Defining Antiretroviral Pharmacology Within HIV-1 Reservoirs of Males and Females

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ABBREVIATIONS

AAUCMB = average area under the curve minus baseline

ARV = antiretroviral

AUC = area under the curve

BP = blood plasma

CL/F = oral clearance of drug

C_{max} = maximum drug concentration

C_{min} = minimum drug concentration

CNS = central nervous system

CSF = cerebrospinal fluid

DTG = dolutegravir

DTG C_{t24h} = DTG total concentration 24 hours post dosing

E_{max} = maximum drug effect

EC_{90} = DTG C_{t24h} required to produce 90% of the maximum drug effect

EC_{95} = DTG C_{t24h} required to produce 95% of the maximum drug effect

HAART = highly active antiretroviral therapy

HPLC-MS/MS = high performance liquid chromatography – tandem mass spectroscopy

INSTI = integrase strand transfer inhibitor

LPV/RTV = lopinavir/ritonavir

PBMCs = peripheral blood mononuclear cells

PK = pharmacokinetic

PD = pharmacodynamic

RT-PCR = real time-polymerase chain reaction

TDF/FTC = tenofovir disoproxil fumarate/emtricitabine

T_{max} = time to C_{max}

T_{min} = time to C_{min}

$T_{1/2}$ = half life

VD = viral dynamic

WIHS = Women's Interagency HIV Study

ABSTRACT

Emerging evidence indicates that sites outside of blood plasma, such as peripheral blood mononuclear cells (PBMCs) and gut-associated lymphoid tissue, remain reservoirs for HIV and play critical roles in viral persistence despite long-term potent combination antiretroviral therapy (cART). Suboptimal ARV drug concentrations within these reservoirs are thought to contribute to our inability to fully eradicate HIV. In addition, host factors such as sex have been found to impact ARV drug exposure within these sites and effect key outcomes such as time to virologic suppression. Our understanding of reservoir site pharmacology and the impact on sex is limited due to several barriers: 1) Difficulty in sampling reservoir sites intensively and 2) Scarcity of women enrolled in HIV clinical research studies. Optimal drug concentrations are ideally determined early in drug development by dose-ranging studies that require intensive blood plasma sampling; this methodology is impractical to employ within tissue sites. To mitigate these barriers, we propose to study the pharmacology of the integrase strand transfer inhibitor dolutegravir (DTG) within three body compartments: blood plasma, PBMCs, and rectal tissue using a novel integrated population pharmacokinetic-viral dynamic (PK-VD) modeling approach. This PK-VD modeling strategy generates concentration-response relationships with limited sampling and dosing simulations ideally suited to reservoir sites. Additionally, we will prioritize the exploration of sex differences by leveraging our extensive research infrastructure, including the clinical core of the Emory Center for AIDS Research (CFAR), the Atlanta Clinical and Translational Science Institute (ACTSI), and the motivated cohort of the Atlanta Women's Interagency HIV Study (WIHS). We hypothesize that integrated population PK-VD modeling will show that optimal drug doses needed to suppress HIV will be higher in PBMC and rectal tissue reservoirs compared to blood plasma and will be different between males and females.

Our proposed aims are: 1) Compare descriptive data generated from our DTG PK-VD blood plasma model to existing dose-ranging study data for validation, 2) Extend the development of DTG PK-VD models to PBMC and rectal tissue reservoirs, and 3) Investigate sex differences in DTG PK-VD in all three body sites. To accomplish this, 20 HIV-infected males and 20 HIV infected females (total n = 40) will be enrolled in a 168 day (6 month) longitudinal study whereby blood plasma, PBMCs, and rectal tissue will be serially sampled during six (6) study visits to measure DTG concentrations, HIV-1 RNA viral load, and perform 3 different assays to measure the HIV reservoir, including total cell-associated HIV DNA, 2-LTR unintegrated HIV DNA circles, and a modified rapid low cell quantitative viral outgrowth assay (QVOA). From these data, integrated population PK-VD models will be constructed and dosing simulations used to determine optimal drug exposure needed to attain virologic suppression at all three sites within males and females. With support from a multi-disciplinary team of mentors, completion of this work will uniquely position me for an independent research career integrating pharmacologic interventions within HIV cure stratagems.

Duration

Enrollment is expected to take 36 months, with completion of all study visits in 40 months. Data collection will be completed after sample collection of the last enrolled participant at their Day 168 visit.

Sample size

A sample size of 20 HIV-infected women and 20 HIV-infected men will be enrolled in this study.

Population

HIV-1 infected women enrolled in the Emory Women's Interagency HIV Study (WIHS), Ponce de Leon Center, or other community HIV clinic and men enrolled at the Ponce de Leon Center or other community HIV clinic.

LAY SUMMARY

Even with strong HIV medications, there is no known cure for HIV infection. When a person is infected with HIV, studies have shown that the virus enters many different parts of the body, including the brain, lymph nodes, the gut (including the rectum), and special cells in the blood. HIV medications are very good at getting into blood plasma and killing the virus, but may have limited ability to get into some of these other places. In sites such as special blood cells and the gut, the virus can hide from HIV medications.

The dose of HIV medication needed for good HIV control is decided by studies that look at drug levels in the blood plasma of patients. While the dose of medication may be good enough to control HIV infection in blood plasma, it may not be good enough to control HIV infection in these other hard-to-reach sites.

