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HIV RNA persists in rectal tissue despite rapid plasma virologic suppression with dolutegravir-based therapy

Cecile D. LAHIRI, MD, MS^{1,2}, Nakita L. BROWN, MS¹, Kevin J RYAN, BS³, Edward P ACOSTA, PharmD³, Anandi N SHETH, MD, MS^{1,2}, C Christina MEHTA, PhD, MSPH⁴, Jessica INGERSOLL, MS⁵, and Ighovwerha OFOTOKUN, MD, MS^{1,2}

¹Emory University School of Medicine, Department of Medicine, Division of Infectious Diseases, Atlanta, Georgia, USA

²Grady Healthcare System Infectious Diseases Program, Atlanta, Georgia, USA

³University of Alabama at Birmingham School of Medicine, Division of Clinical Pharmacology, Birmingham, Alabama, USA

⁴Emory University, Rollins School of Public Health, Department of Biostatistics and Bioinformatics, Atlanta, Georgia, USA

⁵Emory Center for AIDS Research, Virology and Molecular Biomarkers Core, Atlanta, Georgia, USA

Abstract

Objectives—Despite plasma virologic suppression with antiretroviral therapy (ART), HIV persists in gut tissue. The objectives of this study were to compare plasma and rectal tissue (RT) HIV RNA dynamics and to assess relationships with dolutegravir (DTG) plasma and tissue concentrations.

Design—A longitudinal cohort study of HIV-infected treatment-naïve individuals initiating DTG-based ART was conducted over 12 weeks with plasma and RT sampling ([Clinicaltrials.gov:NCT02924389](https://clinicaltrials.gov/NCT02924389)).

Corresponding author and requests for reprints: Cecile D. Lahiri, MD, MS, 49 Jesse Hill Jr Drive SE, Atlanta, Georgia 30303, cdelill@emory.edu, Phone: 404 616 6306, Fax: 404 616 9702.

Current affiliations: Nakita L. BROWN: Emory University School of Medicine, Winship Cancer Center, Atlanta, GA, USA.

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Author Contributions

CD Lahiri designed study; assisted with recruitment and enrollment of participants; collected, analyzed, and interpreted data; created all tables and figures; wrote all drafts of the manuscript; **N Brown** recruited and enrolled participants; collected and interpreted data; reviewed and edited manuscript; **KJ Ryan** developed and validated quantitative dolutegravir assay in rectal tissue; analyzed all pharmacokinetic samples; assisted with data interpretation; wrote portions of the methodology; reviewed and edited the manuscript; **EP Acosta** designed pharmacokinetic study; analyzed and interpreted pharmacokinetic data; assisted with creation of figures; reviewed and edited the manuscript; **AN Sheth** assisted with study design, conduct, and data collection; helped interpret the data; reviewed and edited the manuscript; **CC Mehta** designed statistical analysis plan; conducted statistical analyses and helped interpret data; reviewed and edited the manuscript; **J Ingersoll** created and validated HIV RNA assay for rectal tissue; performed all quantitative HIV RNA assays on plasma and rectal tissue; wrote portions of the methodology; reviewed and edited the manuscript; **I Ofotokun** designed study; assisted in study conduct, data collection, and interpretation of results; reviewed and edited the manuscript.

Methods—HIV RNA and DTG concentrations were quantified in plasma and RT samples collected pre-ART (baseline) and post-ART at weeks 2, 6, and 12 using Abbott Real-Time HIV-1 assays and high-performance liquid chromatography tandem mass spectroscopy, respectively. Relationships between RT RNA and DTG concentrations were modeled using binary logistic regression, controlling for repeated measures.

Results—Twelve participants were enrolled: 6(50.0%) females, 9(75.0%) black, median age 42.0 years (Q1 31.2, Q3 52.0). All attained plasma virologic suppression by Week 6. 11/12 (91.7%) had detectable RT HIV RNA at baseline, and only 3/11(27.3%) achieved RT virologic suppression at any time-point. Compared to RT non-suppressors, 3/3(100.0%) of RT suppressors were female, had higher BMI, 35.9 kg/m²(range 24.9- 38.5) versus 20.6 (17.7-29.9), $p=0.05$, and lower baseline log plasma HIV RNA: 3.7 cop/mL(range 3.6-4.4) versus 4.7(3.8-5.4), $p=0.02$. No significant relationships between RT RNA suppression and DTG concentrations were seen.

