PHARMACOKINETICS AND PHARMACODYNAMICS OF PRE-EXPOSURE PROPHYLAXIS AGAINST HIV

EDITED BY: Max Von Kleist, Peter L. Anderson, Gerardo Garcia-Lerma and Albert Liu

PUBLISHED IN: Frontiers in Pharmacology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-096-4
DOI 10.3389/978-2-88966-096-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

PHARMACOKINETICS AND PHARMACODYNAMICS OF PRE-EXPOSURE PROPHYLAXIS AGAINST HIV

Topic Editors:
Max Von Kleist, Robert Koch Institute (RKI), Germany
Peter L. Anderson, University of Colorado Anschutz Medical Campus, United States
Gerardo Garcia-Lerma, Centers for Disease Control and Prevention (CDC), United States
Albert Liu, San Francisco Department of Public Health, United States

Citation: Von Kleist, M., Anderson, P. L., Garcia-Lerma, G., Liu, A., eds. (2020). Pharmacokinetics and Pharmacodynamics of Pre-Exposure Prophylaxis Against HIV. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-096-4

Table of Contents

frontiers in Pharmacology

EDITORIAL
published: 26 August 2020
doi: 10.3389/fphar.2020.01288

Editorial: Pharmacokinetics and Pharmacodynamics of Pre-Exposure Prophylaxis Against HIV

Max von Kleist[1,2*], *J. Gerardo García-Lerma*[3], *Albert Liu*[4] *and Peter L. Anderson*[5]

[1] MF1 Bioinformatics, Methods Development and Research Infrastructure (MF), Robert Koch Institute, Berlin, Germany, [2] Systems Pharmacology & Disease Control, Department of Mathematics, Freie Universität Berlin, Berlin, Germany, [3] Laboratory Branch, Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, GA, United States, [4] Bridge HIV, San Francisco Department of Public Health, San Francisco, CA, United States, [5] Anschutz Medical Campus, University of Colorado, Denver, CO, United States

Keywords: PrEP, adherence, modeling, translational pharmacology, implementation

Editorial on the Research Topic

Pharmacokinetics and Pharmacodynamics of Pre-Exposure Prophylaxis Against HIV

BACKGROUND

In 2018 about 1.7 million individuals became infected with the human immunodeficiency virus (HIV)[1]. While therapies are highly effective in suppressing virus replication and reducing transmission, viral rebound generally occurs within weeks after treatment discontinuation (Chun et al., 2015). The establishment of a latent virus reservoir early in infection poses challenges for identifying effective HIV cure strategies. Vaccines have had limited success to date (Rerks-Ngarm et al., 2009; Caskey et al., 2019) although some promising strategies are under evaluation. While a major success in HIV research has been the development of highly effective antiretrovirals, a fruitful idea is to re-purpose those drugs for HIV prevention. Substantial progress has been made developing antiretroviral (ARV)-based strategies to prevent HIV transmission, including pre-exposure prophylaxis (PrEP) (Grant et al., 2010). PrEP with oral FTC in combination with tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) is an established prevention strategy to protect certain populations at risk of HIV acquisition.

By inhibiting early virus replication, PrEP drugs increase the chance of virus elimination before a new host is irreversibly infected. However, viral inhibition—and thus PrEP efficacy—largely depends on the drugs' concentration at the target site. While initial PrEP trials with once daily oral FTC/TDF estimated a moderate efficacy based on an intent-to-treat analysis (see[2] for an overview), subsequent analyses indicated that if individuals adhere to the once-daily regimen, protection levels of 80–99% may be reached (Grant et al., 2014). These analyses revealed a certain level of pharmacologic forgiveness with variable adherence, which was evident in the IPERGAY trial that showed high efficacy for evident-driven dosing (Antoni et al., 2020).

This Research Topic compiles articles addressing pharmacokinetic/pharmacodynamic (PK/PD) aspects of PrEP. A particular focus is on PrEP adherence, on translational research to predict PrEP efficacy, as well as innovative approaches to dispensing clinical trial PrEP drugs.

[1] UNAIDS: FACT SHEET - GLOBAL AIDS UPDATE 2019 (https://www.unaids.org/en/resources/fact-sheet).
[2] Global Advocacy for HIV prevention, https://www.avac.org/sites/default/files/u3/By_The_Numbers_PrEP.pdf.

OPEN ACCESS

Edited and reviewed by:
Alastair George Stewart,
The University of Melbourne, Australia

****Correspondence:***
Max von Kleist
kleistm@rki.de

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *09 July 2020*
Accepted: *04 August 2020*
Published: *26 August 2020*

Citation:
von Kleist M, García-Lerma JG, Liu A and Anderson PL (2020) Editorial: Pharmacokinetics and Pharmacodynamics of Pre-Exposure Prophylaxis Against HIV. Front. Pharmacol. 11:1288. doi: 10.3389/fphar.2020.01288

PREP ADHERENCE

Mallayasamy et al. analyzed data from 920 individuals to identify demographic, as well as socio-behavioral factors associated with PrEP adherence in sero-discordant couples in East Africa. They found that older age, female gender, and sexual activity were associated with increased adherence to FTC/TDF, whereas having a partner on ART >6 months, being in the study for >6 months, and problematic alcohol use were associated with lower adherence. These data were gathered objectively using electronic adherence monitoring systems, which may not be available in other PrEP implementation studies and broader roll-out. How can adherence be assessed in real-world settings to interpret and analyze PrEP outcomes? Blumenthal et al. investigated, in a group of HIV-uninfected men who have sex with men (MSM) from the TAPIR study, which self-reported adherence questions correlate with objective measures of drug adherence, as measured by tenofovir diphosphate (TFV-DP) concentrations in dried blood spots (DBS). They found that answers to the question "Thinking about the past 4 weeks, what percentage of the time were you able to take all your PrEP medications" were most strongly associated with objective adherence measurements in this cohort. Pyra et al. analyzed whether TFV-DP levels in DBS, which can be conveniently collected and stored, correlate well with the history of drug intake measured by electronic adherence systems. Lalley-Chareczko et al. analyzed whether tenofovir (TFV) levels in urine after administration of TAF may be a good indicator of recent drug adherence. TAF is a tenofovir prodrug approved for PrEP in combination with FTC for high-risk men and transgender women who have sex with men. Lalley-Chareczko et al. found that urine levels of TFV persist at detectable concentrations in participants taking TAF for at least 7 days despite largely undetectable plasma TFV concentrations, suggesting that urine may be a good marker for recent drug adherence.

TRANSLATIONAL PREP RESEARCH

Quantifying PrEP efficacy from clinical data requires determining the relative rate of seroconversion in the intervention *vs.* control arm. A major statistical challenge arises from the fact that sexual HIV transmission probabilities are extremely low (Royce et al., 1997) and hence the number of seroconversions in a clinical trial, which is used to calculate efficacy is prone to statistical errors (Dunn and Glidden, 2016). Consequently, PrEP development requires making use of the entire translational research toolbox. Herrera discussed the toolbox of *in vitro* and *ex vivo* models used to screen PrEP compounds, characterize their pharmacology, evaluate their safety, and determine target drug levels. Additionally, computational modeling approaches offer flexible and powerful tools to study drug behavior, integrate different data sources, and predict clinical endpoints. Duwal et al. used an integrated pharmacokinetic-viral dynamic computer model (Duwal et al., 2018). They first developed a pharmacokinetic (PK) model for efavirenz based on *in vitro* and *clinical* data from the ENCORE-1 study. Subsequently, they integrate this PK model with a stochastic virus response model to predict the prophylactic efficacy of efavirenz following different dosing schemes. Based on these simulations, they propose that low-dose efavirenz could have high clinical efficacy as PrEP. Straubinger et al. give an overview of mathematical modeling efforts. They summarize approaches for approved and currently developed PrEP drugs from a PK/PD perspective, as well as approaches that connect pharmacology and viral dynamics to ultimately predict PrEP efficacy in relation to drug dosing and provide a short outlook on epidemiological modeling of PrEP efficacy.

TRIAL DESIGN

Lastly, Lal et al. studied in Australia, whether community based pharmacies, which offer more convenience for study participants, can undergo training and modifications to achieve good clinical practice compliance to dispense clinical trial study drugs. Overall, they recorded very few deviations from study protocols, indicating that community-based pharmacies should be considered in HIV prevention trials.

CONCLUSION

PrEP is a very active field of investigation at the intersection of pharmacology, behavior, and public health. When taken as prescribed, oral PrEP is highly effective although inadequate adherence reduces efficacy and public health benefit. The current issue provides a snapshot of current research activities with a focus on PK/PD aspects. As the PrEP field continues to evolve, interest is now shifting to long-acting drug formulations and sustained drug delivery systems that can overcome some of the adherence issues associated with daily PrEP.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

MK acknowledges support from the BMBF projects "Meth4SysPharm," as well as "Trans-PrEP" (grant numbers 031A307, 01KI2016). AL is supported by several grants from the National Institutes of Health and the California HIV Research Program. He has also received research funding from Gilead Sciences and Viiv Healthcare to conduct investigator sponsored

research and has led studies in which Gilead had donated study drug. He has also received support from IAS-USA for manuscript preparation and Practice Point Communications for conducting continuing education activities. PA is supported by NIH R01 AI122298.

REFERENCES

Antoni, G., Tremblay, C., Delaugerre, C., Charreau, I., Cua, E., Rojas Castro, D., et al. (2020). On-demand pre-exposure prophylaxis with tenofovir disoproxil fumarate plus emtricitabine among men who have sex with men with less frequent sexual intercourse: a post-hoc analysis of the ANRS IPERGAY trial. *Lancet HIV* 7 (2), e113–e120. doi: 10.1016/S2352-3018(19)30341-8

Caskey, M., Klein, F., and Nussenzweig, M. C. (2019). Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic. *Nat. Med.* 25 (4), 547–553. doi: 10.1038/s41591-019-0412-8

Chun, T.-W., Moir, S., and Fauci, A. S. (2015). HIV reservoirs as obstacles and opportunities for an HIV cure. *Nat. Immunol.* 16 (6), 584–589. doi: 10.1038/ni.3152

Dunn, D. T., and Glidden, D. V. (2016). Statistical issues in trials of preexposure prophylaxis. *Curr. Opin. HIV AIDS* 11 (1), 116–121. doi: 10.1097/COH.0000000000000218

Duwal, S., Dickinson, L., Khoo, S., and von Kleist, M. (2018). Hybrid stochastic framework predicts efficacy of prophylaxis against HIV: An example with different dolutegravir prophylaxis schemes. *PloS Comput. Biol.* 14 (6), e1006155. doi: 10.1371/journal.pcbi.1006155

Grant, R. M., Lama, J. R., Anderson, P. L., McMahan, V., Liu, A. Y., Vargas, L., et al. (2010). Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl. J. Med.* 363 (27), 2587–2599. doi: 10.1056/NEJMoa1011205

Grant, R. M., Anderson, P. L., McMahan, V., Liu, A., Amico, K. R., Mehrotra, M., et al. (2014). Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect. Dis.* 14 (9), 820–829. doi: 10.1016/S1473-3099(14)70847-3

ACKNOWLEDGMENTS

We would like to thank the Frontiers editorial staff, all the authors who contributed excellent papers, and the reviewers whose work has made publication of this Research Topic possible.

Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., Kaewkungwal, J., Chiu, J., Paris, R., et al. (2009). Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl. J. Med.* 361 (23), 2209–2220. doi: 10.1056/NEJMoa0908492

Royce, R. A., Seña, A., Cates, W. Jr, and Cohen, M. S. (1997). Sexual transmission of HIV. *N Engl. J. Med.* 336 (15), 1072–1078. doi: 10.1056/NEJM199704103361507

Conflict of Interest: GG-L is named in US Government patents on "Inhibition of HIV infection through chemoprophylaxis" and in US Government patent applications on "HIV post-exposure prophylaxis" and "HIV pre-exposure prophylaxis." The findings and conclusions of this manuscript are those of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention. AL has received research funding from Gilead Sciences and Viiv Healthcare to conduct investigator sponsored research and has led studies in which Gilead had donated study drug. PA has received personal fees and research funding from Gilead Sciences.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 13 March 2019
doi: 10.3389/fphar.2019.00199

The Utility of Efavirenz-based Prophylaxis Against HIV Infection. A Systems Pharmacological Analysis

Sulav Duwal[1†], *Daniel Seeler*[1†], *Laura Dickinson*[2], *Saye Khoo*[2] *and Max von Kleist*[1*]

[1] *Department of Mathematics and Computer Science, Systems Pharmacology and Disease Control, Institute of Bioinformatics, Freie Universität Berlin, Berlin, Germany,* [2] *Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom*

Pre-exposure prophylaxis (PrEP) is considered one of the five "pillars" by UNAIDS to reduce HIV transmission. Moreover, it is a tool for female self-protection against HIV, making it highly relevant to sub-Saharan regions, where women have the highest infection burden. To date, Truvada is the only medication for PrEP. However, the cost of Truvada limits its uptake in resource-constrained countries. Similarly, several currently investigated, patent-protected compounds may be unaffordable in these regions. We set out to explore the potential of the patent-expired antiviral efavirenz (EFV) as a cost-efficient PrEP alternative. A population pharmacokinetic model utilizing data from the ENCORE1 study was developed. The model was refined for metabolic autoinduction. We then explored EFV cellular uptake mechanisms, finding that it is largely determined by plasma protein binding. Next, we predicted the prophylactic efficacy of various EFV dosing schemes after exposure to HIV using a stochastic simulation framework. We predicted that plasma concentrations of 11, 36, 1287 and 1486ng/mL prevent 90% sexual transmissions with wild type and Y181C, K103N and G190S mutants, respectively. Trough concentrations achieved after 600 mg once daily dosing (median: 2017 ng/mL, 95% CI:445–9830) and after reduced dose (400 mg) efavirenz (median: 1349ng/mL, 95% CI: 297–6553) provided complete protection against wild-type virus and the Y181C mutant, and median trough concentrations provided about 90% protection against the K103N and G190S mutants. As reduced dose EFV has a lower toxicity profile, we predicted the reduction in HIV infection when 400 mg EFV-PrEP was poorly adhered to, when it was taken "on demand" and as post-exposure prophylaxis (PEP). Once daily EFV-PrEP provided 99% protection against wild-type virus, if ≥50% of doses were taken. PrEP "on demand" provided complete protection against wild-type virus and prevented ≥81% infections in the mutants. PEP could prevent >98% infection with susceptible virus when initiated within 24 h after virus exposure and continued for at least 9 days. We predict that 400 mg oral EFV may provide superior protection against wild-type HIV. However, further studies are warranted to evaluate EFV as a cost-efficient alternative to Truvada. Predicted prophylactic concentrations may guide release kinetics of EFV long-acting formulations for clinical trial design.