The purpose of this study is to find out how well the HIV medication dolutegravir (DTG/Tivicay), a FDA approved HIV medication for the treatment of HIV infections, gets into different parts of the body: including blood plasma, special blood cells, and rectal tissue. Specifically, we want to compare how fast dolutegravir (DTG) lowers the HIV viral load in these three different sites. In addition, we want to see if there are any differences in how dolutegravir acts in males and females. Results of this study will give us more information about HIV medications and their limitations. In the future, this could help us create better HIV medications that can get into these hard-to-reach places and eventually cure HIV infection.

1. STUDY AIMS AND HYPOTHESES

1.1 Aim 1

The primary aim of this study is to validate the integrated population PK-VD model that quantitatively describes the relationship between DTG exposure and HIV viral decay in BP.

Twenty (20) HIV-infected women and twenty (20) men will undergo BP sampling for HIV-1 RNA and DTG concentrations following first dose oral administration of Triumeq (DTG 50 mg/Abacavir, ABC 600 mg/ Lamivudine, 3TC 300 mg) or DTG (50 mg) plus standard dose of co-formulated tenofovir disoproxil fumarate (TDF)/ emtricitibine (FTC) on days 14, 42, 168, and repeat intensive sampling over 24 hours following observed dosing at day 84 for steady state parameter estimates. Integrated population PK-VD modeling and simulation will be used to estimate the EC_{90}, defined as the DTG C_{t24h} (DTG total concentration 24 hours post dosing) required to produce 90% of the maximum drug effect (E_{max}), and other PK-VD parameters.

Hypothesis: A population PK-VD model with exposure-response simulations will show that optimal DTG doses needed for virologic suppression are higher in PBMCs and rectal tissue compared to BP.

1.2 Aim 2

The second aim of this study is to develop an integrated population PK-VD model to describe the relationship between DTG exposure and HIV viral decay in PBMCs and rectal tissue reservoir sites.

HIV-1 infected subjects initiating therapy with a DTG-based HAART regimen will undergo collection of PBMCs at Baseline and on Days 14, 42, 84, and 168. Rectal tissue will be collected within 7 days of ART initiation, and on Days 42, 84, and 168 for DTG concentrations and quantitation of the HIV reservoir by measuring HIV RNA, total cell-associated HIV DNA, 2-LTR DNA circles, and by performing a modified QVOA. Integrated population modeling and simulation will be used to estimate EC_{90} at each site.

Hypothesis: A population PK-VD model will accurately estimate DTG EC90, Emax (concentration required for maximum effect), and optimal dose needed for virologic suppression in BP.

1.3 Aim 3

The third aim is exploratory and will investigate sex differences in DTG penetration into blood plasma, PBMC and rectal tissue reservoirs as well as its impact on the rectal microbiome.

Integrated population modeling and simulation will be used to estimate EC_{90} at each site.

Hypothesis: A population PK-VD model with exposure-response simulations will show that DTG exposure differs between males and females and is associated with both viral dynamic changes within PBMC and rectal tissue reservoirs as well changes within the rectal microbiome.

INTRODUCTION

1.4 Background

Emerging evidence suggests that sites such as gut tissue and peripheral blood mononuclear cells (PBMCs) remain reservoirs for HIV-1 and play critical roles in viral persistence despite combination antiretroviral therapy (cART)[1-11]. Leading theories to explain barriers to HIV eradication from reservoirs include 1) ongoing proliferation of latently infected cells invulnerable to cART and 2) continuing viral replication due to suboptimal antiretroviral (ARV) tissue concentrations[8, 12, 13]. In response, the HIV cure agenda has focused on strategies such as "shock and kill", whereby latently infected cells are activated ("shocked"), making them susceptible to killing by immune attack and/or cART[14]. To achieve killing by cART, drugs must be capable of penetrating reservoirs. This approach requires a clear understanding of drug pharmacokinetics (PK) and HIV viral dynamics (VD) specifically in these affected sites. Drug and host characteristics, including factors modulated by patient's sex [15-18], result in drug distribution and disposition that differs between blood plasma (BP) and other body compartments [19, 20]. For example, lower ARV concentrations in lymphatic tissues were found to be associated with persistent HIV replication [13]. Likewise, sex differences have been noted in PK parameters of multiple ARV drugs within BP[21-25] and PBMCs[26-29], with females showing more rapid HIV viral load decay and shorter time to plasma virologic suppression than males[25, 29]. Most of these prior studies are limited by examination of sex differences only in secondary analyses. **Improved understanding of ARV PK-VD within HIV reservoirs of males and females will have far-reaching public health implications by informing the development of sex-specific pharmacologic HIV cure strategies.**

