized to enter the study in either the control or treatment phase. Following completion of the first phase, subjects will crossover to the next phase after a washout period of at least 6 weeks.



Pre-Screening Recruitment: Subjects interested in study participation will be pre-screened by a member of the study team. Each potential subject will be provided standardized information relating to the general study purpose, inclusion/exclusion criteria, time commitment, scheduling, and study expectations. This information will be provided via a standard recruitment script. Each subject will be asked to complete a pre-screening questionaire. Based upon the pre-screening conversation and questionaire, those individuals believed to be eligible for participation will then be scheduled for screening.

Screening: A copy of the informed consent document will be provided to each subject and each subject will have adequate time to review and formulate questions about the study. Each subject will be allowed to review the consent document in a private room or office. A member of the study team will then review the informed consent document, ensure subject understanding, and answer questions that the subject might have about participating in the study. Following this process, the subject will be asked if they agree to consent to participate in the study. Subjects will provide a written signature using indelible ink on a hard copy of the consent document. The orginal signed hard copy of the consent document will be retained by the investigator and a copy of the consent document will be given to each subject.

After obtaining informed consent but during the initial screening, a blood sample (~15 ml) will be collected from each subject for laboratory tests and urine for urine analyses. An additional ~10 ml will be obtained from each subject to extract genomic DNA for genotyping purposes. Only those subjects that complete the entire study will have their DNA analyzed.

Control Phase: The control phase will consist of subjects taking a regimen mirroring that of the IPERGAY trial: 600 mg oral TDF/FTC on day 1 (2 tablets) followed by 300 mg (1 tablet) doses on days 2 and 3.

Treatment Phase: The treatment phase will consist of subjects taking 600 mg oral TDF/FTC (2 tablets) along with a 2 gram oral probenecid (PRO) dose (4 tablets) on day 1.

5.3 Study Calendar:

Day 1:

- Eligible subjects will be requested to arrive in the morning at the ICRC (about 7 a.m.) on the first study day after fasting overnight. On the evening before admission to the ICRC inpatient study day (day 1), it is important that subjects have nothing to eat or drink after 11 pm except for water.
- Vital signs (e.g., sitting blood pressure, respiration rate, pulse rate, oral temperature) will be obtained prior to initiating study procedures.
- A sterile intravenous catheter will be inserted in one arm for blood collection.
- Prior to administration of study drugs, a baseline blood (6 ml) and urine sample will be collected.
- Subjects will receive 600 mg TDF/FTC (2 tablets) alone (control phase) or with probenecid (treatment phase) by mouth with ~250 mL water. A standard meal will be served approximately 4 hours after drug dosing.

- Samples will be collected as outlined below.

Day 2:

- After the 24 hour blood draw and urine collection, the venous catheter will be removed and subjects will be discharged to go home. One urine collection container will be provided to them to collect urine over the period of 24-48 hours. They will be instructed to bring the collected urine with them on day 3 when they visit the ICRC.
- During the control phase subjects will receive a second TDF/FTC tablet immediately following the 24 hour sample collection.

Day 3:

- Subjects will be requested to return to the ICRC on the morning day 3 (48 hours post dose) for a brief outpatient visit and single blood draw. The outpatient blood sample (6 mL) will be collected via a peripheral venipuncture. An additional urine collection container will be provided to them to collect urine over the period of 48-72 hours. They will be instructed to bring the collected urine with them on day 4 when they visit the ICRC.
- During the control phase subjects will receive a third TDF/FTC tablet immediately following the 48 hour sample collection.