Keywords: PrEP, modeling, PK-PD, translation, repurposing, resource-constrained, cost-efficient, PEP

OPEN ACCESS

Edited by:
Lei Xi,
VCU School of Medicine, Virginia Commonwealth University, United States

Reviewed by:
Collet Dandara,
University of Cape Town, South Africa
Ayyappa Chaturvedula,
University of North Texas Health Science Center, United States

***Correspondence:**
Max von Kleist
max.kleist@fu-berlin.de

[†]These authors share first authorship

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *14 December 2018*
Accepted: *18 February 2019*
Published: *13 March 2019*

Citation:
Duwal S, Seeler D, Dickinson L, Khoo S and von Kleist M (2019) The Utility of Efavirenz-based Prophylaxis Against HIV Infection. A Systems Pharmacological Analysis. Front. Pharmacol. 10:199. doi: 10.3389/fphar.2019.00199

1. INTRODUCTION

The ambitious goals formulated by UNAIDS are to end AIDS by 2030 (UNAIDS, 2017). However, unlike many other infections, no cure is available to clear HIV infection. Ending AIDS therefore heavily relies on strategies to reduce the number of new HIV infections from an estimated 2.1 million in 2014 (UNAIDS, 2015) to 500,000 cases by 2020 and to fewer than 200,000 by 2030 (UNAIDS, 2016). While a vaccine would be the ideal tool for the purpose, intrinsic difficulties have so far precluded the development of an effective vaccine against HIV. Despite these setbacks, the development of about 30 antiviral compounds to stop HIV replication has been an overwhelming success (Gulick, 2018) in HIV research.

In light of the current situation, recent years have seen an increasing interest in utilizing antivirals not only for treatment, but also to prevent HIV transmission. Two general strategies are currently investigated for this purpose:

(i) Treatment-as-prevention (TasP) intends to put individuals with an HIV diagnosis immediately on treatment, which essentially makes them non-contagious (Cohen et al., 2011). However, a major limitation of this approach is that HIV is typically transmitted early after infection (Brenner et al., 2007; Yousef et al., 2016), when the recently infected individual is unaware of his/her HIV status and has consequently not initiated TasP. Thus, maximizing the epidemiological impact of TasP also requires to improve HIV diagnosis, which is a central component of the 90-90-90 strategy (UNAIDS, 2017).

(ii) Pre-exposure prophylaxis (PrEP) acts on the viral dynamics in the virologically challenged individual immediately after virus exposure. Akin to a vaccination, PrEP increases the probability that transmitted virus gets cleared, protecting individuals from becoming irreversibly infected. However, unlike vaccination, PrEP protection is a direct function of the concentration of prophylactic drugs at the target site.

Once-daily oral PrEP with the drug combination Truvada (tenofovir disoproxil fumarate-emtricitabine) has been approved since 2012 in the US and since 2016 in the EU. Initial clinical studies with Truvada demonstrated its utility as a PrEP agent (Grant et al., 2010), while subsequent studies indicated that the efficacy of Truvada-based PrEP was highly dependent on the individual's adherence to the once daily regimen. While it is difficult to quantify PrEP adherence clinically (Haberer et al., 2015), efficacy estimates in apparently highly adherent individuals were 86–100% in the IPrEx OLE study, 58–96% in the PROUD study and 96% in the Partners PrEP OLE study (Grant et al., 2014; McCormack et al., 2016). The VOICE and FEM-PrEP studies indicated that Truvada may not prevent infection in poorly adherent individuals, i.e., if 30% of individuals had detectable drug in their blood plasma (Van Damme et al., 2012; Marrazzo et al., 2015). Mathematical modeling of Truvada-based PrEP (Duwal et al., 2016) established the precise relationship between drug pharmacokinetics and prophylactic efficacy confirming many clinical observations (i.e., quantifying the prophylactic efficacy to be $\approx$ 96% in fully adherent individuals).

While adherence is a major current concern that motivates the identification of novel long-acting drug candidates and optimized deployment strategies (AVAC, 2019), a currently neglected factor is the cost of PrEP, with the majority of HIV infections occurring in resource-constrained countries (UNAIDS, 2016). Keller and Smith (2011) noted that the price of Truvada currently undermines the advancement of pre-exposure prophylaxis, particular in resource-constrained settings. Yet regrettably, current PrEP research focusses entirely on patent-protected compounds (AVAC, 2019). This makes it unlikely that a current, or next-generation PrEP regimen will become broadly implemented in resource-constrained regions where they could benefit most. Moreover, PrEP is the only strategy by which women can protect themselves against HIV infection, making PrEP highly relevant in regions like sub-Saharan Africa, where young women are the most relevant target group to halt the ongoing spread of HIV (Dellar et al., 2015; Maxmen, 2016), accounting for $\approx$ 7000 infections per week (Mathur et al., 2016).

A natural progression would therefore be whether currently neglected, patent-expired compounds might make good candidates for PrEP repurposing. Based on an initial computational assessment of potential candidates (Duwal et al., 2019), we focus herein on the patent-expired non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV), which is successfully used in HIV treatment, particularly in resource-constrained settings, where it costs as little as 0.1US$ per day. To this end, we assess efavirenz pharmacokinetics, consider its mode of action and establish the relationship between pharmacokinetics and prophylactic efficacy. Since reduced-dose (400 mg) efavirenz has a considerably improved safety profile, we assess the prophylactic efficacy of 400 mg oral EFV when used in chronic PrEP, PrEP on demand and post-exposure prophylaxis (PEP).

2. PATIENTS

A previously developed population pharmacokinetic (PK) model, constructed using data collected as part of ENCORE 1 was used. ENCORE 1 was a multi-center, double-blind, placebo-controlled trial designed to compare standard dose efavirenz (600 mg once daily) to a reduced dose (400 mg once daily) in HIV-infected, treatment-naive adults. Patients recruited at sites across Africa, Asian, South America, Europe and Oceania were randomized (1:1) to receive efavirenz 600 or 400 mg once daily in combination with tenofovir disoproxil fumarate/emtricitabine (Truvada, 300/200 mg once daily) (ENCORE1 Study Group, 2014; ENCORE1 Study Group et al., 2015).

At weeks 4 and 12 of therapy, single random blood samples were drawn between 8-16 hours post-dose, additionally intensive sampling was undertaken in a subgroup of patients between weeks 4 and 8 [pre-dose (0 h), 2, 4, 8, 12, 16 and 24 h post-dose]. Plasma efavirenz was quantified using a validated HPLC-MS/MS method (Amara et al., 2011). Overall, 606 patients (n=131, 32% female) randomized to efavirenz 600 mg (n = 311) and 400 mg once daily (n = 295) contributed 1491 samples for model development [median (range) 2 (1–9) per patient].

Median (range) age and weight were 35 years (18–69) and 65kg (39–148) and baseline viral load ranged between 162 and 10,000,000 copies/mL. The majority of patients were of African and Asian ethnicity (37 and 33%, respectively) with the remainder identifying as Hispanic (17%), Caucasian (13%) and Aboriginal and Torres Strait Islander (0.2%).

3. METHODS

3.1. Efavirenz Pharmacokinetics

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor that is frequently used in first-line therapy in resource-constrained regions in combination with emtricitabine (FTC) and tenofovir disoproxil fumerate (TDF) for treatment of HIV infection. EFV is a small (molecular mass: 315.6 g/mol) lipophilic (LogP ≈ 4) compound that is highly bound to plasma proteins (human serum albumin and α-1-acid glycoprotein). The unbound fraction of the drug in human plasma (f_u) is $<$ 1% (Almond et al., 2005; Fayet et al., 2008; Burhenne et al., 2010; Avery et al., 2011, 2013a). Efavirenz is a known inducer of various CYP-P450 enzymes (Fichtenbaum and Gerber, 2002), including *CYP2B6*, which is the main enzyme mediating its own metabolism (Ward et al., 2003; Ogburn et al., 2010). Moreover, it is known that CYP-P450 polymorphisms, in particular *CYP2B6* can lead to large inter-individual variations in EFV concentrations (Orrell et al., 2016). We derived statistical models for the inter-individual variability in plasma pharmacokinetic profiles, particularly taking CYP P450 polymorphisms (*CYP2B6* and *CYP2A6*) in a representative population (ENCORE 1) into account. Furthermore, we modeled metabolic autoinduction and established the relationship between plasma- and target-site concentrations.

3.1.1. Pharmacokinetic Model Building

The population pharmacokinetic analysis of ENCORE 1 has previously been reported (Dickinson et al., 2015, 2016). Briefly, nonlinear mixed effects modeling using NONMEM (v. 7.2; ICON Development Solutions, Ellicott City, MD, USA) was applied to the efavirenz concentration-time data using FOCE-I. The impact of the following covariates on efavirenz apparent oral clearance (CL/F_{bio}) was investigated: age, weight, fat-free mass (FFM), body mass index (BMI), sex, ethnicity and CYP P450 genotypes *CYP2B6* 516G>T, *CYP2B6* 983T>C, *CYP2B6* 15582C>T, *CYP2A6**9B, *CYP2A6**17, *CYP3A4**22, *NR1I3* 540C>T and *NR1I3* 1089T>C. Specifically, of the 606 patients with PK data, 95% had a blood sample for genotyping (n=574), although amplification failed for a small number of individuals (*CYP2B6* 15582C>T and *CYP3A4**22, n=1; *CYP2A6**17, n=2; *CYP2A6**9B, n=4). To drive the PrEP simulations, the final model was used to simulate PK parameters of 1000 virtual patients receiving efavirenz using the same distribution of significant covariates as the original dataset. PK parameters of all virtual patients are summarized in **Supplementary Table 1**.

Efavirenz concentrations over time were best described by a 1 compartment model parameterized by apparent oral clearance [population value of CL/F_{bio}; estimate (RSE%): 11.9L/h (2.4%) for the reference (wild-type) CYP genotype for all four SNPs; *CYP2B6*: 516G>T/983T>C/*CYP2A6**9B/*17 of a 70kg weighing individual], apparent volume of distribution [population mean V/F_{bio}; 282 L (5.2%)] and absorption rate constant k_a fixed to a value of $0.6h^{-1}$ (Arab-Alameddine et al., 2009):

$$\frac{d}{dt}Z_1 = -k_a \cdot Z_1 \tag{1}$$

$$\frac{d}{dt}D_{i,j} = \frac{k_a \cdot Z_1}{V_i/F_{bio}} - \frac{CL_{i,j}(t)/F_{bio}}{V_i/F_{bio}} \cdot D_{i,j} \tag{2}$$

whereby Z_1 denotes the amount of drug in the dosing compartment. The variable of interest is the concentration in the blood plasma (central compartment), i.e., D. Dosing events were modeled as impulse inputs, with

$$Z_{1,t} = Z_{1,t} + dose_k, \tag{3}$$

whenever the current simulation time t coincided with a dosing event τ_k. In the equations above, $CL_{i,j}(t)/F_{bio}$ denotes the bioavailability-adjusted, individual drug clearance at occasion j and k_a denotes the rate of drug uptake. The term V_i/F_{bio} is the bioavailability-adjusted volume of distribution of individual i. Interindividual and interoccasion variability was supported on CL/F_{bio} [36.6% (10.8%) and 21.0 (27.7%), respectively] and residual error was defined by a proportional model [20% (8.6%)]. CL/F_{bio} and V/F_{bio} were allometrically scaled by weight (centered on 70 kg) and *CYP2B6* 516G>T/983T>C/*CYP2A6**9B/*17 composite genotype significantly reduced efavirenz CL/F_{bio} between 4.5-82% , depending on allele combinations, compared to the reference genotype. Pharmacokinetic parameters for a 70 kg individual with reference genotype are summarized in **Table 1.** Overall, there were 16 genotype subgroups (**Supplementary Table 2**). Grouping of patients as extensive, intermediate or slow metabolisers (see below) as part of the modelling process (or after the final model was obtained) did not impact individual parameter estimates. The reduced genotype groups were defined as follows: (i) extensive metabolisers, (ii) intermediate metabolisers and (iii) slow metabolisers as detailed in Dickinson et al. (2015).

For initial model building clearance was assumed to reflect values *after* metabolic autoinduction since pharmacokinetic data was collected at weeks 4 and 12 of therapy. In the following, we consider the autoinduction explicitly, since it affects PrEP efficacy shortly after its initiation (e.g., "PrEP on demand").

3.1.2. Metabolic Autoinduction

In our work, we modeled metabolic autoinduction similarly to the model proposed by Zhu et al. (2009). We defined the term α as the ratio of the mean clearance on day 1 to the mean clearance at steady state (after autoinduction). The clearance ratio α is then computed as $\alpha = \frac{\mathbb{E}_i(CL_{i,t_0})}{\mathbb{E}_i(CL_{i,SS})}$ where the *average* clearance on the first day $\mathbb{E}_i(CL_{i,t_0}) = 5.76$L/h was taken from Zhu et al. (2009) and the *average* clearance at steady state $\mathbb{E}(CL_{i,SS}) = 9.86$L/h was computed from the virtual patient population (**Supplementary Table 1**), deriving $\alpha = 0.58$. For each virtual patient generated from the population pharmacokinetic model,

TABLE 1 | Pharmacokinetic parameters.