A key obstacle to reservoir PK-VD assessment is the challenge of repeated tissue sampling, which is necessary to establish pharmacologic parameters in traditional dose-ranging studies. This is further compounded by paucity of PK-VD data in women who account for >50% of the 36.9 million people living with HIV worldwide[30], yet represent < 18% of participants in clinical studies of HIV cure[31]. Integrated population PK-VD modeling uses mathematical modeling and dose-ranging simulations to simultaneously describe drug exposure and VDs and generate concentration-response relationships. We have recently shown that a PK-VD modeling and simulation tool can accurately predict the PK-VD relationship for a fixed dose of LPV/RTV in a small number of patients[32]. We propose to test this novel PK-VD modeling approach to generate concentration-response relationships using limited tissue sampling combined with dosing simulations for dolutegravir (DTG) to test a more practical approach for pharmacologic assessment of deep-seated reservoirs. Leveraging our extensive translational research infrastructure supported by the clinical core of the Emory Center for AIDS Research, the Atlanta Clinical and Translational Science Institute, and access to the motivated female cohort in the Atlanta Women's HIV Interagency Study, we will apply integrated PK-VD models to assess relationships between DTG exposure and HIV viral dynamics in 3 compartments, BP, PBMCs, and rectal tissue, in HIV-infected females and males. DTG was chosen because of its potent antiviral activity, excellent safety profile, and published robust dose-ranging study data in BP.

1.5 Potential significance

1. We will investigate the utility of a novel strategy, integrated population PK-VD modeling, to predict DTG exposure needed for virologic suppression in BP. This model differs from early phase PK-PD models in that it utilizes data from participants on fixed-dose drug instead of different doses. In place, dosing simulations will be employed to predict optimal dose needed for virologic suppression. This approach will complement efforts to generate PK-VD data for clinical decision making for drugs where data are lacking.

2. Applying this model to predict PK-VD parameters in PBMCs and rectal mucosa is innovative.

3. The recruitment of women, via the resources of an established cohort of women in the Atlanta WIHS, will be a priority enabling the study of sex differences. This will provide novel PK-VD data on females in an area where relevant sex-specific data are not available.

4. Using multiple different assays to measure the HIV reservoir, including HIV RNA, total cell associated HIV DNA, 2-LTR DNA circles, and modified QVOAs allows a multi-faceted mechanism to evaluate the reservoir so that we can better understand the overall size, replication competence of latent virus, and the cell subset origin of latent virus. Together, this information provides key insight into understanding where the viral reservoir is located, which subsets harbor the virus, which latent cells are capable of producing replication-competent virus, and differences across tissues and sex.

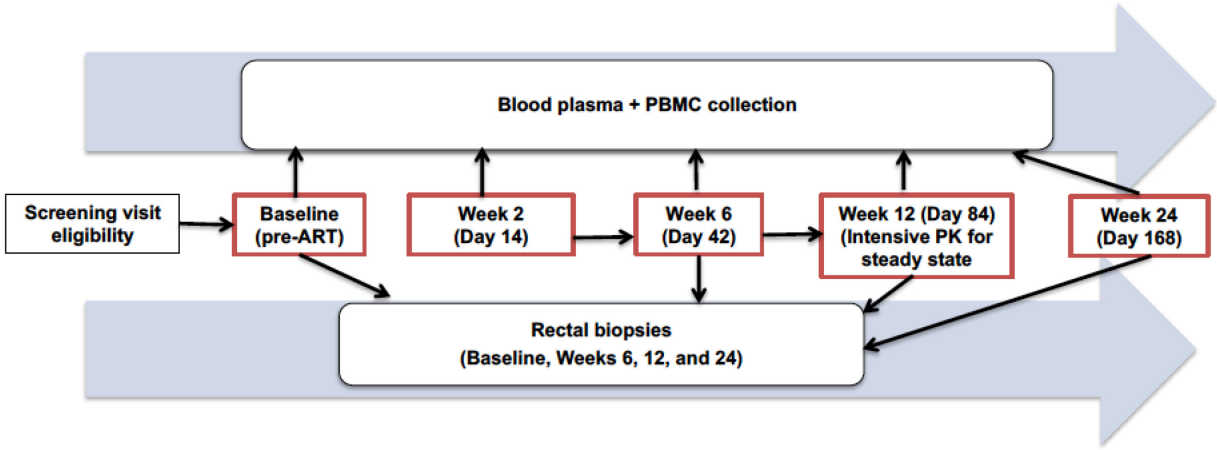
2. STUDY DESIGN

3.1 Study design overview

The primary aim of this study is to determine whether DTG plasma PK-VD parameters can be accurately estimated using an integrated population PK-VD model with data derived from subjects initiating a uniform dose of a DTG-based HAART regimen compared to a standard dose-ranging study. It will be descriptive in nature and will involve HIV-infected women and men prospectively recruited from the Atlanta WIHS cohort, the Ponce de Leon Center, and community HIV clinics. Population PK-VD modeling will be applied. Subjects will undergo intensive blood sampling over a period of 6 months following HAART initiation, as shown in Figure 1. Plasma DTG concentration will be measured by HPLC-MS/MS and plasma HIV RNA will be quantified by RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and PCR amplification. DTG concentrations and viral dynamic data will be modeled to generate blood plasma DTG EC_{90}, E_{max}, and other PK-VD metrics of interest. DTG was selected because of the abundance of dose ranging PK-PD data available in the literature for validation. A 6-month study duration suffices as DTG based HAART regimens are very potent and have steep viral decay slopes with most HIV-infected individuals achieving undetectable plasma viral loads at or before this time point on DTG-based therapy [33].