Conclusions—RT HIV RNA persisted in most participants and was not predicted by DTG concentrations. Impact of host factors, particularly sex, on tissue HIV viral dynamics warrants further exploration.

Keywords

HIV; dolutegravir; antiretroviral pharmacology; rectal tissue; reservoirs

Background

Despite potent combination antiretroviral therapy (ART), HIV eradication has not been accomplished. HIV persists in reservoirs, defined as a cell type or anatomical site where replication-competent virus accumulates and persists[1]. Gut-associated lymphoid tissue (GALT), the largest component of the human lymphoid organ system[2], remains a suspected HIV reservoir.

Standard of care is lifelong ART, achieving HIV plasma viral load suppression, improvement in immunologic function, and reduction of HIV-associated morbidity. First-line ART recommendations by the Department of Health and Human Services (DHHS) for naïve patients include using two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with an integrase strand transfer inhibitor (INSTI)[3]. The second-generation INSTI dolutegravir (DTG, 50 mg daily), is recommended for naïve patients in three ART regimens: DTG plus tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) or tenofovir alafenamide(TAF)/FTC and the fixed combination DTG/abacavir (ABC)/lamivudine (3TC)[3]. With unboosted daily dosing and high barrier to resistance, DTG remains an attractive option for providers and patients and is being launched by the World Health Organization as a low-cost once daily generic in low and middle income countries[4].

Despite rapid plasma virologic suppression with INSTIs, HIV RNA viral dynamics within tissues such as the gut have not been well defined. Evidence shows that antiretrovirals (ARVs) may insufficiently penetrate sites outside plasma due to disposition and distribution determined by drug and host factors including sex, physiochemical properties, and membrane transporter effects[5]. For example, rectal tissue (RT) concentrations of DTG

were <20% of plasma in HIV seronegative men[6]. There is conflicting evidence as to whether suboptimal ARV drug penetration impacts reservoir viral dynamics: one study showed an association between lower ARV drug concentrations in lymphatic tissues (including rectum) and persistent HIV replication[7], while another study showed no correlation between drug concentrations and HIV RNA or DNA in GALT[8]. Our study aims were twofold: 1) Compare early HIV RNA viral dynamics in plasma and RT following initiation of DTG-based ART, and 2) Explore relationships between RT HIV RNA decline and DTG exposure in both plasma and RT. We hypothesized that HIV RNA viral decay would be slower in RT than plasma and positively correlated with DTG exposure.

Methods

Study design and eligibility criteria

Data was collected from treatment-naïve HIV-infected males and females enrolled into an ongoing 12-week prospective longitudinal cohort study within the Grady Infectious Diseases Program (IDP) in Atlanta, Georgia, U.S.A., between February 2015 and September 2017 (NCT 02924389). A 12-week duration was chosen based on prior data showing that >80% of ART-naïve patients initiating a DTG-based regimen achieved plasma virologic suppression at 8 weeks[9]. HIV-1 infected adult males and females were recruited from Grady IDP, other HIV clinics within metropolitan Atlanta, and the Atlanta site of the previously described Women's Interagency HIV Study (WIHS) cohort[10] through fliers and interaction with study staff. Eligibility criteria included: a) Age ≥ 18 years; b) Never having ≥ 30 consecutive days of ART and none in past 6 months; c) Documented plasma HIV-1 RNA >1000 copies/mL; d) Normal renal and liver chemistries within 90 days; e) Able and willing to give informed consent, g) Able and willing to initiate first-line DTG-ART, and h) Willing to undergo serial blood and RT sampling. Exclusion criteria included: a) Pregnant, b) Severe anal/rectal disease, c) Concurrent medications interacting with ART d) Bleeding diathesis, or e) Medical condition that interfered with study conduct.