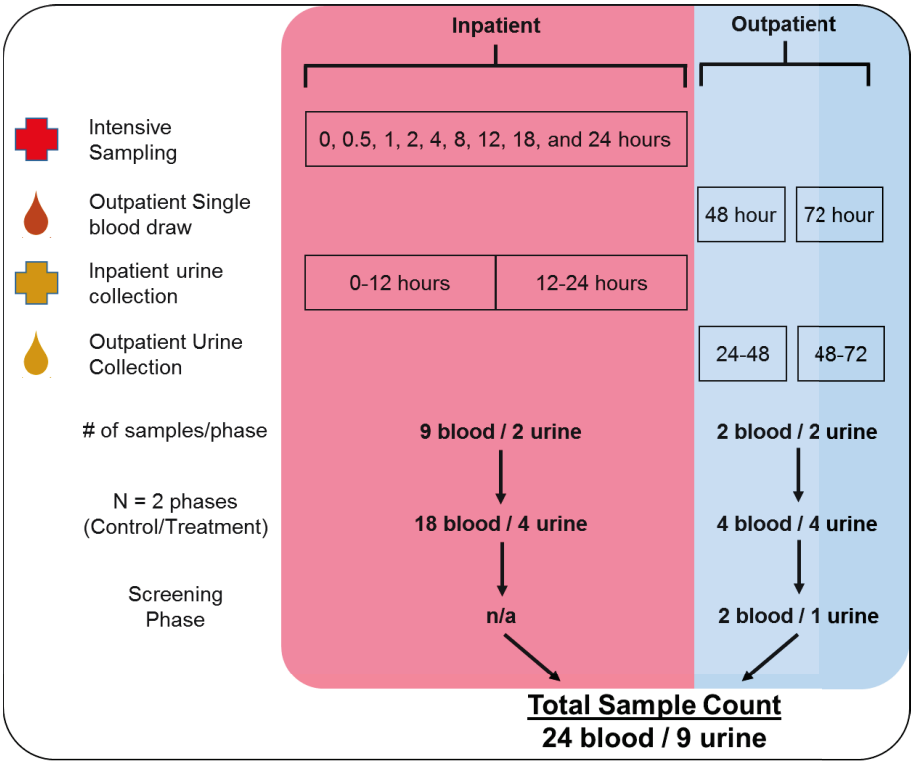
Day 4:

- Subjects will be requested to return to the ICRC on the morning of day 4 (72 hours post dose) for a brief outpatient visit and single blood draw. The outpatient blood sample (6 mL) will be collected via peripheral venipuncture.
- Subjects will be instructed to return to the ICRC for the next study phase following the 6 week washout period.

Control Phase			
Day 1: Inpatient ICRC	**Day 2:** Discharge	**Day 3:** Outpatient	**Day 4:** Outpatient
Administer 2 tablets of TDF/FTC	Obtain 24 hour blood sample	Obtain 48 hour blood sample	Obtain 72 hour blood sample
Obtain blood and urine samples as outlined below	Administer 1 tablet TDF/FTC	Administer 1 tablet TDF/FTC	Collect urine collection containers from subject
	Discharge subject with urine collection container	Discharge subject with urine collection container	
Treatment Phase			
Day 1: Inpatient ICRC	**Day 2:** Discharget	**Day 3:** Outpatient	**Day 4:** Outpatient
Administer 2 tablets of TDF/FTC and probenecid	Obtain 24 hour blood sample	Obtain 48 hour blood sample	Obtain 72 hour blood sample
Obtain blood and urine samples as outlined below	Discharge subject with urine collection container	Discharge subject with urine collection container	Collect urine collection containers from subject

5.4 Sample Collection Schematic: The sample collection schedule (outlined below) will be identical for the control and treatment study phases. The control and test phase will each consist of intensive pharmacokinetic sampling with serial blood draws occurring at baseline (0), 0.5, 1, 2, 4, 8, 12, 18, 24, 48, and 72 hours following administration of

the first dose TDF/FTC dose in each phase. Each phase will be separated by a minimum 6 week washout period to ensure that any residual TFV-DP has been completely eliminated from PBMCs. The blood volume collected during the control and treatment phases will be approximately 66 mL/phase in addition to ~25 mL collected during screening to yield a total blood collection volume of ~157 mL or 2/3 cup per subject during the entire study.



5.5: DNA Genotyping:

Exploratory genotyping efforts will focus on known variants in transporter proteins that may alter the disposition of tenofovir and/or emtricitabine. Variants will include single nucleotide polymorphisms identified in the following genes: SLC22A6 (OAT1), SLC22A8 (OAT3), ABCC2 (MRP2), and ABCC4 (MRP4). Genomic DNA will be extracted from human blood using standard operating procedures. Genotyping for these variants will be performed by use of the predeveloped TaqMan Assay-Reagents Allelic Discrimination Kits (Applied Biosystems, Foster City, CA) according to the supplier's instructions.

5.6: Analyses of Study Drugs

Plasma and Urine Analysis

Tenofovir and Emtricitabine: Within 24 hours following collection, plasma (harvested from blood via centrifugation) and urine samples will be processed using standard operating procedures within the Indiana CTSI Specimen Storage Facility (SSF). Simultaneous determination of tenofovir and emtricitabine concentrations in plasma and urine samples will be accomplished using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay established and routinely performed by co-investigators at the University of Colorado.[10]

Probenecid: We will develop an LC-MS/MS based assay for the determination of probenecid concentrations in plasma and urine samples. It is anticipated that probenecid can be determined in conjunction with tenofovir and emtricitabine by adapting the assay outlined above.