The second aim will be exploratory: we will expand the integrated population PK-VD approach to test its utility in the estimation of PK-VD parameters including EC_{90} and E_{max} in two other reservoir sites: PBMCs and rectal tissue. PBMCs will be collected at Baseline and Days 14, 42, 84, and 168. Rectal tissue will be collected within 7 days of ART initiation and on Days 42, 84, and 168, as shown in Figure 1. Intracellular and rectal tissue DTG concentrations and HIV RNA will be quantified using similar techniques as for blood plasma, but validated for these tissues. As measurement of HIV RNA only provides a very limited view of the total HIV reservoir, we will also measure the reservoir using three additional assays: total cell associated HIV DNA, 2-LTR DNA circles, and a modified QVOA.

Aim 3 seeks to investigate sex differences in penetration of DTG into blood plasma and PBMC and rectal tissue reservoirs, as well as differences in the rectal microbiome.



Study endpoints:

AIM 1: DTG blood plasma EC_{90} and PK-VD metrics
AIM 2: DTG PBMC and rectal tissue EC_{90} and PK-VD metrics
AIM 3: Sex differences between DTG PK-VD metrics

3.2 Study entry and initial screening

- If a person agrees to participate and provides informed consent, a **screening evaluation** will occur at the clinical core of the Emory Center for AIDS Research (CFAR) at the Grady IDP or at the Grady Hospital CRN, a core equipped with state-of-the-art facilities for clinical research. Screening will include informed consent, a review of medical and medication history, and assessment for eligibility. HIV status will be verified with documentation of HIV ELISA test confirmed with western blot or plasma HIV-RNA. Screening laboratory tests, if not already available within 90 days prior to screening, will be conducted at the Grady clinical laboratories on a fee-for-service basis and will include complete blood count (CBC) with platelet and differentials, and full chemistry. Consecutive potential participants in the Atlanta WIHS cohort will be screened until 40 eligible participants are enrolled (20 females and 20 males). Participants will receive $20 in compensation after completion of the screening visit. A preliminary consent-only visit may also be conducted if necessary.
- It is important to know that for this study, the study drug, dolutegravir (DTG/Tivicay) is FDA approved for the treatment of HIV infections and can be given in combination with other antiretrovirals (ARVs), as a standard therapy for HIV. It is also important to know that this study will not provide nor will the study pay for the study medication. Participants will receive from their provider a prescribed supply of the drug, Tivicay® (dolutegravir/DTG), with either Triumeq, or Truvada or Descovy as determined by their primary HIV provider. It will be taken by mouth as prescribed and its dose and its administration will be determined by your provider. Participants will also be responsible for bringing these medications with them to their study visits.

3.3 Study visits

Visit 1: Baseline, Pre-dose

This visit will take place at the Grady IDP clinic and will involve a targeted history and physical, review of reproductive and sexual history, urine dipstick pregnancy test (women) and pre-dose collection of blood plasma, PBMC sampling, and HIV RNA quantification. Subjects will initiate HAART regimen with either Triumeq, DTG 50 mg daily plus Truvada (tenofovir disoproxil fumarate/emtricitabine, TDF/FTC)

300/200 mg daily, or DTG 50 mg daily plus Descovy (emtricitibine/tenofovir alafenamide) 200/25 mg daily. Eligibility to start these regimens will be determined by their primary HIV provider and participants are responsible for acquiring the medication and bringing them to the study visits. Specimens will be processed within one hour to separate the plasma component and PBMCs and stored at -80°C until analyzed for DTG drug concentrations.

In addition, either at this visit or within 7 days of ART initiation, participants will undergo anoscopy with rectal tissue sampling. Rectal mucosa fluid will be collected first using two dry sterile cotton swabs and stored at -80°C for future rectal microbiome analysis using 16S sequencing. Any digital image of the biopsy site may also be taken during the anoscopy procedure to follow healing of the site over time. These de-identified images may be shared with investigators not listed on the study.

Four (4) to 8 pieces of rectal tissue will be collected via punch biopsy during anoscopy for measurement of DTG concentrations, HIV-1 RNA, total cell associated HIV DNA, 2-LTR DNA circles, and modified QVOA. Half of the rectal tissue will be flash-frozen in liquid nitrogen and cryopreserved at -80°C until analysis. The other half will be placed fresh in phosphate-buffered saline and transported to the Laboratory of Biochemical Pharmacology (under the supervision of Drs. Gavegnano and Schinazi) at room temperature within 24 hours of collection for processing and analysis.

Participants will receive $30 for the blood draw and $50 for the rectal biopsy, for a total of $80 compensation for their time and inconvenience if they complete all procedures.

The Screening and Baseline visits can occur on the same day if acceptable to the participant.

Visits 2, 3 : Post-dose population sampling of blood plasma, PBMCs, and rectal tissue.

Visit 2 will occur 14 days after visit 1.

Visit 3 will occur 42 days after visit 1.

During visits 2-3, participants will have a focused history and physical exam. Participants will undergo post-dose population sampling of blood plasma and PBMCs at visits 2-3. Rectal mucosa fluid and tissue will be collected at visit 4 for future rectal microbiome analysis and measurement of DTG concentrations, HIV-1 RNA, total cell-associated HIV DNA, 2-LTR DNA circles, and modified QVOA. Participants will receive $30 for visits requiring only a blood draw, and an additional $50 in compensation at any visit with rectal tissue collection (up to 8 punch biopsies), for a total of $80. A digital image of rectal biopsy sites may be taken during the anoscopy procedure to examine healing of the site and these images may be shared with investigators not listed on the study.

