Effects of Ledipasvir/Sofosbuvir on the Pharmacokinetics and Renal Safety of Tenofovir Alafenamide (TAF)

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Protocol & Statistical Analysis Plan

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Protocol #: 17-0490
Project Title: Effects of ledipasvir/sofosbuvir treatment on the pharmacokinetics and renal safety of tenofovir alafenamide (TAF)
Principal Investigator: Jennifer J. Kiser, PharmD
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I. Hypotheses and Specific Aims:

Hypothesis:

1. Tenofovir plasma concentrations will be lower, tenofovir diphosphate concentrations in peripheral blood mononuclear cells will be higher, and tenofovir diphosphate concentrations in red blood cells (measured as dried blood spots, DBS) will be lower with tenofovir alafenamide relative to tenofovir disoproxil fumarate.
2. Tenofovir plasma and intracellular tenofovir diphosphate concentrations will increase when tenofovir alafenamide is co-administered with ledipasvir/sofosbuvir relative to TAF alone.
3. Markers of renal function (e.g. CrCl, urinary retinol binding protein/creatinine ratio and urinary beta-2 microglobulin/creatinine ratio) will not change when tenofovir alafenamide is coadministered with ledipasvir/sofosbuvir.

Primary Aims:

1. Compare the area under the concentration time curve over the dosing interval (AUC0-24) of tenofovir in plasma when switched from tenofovir disoproxil fumarate (TDF) (Phase 1) to tenofovir alafenamide (TAF 25mg) (Phase 2), and compare the AUC0-24 of tenofovir in plasma given as TDF (Phase 1) to tenofovir in plasma in the form of TAF 25mg concomitantly with ledipasvir (LDV)/sofosbuvir (SOF) (Phase 3)
2. Compare TFV-DP in PBMCs between TDF (Phase 1) and TAF 25mg (Phase 2) and between TDF (Phase 1) and TAF 25mg plus LDV/SOF (Phase 3)

Secondary Aims:

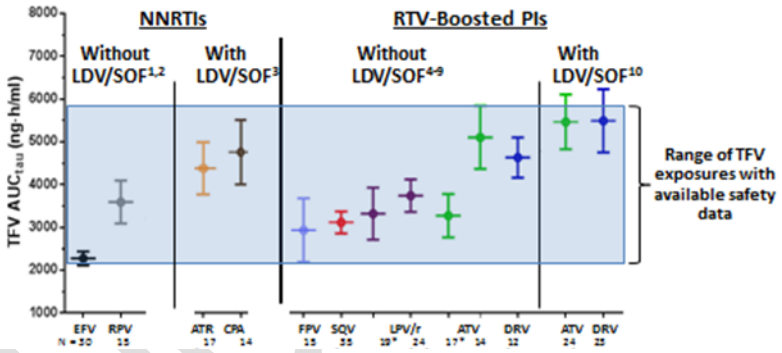
1. Compare TFV-DP in DBS between TDF (Phase 1) and TAF 25mg (Phase 2) and between TDF (Phase 1) and TAF 25mg plus LDV/SOF (Phase 3)
2. Compare CrCl, urinary retinol binding protein/creatinine ratio, urinary beta-2 microglobulin/creatinine ratio between TDF (Phase 1) and TAF 25mg (Phase 2) and between TDF (Phase 1) and TAF 25mg plus LDV/SOF (Phase 3)

II. Background and Significance:

Hepatitis C virus (HCV) infection is common among persons infected with HIV due to their shared routes of transmission.(1) On average, about 30% of individuals living with HIV are co-infected with HCV. HCV is associated with an increased liver-diseases-related morbidity and mortality in persons with HIV.(2) Thus, there is a great need to treat HCV in individuals co-infected with HIV.

Direct-acting antiviral agents (DAAs) have revolutionized the treatment of HCV, but drug interactions remain a substantial challenge.(3, 4) Ledipasvir (LDV) is an inhibitor of the HCV NS5A enzyme. Sofosbuvir (SOF) is an inhibitor of the HCV NS5B polymerase enzyme. These drugs are co-formulated in a single tablet (Harvoni®, Gilead Sciences, Foster City, CA) for once daily administration. Just 12 weeks of LDV/SOF has achieved cure rates of >95% in HCV treatment naïve and treatment experienced patients including in patients coinfected with HIV.(5, 6) LDV/SOF is one of the preferred treatment options for HCV because it is highly efficacious, well tolerated, has a low pill burden, a short treatment duration of between 8 and 24 weeks, and a low potential for drug interactions with antiretroviral agents. The main concern with use of LDV/SOF in co-infected patients is that LDV/SOF can raise concentrations of the HIV drug tenofovir. Tenofovir disoproxil fumarate (TDF) is one of the most widely prescribed antiretroviral agents. It is a well-tolerated drug, but its use has been shown to be associated with renal toxicity including declines in glomerular filtration rate, proximal tubular damage, and acute kidney injury.(7-9) Studies suggest the renal toxicities from TDF are concentration-dependent (i.e., higher tenofovir concentrations are associated with a greater risk for renal toxicity).(10) LDV/SOF, when given concomitantly with TDF and a ritonavir-boosted protease inhibitor, has been shown to increase the tenofovir area under the concentration time curve (AUCtau) in healthy volunteers to levels that exceed the tenofovir levels for which there is established safety data (Figure 1).(11) When patients on antiretroviral regimens that include TDF and a booster are treated with LDV/SOF, they have a "double whammy" in terms of the increase in tenofovir exposures.