Parameter	Value	Unit	Parameter	Value	Unit
CL_{ss}/F_{bio}	11.9	L/h	V/F_{bio}	282	L
α	0.58	–	σ	0.20	–
$CV_{IIV}(CL_{ss}/F_{bio})$	36.6	%	$CV_{IOV}(CL_{ss}/F_{bio})$	21.0	%

*The table displays the pharmacokinetic parameter estimates for a 70 kg individual with reference genotype (reference: CYP2B6, pos. 516:GG, pos. 516:TT and for CYP2A *9B and *17: CC/CC or CC/CT or CC/TT or CA/CC or CA/CT or AA/CC or AA/CT Dickinson et al., 2015). Inter-individual variability (IIV), as well as inter-occasional variability (IOV) was included on drug clearance CL/F_{bio}. These parameters were log-normal distributed with coefficient of variation [%] $CV = 100 \cdot \sqrt{e^{\sigma^2} - 1}$, where σ^2 is the variance of the associated normal distribution. Weight was considered to affect $CL_{ss}(i)/F_{bio} = CL_{ss}/F_{bio} \cdot (\text{weight}(i)/70)^{0.75}$ and the volume of distribution $V(i)/F_{bio} = V/F_{bio} \cdot (\text{weight}(i)/70)$ through allometric scaling. Residual variability was described by a proportional error model ($\sigma = 0.2$).*

the individual clearance at steady state was available and the clearance at day 1 was computed using $CL_{i,t_0} = \alpha \cdot CL_{i,SS}$. Zhu et al. (2009) proposed a model for time-dependent autoinduction that we used herein

$$CL_i(t) = CL_{i,t_0} + \left(CL_{i,SS} - CL_{i,t_0}\right) \cdot \frac{t - t_0}{(t - t_0) + T_{50}} \qquad (4)$$

where $CL_i(t)$ is the individual clearance rate at the time t and t_0 is the time of the first EFV dose. $CL_i(0)$ and $CL_{i,SS}$ represent the clearance rates at day 1 and at steady state. The term $T_{50} = 245$h (Zhu et al., 2009) is the time where the clearance rate reaches half of its steady-state value.

3.1.3. Target-site Concentrations

The general perception is that only the free/unbound intracellular concentration at the site of action (intracellular space) is available to exert an antiviral effect (Smith et al., 2010). For highly lipophilic drugs like EFV, passive diffusion may be the dominating transport mechanisms and therefore the *unbound/free* drug concentrations are identical on both sides of biomembranes, whereas the relation between the *total* concentrations can be computed by considering unspecific drug retention by e.g. binding to plasma proteins or lipids. These assumptions are implemented in so called partition coefficient models commonly used in physiologically based pharmacokinetic modeling, see von Kleist and Huisinga (2007) for an overview. To test whether EFV is dominantly transported into cells by passive diffusion/equilibrating transport we implemented partition coefficient models and compared the predictions with intracellular concentration measurements in **Supplementary Text 1**. We found overwhelming evidence for passive diffusion/equilibrating transport as the dominating mechanism of cellular drug uptake. Moreover, under passive diffusion and unspecific drug retention, there is a direct proportionality between plasma concentrations and concentrations at the site of action. This proportionality implies that we can model the effect of EFV based on plasma drug concentrations (derivations in **Supplementary Text 1**).

3.2. Direct Effects

We modeled the *direct* effect of efavirenz using the sigmoidal Emax-equation (Chou, 2006)

$$\eta_D(t) = \frac{D_t^m}{IC_{50}^m + D_t^m}, \qquad (5)$$

TABLE 2 | Pharmacodynamic parameters.

Strain	IC_{50} (± sd)	m (± sd)	*f*
wild type	5.4 (± 0.9)	1.69 (±0.08)	1
Y181C	$2.8 \cdot IC_{50}$(wt)	$0.9 \cdot m$(wt)	0.78
K103N	$89.1 \cdot IC_{50}$(wt)	$0.83 \cdot m$(wt)	0.74
G190S	$72.1 \cdot IC_{50}$(wt)	$0.6 \cdot m$(wt)	0.24

*The table displays the pharmacodynamic parameters for wild type (Shen et al., 2008) and different viral mutants (Sampah et al., 2011). The hill coefficient m (unit less) was assumed to be normal distributed and IC_{50} values (nmol/L) were assumed to be log-normal distributed (Jilek et al., 2012). Parameters were corrected for protein binding as outlined in **Supplementary Text 1**. Parameter f (unit less) denotes the fitness of the respective strains. The respective parameter distributions for the mutants (IC_{50}, m) were computed by assuming an identical coefficient of variation as compared to the wild type.*

where D_t is the plasma concentration of the drug at time t, which is directly proportional to the target-site concentration (previous section and **Supplementary Text 1**) and the term IC_{50} and m denote the plasma concentration at which the targeted process is inhibited by 50% and a hill coefficient (Shen et al., 2008), respectively. Parameters are displayed in **Table 2** for wild type, K103N, Y181C and G190S mutants together with their standard deviation. Note that the equation above couples the stochastic viral dynamics (below) to the deterministic pharmacokinetics (above). The hill coefficient m and 50% inhibitory concentration IC_{50} have been measured *ex vivo* using single-round infection assays in primary human peripheral blood mononuclear cells, supplemented with 50% human serum for *wild-type* HIV and various resistance mutations (K103N, Y181C and G190S) (Shen et al., 2008; Sampah et al., 2011). Since the *ex vivo* assay was performed with 50% human serum, the measured IC_{50} has to be corrected for protein content, since the drugs' potency might otherwise be overestimated, particularly for highly protein bound drugs like EFV. The IC_{50} correction is demonstrated in **Supplementary Text 1**, together with a sensitivity analysis with regard to uncertainties in measuring the unbound fraction of EFV in human blood plasma.

3.3. Viral dynamics.

We adopted the viral dynamics model described in von Kleist et al. (2010) and von Kleist et al. (2011). Long-lived and latently infected cells are only implicitly considered (outlined at the end of the section), motivated by the observation that

transmitted viruses are not macrophage-tropic (Isaacman-Beck et al., 2009; Ping et al., 2013) and in line with related modeling approaches (Tan and Wu, 1998; Stafford et al., 2000; Perelson, 2002; Tuckwell et al., 2008; Conway et al., 2013). Although this model is a simplified representation of the molecular events happening during virus replication, it allows to accurately and mechanistically describe the effect of all existing antiretroviral drug classes on viral replication, as previously reported in (e.g., Duwal and von Kleist, 2016), and can be parameterized by *in vitro* and *clinical* data, **Table 3**. The modeled viral replication cycle consists of free infectious viruses V, uninfected T-cells (T_u), early infected T-cells (T_1) and productively infected T-cells (T_2). Early infected T-cells (T_1) and productively infected T-cells (T_2) denote T-cells prior- and after proviral integration, respectively, where the latter produces virus progeny. During the onset of infection the number of viruses is relatively low and the number of uninfected T-cells T_u is fairly unaffected by viral dynamics (Perelson et al., 1993; Tan and Wu, 1998; Pearson et al., 2011). We thus consider $T_u = \lambda_T/\delta_T$ to be constant over the course of simulations. The stochastic dynamics of viral replication after virus exposure are then defined by six reactions:

$$
\begin{aligned}
a_1(D_t) &= \left(\mathrm{CL_V} + \mathrm{CL_T}(D_t, \mathrm{mut}) \cdot \mathrm{T_u}\right) \cdot V_t && \text{(clearance of free virus; } V \to *) && (6)\\
a_2 &= \left(\delta_{\mathrm{PIC}} + \delta_{\mathrm{T_1}}\right) \cdot \mathrm{T}_{1,t} && \text{(clearance of early infected cell; } \mathrm{T_1} \to *) && (7)\\
a_3 &= \delta_{\mathrm{T_2}} \cdot \mathrm{T}_{2,t} && \text{(clearance of late infected cell; } \mathrm{T_2} \to *) && (8)\\
a_4(D_t) &= (1 - \eta_D(t)) \cdot \beta \cdot f(\mathrm{mut}) \cdot \mathrm{T_u} \cdot V_t && \text{(infection of a susceptible cell; } V \to \mathrm{T_1}) && (9)\\
a_5 &= k \cdot \mathrm{T}_{1,t} && \text{(proviral integration; } \mathrm{T_1} \to \mathrm{T_2}) && (10)\\
a_6 &= \mathrm{N_T} \cdot \mathrm{T}_{2,t} && \text{(production of virus; } \mathrm{T_2} \to \mathrm{V} + \mathrm{T_2}), && (11)
\end{aligned}
$$

with $\mathrm{CL_T}(D_t, \mathrm{mut}) = \left(\frac{1}{\rho_{\mathrm{rev}}} - (1 - \eta_D(t))\right) \cdot \beta \cdot f(\mathrm{mut})$ in Equation (6), as outlined in von Kleist et al. (2010) where $\rho_{rev} = 0.5$ denotes the probability to successfully complete reverse transcription in the absence of inhibitors (Pierson et al., 2002; Zhou et al., 2005) and $f(\mathrm{mut})$ denotes the fitness of the mutant. Free viruses can be cleared within T-cells during unsuccessful infection with rate $\mathrm{CL_T}$ by destruction of essential viral components of the reverse transcription-, or pre-integration complex (Pierson et al., 2002; Zhou et al., 2005) or it may get cleared by the immune system with a rate constant $\mathrm{CL_V}$. Further, the term β represents the lumped rate of infection of T-cells, including the processes of virus attachment to the cell, fusion and reverse transcription, leading to an early infected cell T_1, before proviral integration. The term k denotes the rate by which early infected T_1 cells are transformed into productively infected T_2 cells, involving proviral integration and cellular reprogramming. The term $\mathrm{N_T}$ denotes the rate of production of infectious virus progeny by productively infected T_2 cells. The terms $\delta_{\mathrm{T_1}} < \delta_{\mathrm{T_2}}$ denote the rates of clearance of T_1 and T_2 cells, respectively, and δ_{PIC} denotes the rate of intracellular destruction of the pre-integration complex. Parameters for the viral model are summarized in **Table 3**. In this article, we study distinct prophylactic schemes with the non-nucleoside reverse transcriptase inhibitor efavirenz. Reverse transcriptase inhibitors act intracellularly on reverse transcription. In our viral dynamics model this translates into an increase of propensity function a_1 and a proportional decrease in propensity function a_4. Derivations and motivation of this mechanisms of action from first principles are given in von Kleist et al. (2010) (Supplementary Methods therein).

3.4. Virus Exposure

Initial viral exposure after sexual intercourse occurs at tissue sites typically not receptive for establishing and shedding HIV infection (e.g., mucosal tissues). Hence, the virus needs to pass several physiological barriers to reach a replication enabling (target-cell) environment where infection can be established and from where it can shed systemically (Joseph et al., 2015).

To determine realistic inoculum sizes after sexual exposure to HIV (initial states for hybrid stochastic simulations), we previously developed a data-driven statistical model linking plasma viremia in a transmitter (VL) to the initial viral population Y_0 in a replication-enabling environment (Duwal et al., 2016) (Supplementary Note 4 therein for details) precisely capturing average per contact transmission rates for various types of exposure. In brief, we assume a binomial model

$$P(Y_0 = V | \mathrm{VL} = \nu) = \binom{[\nu^m]}{n} \cdot r^n \cdot (1 - r)^{[\nu^m] - n} \qquad (12)$$

where $[\cdot]$ is the nearest integer function, $m = \log_{10}(2.45)$ is given by Wilson et al. (2008) and the *success probability* r was estimated in a previous work (Duwal et al., 2016) (Supplementary Note 4 therein), e.g., $r_{\mathrm{homo}} = 3.71 \cdot 10^{-3}$ for homosexual- and $r_{\mathrm{hetero}} = 3.63 \cdot 10^{-4}$ for heterosexual exposure. The parameter

TABLE 3 | Parameters used for the viral dynamics model.

Parameter	Value	References	Parameter	Value	References
λ_T	$2{\cdot}10^9$	Wei et al., 1995	k	0.35	Zhou et al., 2005
δ_T, δ_{T_1}	0.02	Sedaghat et al., 2009	β	$8{\cdot}10^{-12}$	Sedaghat et al., 2008
δ_{T_2}	1	Markowitz et al., 2003	N_T	670	Sedaghat et al., 2009; von Kleist et al., 2010
δ_{PIC}	0.35	Zhou et al., 2005; Koelsch et al., 2008	CL_V	2.3	Tan and Wu, 1998; Tuckwell et al., 2008

Excerpt from von Kleist et al. (2010), except for CL_V*, which assumed that virus clearance is smaller in virus-naive individuals compared to infected individuals, in line with Frank et al., 2011; Duwal et al., 2012. All parameters refer to the absence of drug treatment ∅. All parameters in units (1/day), except for λ (cells/day) and β (1/(day · virus)).*

VL denotes the viral load in a potential transmitter (assumed to be log-normal distributed with $\mu = 4.51, \sigma = 0.98$ (Duwal et al., 2016)). In this model, the *success probability r* summarizes both the extent of local exposure, as well as the probability of passing all bottlenecking physiological barriers and reaching a replication enabling target cell compartment. Herein, we used the "exposure model" to compute drug efficacy estimates after sexual exposure presented in **Figures 3**, **4**.