Visit 4: Steady state 24 hour PK-VD sampling

Visit 4 will occur 84 days (12 weeks) after visit 1.

This visit will involve an 8-hour stay either at the Grady or the Emory site of the Georgia Clinical and Translational Science Alliance (CTSA) for intensive blood plasma and PBMC sampling for ARV concentrations and HIV RNA quantification.

During visit 4, participants will have a focused history and physical exam and provide urine for pregnancy testing and blood for CD4+ T cells (add-on: CD3), CBC (add-ons: BI smear w/diff; RBC morphology) with differential and comprehensive chemistries.

Visit 4 will represent steady-state PK. DTG has an elimination half-life of 14 hours [34], therefore steady state plasma concentration is achieved after 5 days of oral administration. Adherence to previous DTG doses will be documented. Participants are responsible for bringing in their prescribed medication. Blood samples will be drawn 15 minutes prior to DTG administration (time 0) and 1, 2, 3, 4, 6, 8 and 24 hours following observed drug intake to provide steady-state PK-VD data. HIV-RNA sampling will be done once as subjects are expected to be virologically suppressed in the blood plasma at this stage of HAART therapy. In addition, each participant will undergo PBMC and rectal mucosa fluid collection and

tissue sampling (up to 8 punch biopsies) 24 hours post-dose for measurement of rectal microbiome, DTG concentrations,HIV-1 RNA, total HIV-associated HIV DNA, 2-LTR DNA circles, and modified QVOA. Participants will be compensated $150 for this visit.

Visit 5:

Visit 5 will occur 168 days (6 months) after visit 1. During visit 5, participants will have a focused history and physical exam. Participants will undergo post-dose population sampling of blood plasma and PBMCs. Rectal mucosa fluid and tissue will be collected for future rectal microbiome analysis and measurement of DTG concentrations, HIV-1 RNA, total cell-associated HIV DNA, 2-LTR DNA circles, and modified QVOA. A digital image of rectal biopsy sites may be taken during the anoscopy procedure to examine healing of the site and these images may be shared with investigators not listed on the study.

Participants will be compensated $30 for the blood draw and $50 for the rectal biopsy, for a total of $80 compensation if they complete all procedures.

Table 1 illustrates the schedule of events during each study visit:

Table 1. SCHEDULE OF EVENTS

	Screening visit	Baseline Visit[a] (pre-dose)	Day 14	Day 42	Day 84 (24 hr visit, steady state)	Day 168
Screening and Baseline Evaluation						
Informed consent	☐					
Documentation of HIV status	☐					
Targeted H&P		☐	☐	☐	☐	☐
Chem 14[c]	☐				☐	
CBC w/ diff[c] (add-ons: Bl smear w/diff; RBC morphology)	☐				☐	
CD4 T cell count (add-on: CD3)					☐	
Determine DTG EC_{90} in blood plasma						
Plasma DTG concentration		☐	☐	☐	☐	☐
Plasma HIV-1 RNA		☐	☐	☐	☐	☐
Determine DTG EC_{90} in PBMCs and rectal tissue (RT)[b]						
DTG concentrations		☐	☐	☐	☐	☐
HIV-1 RNA		☐	☐	☐	☐	☐
Total cell-associated DNA		☐	☐	☐	☐	☐
2-LTR DNA circles		☐	☐	☐	☐	☐
Modified QVOA		☐	☐	☐	☐	☐
Examine rectal mucosa microbiome						
Rectal mucosa microbiome analysis		☐		☐	☐	☐

[a]Day = Number of days since initiation of cART with DTG-based regimen. Day 0 = day of 1st dose cART

[b]Rectal tissue assays will be performed in all participants within 7 days of first dose, and on Days 42, 84, and 168.

[c]CBC with diff and Chem 14 will only be done at screening visit if they have not been done in last 90 days.

NOTE: *Pregnancy tests will continue to be performed at each visit.*

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion criteria

- Age ≥ 18 years

- No ARVs in the last 6 months (from date of screening)
- No documented or suspected resistance to integrase inhibitors (dolutegravir, elvitegravir, raltegravir, or bictegravir).
- Creatinine Clearance >50 mL/min, as calculated by the Cockcroft-Gault equation within 90 days of screen
- Liver function testing, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase < 5 times upper limit of normal within 90 days of screen
- Intact gastrointestinal tract
- Able and willing to give informed consent
- Willing and eligible to initiate ARV therapy with Triumeq, DTG + Truvada (TDF/FTC), or DTG + Descovy (FTC/TAF)
- Agree to receive from their provider and pay for a prescribed supply of the drug, Tivicay® (dolutegravir/DTG), with either Triumeq, or Truvada or Descovy as determined by their primary HIV provider.
    - Agree to take the prescribed medication by mouth.
    - Agree that they (the participant) is responsible for bringing these medications with them to their study visits.
- Willing to undergo serial blood and rectal tissue sampling
- Female participants' must be willing to have a pregnancy test done at each visit.
  Female participants of childbearing potential (FCB) must agree to either commit to continued abstinence from heterosexual intercourse or to use a reliable form of birth control such as oral contraceptive pills, intrauterine device, Nexplanon, DepoProvera, permanent sterilization, or another acceptable method, as determined by the investigator for the duration of the study. FCB are defined as sexually mature women who have not undergone hysterectomy or bilateral oophorectomy or have not been naturally postmenopausal for at least 24 consecutive months (i.e have had menses at any time in preceding 24 months)