Figure 1. Tenofovir area under the concentration time curve (AUCtau) in plasma with various antiretroviral agents in the presence and absence of LDV/SOF in studies of healthy volunteers. The upper range of tenofovir exposures observed in healthy volunteers given the combination of LDV/SOF, tenofovir disoproxil fumarate, and a ritonavir-boosted protease inhibitor (either ritonavir-booted atazanavir or darunavir) exceeds tenofovir exposures with established safety data.



EFV=efavirenz; RPV=rilpivirine; ATR=the fixed dose combination product of tenofovir disoproxil fumarate, emtricitabine, and efavirenz; CPA=the fixed dose combination product of tenofovir disoproxil fumarate, emtricitabine, and rilpivirine; FPV=fosamprenavir; SQV=ritonavir-boosted saquinavir; LPV/r=ritonavir-boosted lopinavir; ATV=ritonavir-boosted atazanavir DRV=ritonavir-boosted darunavir

There is a new formulation of tenofovir called tenofovir alafenamide (TAF) which provides a lower risk of interactions and less renal toxicity. Because it circulates predominantly in pro-drug form, TAF maintains 90% lower circulating plasma tenofovir levels compared to TDF.(12, 13) TAF is also not a substrate for renal uptake transporters. Thus, TAF is thought to have a significantly reduced potential for renal toxicities.(12, 13) TAF is a very reasonable alternative to TDF in individuals on ritonavir or cobicistat who require HCV treatment with LDV/SOF, but there are currently no data evaluating the pharmacokinetics and safety of the combination of LDV/SOF, TAF and a ritonavir or cobicistat-boosted protease inhibitor when dosed as 25mg of TAF. TAF was FDA approved in two different strengths, 10mg which is only available co-formulated with elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide 10mg (Genvoya®, Gilead Sciences, Foster City, CA) and 25mg which is co-formulated with emtricitabine and tenofovir alafenamide 25mg (Descovy®, Gilead Sciences, Foster City, CA). While favorable pharmacokinetic data are available on the interaction between elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide 10mg with

LDV/SOF in healthy volunteers(14), there is an absence of data with ritonavir-boosted protease inhibitors and with the 25mg strength of TAF.

There are several alternatives to LDV/SOF for HCV treatment (e.g., sofosbuvir plus the HCV NS5A inhibitor, velpatasvir (a combination referred to as SOF/VEL), or Zepatier® which contains elbasvir, a hepatitis C virus (HCV) NS5A inhibitor, and grazoprevir, an HCV NS3/4A protease inhibitor. All of these alternatives have drawbacks clinically. Additionally, simply avoiding the use of ritonavir or cobicistat with TDF as a component of antiretroviral therapy is not possible in all patients.(15, 16) Thus, patients may receive the combination of LDV/SOF, tenofovir, and ritonavir or cobicistat and data are desperately needed to determine the magnitude of the interaction between tenofovir and LDV/SOF and examine potential alternatives, such as TAF, that might be renal-sparing. The purpose of this study is to determine the magnitude of the interaction between tenofovir in the form of TAF and LDV/SOF and examine the effect of this interaction on renal function.

Several renal biomarkers have routinely been used in clinical research, specifically in HIV infected patients to determine the presence or absence of proximal tubular dysfunction.(12-14) An increase in low molecular weight proteins such as urinary retinol binding protein and beta-2 microglobulin have been shown to be indicative of tubular dysfunction, with the urinary concentration being correlated with the severity of the tubular dysfunction.(12) Early studies in HIV infected individuals have shown that the presence of HIV alone increases the urinary concentrations of retinol binding protein and beta-2 microglobulin by 3-10 fold when compared to healthy controls(15) and that patients who have tenofovir-induced Fanconi syndrome tend to have very high levels of these markers.(16) Both retinol binding protein and beta-2 microglobulin have been used to assess renal proximal tubule injury in HIV infected individuals who are taking tenofovir, thus we have chosen to use these biomarkers to study the renal safety of tenofovir alafenamide and LDV/SOF.