Intracellular PBMC Analysis

Tenofovir Diphosphate and Emtricitabine: Within 24 hours following collection, PBMCs will be recovered and counted from whole blood using standard operating procedures within the Indiana CTSI SSF. Due to extensive processing time and cost PBMCs will be isolated only from samples at selected time points. Isolated PBMC samples will be shipped (de-identified) to the University of Colorado for analysis using a validated and routinely performed LC-MS/MS assay.[11]

Dry Blood Spot Analysis

Dry blood spot (DBS) samples will be collected from whole blood prior to harvesting of plasma at each time point. DBS samples will be prepared according to standard operating procedures within the Indiana CTSI SSF and analyzed via LC-MS/MS using a validated method established by co-investigators at the University of Colorado.[12]

Following processing, all samples will be stored at –80°C pending analysis.

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

6.1 Risk Summary:

Venipuncture: Blood samples will be collected through puncture of a vein by a needle. This may cause slight pain, bruising, and bleeding from the site of the needle puncture into the vein, and in rare cases fainting and infection. To minimize these discomforts and risks, only trained and experienced nurses or physicians will place the intravenous catheter in the vein and collect blood samples. Aseptic technique and hospital/nursing standards will be followed.

Blood Loss: The maximum blood volume that will be collected from each subject over the course of the entire study (duration is ~50 days) is expected to be <200 mL. The total amount includes potential for repeat screening and exit laboratory tests which may be needed for safety reasons as deemed by the study physician. To minimize the impact of blood loss, subjects accepted into the study must have a hemoglobin count of 12.0 g/dl or above. Given the total volume of blood sampling and the duration of the study, we would expect that the body will adequately replace this blood and should pose no risk to a healthy subject.

Study Drugs: All drugs to be used in this study (TDF/FTC, probenecid) are FDA approved, commercially available, and are under wide clinical use. Study drugs will be purchased and handled through the Indiana University Hospital Pharmacy, Investigational Drug Service section. Potential risks associated with each study drug are outlined below.

Tenfovir Disoproxil Fumurate / Emtricitabine

Tenofovir disoproxil fumurate (TDF) / Emtricitabine (FTC) is administered as a fixed dose combination tablet containing 300 mg TDF and 200 mg FTC. Truvada is the tradename for this FDA-approved fixed-dose combination. Truvada belongs to a class of antiretroviral drugs known as nucleoside/nucleotide reverse transcriptase inhibitors, commonly known as NRTIs. The most common risks and side effects from NRTIs include fatigue (tiredness), headache, mild stomach discomfort, nausea (upset stomach), insomnia (difficulty sleeping), rash, vomiting, and diarrhea.

Rare but more serious risks and side effects include pancreatitis, severe liver problems, anemia (low red blood cells), lactic acidosis (buildup of lactate in the body), peripheral neuropathy (pain, tingling, and/or numbness in the hands and/or feet), lipodystrophy (alterations in fat distribution in your body), decreased kidney function, and metabolic disorders (changes in lipid and sugar levels in your body).

Probenecid

"Probenecid has been administered millions of times to hundreds of thousands of people with very few adverse effects and almost no serious adverse effects".[3] When used with cidofovir at high doses (2 g before the cidofovir infusion, then 1g at 2 and 8 hours after the cidofovir infusion) for the treatment of cytomegalovirus in persons with AIDS, nausea, vomiting, fever, headache, or rash occur in 44%-56% of patients and result in treatment discontinuations in 4%-7%.[13,14] However, these side effects may be reflective of cidofovir use or underlying medical conditions rather than PRO itself. The safety profile of PRO in the setting of TDF-boosting in healthy individuals has not been determined. This may be important if the increased nausea and vomiting seen with TDF vs. placebo in other PrEP studies may be exacerbated with PRO.[15] Furthermore, the effectiveness of PrEP using a PRO-boosted TDF strategy may be limited by tolerability. Thus, the proposed study will assess the short-term adverse event profile of PRO and inform future studies on the acceptance of this drug combination. Patients will be advised not to use salicylates (e.g. aspirin) during the course of the study due to a potential drug-drug interaction with probenecid. Subjects who require mild analgesia during the study will be advised to use acetaminophen (brand name: Tylenol).

Other Possible Risks: The study drug treatment combination may involve risks that are currently unknown or unpredictable. Important new findings uncovered during the course of this study that may impact subject safety or willingness to participate will be provided to subjects. Study investigators and ICRC staff will routinely ask subjects about any potential side effects.

Efforts will be made to keep all personal information confidential. However, a risk of loss of confidentiality still exists.