Type of DTG-ART was selected and prescribed by the participant's personal HIV provider. Participants were instructed to fill ART at their preferred pharmacy but refrain from starting medication until their first visit. All participants brought their own ARVs to each visit. Participants underwent paired plasma and RT sampling at four time points: baseline (pre-ART), and post-ART at weeks 2 (Day 7, 10, or 14), 6 and 12. Paired RT and plasma samples were collected at random times post-ART dosing: Sixteen of 32 (50%) pairs were collected within 30 minutes to 6 hours post-dose, 16/32 (50%) of pairs were collected between 12-24 hours post dose. In addition, intensive plasma pharmacokinetic (PK) sampling was performed at DTG first dose and steady state (Week 12) at hours 1, 2, 3, 4, 6, 8, 24. Participants were counseled not to place anything per rectum for at least 7 days post-biopsy. Complete blood counts, comprehensive chemistries, and CD4+ lymphocyte counts were performed at baseline and week 12. The Emory University Institutional Review Board (IRB) and the ethics committee for Grady Memorial Hospital (Grady Research Oversight Committee) approved this study. Informed consent was obtained from all study participants prior to study procedures.

Blood and rectal tissue sampling and processing

Study visits occurred either at Grady IDP or Grady Memorial Hospital within the Clinical Research Unit of the Georgia Clinical and Translational Science Alliance. All participants had witnessed administration of DTG-ART at baseline and week 12. At weeks 2 and 6, adherence to ART was self-reported. Blood was obtained via venipuncture in four 10 mL sodium citrate cell preparation tubes and spun at 2300 rpm for 20 minutes. Plasma was centrifuged again at 2300 rpm for 10 minutes to remove erythrocytes, then stored at -80°C . To acquire rectal tissue, a 10 cm plastic anoscope was inserted, rectal mucosa was visualized using a colonoscope, and up to eight random punch biopsies (2.3×4.2 mm) were obtained using mini-Tischler forceps. RT was placed immediately in cryovials, snap frozen in liquid nitrogen, and stored at -80°C .

HIV-1 RNA quantitation

Plasma and RT HIV-1 RNA quantitation was performed at the Emory Center for AIDS Research Virology and Molecular Biomarkers Core Laboratory using the Abbott Real-Time HIV-1 Assay with detectable range of 40-10,000,000 copies/mL. The RT HIV RNA assay was developed and optimized with methodologies used previously in other biological matrices[11]. RT was weighed, combined with 500 μL of Promega lysis buffer and 25 μL Abbott Proteinase K, and incubated at 56°C until dissolved. The supernatant was removed, volume brought up to 1 mL with phosphate-buffered saline, and run on the Abbott Realtime HIV-1 assay with results reported as RNA copies per gram.

Dolutegravir concentrations

DTG plasma and RT concentrations were measured at the University of Alabama-Birmingham's (UAB) Clinical Pharmacology Laboratory with high performance liquid chromatography-tandem mass spectroscopy (HPLC-MS-MS). DTG plasma concentrations were measured using methods previously developed by our team[12]. DTG and labeled DTG ($^{15}\text{N}_2\text{H}_7$, the internal standard, IS) were extracted from 50 μL human tissue homogenate using protein precipitation with acetonitrile. Extracts were analyzed by reverse-phase chromatography using an ACE Excel 3 C18-PFP column under isocratic conditions at a flow rate of 500 $\mu\text{L}/\text{minute}$ at 40°C with mobile phase A and B consisting of 0.1% formic acid in water and 0.1% formic acid acetonitrile, respectively. A triple quadrupole mass spectrometer (AB Sciex 5000) equipped with TurboV IonSpray[®] operating in positive-ion mode was used. Column effluents were analyzed by multiple reaction monitoring (MRM). The precursor/product transitions were 420.1 $\rightarrow$ 277.3 m/z for DTG and 428.1 $\rightarrow$ 283.3 m/z for IS. The calibration curve was fit using weighted ($1/x^2$) linear regression analysis of DTG/IS peak area ratio versus DTG concentration from 5.00 – 5,000 ng/mL. Assays were validated using the Guidance for Industry for U.S. Federal Drug Administration (FDA) regulated studies with 5.5% coefficient of variation and lower and upper limits of quantitation 5-5,000 ng/mL or ng/g.