Good adherence to study medications is important in the context of a pharmacokinetic / drug interaction study. There are a variety of tools that can be used to assess adherence including pill counts, refill histories, digital ingestible sensor, and electronic monitoring. To monitor participants adherence in this study, we will use the Wisepill portable medication dispenser (Wisepill Technologies, Cape Town, South Africa) or directly observed therapy via video-streaming methods (e.g., FaceTime, Google Hangouts, TimeStamp). Wisepill transmits a cellular signal (real-time) when opened to a central management system (Wisepill Web Server). The dispenser is slightly larger than a cell phone and small enough to fit in a pocket. The battery lasts 4 months without charging. Participants will switch out 7-day medication cartridge once weekly - which takes seconds to perform.(17) We are using this device in an ongoing study of LDV/SOF in HIV/HCV coinfected persons who use drugs. Directly observed therapy via video-streaming methods may be used in place of the Wisepill device, approaches of which have also been used in multiple studies through the CAVP laboratory.

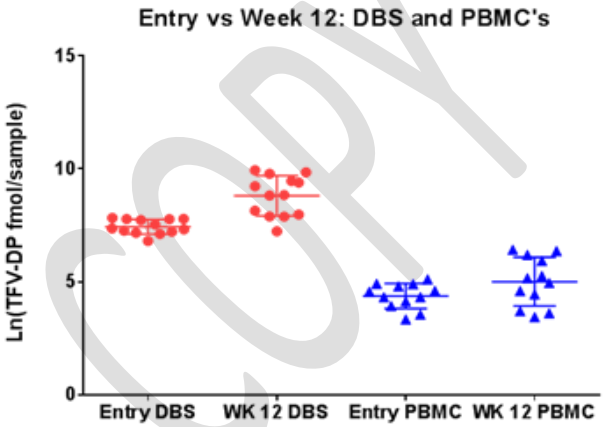
III. Preliminary Studies/Progress Report:

Analytical approaches to be used in this research:
Tenofovir will be quantified in plasma using a validated LC-MS/MS method with a dynamic range of 10ng/mL to 1500 ng/mL.(18) Intracellular tenofovir diphosphate (TFV-DP) will be measured in

peripheral blood mononuclear cells (PBMC) using a validated LC-MS/MS method with a dynamic range of 2.5 fmol/sample to 2000 fmol/sample.(19)

Our group is well-versed in these techniques as shown in the following studies:
We have been performing studies on the pharmacology of tenofovir for more than 10 years, (18, 20-25) and recently identified a drug interaction between individuals taking TDF in combination with SOF/ribavirin.(26)

As Figure 2 indicates, TFV-DP was increased 4-fold in dried blood spots (DBS) after 12 weeks of SOF and ribavirin (RBV) treatment. This interaction was also observed in peripheral blood mononuclear cells (PBMC), where TFV-DP concentrations at week 12 of SOF/RBV treatment were double those observed before (study entry) and 12 weeks following the completion of SOF/RBV treatment.(26)



Clinical Considerations:
We have established collaborations with study co-investigator Dr. Jose Castillo Mancilla who cares for HIV-positive patients in the Infectious Disease Group Practice (IDGP) Clinic at the University of Colorado Hospital. Dr. Castillo-Mancilla recently completed a large study in HIV-infected patients on TDF in the IDGP clinic, thus he has a research-relationship with potentially eligible participants.

IV. Research Methods

A. Outcome Measure(s):

Primary Outcome:

1. Area under the concentration-time curve over the dosing interval (AUC0-24) of tenofovir in plasma when switched from TDF (Phase 1) to TAF 25mg (Phase 2), and once on TAF 25mg, compare the AUC0-24 of tenofovir in plasma given as TDF (Phase 1) to TAF 25mg concomitantly with LDV/SOF (Phase 3)
2. TFV-DP in PBMCs between TDF (Phase 1) and TAF 25mg (Phase 2) and between TDF (Phase 1) and TAF 25mg plus LDV/SOF (Phase 3)

Secondary Outcomes:

1. Compare TFV-DP in DBS between TDF (Phase 1) and TAF 25mg (Phase 2) and between TDF (Phase 1) and TAF 25mg plus LDV/SOF (Phase 3)
2. Compare CrCl, urinary retinol binding protein/creatinine ratio, urinary beta-2 microglobulin/creatinine ratio between TDF (Phase 1) and TAF 25mg (Phase 2) and between TDF (Phase 1) and TAF 25mg plus LDV/SOF (Phase 3)

B. Description of Population to be Enrolled:

This study will enroll HIV monoinfected individuals receiving TDF + a ritonavir or cobicistat boosted protease inhibitor (PI). TAF 25mg/emtricitabine 200mg and LDV 90mg/SOF 400mg will be provided by the study. Study subjects will also be given a Wisepill device which is a pillbox that monitors adherence by tracking when the pillbox is opened.