6.2 Minimizing Potential Risks: Any changes in subject status that might be judged unsafe to the subject will immediately lead to discontinuation of therapy and early termination of the study for that individual. The research nursing staff, investigators, and subjects will not be blinded to treatment for reasons of safety. The patients will be asked to report any intolerance or bothersome side effects that occur during the study. All subjects participating in this study will be informed in detail about potential risks, followed closely, and encouraged to report any adverse reactions immediately. During

each inpatient clinical day advanced cardiac life support equipment and oxygen will be available to treat hypoventilation or apnea and intravenous fluids will be available to treat hypovolemia. Low wall suction equipment and resuscitation equipment including chemical code drugs will be available at the ICRC. In addition, medication orders for anti-emetic therapy will be provided in the event that any subject experiences nausea and/or vomiting as a result of the study medications.

6.3 Adverse Event Reporting: Patients will be closely monitored for any evidence of adverse events. Any adverse effects associated with the study intervention (as deemed by the PI and/or study physician) will be documented and reported to appropriate bodies (IRB, FDA) using standard procedures. The subjects participating in this study will be informed in detail about the potential risk and encouraged to report any adverse reactions immediately.

Adverse events will be assessed using the NCI Common Toxicity Criteria guidelines listed in (http://ctep.cancer.gov/forms/CTCAEv3.pdf) along with potential study drug specific toxicities as outlined above.

Adverse events will be graded as follows:

Grade 0 – No adverse effects
Grade 1 - Mild adverse events
Grade 2 – Moderate adverse events
Grade 3 – Severe adverse events
Grade 4 – Life threatening or disabling adverse events
Grade 5 – Death related to adverse events

Any serious and unexpected adverse events associated with the study intervention will be reported to Research and Sponsored Programs and the ICRC within 3 working days of notification of the event. Deaths will always be reported if they occur within 30 days of study intervention. The principal investigators will be responsible for reporting the adverse effects to the IRB and other relevant bodies. All adverse events that do not meet immediate reporting requirements will be submitted to the IRB at time of continuing review.

6.4 Data and Safety Monitoring Plan: A Data and Safety monitoring board will created to provide additional study oversight and to ensure patient safety. The DSMB will be chaired by Michael Eadon, MD. Dr. Eadon is board-certified in internal medicine, nephrology, and clinical pharmacology. These credentials make Dr. Eadon an excellent individual to assess the safety of this study which focuses on modulation of renal drug transport. Additional members will include Samir Gupta, MD, MD, Brandon Gufford, PharmD, PhD, and Zeruesenay Desta, PhD. The DSMB will be charged with: (1) review of the research protocol and establishment of plans for data and safety monitoring, (2) study progress evaluation such as assessments of data quality, timeliness, subject recruitment, accrual, retention, and subject risk vs. benefit, (3) reviewing scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study, (4) reviewing major proposed modifications to the study prior to their implementation, including termination, dropping an arm of the study, or increasing the target sample size, (5) submitting reports of recommendations to the IRB or funding foundation that include the current status of the study, summary reports of adverse

events resulting from participation in the study, and determination of whether the study should continue as originally designed, should be changed, or should be terminated.

The first DSMB meeting will be held once three subjects have completed the study in order to evaluate the data produced. Data quality, subject recruitment, accrual, adverse events, and results of related studies that impact subject safety will be evaluated. The voting members of the DSMB will determine the frequency of future DSMB meetings. The frequency may be based on accrual rates (i.e. after 8 volunteers are enrolled) or elapsed time (i.e. every two months).

7.0 Study Withdrawal/Discontinuation

Taking part in this study is voluntary. Subjects can choose not to take part or to withdraw from this study at any time without penalty. Leaving the study will not result in any penalty or loss of benefits to which the subject is entitled. The decision of any subject to participate or not to participate will not affect: current or future relationship with the research team; the status as a volunteer, employee, student, etc; or medical care. The principal investigator reserves the right to withdraw a subject from the study without regard to subjects consent in the following circumstances: if risks in participating in this study outweigh their best interest in terms of safety; or if subject is unable to adhere to the study requirements. In addition, this study may be terminated by the IRB at their discretion.

Subjects have the option of participating or not participating in the study. There are no other alternative procedures or courses of treatment that may be advantageous to them. Subjects will receive $225 for each 24 hour overnight stay (Day 1 of the Control and Treatment phase) and $50 for each outpatient visit (Days 3 and 4 of the Control and Treatment phase). Subjects who complete all phases of the study will receive a total of $650. Individuals that withdraw from the study (voluntarily or at the discretion of the PI) will be compensated for the portions of the study which were completed. Compensation will be issued at the completion/withdrawal of the study.