Statistical analyses

Clinical and demographic characteristics were summarized by descriptive statistics with counts and percentages for categorical variables and medians and first and third quartiles

(Q1, Q3) or range (min-max) for continuous variables. Non-compartmental PK analyses were performed for first dose and steady-state DTG using Phoenix Win Nonlin v8.0 (Certara, L.P, St. Louis, Missouri). The area under the plasma concentration curve over the 24-hour dosing interval (AUC_{0-24h}) was computed using the linear trapezoidal method. Maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), and plasma concentration at the end of the dosing interval (C_{τ}) were determined by visual inspection of the plasma concentration time curve. 24-hour DTG concentrations were missing for three participants, and interpolated values were determined using the calculated slope of the terminal linear phase. Results below the assay's limit of detection were imputed as zero for first dose PK and half the lower limit of quantitation for steady-state PK. Correlation between repeated measures variables by site were computed from mixed models with random and repeated effects for site.

To calculate DTG rectal:plasma concentration ratios, rectal DTG concentrations were converted from ng/g to ng/mL assuming a tissue density of 1.06 ng/mL, then divided by plasma DTG concentrations. Median RT DTG concentrations were compared at Weeks 2, 6, and 12 using Wilcoxon Signed Rank test.

To determine whether DTG concentrations predicted RT RNA suppression, we performed two different analyses. First, we developed binary logistic regression models accounting for repeated measures where the event is rectal RNA suppression. A variance components covariance structure was assumed, and log transformed plasma, rectal, and rectal:plasma DTG concentration ratios were evaluated separately as predictors. Second, we compared PK parameters, including 1st dose and steady state DTG AUC_{0-24} , and steady-state RT concentrations at Weeks 2, 6, and 12, between those who had baseline detectable RT HIV RNA and achieved RT HIV-1 RNA suppression at any time point (RT suppressors) versus participants with persistent detectable RT HIV-1 RNA (RT non-suppressors) using Wilcoxon Rank Sum Tests. Finally, demographic and clinical characteristics between RT suppressors and non-suppressors were compared using Chi-square or Fisher's exact tests for categorical variables and Wilcoxon Rank Sum Test for continuous variables.

All statistical analyses were performed using SAS version 9.4 software (Cary, NC), with an alpha level of 0.05.

Role of the funding source

The study sponsors had no role in study design; collection, analysis, and interpretation of data; writing of the report; or in the decision to submit the paper for publication.

Results

Demographic and clinical characteristics

Seventy-one (71) individuals were assessed for eligibility; 21 were ineligible (12 not ART-naïve, 8 had severe anal/rectal disease, 1 desired other ART), 37 decline to participate, and thirteen were enrolled. Twelve (12) participants completed 2 study visits and were included in the analyses. Table 1 summarizes the clinical and demographic characteristics of the study population: 6 of 12 (50.0%) were female, 9/12 (75.0%) black, median age was

42.0 years (Q1 31.2, Q3 52.0), and 7/12 (58.3%) were diagnosed with HIV within the last year. Most common risk factors for HIV acquisition included unprotected heterosexual sex (8/12, 66.7%) and men who have sex with men (5/12, 41.6%). Median baseline BMI was 23.3 (Q1 20.2, Q3 29.7), CD4 279 cells/mm³ (Q1 150, Q3 371), and plasma HIV RNA was 4.5 log cop/mL (Q1 3.8, Q3 4.9). Most participants (58.3%) received TDF/FTC as their NRTI backbone.

HIV-1 RNA Quantitation

Forty-two (42) paired plasma and RT samples were available for HIV RNA analysis, with each participant providing at least 2 pairs. Fifty percent (6/12) of participants had HIV RNA below the assay's limit of detection in plasma by Week 2, and all participants (100%) were undetectable in plasma by Week 6, Figure 1a. In contrast, only four of 12 (33.3%) participants achieved undetectable HIV RNA in RT at any time point: Participant 1 became undetectable at Week 12, Participant 5 was undetectable at Week 6 but had rebound detectable rectal RNA at Week 12, and Participants 4 and 9 had persistent undetectable rectal RNA at three consecutive time points, Figure 1b. Median log RT RNA at weeks 6 and 12 was 5.4 cop/g (Q1, 1.3, Q3, 5.7) and 4.7 (2.0-5.5), respectively.