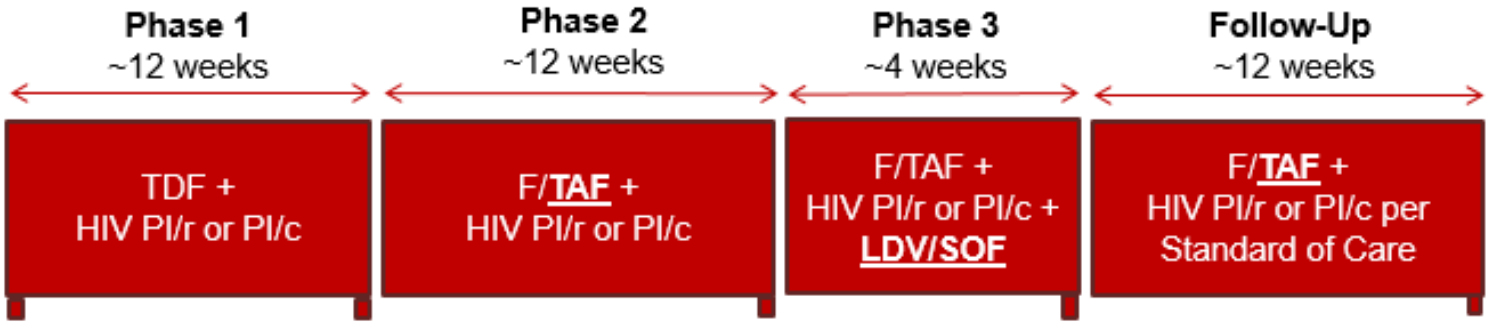
Inclusion criteria:

1. Participants must be between 18-70 years of age
2. Have been taking TDF and a ritonavir- or cobicistat-boosted PI, as part of standard care for treatment of HIV

Exclusion criteria:

1. Glomerular filtration rate < 30 mL/min/1.73 m2
2. Pregnant or planning pregnancy
3. Breastfeeding
4. Any laboratory value or uncontrolled medical conditions, including mental health, that, in the opinion of the investigators, would interfere with participant safety or protocol compliance such as but not limited to, heart disease, autoimmune disorders, low bone density, drug/alcohol addiction, and/or cancer
5. Signs or symptoms of decompensated liver disease (i.e., ascites, history of esophageal variceal bleeding, hepatic encephalopathy)
6. Hepatitis B virus infection
7. Pathologic fracture or other risk factors of bone loss or osteoporosis
8. Medications not recommended per the LDV/SOF or F/TAF 25mg prescribing information that at the discretion of the investigator may cause unwanted drug interactions (e.g., Pgp inducers such as tipranavir)
9. Unwillingness or inability to comply with study procedures, including the wisepill device and clinic visits
10. Chronic HCV infection (i.e., detectable HCV RNA)

C. Study Design and Research Methods



Visit 1: Screening
- HIV-1 RNA
- HCV RNA
- CBC
- CMP
- Vitals
- Wisepill device dispensing or video DOT instructions
- Pregnancy test (if applicable)

Visit 2: 4-hour PK Visit
- TFV PK at pre-, 1, and 4 hours
- TFV-DP in PBMCs and DBS at pre-dose
- hDNA
- HIV-1 RNA
- CBC
- CMP
- Vitals
- Urinary beta 2-microglobulin, urinary retinol binding protein, protein:Cr ratio
- Medication dispensing (12 weeks of F/TAF (Descovy)
- Pregnancy test (if applicable)

Visit 3: 4-hour PK Visit
- TFV and TAF PK at pre-, 1, and 4 hours
- TFV-DP in PBMCs and DBS at pre-dose
- HIV-1 RNA
- HCV RNA
- CBC
- CMP
- Vitals
- Urinary beta 2-microglobulin, urinary retinol binding protein, protein:Cr ratio
- Medication dispensing (4 weeks of F/TAF (Descovy) and LDV/SOF (Harvoni))
- Pregnancy test (if applicable)

Visit 4: 4-hour PK Visit
- TFV and TAF PK at pre-, 1, and 4 hours
- TFV-DP in PBMCs and DBS at pre-dose
- HIV-1 RNA
- CBC
- CMP
- Vitals
- Urinary beta 2-microglobulin, urinary retinol binding protein, protein:Cr ratio

Visit 5: Off-Study
- TFV in plasma
- TFV-DP in PBMCs and DBS
- HIV-1 RNA
- CBC
- CMP
- Urinary beta 2-microglobulin, urinary retinol binding protein, protein:Cr ratio

Up to fifteen HIV monoinfected individuals already taking TDF + a ritonavir or cobicistat boosted PI will be invited to participate in this study. Participants from the University of Colorado Infectious Diseases Group Practice (IDGP) clinic and Denver Health will be targeted for enrollment. Participants may also be referred by other providers in the greater Denver-area.

The study consists of five study visits: a screening visit, three abbreviated 5-hour pharmacokinetic visits, and one “off study” visit of Descovy (see schedule of events). The study visits will take place in the IDGP Clinic at University of Colorado Hospital or the University of Colorado Clinical & Translational Research Center (CTRC). Subjects will receive $75 for the baseline visit, $225 for each 5-hour intensive pharmacokinetic visit, and $75 for the end-of-study visit for a total of $825, and free F/TAF and LDV/SOF throughout the study.