3.5. Numerical Simulation

We use the exact numerical simulation scheme proposed in Duwal et al. (2018). Briefly, the modeled system is split into stochastic reactions describing viral dynamics and a set of ordinary differential equations describing individual EFV pharmacokinetics after drug administration, including covariates (e.g., CYP2B6 polymorphisms), autoinduction and the relationship between plasma- and target-site concentrations outlined above. In our approach EFV pharmacokinetics affect certain stochastic reaction propensities as outlined in Equations (6), (9). This hybrid system is then simulated using the numerically exact EXTRANDE algorithm (Voliotis et al., 2016) and hybrid trajectories are classified as extinction events when all viral compartments are cleared. On the other hand, trajectories were considered infections if (i) either long-lived- or latently infected cells emerged, or if (ii) the trajectories left an *extinction simplex* ($\epsilon = 0.0001$), meaning that it becomes unlikely (probability $\leq \epsilon$) that the virus will eventually be cleared (details provided in Duwal et al., 2018).

3.6. Prophylactic Efficacy of a Drug Regimen

Our goal is to estimate the prophylactic efficacy φ of a particular medication regimen S_D. The prophylactic efficacy denotes the reduction in infection risk *per contact*, with φ =100% indicating complete protection and φ =0% indicating no change in infection risk.

$$\varphi(Y_0, S_D) = 1 - \frac{P_{\mathrm{I}}(Y_0|S_D)}{P_{\mathrm{I}}(Y_0|\varnothing)} \quad \text{(prophylactic efficacy)}, \qquad (13)$$

where $P_{\mathrm{I}}(Y_0|S_D)$ and $P_{\mathrm{I}}(Y_0|\varnothing)$ denote the virus infection probabilities for a particular prophylactic scheme S_D and in the absence of prophylactic drugs ($\varnothing$), respectively, for initial state $Y_0 = [V, \mathrm{T}_1, \mathrm{T}_2]^T$ (number of viral particles, early- and late infected cells in a replication-enabling compartment). The probabilities $P_{\mathrm{I}}(Y_0|S_D)$ are approximated by the number of simulations that were classified as *infection events* divided by the *total* number of hybrid stochastic simulation runs for each particular prophylaxis scheme S_D during PrEP, PrEP "on demand" and PEP simulations. $P_{\mathrm{I}}(Y_0|\varnothing)$ can be computed using the analytical formulas derived in Duwal et al. (2019).

Simulation of Pre- and Post-Exposure Prophylaxis

Codes were written in MATLAB R2018b (MathWorks, Natick, MA; v. 9.5, including the statistics toolbox). Individual pharmacokinetic model parameters were drawn from the distributions defined by the parameter estimates from the final efavirenz population pharmacokinetic model (**Table 2**), generating 1000 virtual patients (**Supplementary Table 1**). We then simulated individual pharmacokinetic profiles for the prophylactic schedule S_D under consideration using ode113 in MATLAB. To simulate different adherence levels, a sequence of uniformly distributed random numbers with $r_i \sim \mathcal{U}(0, 1)$ was drawn and the ith dose was missed if $r_i >$ adherence level.

The number of viruses to be inoculated was drawn from the virus exposure model, where we first sampled the viral load in a potential transmitter ($\log_{10}\mathrm{VL} \sim \mathcal{N}(4.51, 0.98)$) and then used the virus load in the transmitter to determine the number of viruses V_0 entering a replication-competent compartment in the virus-exposed individual using Equation. (12). Samples with $V_0 = 0$ were rejected (they do not contribute to the infection risk). For once-daily PrEP simulations with different adherence levels, a time of virus exposure was randomly drawn within a 3 month interval starting at day 31 after PrEP initiation. The corresponding drug concentrations at this time and the number of transmitted viruses reaching a target cell compartment were used as the initial states for EXTRANDE and simulated until stopping criteria were satisfied (either virus clearance or infection). For "PrEP on demand" simulations, the time of virus exposure was fixed as indicated in the corresponding graphics. In the case of PEP, virus was inoculated as stated above and the stochastic viral dynamics were simulated in the absence of drugs until the time of PEP initiation (to determine the initial condition of the system), and henceforth simulated until a stopping criterium was reached.

In total, for each prophylactic scenario, 10000 simulations were run and $P_{\mathrm{I}}(Y_0|S_D)$ was computed as the fraction of simulations that resulted in infection.

4. RESULTS

4.1. Pharmacokinetics

The standard EFV dose used in treatment is 600 mg once daily taken orally. However, this dose is associated with neurotoxic effects (Rakhmanina and van den Anker, 2010; Apostolova et al., 2015), which could be prohibitive when using EFV as prophylaxis. Notably, neurotoxicity is associated with EFV plasma concentrations (and CYP2B6 polymorphism) (Rakhmanina and van den Anker, 2010). Therefore, a reduced, 400 mg dose has recently been explored, significantly reducing the risk of neurotoxicity while maintaining sufficient antiviral effects (ENCORE1 Study Group, 2014; ENCORE1 Study Group et al., 2015).

In **Figures 1A,B** we depict simulated pharmacokinetics of once daily oral EFV with 400 and 600 mg. EFV was quickly absorbed with a median $t_{\max} \approx$ 5.90h (95% CI: 4.56–7.88) and has a long median half life $t_{1/2} \approx$ 35.57h (CI: 14.28–125.26) at day 1 and a median half life $t_{1/2} \approx$ 20.77h (CI: 8.34–73.15) after metabolic autoinduction, in agreement with the literature (Avery et al., 2011, 2013a; Dickinson et al., 2015). Due to its linear pharmacokinetics, the dose reduction 600 $\rightarrow$ 400 mg resulted in a concentration reduction of $\approx 2/3$ for the 400mg dosing regime. In **Figures 1C,D**, we show the long-term pharmacokinetics after

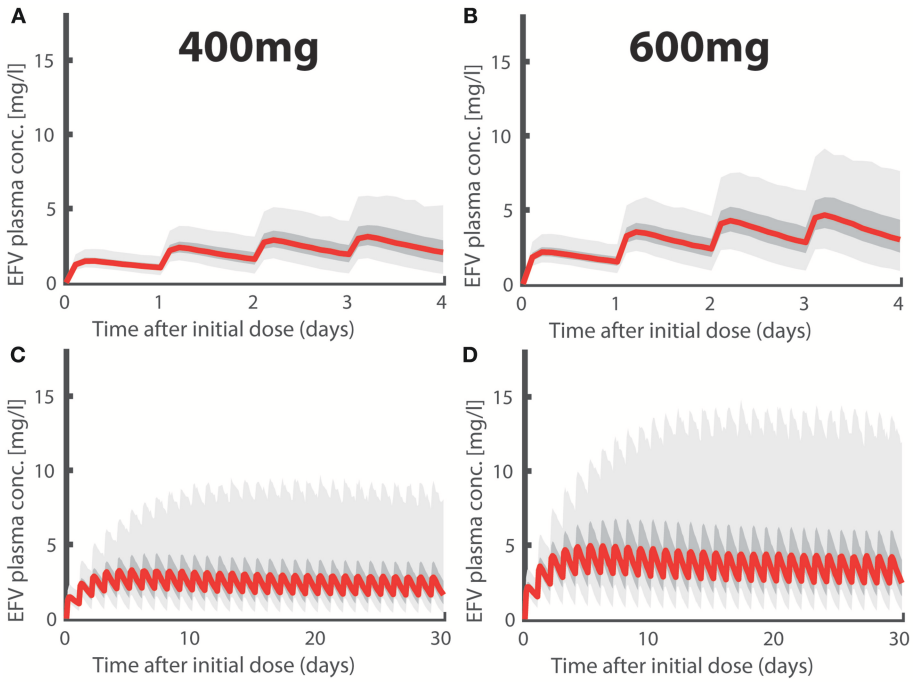

FIGURE 1 | Efavirenz Pharmacokinetics. Population plasma pharmacokinetics for the first four days after intake of **(A)** 400- and **(B)** 600-mg daily oral EFV. Long-term pharmacokinetics of **(C)** 400- and **(D)** 600-mg daily oral EFV due to metabolic autoinduction. Light gray regions encompass 95% of individual PK predictions, whereas dark gray areas encompass 50% of the predictions (quartiles). The thick red line marks the median pharmacokinetic profiles.

multiple dosing. Two things come to mind: (i) after an initial plateau phase (4–5 doses), concentrations tend to decrease, due to metabolic autoinduction, reaching median trough levels of $\approx$ 1.35 and $\approx$ 2.02 mg/L (95% CI: 0.30–6.55 and 0.45–9.83mg/L) in the 400- and 600mg dosing regime, respectively. (ii) The variability in the predicted pharmacokinetic profiles increases after multiple dosing with some individuals achieving concentrations $>$ 10 (mg/L) (light grey area indicating the 95% range). This observation is attributable to genetic polymorphisms affecting some individuals of our virtual patient cohort that slowly metabolize EFV. Interestingly, there is clinical evidence that some individuals, particularly slow metabolisers, achieve concentrations $>$ 10 (mg/L), and that the proportion of these individuals is much higher for the 600 mg regimen (Dickinson et al., 2015). In our simulations, 11.3% in the 600 mg group eventually exceed concentration of 10 mg/L, whereas it is only 2.5% in the 400 mg group. If EFV toxicity is proportional to exposure, as suggested by Rakhmanina and van den Anker (2010), this may indicate that dose reduction could significantly reduce the risk of adverse effects. But is it also protective against infection?

4.2. Concentration-prophylaxis Profile

We used the analytical solutions presented in Duwal et al. (2019) to compute concentration-prophylaxis profiles $\varphi(Y_0, S_D)$ assuming a single virus particle enters a replication-enabling compartment ($Y_0 = [1, 0, 0]^T$), see **Figure 2A**. The reason is that the virus exposure model (Methods section) predicts that in most cases only a single virus enters a replication-competent compartment after (homo-/hetero-)sexual exposure, if a virus manages at all to pass the various bottlenecking physiological barriers after sexual exposure. Besides the *wild-type* virus, we also show the prophylaxis profile against transmitted drug resistance with viruses carrying EFV resistance mutations G190S, K103N and Y181C (Rhee et al., 2003). As a visual guide, the shaded areas mark the 95% trough (pre-dose) concentration ranges achieved at plateau and after metabolic autoinduction for once daily 400 mg efavirenz (computed using the POP-PK model).

Figure 2A suggests that once daily EFV (with 400 mg) provides complete protection against HIV infection after exposure to *wild-type* virus and resistant viruses carrying the Y181C mutation. After exposure to the G190S and K103N mutants, $>>$50% protection is provided by once daily 400 mg EFV and $>>$60% protection by the 600 mg regime. Since selection of drug resistant variants is a major concern, we evaluated the relative transmissibility of mutant viruses when compared with *wild-type* virus as $\varphi_{\text{wt}} - \varphi_{\text{mut}}$ in **Figure 2B**. The figure can be interpreted as follows: At low concentrations there is no reduction in infection if an individual was exposed to *wild-type* and/or mutant virus. At an intermediate concentration range (between 0.001 and 0.1 for Y181C and between 0.001 and 1 mg/L for K103N, G190S, respectively), infections with the *wild type* would be prevented, while the prophylaxis cannot, or only partially reduce the infection risk after exposure to mutant virus. The maximum corresponds to the maximal difference in risk reduction, meaning that resistant virus is more likely

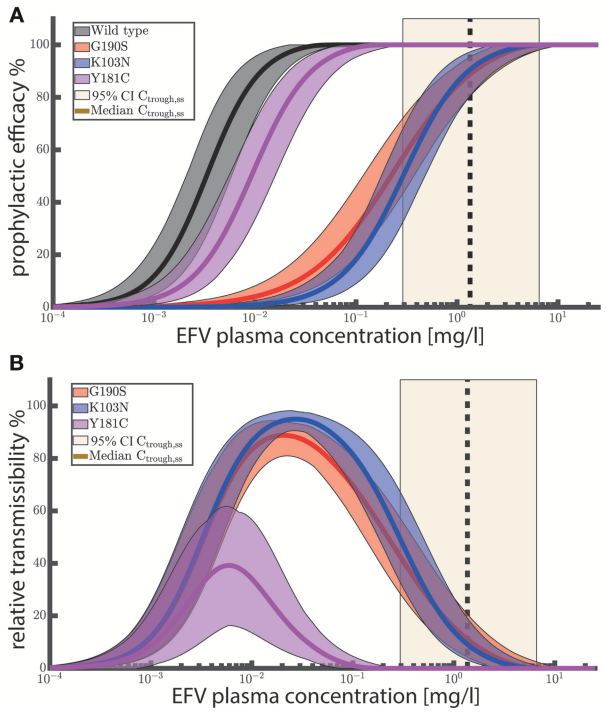

FIGURE 2 | (A) Prophylactic efficacy against different viral genotypes. Predicted efficacy ranges are depicted as colored shaded areas, with superimposed mean efficacy estimates as solid lines. Ninety-five percent confidence intervals of steady-state EFV trough (pre-dose) concentrations $C_{trough,ss}$ for 400 mg EFV after once daily dosing are depicted as light yellow areas with the vertical black dashed line marking the median trough concentration. **(B)** Relative transmissibility $\varphi_{wt} - \varphi_{mut}$ of mutant viruses. Colored areas depict the ranges of relative transmissibility and solid lines indicate the mean relative transmissibility.Yellow shaded areas depict the 95% CI of EFV trough (pre-dose) concentrations after 400 mg once daily at steady state. Black dashed vertical lines indicate the corresponding median trough concentrations. The prophylactic efficacy against a single virus was computed using the analytical solutions provided in Duwal et al. (2018) (Equation 19–21 therein) for 1000 logarithmically spaced concentrations between 10^{-4} and 25 mg/L using 1000 sampled values for IC_{50} and m per viral genotype (**Table 2**). For each viral genotype, $IC_{50_{assay}}$ and m were sampled from a log-normal and normal distribution respectively as stated in the caption of **Table 2**. $IC_{50_{assay}}$ were corrected for plasma binding using $f_{u,plasma} = 0.2\%$ to obtain $IC_{50_{plasma}}$, as outlined in **Supplementary Text 1**. Changes in drug sensitivity for the mutants considered the fold changes stated in **Table 2**. Fitness defects of the mutants were considered using $\beta(\text{mut}) = f(\text{mut}) \cdot \beta(\text{wt})$.

transmitted than the *wild type*. At very high EFV concentrations, the infection risk with both *wild type* and mutant is reduced. Importantly, when inspecting (population) *median* EFV trough (pre-dose) concentrations after 400 mg once daily dosing (dashed vertical black line in **Figure 2B**) , we can see that the relative transmissibility of the Y181C mutant is zero, while the relative transmissibilities of the G190S and K103N mutants are less than 20%. The analysis suggests that the typical concentration ranges achieved after once daily EFV do not, or just slightly, favor resistance transmission over *wild type* for the considered single-substitution mutants. Note that these mutations decrease EFV susceptibility by ≈ 90 fold, **Table 2**. However, clinically derived isolates may contain multiple substitutions and confer even higher levels of EFV resistance.