There was discordance of HIV RNA detection in 17/42 (40.5%) pairs: 15/17 (88.2%) of those pairs had undetectable plasma RNA and detectable rectal RNA and only 2/17 (11.8%) had detectable plasma RNA but undetectable rectal RNA. There was a moderate positive correlation between paired plasma and rectal tissue HIV RNA viral loads, $r = 0.59$.

Dolutegravir plasma and rectal tissue quantitation

First-dose DTG pharmacokinetic data was available for all 12 participants: median C_{max} was 2005 ng/mL (Q1 1170, Q3 2315), T_{max} was 1.5 hours (Q1 1.0, Q3 3.8), and AUC_{0-24h} was 22,301 h*ng/mL (Q1 15,133, Q3 30,845). Steady-state plasma PK data was available for 9 of 12 participants: C_{max} was 2337 ng/mL (Q1 1842-3035), T_{max} 3.0 hours (Q1 3.0-4.0), C_{τ} 560 ng/mL (Q1 435.6, Q3 1210), and AUC_{0-24h} was 27,240 h*ng/mL (Q1 24,475, Q3 48,075).

Thirty-two (32) of 42 plasma and RT pairs were collected after at least seven days of daily DTG administration and analyzed for steady-state DTG concentrations, with each participant providing at least one pair. DTG was detectable in 31/32 pairs (96.9%) of plasma and RT samples. Median DTG concentrations were significantly higher in plasma compared to RT at Week 2: 1880 ng/mL (Q1 1106, Q3 3921) vs 807 ng/mL (Q1 646, Q3 1420), $p=0.02$, and Week 6: 2810 ng/mL (Q1 697, Q3 4010) vs 661 ng/mL (Q1 271, Q3 1452), $p=0.007$, and non-significantly higher at Week 12: 1142 ng/mL (Q1 614, Q3 1484) vs 365 ng/mL (Q1 163, Q3 510), $p=0.06$. There was a moderate to strong positive correlation between paired BP and RT DTG concentrations: $r = 0.76$. There were no statistically significant differences in DTG RT, plasma, or RT:plasma concentration ratios at time points where RT RNA was undetectable versus detectable (Table 2).

Rectal tissue RNA suppressors versus non-suppressors

Characteristics of participants with baseline detectable RT HIV RNA who achieved RT HIV RNA suppression at any time point, $n = 3$, were compared to RT HIV RNA non-suppressors, $n = 8$. All 3 (100.0%) RT suppressors compared to three of 8 (37.5%) non-suppressors were females. Compared to RT non-suppressors, RT suppressors were older, with a median age of 52.4 years (range 50.5-54.2) vs 36.9 (24.3-53.6), $p=0.05$, had higher median baseline BMI, 35.9 kg/m²(range 24.9-38.5) versus 20.6 (17.7-29.9), $p=0.05$, and lower baseline log plasma HIV RNA: 3.7 cop/mL(range 3.6-4.4) versus 4.7(3.8-5.4), $p=0.02$. There was a trend toward lower baseline log RT HIV RNA in RT suppressors compared to non-suppressors: 5.4 log cop/mL (range 4.4-5.6) vs 6.8 (4.9-8.3), $p=0.08$, Table 3. No statistically significant differences were seen between RT suppressors and non-suppressors with regard to 1st dose or steady state plasma AUC_{0-24h}, Figure 2, or steady-state RT concentrations, Table 3.