Real-Time Adherence Monitoring: Participants will be provided the Wisepill portable medication dispenser or counseled on video-streaming methods for directly observed therapy during Visit 1. In those receiving the Wisepill device, on a weekly basis, participants will fill the 7-day cartridges with their ritonavir or cobicistat-boosted protease inhibitor plus TDF (Phase 1), F/TAF (Phase 2), or F/TAF and LDV/SOF (Phase 3). Each Wisepill device contains a SIM card with 3G wireless capabilities that records and transmits the time and date of opening the medication dispenser. The SIM card gives each device a unique signal. Power failure with the Wisepill device is mitigated by a signaling subsystem which maintains data for later transmission if connectively is lost. In addition to recording device openings, the Wisepill signal reports the remaining battery power for the device and airtime left on the SIM card so there is typically advanced notice of potential problems.(17) For the video-streaming methods, participants that already own a smartphone will be shown how to install and use the video-streaming application of their choice (e.g., FaceTime, Google Hangouts, TimeStamp). For those who do not own a smartphone, the study team will provide one with the video-streaming methods pre-installed, and participants will be educated on the appropriate use of the device. Participants will then record or livestream ingestion of their medications daily for review by study personnel. Study personnel will then record the date and time of drug ingestion.

Visit 1 (Week 0): Screening Visit
This visit will take place in either the IDGP clinic or the CTRC. During this visit, subject's informed consent will be conducted, and subjects will have the opportunity to sign the informed consent. Following informed consent subjects will have their blood drawn for safety and eligibility specifically obtaining a HIV 1-RNA, HCV-RNA, CBC and CMP. Vitals will be collected. Women of childbearing potential will have a urine pregnancy test. Those that qualify for the study will receive a Wisepill device for tracking adherence, or be instructed on the use of video DOT methods. Participants that are initially started on the DOT video-streaming methods may be transitioned to the Wisepill device at a subsequent study visit.

Visit 2 (Week 12, ±3 days): Phase 1 Measurements
This visit will take place only after the participant has been taking TDF for 12 weeks. Measurements during this visit will include: an abbreviated PK visit (pre dose, 1 hour and 4 hour post dose samples will be obtained) as well as safety labs, renal biomarkers, HIV-1 RNA. A whole blood sample for DNA isolation will also be collected. Vitals will be collected. Women of childbearing potential will have a urine pregnancy test. Participants will be instructed on switching from TDF to F/TAF 25.

Visit 3 (Week 24, ±3 days): Phase 2 Measurements
Twelve weeks following the switch to F/TAF 25, subjects will undergo the Phase 2 measurements. This visit will take place after the participant has been taking F/TAF 25mg for 12 weeks. Measurements during this visit will include: an abbreviated PK visit (pre dose, 1 hour and 4 hour post dose samples will be obtained) as well as safety labs, renal biomarkers, HCV-RNA, and an HIV-1 RNA. Vitals will be collected. Women of childbearing potential will have a urine pregnancy test. 4 weeks of LDV/SOF will be provided and instructions regarding when to initiate taking LDV/SOF

Visit 4 (Week 28, ±3 days): Phase 3 Measurements
After taking LDV/SOF for 4 weeks, subjects will undergo the Phase 3 measurements which will include: an abbreviated PK visit (pre dose, 1 hour and 4 hour post dose samples will be obtained) as well as safety labs, renal biomarkers, and an HIV-1 RNA. Vitals will be collected.

Visit 5 (Week 40, ±3 days): Off-Study 12 weeks after completion of LDV/SOF
This visit will take place in either the IDGP clinic or the CTRC and will assess how the patient is doing following completion of LDV/SOF. At this visit, safety labs will be obtained evaluating HIV-1 RNA, CBC, CMP, TFV in plasma, TFV-DP in PBMC's and RBCs and renal safety markers.

Schedule of Events (SOC = standard of care)

	Visit 1: Screening	Visit 2: TDF for 12 weeks "Phase 1"	Visit 3: F/TAF for 12 weeks " Phase 2"	Visit 4: F/TAF + LDV/SOF for 4 weeks "Phase 3"	Visit 5: 12 weeks after completion of LDV/SOF "Off Study"
Visit Type	Research	Research	Research	Research	Research
Informed Consent	X				
Standardized Breakfast		X	X	X	
Observed Dosing		X	X	X	
Vitals	X	X	X	X	
PK blood draws at pre dose, 1 and 4 hours post-dose[a]		X	X	X	
Single convenience PK blood draw[b]					X
hDNA		X			
HIV -1 RNA	X	X	X	X	X
HCV RNA	X		X		
CBC	X	X	X	X	X
CMP	X	X	X	X	X
Urinalysis (protein: Cr ratio)		X	X	X	X
Urinary Beta 2-microglobulin: Cr ratio)		X	X	X	X
Urinary retinol binding protein/creat ratio		X	X	X	X
Pregnancy Test (in women of childbearing potential)	X	X	X		

[a]Includes SCr for CrCl estimate [b]TFV in plasma and TFV-DP in PBMCs and DBS will be measured from blood PK samples