Since poor drug adherence may give rise to lower EFV concentrations and since it is a major factor confounding the clinical efficacy of Truvada (Haberer et al., 2015), we next set out to test whether similar issues are to be expected for 400 mg oral EFV for pre-exposure prophylaxis, or when EFV is used "on demand" and post-exposure.

4.3. Once-daily PrEP With 400 mg EFV

The predicted prophylactic efficacy of once daily PrEP with 400 mg is shown in **Figure 3A** as a function of adherence after exposure to either *wild-type* virus or after exposure to drug resistant mutants. As can be seen, if at least 75% of doses were taken, complete protection against the *wild-type* virus and against the Y181C mutant was achieved. Notably, for these viral genotypes protection was > 95% if 50% of the pills were taken and ≈ 90% when ≈25% of the pills were taken. In contrast, after exposure to resistant viruses carrying the G190S or K103N mutation, protection was >82% when at least 75% of the once daily 400 mg EFV pills were taken, gradually dropping to ≈ 50% protection when every fourth pill was taken.

We next wanted to assess how quickly the prophylactic protection vanishes, when consecutive EFV doses were missed (illustratively depicted in **Supplementary Figure 1**). In order to do so, we simulated 400 mg EFV-based once daily PrEP with 100% adherence. Subsequently, we computed how long it will take for the concentrations to drop below the respective 50%, or 90% protective levels (EC_{50}, EC_{90}). We computed that a median of 7 (CI: 2–32) consecutive doses need to be missed in order to provide less than 50% protection against *wild-type* virus. Correspondingly, 5 (CI: 1–26) consecutive doses need to be missed to provide less than 90% protection against *wild-type* virus.

4.4. "PrEP on Demand" With 400 mg EFV

Next, we evaluated whether 400 mg EFV "on demand" would protect against HIV infection. We tested an "on demand" dosing scheme similar to the one recently tested for Truvada-based PrEP (Molina et al. , 2015): The first EFV dose was taken within a time window of 1–23 h *prior* to virus exposure and followed by two more doses, 24- and 48- hours after the initial dose. Our predictions indicate that EFV-based "PrEP on demand" provides complete protection against *wild-type* virus and against the Y181C single mutant, when initiated 1–23 h prior to virus exposure. Protection against the single mutants G190S and K103N was still > 81% for 400 mg "PrEP on demand." This surprisingly superior prophylactic efficacy of EFV "on demand" can be attributed to its rapid uptake and slow elimination. Particularly the latter ensures that virus gets eliminated when EFV is taken as "PrEP on demand." The comparatively higher efficacy of "PrEP on demand," when compared to once daily PrEP with low adherence can be explained as follows: In the case of once-daily PrEP, several *consecutive* dose intakes may be missed, which allows the EFV concentrations to fall below their respective EC_{50}, EC_{90}. If virus exposure occurs during

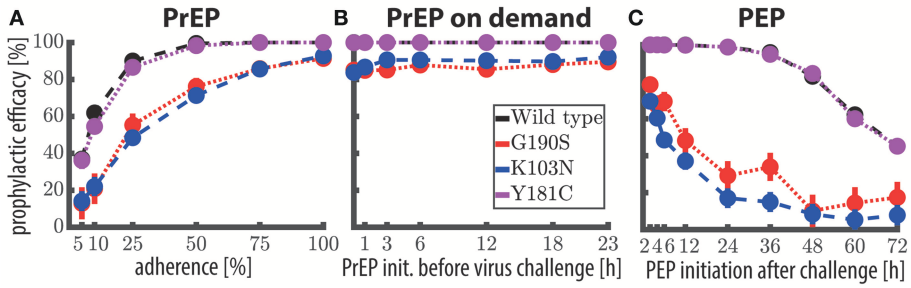

FIGURE 3 | Prophylactic efficacy of EFV against the *wild-type* and resistant virus using different prophylaxis schemes. **(A)** Predicted prophylactic efficacies for once daily 400 mg oral EFV PrEP with different levels of adherence. For example in the 25% adherence scheme, each dosing event was randomly missed with 75% chance. Colored dots mark the median predicted prophylactic efficacy and error bars mark the 95% confidence interval (computed using Greenwood's formula), considering variabilities in pharmacokinetic, as well as pharmacodynamic parameters. **(B)** Predicted prophylactic efficacy of 400 mg oral EFV during "PrEP on demand" (3 doses) depending on the time of PrEP initiation with respect to viral encounter, respectively. **(C)** Predicted prophylactic efficacy of post-exposure prophylaxis (9 doses) with 400 mg oral EFV as a function of the time of PEP initiation after viral challenge. Simulations were conducted using the hybrid EXTRANDE method outlined in the Methods section. In total, 10,000 stochastic simulations were performed to estimate prophylactic efficacy for each condition (e.g., viral challenge with K103N during chronic PrEP with 5% adherence is one condition).

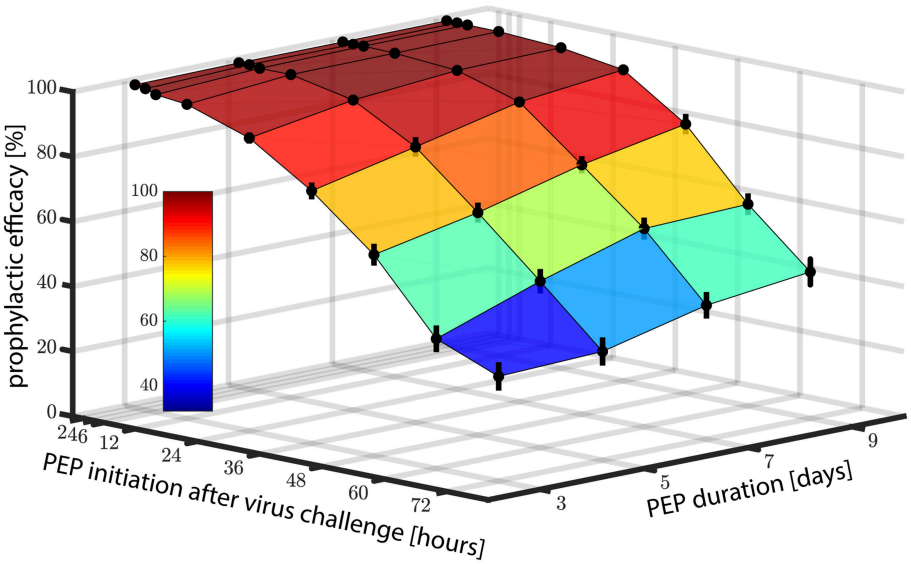

FIGURE 4 | Prophylactic efficacy of *PEP* against *wild type* virus depending on both the time of PEP initiation and the number of subsequent 400 mg EFV doses taken. Error bars denote the 95% confidence intervals, computed using Greenwood's formula. Prophylactic efficacy was estimated for each condition based on 10,000 hybrid EXTRANDE simulations as outlined in the Methods section.

these time windows of low EFV concentrations, infection may occur (illustrated in **Supplementary Figure 1**). In contrast, if all "PrEP on demand" pills are taken, concentrations will be above the EC_{90} at the time of exposure, and, due to the long half life of EFV remain above this value, until the virus is eliminated (which typically would happen $\leq$ 1 week post exposure Konrad et al., 2017).

4.5. Post-Exposure Prophylaxis With 400 mg EFV

Motivated by the promising predictions regarding the use of EFV in pre-exposure prophylaxis, we also wanted to investigate whether EFV could prevent infection, if taken as post-exposure prophylaxis (PEP). In **Figure 4**, we show the predicted prophylactic efficacy of 400 mg oral EFV as a function of both the delay in PEP initiation and the duration of PEP after challenge with *wild-type* virus. In **Figure 4** it becomes evident that it is more critical to initiate PEP early after exposure, than to prolong PEP duration. For example, when PEP is initiated as late as 72 h post virus exposure and the duration of PEP is three days (3 consecutive doses), the prophylactic efficacy was estimated to be ≈20%. If the duration of PEP was increased to 9 days, the prophylactic efficacy increases to only ≈40%. However, if PEP was initiated shortly after virus exposure (e.g., within 2 h), the prophylactic efficacy increases to 100%, even if the PEP duration was only 3 days.

As a midpoint, taking the first PEP dose within 24 h post-exposure resulted in prophylactic efficacies of $>$ 88, $>$ 94, $>$

97 and > 98% against the *wild type* for 3, 5, 7, and 9 dose intakes, respectively.

Next, we wanted to investigate in more detail the sensitivity of PEP efficacy towards the timing of PEP initiation in the *wild type* and drug resistant mutants. To simplify interpretation, we assumed a PEP duration of 9 days (9 doses). Predictions are shown in **Figure 3C**. PEP provided > 98% protection against the *wild type* and the Y181C mutant when started within 12 h after virus exposure. Protection against viruses containing the G190S mutation was > 21% using the same parameters, and > 11% for the K103N mutant. These simulations indicate that EFV may potently protect against infection with *wild type* and the weakly resistant Y181C virus, when initiated within 24 h post expose. The prophylactic efficacy against transmitted, highly resistant viruses carrying the G190S or K103N mutation is insufficient for post-exposure prophylaxis.

5. DISCUSSION

Truvada-based PrEP is being implemented in a number of countries (AVAC, 2019), however, there are two major limitations to its optimal use: (i) its costs (Keller and Smith, 2011), and (ii) its sensitivity to poor medication adherence (Haberer et al., 2015).

Current PrEP research focusses on overcoming adherence-related concerns, either in terms of promoting drug adherence, or through the development of novel long-acting drugs/drug formulations for HIV prophylaxis, that only require monthly drug administration (McGowan et al., 2016; Markowitz et al., 2017; McMillan et al., 2017). However, little has been done to investigate cost-efficient Truvada alternatives that may be affordable in low- and middle-income countries hit hardest by the epidemic.

A recent computational screen of the prophylactic potential of treatment-approved compounds for PrEP repurposing suggested that darunavir, efavirenz, nevirapine, etravirine and rilpivirine may more potently prevent HIV infection than Truvada at clinically relevant concentration ranges (Duwal et al., 2019). Of these candidates we set out to investigate efavirenz in more detail, since it is both inexpensive and readily available in most resource-constrained settings.

However, 600 mg EFV has been associated with neurotoxicity (Rakhmanina and van den Anker, 2010; Apostolova et al., 2015). Decloedt and Maartens (2013) and Siccardi et al. (2012) have previously suggested a connection between EFV metabolism and toxicity, indicating that slow metabolisers, who have higher plasma concentrations, also have a higher tendency to experience adverse effects (associations have been made between the major EFV metabolite and neurotoxicity). The direct association between plasma concentrations and CNS side effects has also been reported in Marzolini et al. (2001). Motivated by these studies, we explicitly considered genetic polymorphisms affecting EFV metabolism. Moreover, since EFV pharmacokinetics are linear, dose reductions would naturally lead to decreased EFV exposure (and consequently toxicity) as investigated in the ENCORE 1 trial (ENCORE1 Study Group, 2014; ENCORE1 Study Group et al., 2015), which suggested non-inferiority of the 400 mg EFV regimen with regard to treatment. Motivated by these results, we set out to investigate the prophylactic potential of 400 mg EFV.

Our simulations strongly suggest that 400 mg efavirenz can potently prevent infection with drug susceptible HIV, when used as once daily PrEP, during "PrEP on demand" and even as PEP, if initiated early enough after exposure (**Figures 3**, **4**). Overall, these simulations suggest that EFV provides a good efficacy margin with respect to incomplete adherence and various event-driven dosing scenarios. Notably, if the association between EFV toxicity and metabolism is evident, it could also be envisioned that individuals that experience adverse effects may even further reduce EFV dosing. For example, using the POP-PK model, we predicted that the number of patients experiencing plasma concentrations of > 10 mg/L following a 200mg once daily dosing regimen is only 0.1%.