8.0 Statistical Considerations

The area under the TFV plasma and TFV-DP PBMC concentration-time curve (AUC) will be calculated via routine non-compartmental approaches. The primary trial outcome will be the geometric mean ratio (GMR) calculated as the test phase/control phase TFV plasma and TFV-DP PBMC AUCs. A two one-sided statistical testing procedure, as recommended by the FDA, will be used for the primary endpoint analysis.[16] If the 90% confidence interval for the geometric mean ratio is contained entirely within the bounds of 0.8-1.25 then the probenecid "boosting" strategy is determined to provide equivalent tenofovir diphosphate concentrations relative to the IPERGAY regimen. Using this study design allows each subject to serve as his/her own control to decrease variability and reduce the required sample size. Power and sample sizes were determined based on a study of daily tenofovir DF/emtricitabine in healthy volunteers by Peter Anderson.[17] At day 30 (steady state), the mean (SD) TFV-DP concentration in the PBMCs of 18 participants was 100.3 (±32.3) fmol/10^6 cells.

To demonstrate equivalence of TFV-DP concentrations in PBMCs with probenecid "boosting" strategies relative to the IPERGAY trial TDF/FTC regimen (i.e. control group) would require 14 total participants in a crossover design. A total of 14 evaluable subjects with complete data will provide 90% power to detect a mean difference of 25% in the primary outcome assuming a Type I error rate of 0.05. Given that dropouts are likely for any PK study of this duration, we estimate that 16 participants will need to be enrolled to achieve the final 14 participants who complete the entire crossover trial. In the event that an individual is withdrawn from the study, an alternate participant will replace that individual using an identical study sequence to maintain the randomized block design. Additional subjects will be screened and enrolled until the target of n=14 subjects have completed the entire study.

9.0 Privacy/Confidentiality Issues

The study data and results are for research purposes only. To the extent allowed by law, information about study subject's personal and medical data is considered confidential. Although we cannot guarantee absolute confidentiality, every effort will be made to keep all subject information strictly confidential. The samples collected from this study will be stored in locked freezers and only authorized personnel will have access to the samples, coded databases, and the results. The samples will be labeled with a code and not with subject names. All subject identified information will be stored in locked cabinets and on the PIs computer that is password protected. It will not be possible for anyone other than the investigators to determine subject specific test results without the explicit written consent of the subject or unless allowed by law. It is possible that data collected during the screening and exit lab tests, and inpatient care may become part of subject's medical record. However, DNA genotyping results will not be recorded in medical records. All DNA samples will be marked with a unique code number and stored in an anonymous fashion in two different secured computer databases; one containing the DNA sample codes and the other with subject information (such as age, sex, ethnic group, health conditions, etc.) to maximize confidentiality.

Personal information may be disclosed if required by law. People or organizations that may review or inspect personal information to verify the quality of the research and data analysis include the study principal investigator and his research team, the Indiana University Institutional Review Board or its designees, the study sponsor which is the Campbell Foundation or their designees, and the Indiana Clinical Research Center (ICRC). In addition, as allowed by law, state or federal agencies, specifically the Office for Human Research Protections (OHRP) and the Food and Drug Administration (FDA), may need to access medical and/or research records.

Samples or information will not be released to anyone other than those listed above without obtaining a written consent from the subjects who participated in the study. When the results of study are published, no names or specific identifiers will be used. In addition, we will not use this sample to characterize subject's genetic history or determine the likelihood that they will experience a disease or not. The genetic information gathered in this study will be used together with information from other participants. Therefore, while the possibility that participation in this study may cause a

loss of privacy cannot be excluded entirely, the steps outlined above will be implemented to significantly minimize the risk of loss confidentiality.

Finally, a description of this clinical trial will be made available on ClinicalTrials.gov, as required by U.S. Law. This Web site does not include information that can identify individual subjects. At most, the site will include a summary of the protocol and results. Subjects have the opportunity to search this freely available online resource at any time.

10.0 Follow-up and Record Retention

This study is expected to be completed within one year. Records (documents) will be kept to the extent allowed by law per NIH recommendations after the study is closed (typically up to 7 years). All documents will be destroyed by shredding any document holding study information or through deletion of computer files using HIPPA compliant digital shredding utilities. Subjects retain (keep) the right to have any remaining biological sample material destroyed at any time by contacting the principal investigator. The principal investigator is responsible for the destruction of biological samples at the subject's request. Any data from this study will be published without subject names.

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