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Description of risks:
We are collecting blood, urine, genetic, and clinical information from participants through this study as well as switching their HIV regimen from TDF to TAF 25mg and using LDV/SOF in HCV seronegative individuals. The potential risks associated with study participation are and are outlined below:

1) Blood will be collected from an arm vein. Risks of blood draws include pain when the needle or lancet pierces the skin, bruising, and/or infection. We are collecting 24 mL of blood at each of the abbreviated pharmacokinetic visits for a total of 72 mL of blood. The blood volumes are well below the maximal volumes set for blood donations and clinical research of 450 to 550 mL in 8 weeks.
2) Privacy risk: As with participation in any clinical trial, privacy cannot be guaranteed. This study will collect clinical information from patients with HIV infection. The video DOT methods may also pose a risk as video sessions may not be encrypted and could be decoded and viewed by third parties, in addition to telephone numbers.
3) Switching from TDF to TAF 25mg. The risks of switching therapy are minimal due to TAF 25mg having lower renal adverse events. The risk include potential virologic failure (e.g. increases in the HIV-1 RNA) or potential adverse reaction to TAF 25mg that was not experienced with TDF including: hypersensitivity, gastrointestinal issues or renal failure.
4) Renal toxicity and adverse events with TDF or TAF 25mg. TDF specifically has been shown to cause renal toxicity including declines in glomerular filtration rate, proximal tubular damage, and acute kidney injury. The FDA labeling information for TDF and TAF 25mg list this as a potential risk. Other adverse events from TDF or TAF include: nausea (13%), diarrhea (21%), fatigue (14%), and increase in total cholesterol ($\geq$ 10%).
5) There are risks associated with Hepatitis C treatment in an HCV seronegative individual. These include development of virologic resistance if participants were to become infected while taking the medication. This could make future Hepatitis C therapies less effective.
6) The risks of taking ledipasvir/sofosbuvir include adverse events of the medication such as: fatigue (13-18%), headache (11-17%), nausea (6-9%), diarrhea (3-7%), trouble sleeping (3-6%) and bilirubin elevations (less than 3%).
7) Pregnancy. The risk of taking ledipasvir/sofosbuvir, TDF and TAF 25mg while pregnant are unknown, thus measures will be taken to not enroll pregnant women and also educate subjects on the importance of not becoming pregnant while on study.
8) Genetic information. This study will collect DNA samples to explore relationships between genetic variability in enzymes and transporters that may affect the pharmacokinetics of the study drugs being examined in the study. There is a risk that a third party could access this information and use it to discriminate against subjects.

Minimizing risks:

1) Blood draws will be performed by certified and experienced phlebotomists.
2) Informed Consent: The study will be reviewed and approved by the IRB prior to initiation of enrollment. Written documentation of informed consent approval will be present prior to initiation of any study related procedures. A consent form will be provided to participants in the clinic. Ample time will be allotted for the consenting process, which will take place in a private room. Subjects will be encouraged to ask questions and take as much time as needed to reach a decision. Subjects will be given a copy of the signed consent document.
3) Participants will be assigned a unique study identification number (SID). This number will be used on case report forms and samples rather than patient identifiers. The link between the SID and patient name and any documents (consent form, demographic form, W9, etc.) with identifying information will be stored in a locked cabinet in Dr. Kiser's locked office in the Skaggs School of Pharmacy building room 4102.

4) Video DOT: every effort will be made to protect subjects' information. Videos will be viewed on a password-protected phone, and only the participant's SID will be programmed into the phone. Live-streamed videos will be observed in a private location, and any recorded videos sent via text will be erased following appropriate documentation of the date and time of drug ingestion.
5) Participants will be managed by their primary infectious disease doctor and monitored for either virologic failure or adverse events that may result from switching from TDF to TAF 25mg.
6) For patients taking either TDF or TAF 25mg with a ritonavir or cobicistat-boosted protease inhibitor, urine parameters will be assessed every 4 weeks and the tenofovir dose altered as indicated for reductions in renal function. Individuals at higher risk for renal toxicity (glomerular filtration rate < 30 mL/min/1.73 m2) are excluded from study. Subjects will also be discontinued if glomerular filtration rate declines to less than 30 mL/min/1.73 m2. We will also follow the Division of AIDS toxicity tables and Grade 3 or higher for Scr (> 1.8 to <3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline) and CrCl (<60 to 30 ml/min or ml/min/1.73 m2 OR $\geq$ 30 to < 50% decrease from baseline) will be reported to Dr. Castillo-Mancilla within 24 hours for management. If any other adverse events arise, they will be managed per standard of care.
7) Subjects will be counseled on the importance of reducing the risk of acquiring HCV while enrolled in the study (i.e., safe sex practices, use of clean needles, etc.). The importance of adherence to Hepatitis C treatment will be stressed to participants to minimize the risk of developing viral resistance. Adherence will also be monitored using the Wisepill device.
8) Adverse events will be monitored as below (Toxicity Management section)
9) Pregnant women will be excluded from the study and pregnancy tests will be done at screening as well as Visits 2 and 3.
10) Genetic information: the subject will be asked for explicit consent within the ICF to collect a genetic sample, and may request for their sample to be withdrawn and destroyed after collection. Collected genetic samples will be de-identified and labeled with the subject's SID. Genetic analyses with the subject's sample will not be included in the patient's medical record and will be kept either in a locked file cabinet or secured server that is only available to study personnel.