However, our simulations also suggested that EFV-based post-exposure prophylaxis (PEP) may insufficiently protect against transmitted, highly resistant strains (K103N, G190S), as depicted in **Figure 3C**. We should also note that circulating resistant viruses may have multiple compensatory mutations that increase fitness and resistance through epistatic effects (Rath et al., 2013). Thus, their phenotypic attributes may deviate from laboratory strains with single point mutations that were evaluated in the present analysis and by Sampah et al. (2011). A recent study (Zazzi et al., 2018) highlighted high levels of NNRTI resistance particularly in South Africa, but it is unclear whether the analyzed NNRTI resistance mutations also confer high level resistance against EFV. Regarding high level resistance mutations, the Stanford database currently reports a prevalence $\pi_{\text{untreated}} << 5\%$ [1] in the *untreated* population in South Africa, mainly conferred by K103N. The prevalence of resistance mutations in *treated* individuals π_{treated} is much higher: K103N $\approx$ 30%, Y181C $\approx$ 20% and G190A/S $\approx$ 15% [2], but comparable to Truvada resistance mutations (M184V: 48–60%; K65R: 4–15%) in *treated* individuals. Notably, the overall risk of exposure to resistant strains would be much smaller than these numbers, as it is both determined by prevalence, as well as the probability of resistance-associated treatment failure in the donor at the moment of virus transmission, e.g., mathematically **Prob.**{exposure to res.} = **Prob.**{untreated.}$\cdot\pi_{\text{untreated}}$ + **Prob.**{treated.}$\cdot$ **Prob.**{failing due to resistance} $\cdot\pi_{\text{treated}}$. The calculations state that resistance exposure from *treated* individuals may only originate from those *treated* individuals that fail on the treatment at the time of exposure, due to resistance emergence (if they are successfully treated at the time of exposure, they are non-contagious Cohen et al., 2011).

Another important aspect that is quantified in **Supplementary Text 2** is resistance emergence in the exposed individual *prior* to PEP initiation. As can be seen in **Supplementary Text 2**, the probability of resistance emergence increases with the delay between virus exposure and PEP initiation. For example, we calculated that if PEP is initiated 3 days after exposure (72 h), the virus had either gone extinct

[1] https://hivdb.stanford.edu/page/surveillance-map/
[2] https://hivdb.stanford.edu/cgi-bin/MutPrevBySubtypeRx.cgi

or developed resistance with 38% probability. This *de novo* resistance may subsequently be selected by EFV, limiting future treatment options. On the other hand, if PEP is initiated within 12 hours, the probability of resistance emergence in the exposed individual *prior* to PEP is <0.01%. Thus, both in terms of lack of efficacy (**Figure 4**), as well as in terms of resistance (**Supplementary Text 2**), the window of opportunity with regards to PEP is short. Based on our simulations, PEP should be initiated as early as possible and is contraindicated if the suspected virus exposure occurred more than 3 days ago. The same considerations also apply for Truvada-based prophylaxis.

Our predictions regarding EFV prophylactic efficacy depend on (i) parameters of EFV potency (IC_{50}) and (ii) the concentrations of EFV at the target site.

Regarding EFV potency, one limitation of our work is that we used parameters determined *ex vivo* (Shen et al., 2008; Sampah et al., 2011) using primary human peripheral blood mononuclear cells (PBMCs). These cell mixtures are commonly used as surrogate markers to determine drug efficacy, since they contain a large proportion of $CD4^+$ T-cells (the primary HIV target cell type). Moreover, utilised parameters are generally in agreement with published values from other sources (Smith et al., 2001; Parkin et al., 2004; Avery et al., 2013b; Hu and Kuritzkes, 2014; Schauer et al., 2014) (after correction for protein binding; **Supplementary Text 1**).

Regarding the *relevant* target-site concentrations of EFV, there has been some debate since the *total* (protein bound and unbound) EFV concentrations in tissues have been reported to be highly heterogeneous (Thompson et al., 2015) and some studies have suggested associations between drug heterogeneity and incomplete HIV suppression (Fletcher et al., 2014), whereas others report high concentrations in tissues related to HIV exposure (Thompson et al., 2015). There are two main mechanisms that could explain heterogeneous drug distribution, which we discuss in detail:

a) Active transport (e.g. P-glycoprotein): In this case, the expression of transporters in particular cell types may cause concentration differences between distinct tissues. Notably, active transport would cause a difference in the *unbound* concentrations, which are available to exert an antiviral effect. As a consequence of active efflux, lower amounts of EFV may be available in some relevant target cells, giving rise to pharmacological sanctuaries relevant to EFV prophylaxis. A detailed analysis of EFV active transport (Burhenne et al., 2010) however revealed that it does not affect EFV intracellular concentrations. Moreover, EFV is a small (molecular mass: 315.6 g/mol) and highly lipophilic (LogP $\approx$ 4) compound that could rapidly cross biomembranes by passive diffusion. Thus, even if EFV was a substrate of cellular transporters, the *dominating* (i.e. fastest) mechanism mediating cellular uptake and efflux is probably passive diffusion. Furthermore, passive diffusion does not imply that the *total* (protein bound and -unbound) concentrations on either side of a biomembrane are equal, but rather implies that the *unbound* concentrations are equal. I.e. at each side of a biomembrane, EFV may be (un-)specifically retained by binding to biomolecules (lipids, proteins, see von Kleist and Huisinga, 2007 for an overview). However, since only the *unbound* concentration is available for drug-target interaction, EFV concentrations exerting antiviral effects would be identical in different cell types under passive diffusion.

b) Protein binding: EFV is highly (> 99%) bound to plasma proteins (Boffito et al., 2003), mainly albumin and $\alpha-1$-acid glycoprotein. Naturally, the concentrations of these proteins are magnitudes lower in tissues, which affects the amount of protein-bound EFV (and consequently the *total* concentrations). Studies that measure *unbound* drug concentrations lend strong support to this hypothesis. Avery et al. reported that the *unbound* EFV concentrations in plasma and semen (Avery et al., 2011) and in plasma and cerebrospinal fluid are nearly identical (Avery et al., 2013a). Importantly, considering albumin concentrations (calculations in **Supplementary Text 1**) in proposed sanctuary sites, we can precisely recover differences in *total* EFV concentrations reported, e.g., semen-to-plasma ratio: 3.4–5 % (Reddy et al., 2002; Avery et al., 2011) and cervical fluid-to-plasma ratio: 0.4% (Dumond et al., 2007). The fact that *unbound* plasma-, cerebrospinal fluid- cervical fluid and semen concentrations are nearly identical also suggests that EFV can cross the blood-brain, blood-testis and blood-uterine barrier.

In summary, these combined observations strongly argue that the distribution of EFV in tissues is governed by passive diffusion and (un-)specific binding to plasma proteins. In terms of PK-PD modeling, this implies that the *unbound* concentration in plasma are representative for the *unbound* concentration within target cells ($CD4^+$ immune cells/T-cells; derivations in **Supplementary Text 1**). When *unbound* concentrations are proportionally related to the *total* concentrations, it also implies that EFV *total* plasma concentrations can be used as a marker of drug efficacy (Marzolini et al., 2001). As a cautionary note we want to add that there could still be additional unaccounted, specific barriers lowering EFV *unbound* concentrations in physiological sites relevant for establishing the initial infection upon sexual exposure to HIV-1 (male genital compartment, female genital compartment and rectum), which warrant further verification. However, based on the discussions above, we would strongly disagree with the statement by Dumond et al., that "agents such as efavirenz that achieve *total* genital tract exposures less than 10% of blood plasma are less attractive PrEP/PEP candidates" (Dumond et al., 2007). This simplistic criterium of selecting drug candidates ignores the drug's individual pharmacology, might only select drugs that are not extensively protein bound, or select highly protein-bound candidates merely as a function of genital albumin concentrations. Our simulations are however in line with a later study from the same group (Dumond et al., 2012), which find that the concentrations at the site of virus exposure (in Dumond et al., 2012 the female genital tract) are proportional to unbound plasma concentrations during *chronic dosing*. However, it is unclear after how many dosing events this equilibrium between plasma and target site concentrations is achieved. While plasma concentrations rapidly peak at a t_{max} of about 5.9 h, there could be a time-delay in building up concentrations at the site of infection, which could impair the efficacy of "PrEP on demand" and PEP (compare **Figures 3B,C**), in the sense that it becomes more important to initiate the respective protocols as early as possible.

Notably, genital tract concentrations measured after the first dose in Dumond et al. (2007) are in line with our predictions, arguing for our modeling approach and for the presumed fast kinetics of cellular uptake by passive diffusion (see also **Supplementary Text 1**).

Another limitation of our study is that the parametrization of the PK model is based on data from HIV-infected individuals, while prophylaxis is intended for healthy individuals. In fact, it is unclear whether there are significant differences with respect to e.g., drug metabolism as a consequence of the infection status. For example, measured EFV plasma concentrations (400 mg once daily) in healthy individuals from Burhenne et al. (2010) are similar to those predicted herein. However, our model predicts large inter-individual variabilities due to pharmacogenomics (CYP 450 C2B6 polymorphisms). This hints toward the fact, that the pharmacokinetic differences between healthy vs. infected individuals could be small in comparison to the variability due to CYP polymorphisms. On the other hand, a study in healthy Ugandan individuals reports EFV concentrations (Mukonzo et al., 2009) that are considerably larger than predicted by our model. At the moment it is unclear whether differences are due to the infection status, or contributed to differences in ethnicity, weight, or co-medications: I.e. ethnicity ("black") has been associated with lower EFV clearance (Barrett et al., 2002). However, it is unclear whether concentration differences are due to a higher proportion of poor metabolisers in Ugandans, as suggested by Mukonzo et al. (2009), or other factors. It is interesting to note here that "gender" was a significant co-variate in the Ugandan study whereas it was not associated with changes in EFV PK in the ENCORE 1 study (Dickinson et al., 2015). While the drug's half life is similar for ENCORE 1 patients (35.57h; CI: 14.28–125.26) and healthy men in the Ugandan study (Mukonzo et al., 2009) (37.3h in wild type and 54.7h in slow metabolisers), the drug's terminal half life in females in the Ugandan study (Mukonzo et al., 2009) was twice as large as that for men. For comparison, a meta-analysis of 16 phase I studies reports a difference of only 10% (Barrett et al., 2002), warranting further research to clarify the mechanistic sources of the discrepancy between the results from the phase I studies (Barrett et al., 2002), the Ugandan study (Mukonzo et al., 2009) and the data from ENCORE 1 (Dickinson et al., 2015).

Regarding possible co-medications, it is worth mentioning that efavirenz has a large drug interaction potential. For example, it has been shown in Fan et al. (2009) that certain herbal medicines might compete for CYP2B6 metabolisms raising plasma levels, potentially up to toxic ranges. In any case, toxicity in the context of EFV-PrEP remains to be elucidated clinically and it remains to be elucidated if even further dose reductions would be suitable for PrEP in particular populations. The present work provides a good starting point to support these decisions, e.g., based on the concentration-prophylaxis profiles presented in **Figure 2**.

Moreover, EFV is an inducer of many CYP enzymes (Fichtenbaum and Gerber, 2002), possibly altering the pharmacology of co-medications. Thus, co-medication with EFV-based PrEP might require careful monitoring. The Liverpool drug-interaction database provides an excellent overview over known effects of EFV on various co-medications (https://www.hiv-druginteractions.org/).

Overall, this mathematical modelling study argues for the experimental investigation of EFV as a cost-efficient alternative PrEP candidate based on its superior prophylactic efficacy and forgiveness to incomplete adherence and event-driven usage. However, further analysis emphasising on the safety of EFV in the context of PrEP/PEP is warranted.

DATA AVAILABILITY

All data is contained in the manuscript.

AUTHOR CONTRIBUTIONS

SD and MvK conceptualized and designed the study. SD and DS performed the analysis on modeling prophylactic efficacy. LD performed the PK analysis. MvK wrote the manuscript with inputs from SD, DS, LD, and SK. All authors contributed to the analysis of the results.

FUNDING

MvK acknowledges financial support from the BMBF e:Bio junior research group Systems Pharmacology & Disease Control, grant number 031A307.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00199/full#supplementary-material

Supplementary Figure 1 | The figure shows an example of a concentration-time profile for chronic PrEP with 400 mg oral EFV and 25% adherence, where a temporal window for infection arises and EFV concentrations are insufficient for protection.

Supplementary Text 1 | The supplementary text contains an in-depth analysis of EFV cellular uptake, providing support for the free drug hypothesis and for equilibrative transport or passive diffusion as the main cellular uptake mechanisms of EFV. Furthermore, it analyses whether the drug potency is sensitive to uncertainty in drug binding.

Supplementary Text 2 | This supplementary text quantifies the probability of EFV resistance emergence prior to PEP initiation. We derive the probability of resistance emergence as a function of the time between virus exposure and start of post-exposure prophylaxis. The aim is to provide decision support on whether to start PEP after suspected exposure or not.

Supplementary Table 1 | The table shows the individual pharmacokinetic parameters (CL_{ss}/F_{bio}, V/F_{bio} and k_a) of all virtual patients.

Supplementary Table 2 | The table shows the considered single nucleotide polymorphisms associated with EFV metabolism and how they were modeled to affect efavirenz apparent oral clearance in the population pharmacokinetic model.

REFERENCES

AIDS Vaccine Advocacy Coalition. (2019) *Pre-Exposure Prophylaxis (PrEP).* Available online at: http://www.avac.org/sites/default/files/resource-files/prep_BTN_aug.pdf (Accessed dec 14, 2019).

Almond, L. M., Hoggard, P. G., Edirisinghe, D., Khoo, S. H., and Back, D. J. (2005). Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. *J. Antimicrob. Chemother* 56, 738–744. doi: 10.1093/jac/dki308

Amara, A., Tjia, J., Dutton, J., Else, L., Back, D., and Khoo, S. (2011). "Development and validation of a hplc-ms/ms assay to quantify the antiretroviral (arv) drug, efavirenz and its major metabolites in plasma," in *British Mass Spectrometry Society Meeting, Abstract BMS S11-1240* (Cardiff).