4.2 Exclusion criteria

- Pregnant or attempting to conceive now or during the course of the study
- Self-reported or documented current anal or rectal disease prohibiting safe anoscopy and biopsies, in investigator's opinion.
- Taking concurrent medications that interfere with DTG (see Table 2)
- Bleeding diathesis
- Platelet count <50,000 mm^3
- Medical condition that interferes with conduct of study, in investigator's opinion

4.3 Study enrollment procedures

The study protocol and protocol consent form will be approved by the Emory University institutional review board (IRB) and the Grady Health System Research Oversight Committee prior to implementation of this study.

Candidates will be notified of the study via a flier (*Appendix A).* Once a candidate has been identified, the details of the study will be carefully discussed with them. Prior to undergoing any study procedure, the candidate will be asked to read and sign the study protocol consent form that was approved by the Emory IRB. The subject will be given a copy of the signed informed consent form for their records, and a second copy will be kept in the subject's research record.

4.4 Prohibited and precautionary medications

Any participant actively taking any of the prohibited or precautionary medications listed in Table 2 will not be eligible to participate in this study.

Table 2: Prohibited and precautionary medications

Concomitant drug class: drug name	Effect of concentration on Dolutegravir and/or concomitant drug	Clinical comment
HIV antiviral agents		
Non-nucleoside reverse transcriptase inhibitor (NNRTI): etravirine	↓ Dolutegravir	Do not coadminister without also giving darunavir/ritonavir, atazanavir/ritonavir, or lopinavir/ritonavir
NNRTI: Efavirenz	↓ Dolutegravir	Dose adjustment of DTG to 50 mg bid for treatment naïve
NNRTI: Nevirapine	↓ Dolutegravir	Avoid coadministration: insufficient data for dosing recommendations
Protease inhibitors: Fosamprenavir/ritonavir Tipranavir/ritonavir	↓ Dolutegravir	Dose adjustment of DTG to 50 mg bid for treatment naïve
Other agents		
Psychotropic agents: Oxcarbazepine Phenytoin Phenobarbital Carbamazepine St. John's Wort	↓ Dolutegravir	Avoid coadministration: insufficient data for dosing recommendations
Medications with polyvalent cations: Cation-containing antacids or laxatives Sucralfate Buffered medications	↓ Dolutegravir	Must administer Dolutegravir 2 hours before or 6 hours after these medications
Hypoglycemic: Metformin	↑ Metformin	Consider metformin dose reductions
Antibiotic: Rifampin	↓ Dolutegravir	Dose adjustment of DTG to 50 mg bid for treatment naïve

Adapted from Table 5 in Tivicay (dolutegravir) [package insert]. ViiV Healthcare: Research Triangle Park, NC; 2014 [34].

5. Definitions for Schedule of Events- timing of study visits

5.1.1 Screening Visit

Screening evaluations will determine eligibility and will occur prior to the participants' enrollment into the study.

5.1.2 On Study Visits

The first on-study visit will occur after the screening visit, but within a month (30 days) of screening visit (unless otherwise specified). All other on-study visits that occur after the first on-study visit will occur 14 days, 42 days, 84 days, and 168 days after the first visit.

The window period for visits on day 14 can be +/- 3 days.

The window period for visits on day 42 and 84 can be +/- 7 days.

The window period for the visit on day 168 can be +/- 14 days.

Please see Table 1 for schedule of clinical and laboratory evaluations.

5.2 Definitions of evaluations

5.2.1 Consent Form
A signed and dated consent form approved by the Emory IRB is required prior to participation in this study.

5.2.2 Documentation of HIV
HIV-1 infection, as documented by any licensed serologic test confirmed by western blot or by positive plasma HIV-1 RNA performed by any laboratory that has a CLIA certification.

5.2.3 Medication History
Current medications will be recorded from the patient's self-report and medical record including doses, dosing frequency, and duration of treatment.

5.2.4 Screening Clinical Assessment
Screening clinical assessment will include a review of medical diagnoses, current medications, and eligibility criteria.

5.2.5 Screening Laboratory Evaluations
At screening, most recent HIV-1 RNA PCR (if available) and CD4 T-cell counts will be recorded from the medical record. Absolute CD4+/CD8+ counts and percentages performed by flow cytometry at a CLIA-certified or equivalent laboratory will be recorded. Study eligibility will be determined based on medical history review and laboratory testing as described below:

- Urine collected at this visit in all female participants will be tested by dipstick for beta-hCG to assess for pregnancy. If this test indicates pregnancy, the patient will be notified and referred to their primary care provider in the clinic for further evaluation.
- Platelet count within the last 90 days will be reviewed from the medical record. Participants with platelets <50,000 are not eligible to participate.
- Renal and liver function testing within the last 90 days will be reviewed from the medical record. Participants with creatinine clearance <50 mL/min by Cockcroft-

Gault equation or AST, ALT, alkaline phosphatase > five (5) times upper limit of normal are not eligible to participate.