Discussion

To our knowledge, this is the first study to show detailed early HIV RNA viral dynamics in plasma and RT following initiation of DTG-based ART in naïve men and women. Plasma virologic suppression with DTG-based therapy was rapid in all participants, consistent with what we know regarding INSTI potency.[13] In contrast, two-third of participants had persistent detectable HIV RNA in RT, even after achieving plasma virologic suppression. This is consistent with prior data showing recovery of HIV RNA from RT (ranging from 4.0 to 7.0 log copies RNA/gram) at 3 months despite plasma suppression in non-INSTI ART regimens.[7] One third of participants achieved HIV RT suppression at some point within the first 12 weeks of DTG-ART. In contrast, Asmuth et al[8] reported a higher proportion (15/25, 60%) of viral suppression in RT, but this study evaluated patients after 9 months of ART. Taken together, these data suggest that tissue RNA suppression is possible, albeit at a slower rate than plasma. While the clinical implications of time-to-virologic-suppression within tissue remains unclear, it is possible that prolonged HIV persistence in tissue impacts immune activation and inflammatory cascades, potentially contributing to increased morbidity in an aging HIV cohort.

First dose and steady-state DTG AUC_{0-24h} were lower than previously reported data[6, 14, 15], which may be due to our cohort being older with higher BMI, two factors associated with lower DTG exposure in a population PK study[16]. Median RT DTG concentrations were lower than paired plasma concentrations at all time points, consistent with literature reporting low RT concentrations relative to plasma in HIV seronegative men[6]. Effective distribution of drug into lymphatic tissues is dependent on drug physiochemical properties and intestinal expression of efflux transporters. First, hydrophobic drugs with log octanol water partition coefficient, log P, >5 and high molecular weight have greater absorption via the gastrointestinal lymphatic system[17]. DTG lacks these characteristics, with a low molecular weight of 441.36 g/mol and log P of 2.16 [18]. Secondly, the intestinal expression of drug transporters changes with both HIV infection and ART initiation: Gene and protein expression of p-glycoprotein is significantly increased in HIV-infected individuals receiving ART[19]. As a result, substrates for this transporter, such as DTG may have reduced transport into intestinal lymphatic tissues.

Despite low DTG penetration into RT, neither DTG RT concentrations nor rectal:plasma concentration ratios were statistically significant predictors of RT RNA suppression, consistent with findings examining other primarily non-INSTI medications.[8] Since drug concentration assessments at single time points are limited snapshots of tissue drug exposure, we cannot completely rule out the possibility that suboptimal DTG concentrations affected RT viral dynamics in this study. Ideally, tissue drug AUC₍₀₋₂₄₎ over a single dosing interval would be measured, however given the invasiveness of repeated tissue sampling, this type of analysis would only be possible using a population approach.

Our findings that RT non-suppressors had higher baseline plasma and RT RNA viral loads than RT-suppressors suggest that these compartments are linked. Higher baseline plasma viral loads (>100,000 copies/mL) are associated with residual low-level viremia detectable by single-copy PCR assays[20], and it is reasonable that this relationship would hold true in tissue sites. The finding of higher BMI in RT suppressors compared to RT non-suppressors is new. Being overweight was associated with lower risk of HIV progression in both HIV+ women and men,[21, 22] and higher BMI in women was associated with higher CD4 count and lower mean log viral load[22]. One theory is that the protective effect of obesity may be mediated by the immunomodulatory properties of the hormone leptin[23]. Leptin levels are directly proportional to fat cell mass and tend to be higher in women than men, even at comparable body fat levels[24]. Interestingly, all 3 RT suppressors in this study were women. While studies have shown that females have lower baseline plasma viral loads and faster time to plasma virologic suppression than males,[25] sex differences in HIV viral dynamics within tissues such as gut have not been well studied, largely due to the scarcity of women in HIV clinical research[26]. Mechanisms behind these differences have not been elucidated but theories include interactions between sex steroids and the immune system[27]. While our sample size was too small to conduct sex-stratified analyses, future studies powered to examine sex differences in HIV viral dynamics within tissues are planned.

Our study had several limitations. First, sample size was small with $n = 12$, limiting our ability to conduct multivariate analyses; nonetheless, participants provided 42 pairs of blood and tissue samples, allowing for robust assessments of viral dynamics and drug exposure not previously assessed in other studies. Second, adherence was self-reported in 50% of study visits, which is prone to recall bias. However, given that all participants attained plasma virologic suppression by week 6 with detectable DTG in 97% of blood and RT sample pairs, we can conclude that participants were routinely taking ART. Third, insufficient sampling of tissue could affect the ability to detect HIV RNA. While this is theoretically possible, we quantified HIV RNA in 35/42 (83%) of RT samples, and two participants had undetectable rectal HIV RNA at 3 sequential time points. These consistent results suggest our findings were accurate and less likely due to inadequate sampling.