Apostolova, N., Funes, H. A., Blas-Garcia, A., Galindo, M. J., Alvarez, A., and Esplugues, J. V. (2015). Efavirenz and the cns: what we already know and questions that need to be answered. *J. Antimicrob. Chemother* 70, 2693–708. doi: 10.1093/jac/dkv183

Arab-Alameddine, M., Di Iulio, J., Buclin, T., Rotger, M., Lubomirov, R., Cavassini, M., et al. (2009). Pharmacogenetics-based population pharmacokinetic analysis of efavirenz in HIV-1-infected individuals. *Clin. Pharmacol. Ther.* 85, 485–94. doi: 10.1038/clpt.2008.271

Avery, L. B., Bakshi, R. P., Cao, Y. J., and Hendrix, C. W. (2011). The male genital tract is not a pharmacological sanctuary from efavirenz. *Clin. Pharmacol. Ther.* 90, 151–156. doi: 10.1038/clpt.2011.99

Avery, L. B., Sacktor, N., McArthur, J. C., and Hendrix, C. W. (2013a). Protein-free efavirenz concentrations in cerebrospinal fluid and blood plasma are equivalent: applying the law of mass action to predict protein-free drug concentration. *Antimicrob. Agents Chemother* 57, 1409–14. doi: 10.1128/AAC.02329-12

Avery, L. B., Zarr, M. A., Bakshi, R. P., Siliciano, R. F., and Hendrix, C. W. (2013b). Increasing extracellular protein concentration reduces intracellular antiretroviral drug concentration and antiviral effect. *AIDS Res. Hum. Retrovirus.* 29, 1434–1442. doi: 10.1089/aid.2013.0031

Barrett, J. S., Joshi, A. S., Chai, M., Ludden, T. M., Fiske, W. D., and Pieniaszek Jr, H. J. (2002). Population pharmacokinetic meta-analysis with efavirenz. *Int. J. Clin. Pharmacol. Ther.* 40, 507–519. doi: 10.5414/CPP40507

Boffito, M., Back, D. J., Blaschke, T. F., Rowland, M., Bertz, R. J., Gerber, J. G., et al. (2003). Protein binding in antiretroviral therapies. *AIDS Res. Hum. Retrovirus.* 19, 825–835. doi: 10.1089/088922203769232629

Brenner, B. G., Roger, M., Routy, J.-P., Moisi, D., Ntemgwa, M., Matte, C., et al. (2007). High rates of forward transmission events after acute/early HIV-1 infection. *J. Infect. Dis.* 195, 951–959. doi: 10.1086/512088

Burhenne, J., Matthée, A.-K., Pasáková, I., Röder, C., Heinrich, T., Haefeli, W. E., et al. (2010). No evidence for induction of ABC transporters in peripheral blood mononuclear cells in humans after 14 days of efavirenz treatment. *Antimicrob. Agents Chemother.* 54, 4185–4191. doi: 10.1128/AAC.00283-10

Chou, T.-C. (2006). Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacolog. Rev.* 58, 621–681. doi: 10.1124/pr.58.3.10

Cohen, M. S., Chen, Y. Q., McCauley, M., Gamble, T., Hosseinipour, M. C., Kumarasamy, N., et al. (2011). Prevention of HIV-1 infection with early antiretroviral therapy. *N. Engl. J. Med.* 365, 493–505. doi: 10.1056/NEJMoa1105243

Conway, J. M., Konrad, B. P., and Coombs, D. (2013). Stochastic analysis of pre- and postexposure prophylaxis against HIV infection. *SIAM J. Appl. Math.* 73, 904–928. doi: 10.1137/120876800

Decloedt, E. H., and Maartens, G. (2013). Neuronal toxicity of efavirenz: a systematic review. *Expert Opin. Drug Saf.* 12, 841–846. doi: 10.1517/14740338.2013.823396

Dellar, R. C., Dlamini, S., and Karim, Q. A. (2015). Adolescent girls and young women: key populations for HIV epidemic control. *J. Int. AIDS Soc.* 18(2 Suppl. 1):19408. doi: 10.7448/IAS.18.2.19408

Dickinson, L., Amin, J., Else, L., Boffito, M., Egan, D., Owen, A., et al. (2015). Pharmacokinetic and pharmacodynamic comparison of once-daily efavirenz (400 mg vs. 600 mg) in treatment-naïve HIV-infected patients: results of the ENCORE1 study. *Clin. Pharmacol. Ther.* 98, 406–416. doi: 10.1002/cpt.156

Dickinson, L., Amin, J., Else, L., Boffito, M., Egan, D., Owen, A., et al. (2016). Comprehensive pharmacokinetic, pharmacodynamic and pharmacogenetic evaluation of once-daily efavirenz 400 and 600 mg in treatment-naïve HIV-infected patients at 96 weeks: Results of the encore1 study. *Clin. Pharmacokinet.* 55, 861–873. doi: 10.1007/s40262-015-0360-5

Dumond, J. B., Nicol, M. R., Kendrick, R. N., Garonzik, S. M., Patterson, K. B., Cohen, M. S., et al. (2012). Pharmacokinetic modelling of efavirenz, atazanavir, lamivudine and tenofovir in the female genital tract of HIV-infected pre-menopausal women. *Clin. Pharmacokinet.* 51, 809–822. doi: 10.1007/s40262-012-0012-y

Dumond, J. B., Yeh, R. F., Patterson, K. B., Corbett, A. H., Jung, B. H., Rezk, N. L., et al. (2007). Antiretroviral drug exposure in the female genital tract: implications for oral pre- and post-exposure prophylaxis. *AIDS* 21, 1899–1907. doi: 10.1097/QAD.0b013e328270385a

Duwal, S., Dickinson, L., Khoo, S., and von Kleist, M. (2018). Hybrid stochastic framework predicts efficacy of prophylaxis against HIV: an example with different dolutegravir prophylaxis schemes. *PLoS Comput. Biol.* 14:e1006155. doi: 10.1371/journal.pcbi.1006155

Duwal, S., Dickinson, L., Khoo, S., and von Kleist, M. (2019). Mechanistic framework predicts drug-class specific utility of antiretrovirals for hiv prophylaxis. *PLoS Comput. Biol.* 15:e1006740. doi: 10.1371/journal.pcbi.1006740

Duwal, S., Schütte, C., and von Kleist, M. (2012). Pharmacokinetics and pharmacodynamics of the reverse transcriptase inhibitor tenofovir and prophylactic efficacy against HIV-1 infection. *PLoS ONE* 7:e40382. doi: 10.1371/journal.pone.0040382

Duwal, S., Sunkara, V., and von Kleist, M. (2016). Multiscale systems-pharmacology pipeline to assess the prophylactic efficacy of NRTIs against HIV-1. *CPT Pharmacometr. Syst. Pharmacol.* 5, 377–387. doi: 10.1002/psp4.12095

Duwal, S., and von Kleist, M. (2016). Top-down and bottom-up modeling in system pharmacology to understand clinical efficacy: an example with NRTIs of HIV-1. *Eur. J. Pharm. Sci.* 94, 72–83. doi: 10.1016/j.ejps.2016.01.016

ENCORE1 Study Group (2014). Efficacy of 400 mg efavirenz versus standard 600 mg dose in HIV-infected, antiretroviral-naive adults (ENCORE1): a randomised, double-blind, placebo-controlled, non-inferiority trial. *Lancet* 383, 1474–1482. doi: 10.1016/S0140-6736(13)62187-X

ENCORE1 Study Group, Carey, D., Puls, R., Amin, J., Losso, M., Phanupak, P., et al. (2015). Efficacy and safety of efavirenz 400 mg daily versus 600 mg daily: 96-week data from the randomised, double-blind, placebo-controlled, non-inferiority ENCORE1 study. *Lancet Infect Dis.* 15, 793–802. doi: 10.1016/S1473-3099(15)70060-5

Fan, L., Wang, J.-C., Jiang, F., Tan, Z.-R., Chen, Y., Li, Q., et al. (2009). Induction of cytochrome p450 2b6 activity by the herbal medicine baicalin as measured by bupropion hydroxylation. *Eur. J. Clin. Pharmacol.* 65, 403–409. doi: 10.1007/s00228-008-0594-3

Fayet, A., Béguin, A., de Tejada, B. M., Colombo, S., Cavassini, M., Gerber, S., et al. (2008). Determination of unbound antiretroviral drug concentrations by a modified ultrafiltration method reveals high variability in the free fraction. *Ther. Drug Monit.* 30, 511–522. doi: 10.1097/FTD.0b013e3181817318

Fichtenbaum, C. J., and Gerber, J. G. (2002). Interactions between antiretroviral drugs and drugs used for the therapy of the metabolic complications encountered during HIV infection. *Clin. Pharmacokinet.* 41, 1195–211. doi: 10.2165/00003088-200241140-00004

Fletcher, C. V., Staskus, K., Wietgrefe, S. W., Rothenberger, M., Reilly, C., Chipman, J. G., et al. (2014). Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc. Natl. Acad. Sci. U.S.A.* 111, 2307–2312. doi: 10.1073/pnas.1318249111

Frank, M., von Kleist, M., Kunz, A., Harms, G., Schütte, C., and Kloft, C. (2011). Quantifying the impact of nevirapine-based prophylaxis strategies to prevent mother-to-child transmission of HIV-1: a combined pharmacokinetic, pharmacodynamic, and viral dynamic analysis to predict clinical outcomes. *Antimicrob. Agents Chemother.* 55, 5529–5540. doi: 10.1128/AAC.00741-11

Grant, R. M., Anderson, P. L., McMahan, V., Liu, A., Amico, K. R., Mehrotra, M., et al. (2014) Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect Dis.* 14, 820–829. doi: 10.1016/S1473-3099(14)70847-3

Grant, R. M., Lama, J. R., Anderson, P. L., McMahan, V., Liu, A. Y., Vargas, L., et al. (2010). Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N. Engl. J. Med.* 363, 2587–2599. doi: 10.1056/NEJMoa1011205

Gulick, R. M. (2018). Investigational antiretroviral drugs: What is coming down the pipeline. *Top Antivir. Med.* 25, 127–132.

Haberer, J. E., Bangsberg, D. R., Baeten, J. M., Curran, K., Koechlin, F., Amico, K. R., et al. (2015). Defining success with HIV pre-exposure prophylaxis: a prevention-effective adherence paradigm. *AIDS* 29, 1277–1285. doi: 10.1097/QAD.0000000000000647

Hu, Z., and Kuritzkes, D. R. (2014). Altered viral fitness and drug susceptibility in HIV-1 carrying mutations that confer resistance to nonnucleoside reverse transcriptase and integrase strand transfer inhibitors. *J. Virol.* 88, 9268–9276. doi: 10.1128/JVI.00695-14

Isaacman-Beck, J., Hermann, E. A., Yi, Y., Ratcliffe, S. J., Mulenga, J., Allen, S., et al. (2009). Heterosexual transmission of human immunodeficiency virus type 1 subtype C: macrophage tropism, alternative coreceptor use, and the molecular anatomy of CCR5 utilization. *J. Virol.* 83, 8208–8220. doi: 10.1128/JVI.00296-09

Jilek, B. L., Zarr, M., Sampah, M. E., Rabi, S. A., Bullen, C. K., Lai, J., et al. (2012). A quantitative basis for antiretroviral therapy for HIV-1 infection. *Nat. Med.* 18, 446–451. doi: 10.1038/nm.2649

Joseph, S. B., Swanstrom, R., Kashuba, A. D. M., and Cohen, M. S. (2015). Bottlenecks in HIV-1 transmission: insights from the study of founder viruses. *Nat. Rev. Microbiol.* 13, 414–425. doi: 10.1038/nrmicro3471

Keller, S. B., and Smith, D. M. (2011). The price of tenofovir-emtricitabine undermines the cost-effectiveness and advancement of pre-exposure prophylaxis. *AIDS* 25, 2308–2310. doi: 10.1097/QAD.0b013e32834d3cab

Koelsch, K. K., Liu, L., Haubrich, R., May, S., Havlir, D., Günthard, H. F., et al. (2008). Dynamics of total, linear nonintegrated, and integrated HIV-1 DNA *in vivo* and *in vitro*. *J. Infect. Dis.* 197, 411–419. doi: 10.1086/525283

Konrad, B. P., Taylor, D., Conway, J. M., Ogilvie, G. S., and Coombs, D. (2017). On the duration of the period between exposure to HIV and detectable infection. *Epidemics* 20, 73–83. doi: 10.1016/j.epidem.2017.03.002

Markowitz, M., Frank, I., Grant, R. M., Mayer, K. H., Elion, R., Goldstein, D., et al. (2017). Safety and tolerability of long-acting cabotegravir injections in HIV-uninfected men (ECLAIR): a multicentre, double-blind, randomised, placebo-controlled, phase 2a trial. *Lancet HIV* 4, e331–e340. doi: 10.1016/S2352-3018(17)30068-1

Markowitz, M., Louie, M., Hurley, A., Sun, E., Mascio, M. D., Perelson, A. S., et al. (2003). A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay *in vivo*. *J. Virol.* 77, 5037–5038. doi: 10.1128/JVI.77.8.5037-5038.2003

Marrazzo, J. M., Ramjee, G., Richardson, B. A., Gomez, K., Mgodi, N., Nair, G., et al. (2015). Tenofovir-based preexposure prophylaxis for HIV infection among african women. *N. Engl. J. Med.* 372, 509–518. doi: 10.1056/NEJMoa1402269

Marzolini, C., Telenti, A., Decosterd, L. A., Greub, G., Biollaz, J., and Buclin, T. (2001). Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS* 15, 71–75. doi: 10.1097/00002030-200101050-00011

Mathur, S., Pilgrim, N., and Pulerwitz, J. (2016). PrEP introduction for adolescent girls and young women. *Lancet HIV* 3, e406–e408. doi: 10.1016/S2352-3018(16)30115-1

Maxmen, A. (2016). Older men and young women drive south african HIV epidemic. *Nature* 535, 335–335. doi: 10.1038/nature.2016.20273

McCormack, S., Dunn, D. T., Desai, M., Dolling, D. I., Gafos, M., Gilson, R., et al. (2016). Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. *Lancet* 387, 53–60. doi: 10.1016/S0140-6736(15)00056-2