Toxicity Management

This study is collecting blood and clinical information. Any side effects observed in the course of the study will be reported to Dr. Castillo-Mancilla, and managed as per standard of care and the toxicity management plan described below:

1. **Assessing adverse events.** Each subject will be evaluated for clinical and laboratory adverse events at each study visit. Dr. Kiser and her study staff will review laboratory results daily, and our University hospital clinical laboratory alerts study personnel immediately for grossly abnormal clinical laboratory results. All adverse events will be graded using the DAIDS tables. The chronicity of the event will be documented as: single occurrence, intermittent, or persistent. The date of adverse event onset, cessation, and report will also be documented. The relationship to study medication(s) will be documented as yes, no, or cannot be ruled out. The action taken will be documented as well (e.g., none, study drugs held, study drugs discontinued, additional laboratory tests required, and/or medications prescribed to manage the adverse effects).
2. **Adverse event attribution scale.** All serious adverse events will be graded as to their expectedness and their outcome (i.e., not-related, possibly-related, probably-related, or definitely-related to the study protocol).
3. **Reporting adverse events.** Any grade 3 or higher clinical adverse events and abnormal laboratory results will be reported to Dr. Castillo-Mancilla within 24 hours of the event. Adverse events grades ≤2 will be managed by Dr. Castillo-Mancilla. Any adverse event that is reported to the PI or her designated research associates by the subject or medical

staff caring for the subject that is (1) unexpected and related to study drug or study treatment or (2) required hospitalization or prolonged hospitalization, caused disability, death, a congenital anomaly or birth defect, or was considered life-threatening will be documented and reported immediately to the Colorado Multiple Institutional Review Board, the University of Colorado Clinical Translational Research Center, and Gilead Sciences.

Individual study stopping criteria

The study interventions that would differ from standard of care include additional clinic visits, blood draws and providing LDV/SOF to individuals without HCV infection. Thus, side effect management is at the discretion of the infectious disease provider (e.g. Dr. Castillo-Mancilla). The following criteria have been developed to indicate when an individual subject would need to discontinue study medications and study participation.

1. Individual Study Stopping Criteria for all subjects:
    a. Non-adherence with study activities at the discretion of the Investigator
    b. Pregnancy
    c. Decrease of glomerular filtration rate by ≥50%, if glomerular filtration rate is ≤ 60 mL/min/1.73m^2 at time of enrollment
    d. Adverse event for which study team deems HCV treatment or study participation to no longer be in subject's best interests
    e. Threatening to clinic or study personnel

Study Stopping Criteria.

The rate of serious adverse events in the Phase 3 LDV/SOF trials was 4-6% and in TAF was 3-4%. If greater than 10% or 2 patients in a row (whichever is smaller) experience a serious adverse event deemed possibly or probably related to LDV/SOF, TAF 25mg treatment or study participation, the study will be stopped and the study team will review all safety and laboratory data and consider making changes to the study vs. discontinuing the study permanently.

E. Potential Scientific Problems:

We would like complete data for 10 subjects for this study. We plan to recruit the study from the University of Colorado IDGP clinic. If after the first 6 months we have not recruited 5 subjects, we will enlist the assistance of other local HIV providers/clinics (e.g., Denver Health, Apex family medicine, Kaiser, Rose Medical Center).

F. Data Analysis Plan:

Analysis Plan. For the primary analysis, Phase 1 will be compared to both Phase 2 and 3. Given two comparisons of interest (tenofovir in plasma and TFV-DP in PBMCs), the primary comparisons will be performed using a significance level of 0.025. We will enroll 15 individuals with the goal of having complete data on 10 HIV monoinfected subjects. Given the small sample size for primary objectives, we will utilize a Wilcoxon signed-rank non-parametric test to detect differences in outcomes between phases. R (http://www.r-project.org/), GraphPad Prism (La Jolla, CA) and SAS (SAS Institute Inc., Cary NC) software will be used for analysis. All power calculations were performed in PASS, NCSS software, Kaysville UT.

AIM-1. AIM-1 is to compare the AUC levels of tenofovir in plasma between Phase 1 and Phase 2 and between Phase 1 and Phase 3. Sample size parameters for Phase 1 were estimated from $AUC_{0\text{-}24}$ levels of tenofovir in plasma from patients on a TDF + lopinavir/ritonavir regimen for a minimum of 4 weeks on study. (25) For these 15 participants, a geometric mean of 4108 ng*hr/mL was observed with a corresponding $\log_e$ transformed mean (SD) = 8.321 (0.320) used for sample size calculations.