Furthermore, we quantitated DTG concentrations from RT homogenates rather than isolating rectal cells. As tissue homogenates are composed of both extracellular and intracellular components, concentrations overestimate exposure in drugs with intracellular modes of action, such as DTG[28]. However, our study paired tissue homogenate drug concentrations with objective measures of effectiveness (i.e. HIV RNA) at multiple time-points for each

patient. Additionally, participants were receiving different NRTI backbones, and we did not measure NRTI drug concentrations. While ideal, enrolling participants receiving identical NRTI backbones would have been impossible given the subsequent approval of DTG/ABC/3TC and TAF/FTC mid-study. While TDF has been shown to attain very high rectal:plasma tissue concentrations compared to other NRTIs[29], 3/5 (60%) participants in our study receiving TDF/FTC had persistent detectable RT RNA. Even so, given the increasing use of TAF, an NRTI with significantly lower RT penetration[30], further studies are needed to assess the impact of TAF exposure on tissue viral dynamics. Finally, we cannot infer from these results whether the HIV RNA recovered is infectious or capable of replication. Studies including quantitative viral outgrowth and infectivity assays are needed and currently underway.

In conclusion, plasma and RT HIV viral dynamics were discordant in the majority of participants initiating DTG-ART. Neither plasma nor RT DTG concentrations were statistically significant predictors of RT HIV RNA suppression, although larger population pharmacokinetic-pharmacodynamic studies are needed to assess the role of tissue drug exposure in tissue viral dynamics or HIV persistence. Future studies should explore mechanisms, particularly sex-mediated, that may explain differences in compartmental HIV viral dynamics and inform strategies to reduce the HIV tissue reservoir.

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Figure 1. HIV-1 RNA viral dynamics over time for n = 12 participants in (a) blood plasma and (b) rectal tissue

Limit of assay detection (dashed black line) was 1.6 log copies/mL or copies/gram. All participants achieved plasma virologic suppression at Week 6 but only 4/12 (33%) achieved rectal tissue virologic suppression at any time-point (Participants 1, 4, 5, and 9).

Figure 2. Median first dose (a) and steady-state (b) dolutegravir (DTG) concentration-time curves in rectal tissue (RT) suppressors versus RT non-suppressors

RT suppressors (n=3) defined as individuals with baseline detectable RT HIV RNA who achieved RT HIV RNA <40 copies/gram at any time point, RT non-suppressors (n=8) were individuals with persistent detectable RT HIV RNA. Steady-state PK data available for 3/3 (100%) RT suppressors and 6/8 (75%) RT non-suppressors. No significant differences in 1st dose or steady-state plasma DTG pharmacokinetic parameters, including maximal concentration (C_{max}), time to maximal concentration (t_{max}), concentration at end of dosing

interval (C_{τ}), or area under the concentration time curve over the 24 hour dosing interval (AUC_{0-24h}) were seen between groups.

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Table 1

Baseline demographic and clinical characteristics (n = 12)

Variable	N (%) or median (Q1, Q3)
Age (years)	42.0 (31.2, 52.0)
Female sex at birth	6 (50.0)
Race	
Black	9 (75.0)
White	3 (25.0)
HIV risk factors ^a	
Men who have sex with men	5 (41.7)
Heterosexual sex	8 (66.7)
Intravenous drug abuse	2 (16.7)
Time since HIV Diagnosis	
< 1 year	7 (58.3)
1-5 years	3 (25.0)
> 5 years	2 (16.7)
HBV or HCV ^b co-infection	3 (25.0)
Body mass index (kg/m ²)	23.3 (20.2, 29.7)
Absolute CD4 count (cells/mm ³)	279 (150, 371)
Plasma HIV RNA log ₁₀ (copies/mL)	4.5 (3.8, 4.9)
NRTI ^c backbone initiated	
TDF/FTC ^d	7 (58.3)
TAF/FTC ^e	3 (25.0)
ABC/3TC ^f	2 (16.7)