5.2.6 HIV-1 RNA in plasma, PBMCs, and rectal tissue

HIV-1 RNA will be quantified in cryopreserved plasma, PBMCs, and rectal tissue by RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and PCR amplification using assays validated for each site at the Emory CFAR virology core on a fee-for-service basis.

5.2.7 HIV-1 total cell associated DNA, 2-LTR DNA circles, and modified QVOA in PBMCs and rectal tissue

Total-cell associated HIV DNA , **2-LTR DNA circles, and modified rapid-low cell QVOA** will be quantified by Dr. Christina Gavegnano within the Laboratory of Biochemical Pharmacology under the supervision of Dr. Raymond Schinazi on a fee-for-service basis. For total HIV DNA and 2-LTR DNA circle assays, PBMCs, at a minimum of 1.0 x 10^5 cells, will be subjected to total cell lysis with an NP-40 based detergent while rectal tissue will be homogenized and then dissolved. Quantitation of 2-LTR DNA circles will be performed using real-time PCR and Taqman technology (Applied Biosystems), as previously described. Plasmids containing LTR sequences will be used as standards for quantitation (NL4-3, subtype B). Total-cell associated HIV DNA will be performed using the Abbott Real-time HIV-1 DNA assay. **The modified rapid-low cell QVOA** is a culture-based assay that detects the outgrowth of replication-competent virus. Ex-vivo PBMCs will be subjected to density gradient centrifugation to obtain total PBMC populations and sorted using fluorescent activated cell sorting (FACS). For PBMCs from tissue, cells will be subjected to tissue homogenization and purification of live, single cell supsensions using existing methods in the BSL 2/3 Laboratory of Biochemical Pharmacology (OctoMacs Pro; Miltenyi Biotec methodology). Sorted cells are cultured with EC_{99} of cART (Raltegravir/(-)-FTC/efavirenz) for 72 hours to prevent virus harbored in sorted cells from spreading. CD3/CD28 and IL-15 are added to induce activation of latent virus from sorted memory cultures; 24 hours post reactivation, cells are stained with anti-p24 fluorescein isothiocyanate mAb and non-dividing p24+ cells are quantified with FACS to measure reservoir size. Supernatant is harvested and virus is quantified with p24 ELISA prior to infection with naïve PBMCs at limiting dilutions of inoculates to determine replication competence.

Total cell-associated HIV DNA and 2-LTR DNA circles quantitation will be performed on cryopreserved PBMCs and rectal tissue. The **modified QVOA** will be performed in both cryopreserved PBMCs and fresh rectal tissue. Fresh rectal tissue will be placed in phosphate-buffered saline and transported at room temperature to the Laboratory of Biochemical Pharmacology within 24 hours of collection for processing.

5.2.8 Dolutegravir concentrations in plasma, PBMCs, and rectal tissue
DTG concentrations will be measured from blood plasma, PBMCs, and rectal tissue under Dr. Edward Acosta's supervision at the University of Alabama-Birmingham Antiviral Laboratory using HPLC-MS/MS, as previously described [35]. To decrease variability, samples will be analyzed in duplicate and resulted as the average.

5.2.9 Any Digital images of biopsy sites will be examined from 2 consecutive visits with digital imaging analysis software. The percentage of the biopsy that has healed over time will be calculated as an exploratory analysis to better understand mucosal healing after injury.

5.2.10 Rectal fluid microbiome
Rectal microbiome analysis will be performed at a future date using 16S sequencing.

6. STATISTICAL CONSIDERATIONS

6.1 Primary endpoint

To estimate the EC_{90} of dolutegravir in blood plasma using an integrated Pharmacokinetic (PK) – Viral Dynamic (VD) model.

6.2 Secondary endpoints

To estimate the EC_{90} of dolutegravir in PBMCs and rectal tissue using an integrated Pharmacokinetic (PK) – Viral Dynamic (VD) model.

To compare the PK-VD determined EC_{90} of dolutegravir in blood plasma, PBMCs, and rectal tissue between males and females.

6.3 Sample size justification

For our primary endpoint, we plan to enroll a total of 40 HIV-1 infected persons (20 men and 20 women into this study. As our primary endpoint is descriptive, our goal is to attain an accurate parameter estimate by achieving a sufficiently narrow confidence interval. Based on a coefficient of variation of 25% [36], a sample size of 17 HIV-1 infected women gives us 80% certainty that the 90% confidence interval for DTG EC_{90} will be no wider than 0.20 units [37].

6.4 Analyses

6.4.1 Demographic and clinical characteristics

Baseline demographic and clinical characteristics will be summarized by descriptive statistics.

6.4.2 Primary objective analysis: Estimate EC_{90} of DTG in blood plasma using integrated PK-VD modeling

Pharmacokinetics: PK data will be used to plot plasma concentration-time curves for DTG at steady-state to determine total drug exposure (AUC) and other PK parameters, including peak concentration (C_{max}), minimum concentration (C_{min}), time to C_{max} (t_{max}), time to C_{min} (t_{min}), half-life ($t_{1/2}$), and estimated clearance (CL/F).