Aim-1 Power: Phase 1 to Phase 2. When patients switch to TAF 25mg during Phase 2, we expect 90% lower AUC_{0-24} levels of tenofovir compared to Phase 1. (12, 13) Conservatively doubling the standard deviation from Phase 1 data and using a sample size of 10, we are fully powered to detect a 90% decrease in AUC_{0-24} levels with a significance level of 0.025 using a two-sided Wilcoxon test assuming that the actual distribution is normal. Powering off a lower than expected decrease in AUC_{0-24} levels, a sample size of 10 achieves ≥ 80% power to detect a 50% decrease in AUC_{0-24} with a significance level of 0.025 using the same two-sided Wilcoxon test above.

Aim-1 Power: Phase 1 to Phase 3. During Phase 3, patients who have been on 12 weeks of F/TAF 25mg + a ritonavir or cobicistat boosted protease inhibitor (Phase 2) will continue on this regimen while LDV/SOF treatment is added. From Phase 2 to Phase 3, we expect a 27% increase in AUC_{0-24} levels of tenofovir compared to Phase 2. This results in an overall expected decrease in AUC_{0-24} levels from Phase 1 to Phase 3 of 87% (90% decrease x 27% increase). With a sample size of 10, a significance level of 0.025 and conservatively doubling the standard deviation from Phase 1 data, we are fully powered to detect an 87% decrease in AUC_{0-24} levels using a two-sided Wilcoxon test assuming that the actual distribution is normal. Using the same two-sided Wilcoxon test above but powering off a lower than expected decrease, a sample size of 10 achieves ≥80% power to detect a 49% decrease in AUC_{0-24} levels from Phase 1 to Phase 3.

AIM-2. AIM-2 is to compare the TFV-DP in PBMCs between Phase 1 and Phase 2 and between Phase 1 and Phase 3. Sample size parameters for TFV-DP in PBMCs for Phase 1 were estimated from 11 subjects on TDF with a ritonavir-containing antiretroviral regimen from Cohort 1 of ACTG 5327. For these individuals, a geometric mean of 74 fmol/million cells was observed with corresponding $\log_e$ transformed mean (SD) = 4.305 (0.529) used for sample size calculations.

Aim-2 Power: Phase 1 to Phase 2. Preliminary data for 10 individuals on a TAF 25mg with cobicistat regimen was used to estimate levels of TFV-DP in PBMCs for Phase 2.(27) The geometric mean for these individuals was 517 fmol/million cells resulting in a difference in TFV-DP between Phase 1 and Phase 2 of 1.94 (0.637) on the $\log_e$ scale, where the estimated standard deviation conservatively assumes no correlation of subjects between phases. Assuming a two-sided Wilcoxon test with a sample size of 10 and a significance level of 0.025, we are fully powered to detect a difference of 443 in geometric means between Phase 1 and Phase 2. The power estimate is robust to increased variability, as we have greater than 99% power when the standard deviation is doubled in the power calculation.

Aim-2 Power: Phase 1 to Phase 3. In Phase 3, LDV/SOF treatment is added to patients taking F/TAF 25mg + a ritonavir or cobicistat boosted protease inhibitor. Data on the interaction between F/TAF 25mg and cobicistat with LDV/SOF shows an increase in TFV-DP in PBMCs in healthy volunteers.(14) As we are ≥99% powered under conservative estimates from phase 1 to phase 2, we expect to be fully powered from Phase 1 to Phase 3 due to the larger detectable difference resulting from the addition of LDV/SOF.

Secondary Objectives. Secondary analyses include: 1) compare TFV-DP in DBS between Phase 1 and Phase 2 and between Phase 1 and Phase 3; 2) compare CrCL, urinary retinol binding protein/creat ratio, urinary beta-2 microglobulin urine per ratio CRT between Phases 1 and 2 and between Phases 1 and 3; 3) compare AUC_{0-24} of tenofovir in plasma between Phase 2 and Phase 3; and 4) compare TFV-DP in PBMCs between Phase 2 and Phase 3. Friedman's test and non-parametric methods will be employed. As secondary objectives are considered hypothesis generating, p-values will be reported unadjusted for multiple comparisons. The percentage of patients achieving SVR will be provided.

G. Summarize Knowledge to be Gained:

LDV/SOF is the preferred treatment for HCV because it is highly efficacious, well tolerated, has a low pill burden, and a short treatment duration of between 8 and 24 weeks. It is a highly desirable treatment option for HIV infected individuals because of its low potential for drug interactions with antiretroviral agents. However, the increase in tenofovir exposures observed in drug interaction studies in healthy volunteers is a significant concern and there is minimal guidance on the appropriate management of patients on tenofovir-based antiretroviral therapy initiating LDV/SOF. Through this study, we will determine the magnitude of the interaction between tenofovir administered in the form of TAF 25mg and LDV/SOF in HIV monoinfected patients and examine the effect of this interaction on renal function.

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