^a Column percentages >100% due to participants reporting multiple risk factors;^b HBV, Hepatitis B Virus, HCV, Hepatitis C Virus;^c NRTI = nucleoside reverse transcriptase inhibitor;^d TDF/FTC = tenofovir disoproxil fumarate/emtricitabine;^e TAF = tenofovir alafenamide;^f ABC/3TC = abacavir/lamivudine

Table 2

Univariable logistic regression: Predictors of RT HIV RNA suppression^a

Predictor	β^b	S.E. ^c	P Value	e ^d (OR) ^d	95% CI ^e for OR
Model 1: log rectal DTG ^f concentration	0.68	0.55	0.24	1.96	0.67-5.78
Model 2: log plasma DTG concentration	0.12	0.38	0.75	1.13	0.54-2.36
Model 3: rectal:plasma DTG ratio	0.76	0.53	0.17	2.13	0.76-5.99

^a Binary logistic regression analysis performed accounting for repeated measures where event of interest = RT (rectal tissue) HIV RNA suppression (event = 1) vs detectable RT HIV RNA (event = 0);

^b β = coefficient;

^c S.E = standard of error of the coefficient;

^d OR = odds ratio;

^e CI = confidence interval;

^f DTG = dolutegravir

Table 3

Characteristics of rectal tissue HIV RNA suppressors vs non-suppressors

Variable	RT suppressor ^a (n = 3), n (%) or median (range)	RT non-suppressor (n = 8), n (%) or median (range)	p value ^c
Age (years)	52.4 (50.5-54.2)	36.9 (24.3-53.6)	0.05
Female sex at birth	3 (100.0)	3 (37.5)	0.18
Race			1.0
Black	3 (100.0)	6 (75.0)	
White	0 (0)	2 (25.0)	
Years since HIV diagnosis	0.4 (0.1-0.8)	0.5 (0.1-7.7)	0.79
Baseline body mass index (kg/m ²)	35.9 (24.9-38.5)	20.6 (17.7-29.9)	0.05
Baseline CD4 count (cells/mm ³)	357 (260-389)	254 (14-424)	0.50
Baseline plasma HIV RNA(log ₁₀ copies/mL)	3.7 (3.6-4.4)	4.7 (3.8-5.4)	0.02
Baseline rectal tissue HIV RNA (log ₁₀ copies/g)	5.4 (4.4-5.6)	6.8 (4.9-8.3)	0.08
NRTI ^d backbone			0.10
TDF/FTC ^e	1 (33.3)	5 (62.5)	
TAF/FTC ^f	0	3 (37.5)	
ABC/3TC ^g	2 (66.7)	0	
Rectal DTG ^{h,i} (ng/mL)			
Week 2	1606 (1420-1791)	749 (608-1559)	0.09
Week 6	724 (102, 1579)	625 (134-1452)	0.83
Week 12	473 (163, 12,369)	373 (2.65-621)	0.67
Rectal:Plasma DTG ratio			
Week 2	1.89 (0.33-3.45)	0.31 (0.12-2.00)	0.40
Week 6	0.26 (0.15-0.32)	0.24 (0.17-1.45)	0.57
Week 12	0.48 (0.17, 7.20)	0.44 (0.12, 1.22)	0.83

^aRT suppressor = rectal tissue suppressor, defined as participant with baseline detectable rectal HIV RNA who developed undetectable rectal tissue HIV RNA (<40 copies/gram) at any time-point;

^cDifferences between groups compared using Wilcoxon rank sum test or chi-square/Fisher exact as appropriate;

^dNRTI = nucleoside reverse transcriptase inhibitor;

^eTDF/FTC = tenofovir disoproxil fumarate/emtricitabine;

^fTAF = tenofovir alafenamide;

^gABC/3TC = abacavir/lamivudine;

^hDTG = dolutegravir, steady-state plasma and rectal samples were collected at random times post dose;

i rectal DTG concentrations were converted from ng/g to ng/mL assuming a tissue density of 1.06 ng/mL

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