Viral dynamics: Viral dynamic determinant of response will be AAUCMB, defined as the decrease in $\log_{10}$ time-averaged under the viral load-time curve from 0 to Day 168 ($AUC_{0\text{-}week\text{-}12}$) minus baseline.

PK-VD Modeling: Population analysis will be used to develop the overall PK-VD model. Because this is a longitudinal study with repeated measures, an expectation-maximization algorithm will be used on a parametric non-linear mixed-effects model to determine maximum likelihood estimates for model parameters, as developed by Shumitzky and Walker [38, 39]. This will be implemented using ADAPT software (version 5, MLEM module) [40]. Baseline covariates, including age, race, body mass index, menstrual cycle, and use of hormonal contraception will be evaluated for their ability to explain inter-individual variability in base model parameters. Prediction-corrected visual checks [41] will be used for model evaluation.

EC_{90} estimate: To estimate EC_{90}, exposure-response simulations using a wide range of DTG doses will be conducted: The DTG C_{min} (exposure) will be linked to the VD

determinant of response, AAUCMB. Median estimates will be reported in ng/mL with interquartile range. 90% confidence intervals will be constructed for the EC_{90} parameter using the bootstrap method, as described by Miller [42].

6.4.3 Secondary objectives analyses: Estimate EC_{90} of DTG in PBMCs and rectal tissue using integrated PK-VD modeling

Analyses similar to those described in 6.4.2 for the primary objective will be used here.

7. DATA COLLECTION AND MONITORING

7.1 Records to be kept

Case report forms (CRFs) will be provided for each subject to collect demographic, clinical, and laboratory data at study entry, and additional clinical data at study visits. These data will be collected from the screening clinical assessment, the subject's medical record, the study entry physical examination, and the subject's medication diary. Subjects will not be identified by name on any CRFs. Subjects will be identified by the participant identification number (PID), which will be provided by the study investigator upon registration.

7.2 Role of Data Management

7.2.1 Instructions regarding the recording of study data on CRFs will be provided by the study investigator

7.2.1 The study investigator will be responsible for assuring the quality of computerized data. This role extends from protocol development to generation of the final study databases.

Data will be entered into Emory Redcap, a secure web application for building and managing online surveys and databases.

7.3 Clinical Site Monitoring and Record Availability

7.3.1 The Emory University IRB, the OHRP, FDA, , or other government regulatory authorities may perform clinical site monitoring. Clinical research sites monitoring may include the review of the individual participant records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts) to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors may also inspect sites' regulatory files to ensure that regulatory requirements are being followed.

7.3.2 The investigator will make study documents (e.g., consent forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB or the OHRP for confirmation of the study data.

8. HUMAN SUBJECTS

8.1 Responsibilities of the Investigator

In implementing this protocol, the investigators will adhere to the basic principles of "Good Clinical Practice" as outlined in CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," CFR 21, part 50 and CFR 21, part 56 and Section 4 of ICH Harmonized Tripartite Guideline for GCP.

8.2 IRB Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the Emory University IRB. A signed and dated, Emory IRB-approved consent form will be obtained from the subject (or parent, legal guardian, or person with power of attorney for subjects who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the signed consent form will be given to the subject, parent, or legal guardian, and this will be documented in the subject's record.

8.3 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by coded number to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB or the OHRP.

8.4 Study Discontinuation

A study participant may elect to discontinue participation in the study at any time. The study may be discontinued at any time by the IRB, the OHRP, or other government agencies as part of their duties to ensure that research subjects are protected.

8.5 Risks to Human Subjects

The risks to study participants include side effects of Triumeq, DTG and TDF/FTC or DTG and FTC/TAF, risk of venipuncture for blood sampling, and risk of rectal tissue biopsies. DTG was approved by the FDA for use in combination with other ARV agents for treatment of HIV-1 infection in treatment-experienced or ARV-naïve persons in 2013. Subjects will be initiating a DTG-based regimen as part of treatment for HIV-infection and therefore will not assume additional ARV-related risk above and beyond standard of care. Clinical evaluation and safety laboratory testing will be performed to monitor participants for development of ARV-related adverse events. Recently, DHHS guidelines have released updated recommendations for the use of DTG in females of child-bearing potential (FCB) due to the potential risk of neural tube defects in infants born to women taking DTG-based regimens[43]. Given this new information, FCB must agree to either commit to continued abstinence from heterosexual intercourse or to use a reliable form of birth control such as oral contraceptive pills, intrauterine device, Nexplanon, DepoProvera, permanent sterilization, or another acceptable method, as determined by the investigator for the duration of the study. Pregnancy tests will continue to be performed each study visit. Common risks of venipuncture include discomfort, bleeding, and bruising. When repeated blood draws are necessary, it will be attempted through peripheral IV to minimize venipuncture.

Common risks of rectal biopsies include discomfort and bleeding. Rare (<1%) complications of anoscopy with rectal biopsies include infection, anal tears, or colonic perforation.

Adverse events will be reported per Emory IRB protocol.

9. Data safety and monitoring plans

Grade 3 and 4 adverse events and study conduct will be reviewed regularly by study investigators. Standard Emory IRB guidelines for reporting adverse events will be followed. Adverse Events and Serious Adverse Events will be reported to the IRB per Emory's guidelines for reportable events.

10. SPONSORS

The NIH/NIAID is sponsoring this study.

11. BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72.

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