McGowan, I., Dezzutti, C. S., Siegel, A., Engstrom, J., Nikiforov, A., Duffill, K., et al. (2016). Long-acting rilpivirine as potential pre-exposure prophylaxis for HIV-1 prevention (the MWRI-01 study): an open-label, phase 1, compartmental, pharmacokinetic and pharmacodynamic assessment. *Lancet HIV* 3, e569–e578. doi: 10.1016/S2352-3018(16)30113-8

McMillan, J., Szlachetka, A., Slack, L., Sillman, B., Lamberty, B., Morsey, B., et al. (2017). Pharmacokinetics of a long-acting nanoformulated dolutegravir prodrug in rhesus macaques. *Antimicrob. Agents Chemother.* 62, e01316-17. doi: 10.1128/AAC.01316-17

Molina, J. M., Capitant, C., Spire, B., Pialoux, G., Cotte, L., Charreau, I., et al. (2015). On-demand preexposure prophylaxis in men at high risk for HIV-1 infection. *N. Engl. J. Med.* 373, 2237–2246. doi: 10.1056/NEJMoa1506273

Mukonzo, J. K., Röshammar, D., Waako, P., Andersson, M., Fukasawa, T., Milani, L., et al. (2009). A novel polymorphism in abcb1 gene, cyp2b6*6 and sex predict single-dose efavirenz population pharmacokinetics in ugandans. *Br. J. Clin. Pharmacol.* 68, 690–699. doi: 10.1111/j.1365-2125.2009.03516.x

Ogburn, E. T., Jones, D. R., Masters, A. R., Xu, C., Guo, Y., and Desta, Z. (2010). Efavirenz primary and secondary metabolism *in vitro* and *in vivo*: identification of novel metabolic pathways and cytochrome p450 2a6 as the principal catalyst of efavirenz 7-hydroxylation. *Drug Metab. Dispos.* 38, 1218–1229. doi: 10.1124/dmd.109.031393

Orrell, C., Bienczak, A., Cohen, K., Bangsberg, D., Wood, R., Maartens, G., et al. (2016). Effect of mid-dose efavirenz concentrations and CYP2B6 genotype on viral suppression in patients on first-line antiretroviral therapy. *Int. J. Antimicrob. Agents* 47, 466–472. doi: 10.1016/j.ijantimicag.2016.03.017

Parkin, N. T., Hellmann, N. S., Whitcomb, J. M., Kiss, L., Chappey, C., and Petropoulos, C. J. (2004). Natural variation of drug susceptibility in wild-type human immunodeficiency virus type 1. *Antimicrob. Agents Chemother* 48, 437–443. doi: 10.1128/AAC.48.2.437-443.2004

Pearson, J. E., Krapivsky, P., and Perelson, A. S. (2011). Stochastic theory of early viral infection: continuous versus burst production of virions. *PLoS Comput. Biol.* 7:e1001058. doi: 10.1371/journal.pcbi.1001058

Perelson, A. S. (2002). Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* 2, 28–36. doi: 10.1038/nri700

Perelson, A. S., Kirschner, D. E., and De Boer, R. (1993). Dynamics of HIV infection of CD4+ T cells. *Math. Biosci.* 114, 81–125. doi: 10.1016/0025-5564(93)90043-A

Pierson, T. C., Zhou, Y., Kieffer, T. L., Ruff, C. T., Buck, C., and Siliciano, R. F. (2002). Molecular characterization of preintegration latency in human immunodeficiency virus type 1 infection. *J. Virol.* 76, 8518–8531. doi: 10.1128/JVI.76.17.8518-8513.2002

Ping, L.-H., Joseph, S. B., Anderson, J. A., Abrahams, M.-R., Salazar-Gonzalez, J. F., Kincer, L. P., et al. (2013). Comparison of viral Env proteins from acute and chronic infections with subtype c human immunodeficiency virus type 1 identifies differences in glycosylation and CCR5 utilization and suggests a new strategy for immunogen design. *J. Virol.* 87, 7218–7233. doi: 10.1128/JVI.03577-12

Rakhmanina, N. Y., and van den Anker, J. N. (2010). Efavirenz in the therapy of HIV infection. *Expert. Opin. Drug Metab. Toxicol.* 6, 95–103. doi: 10.1517/17425250903483207

Rath, B. A., Yousef, K. P., Katzenstein, D. K., Shafer, R. W., Schütte, C., von Kleist, M., et al. (2013). *In vitro* HIV-1 evolution in response to triple reverse transcriptase inhibitors & in silico phenotypic analysis. *PLoS ONE* 8:e61102. doi: 10.1371/journal.pone.0061102

Reddy, Y. S., Gotzkowsky, S. K., Eron, J. J., Kim, J. Y., Fiske, W. D., Fiscus, S. A., et al. (2002). Pharmacokinetic and pharmacodynamic investigation of efavirenz in the semen and blood of human immunodeficiency virus type 1-infected men. *J. Infect Dis.* 186, 1339–1343. doi: 10.1086/344311

Rhee, S.-Y., Gonzales, M. J., Kantor, R., Betts, B. J., Ravela, J., and Shafer, R. W. (2003). Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res.* 31, 298–303. doi: 10.1093/nar/gkg100

Sampah, M. E. S., Shen, L., Jilek, B. L., and Siliciano, R. F. (2011). Dose-response curve slope is a missing dimension in the analysis of HIV-1 drug resistance. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7613–7618. doi: 10.1073/pnas.1018360108

Schauer, G. D., Huber, K. D., Leuba, S. H., and Sluis-Cremer, N. (2014). Mechanism of allosteric inhibition of HIV-1 reverse transcriptase revealed by single-molecule and ensemble fluorescence. *Nucleic Acids Res.* 42, 11687–11696. doi: 10.1093/nar/gku819

Sedaghat, A. R., Dinoso, J. B., Shen, L., Wilke, C. O., and Siliciano, R. F. (2008). Decay dynamics of HIV-1 depend on the inhibited stages of the viral life cycle. *Proc. Natl. Acad. Sci. U.S.A.* 105, 4832–4837. doi: 10.1073/pnas.0711372105

Sedaghat, A. R., Siliciano, R. F., and Wilke, C. O. (2009). Constraints on the dominant mechanism for HIV viral dynamics in patients on raltegravir. *Antivir. Ther.* 14, 263–271.

Shen, L., Peterson, S., Sedaghat, A. R., McMahon, M. A., Callender, M., Zhang, H., et al. (2008). Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. *Nat. Med.* 14, 762–766. doi: 10.1038/nm1777

Siccardi, M., Almond, L., Schipani, A., Csajka, C., Marzolini, C., Wyen, C., et al. (2012). Pharmacokinetic and pharmacodynamic analysis of efavirenz dose

reduction using an *in vitro-in vivo* extrapolation model. *Clin. Pharmacol. Ther.* 92, 494–502. doi: 10.1038/clpt.2012.61

Smith, D. A., Di, L., and Kerns, E. H. (2010). The effect of plasma protein binding on *in vivo* efficacy: misconceptions in drug discovery. *Nat. Rev. Drug Discov.* 9, 929–939. doi: 10.1038/nrd3287

Smith, P. F., DiCenzo, R., and Morse, G. D. (2001). Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. *Clin. Pharmacokinet.* 40, 893–905. doi: 10.2165/00003088-200140120-00002

Stafford, M. A., Corey, L., Cao, Y., Daar, E. S., Ho, D. D., and Perelson, A. S. (2000). Modeling plasma virus concentration during primary HIV infection. *J. Theor. Biol.* 203, 285–301. doi: 10.1006/jtbi.2000.1076

Tan, W. Y., and Wu, H. (1998). Stochastic modeling of the dynamics of CD4+ T-cell infection by HIV and some Monte Carlo studies. *Math. Biosci.* 147, 173–205. doi: 10.1016/S0025-5564(97)00094-1

Thompson, C. G., Bokhart, M. T., Sykes, C., Adamson, L., Fedoriw, Y., Luciw, P. A., et al. (2015). Mass spectrometry imaging reveals heterogeneous efavirenz distribution within putative HIV reservoirs. *Antimicrob. Agents Chemother* 59, 2944–2948. doi: 10.1128/AAC.04952-14

Tuckwell, H. C., Shipman, P. D., and Perelson, A. S. (2008). The probability of HIV infection in a new host and its reduction with microbicides. *Math. Biosci.* 214, 81–86. doi: 10.1016/j.mbs.2008.03.005

UNAIDS (2015). *AIDS by the Numbers 2015*. Technical report. Available online at: http://www.unaids.org/en/resources/documents/2015/AIDS_by_the_numbers_2015 (Accessed Dec 14, 2018).

UNAIDS (2016). *AIDS by the Numbers.* Available online at: (http://www.unaids.org/sites/default/files/media_asset/AIDS-by-the-numbers-2016_en.pdf), (Accessed Dec 14, 2018).

UNAIDS (2017). *Ending Aids: progress Towards the 90-90-90 Targets*. Available online at: (http://www.unaids.org/sites/default/files/media_asset/Global_AIDS_update_2017_en.pdf) (Accessed dec 14, 2018).

Van Damme, L., Corneli, A., Ahmed, K., Agot, K., Lombaard, J., Kapiga, S., et al. (2012). Preexposure prophylaxis for HIV infection among African women. *N Engl. J. Med.* 367, 411–422. doi: 10.1056/NEJMoa1202614

Voliotis, M., Thomas, P., Grima, R., and Bowsher, C. G. (2016). Stochastic simulation of biomolecular networks in dynamic environments. *PLoS Comput. Biol.* 12:e1004923. doi: 10.1371/journal.pcbi.1004923

von Kleist, M., and Huisinga, W. (2007). Physiologically based pharmacokinetic modelling: a sub-compartmentalized model of tissue distribution. *J. Pharmacokinet Pharmacodyn.* 34, 789–806. doi: 10.1007/s10928-007-9071-3

von Kleist, M., Menz, S., and Huisinga, W. (2010). Drug-class specific impact of antivirals on the reproductive capacity of HIV. *PLoS Comput. Biol.* 6:e1000720. doi: 10.1371/journal.pcbi.1000720

von Kleist, M., Menz, S., Stocker, H., Arasteh, K., Schütte, C., and Huisinga, W. (2011). HIV quasispecies dynamics during pro-active treatment switching: impact on multi-drug resistance and resistance archiving in latent reservoirs. *PLoS ONE* 6:e18204. doi: 10.1371/journal.pone.0018204

Ward, B. A., Gorski, J. C., Jones, D. R., Hall, S. D., Flockhart, D. A., and Desta, Z. (2003). The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J. Pharmacol. Exp. Ther.* 306, 287–300. doi: 10.1124/jpet.103.049601

Wei, X., Ghosh, S. K., Taylor, M. E., Johnson, V. A., Emini, E. A., Deutsch, P., et al. (1995). Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 373, 117–122. doi: 10.1038/373117a0

Wilson, D. P., Law, M. G., Grulich, A. E., Cooper, D. A., and Kaldor, J. M. (2008). Relation between HIV viral load and infectiousness: a model-based analysis. *Lancet* 372, 314–320. doi: 10.1016/S0140-6736(08)61115-0

Yousef, K. P., Meixenberger, K., Smith, M. R., Somogyi, S., Gromöller, S., Schmidt, D., et al. (2016). Inferring HIV-1 Transmission dynamics in Germany from recently transmitted viruses. *J. Acq. Immune Defic. Syndr.* 73, 356–363. doi: 10.1097/QAI.0000000000001122

Zazzi, M., Hu, H., and Prosperi, M. (2018). The global burden of HIV-1 drug resistance in the past 20 years. *PeerJ* 6:e4848. doi: 10.7717/peerj.4848

Zhou, Y., Zhang, H., Siliciano, J. D., and Siliciano, R. F. (2005). Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells. *J. Virol.* 79, 2199–2210. doi: 10.1128/JVI.79.4.2199-2210.2005

Zhu, M., Kaul, S., Nandy, P., Grasela, D. M., and Pfister, M. (2009). Model-based approach to characterize efavirenz autoinduction and concurrent enzyme induction with carbamazepine. *Antimicrob. Agents Chemother.* 53, 2346–2353. doi: 10.1128/AAC.01120-08

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 17 April 2019
doi: 10.3389/fphar.2019.00401

Tenofovir-Diphosphate as a Marker of HIV Pre-exposure Prophylaxis Use Among East African Men and Women

Maria Pyra[1,2], Pete Anderson[3], Jessica E. Haberer[4,5], Renee Heffron[1,2], Connie Celum[1,2,6], Stephen Asiimwe[7], Elly Katabira[8], Nelly R. Mugo[2,9], Elizabeth A. Bukusi[2,9,10] and Jared M. Baeten[1,2,6] on behalf of the Partners Demonstration Project Study Team*

[1] Department of Epidemiology, University of Washington, Seattle, WA, United States, [2] Department of Global Health, University of Washington, Seattle, WA, United States, [3] Department of Pharmaceutical Sciences, University of Colorado, Aurora, Aurora, CO, United States, [4] Massachusetts General Hospital Global Health, Boston, MA, United States, [5] Department of Medicine, Harvard Medical School, Boston, MA, United States, [6] Department of Medicine, University of Washington, Seattle, WA, United States, [7] Kabwohe Clinical Research Center, Kabwohe, Uganda, [8] Infectious Diseases Institute, Makerere University, Makerere, Uganda, [9] Kenya Medical Research Institute, Nairobi, Kenya, [10] Department of Obstetrics & Gynecology, University of Washington, Seattle, WA, United States

OPEN ACCESS

Edited by:
Noel Edward Cranswick, The University of Melbourne, Australia

Reviewed by:
Nazareno Paolocci, Johns Hopkins University, United States
Jill Blumenthal, University of California, San Diego, United States

****Correspondence:***
Jared M. Baeten
jbaeten@uw.edu

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *23 April 2018*
Accepted: *01 April 2019*
Published: *17 April 2019*

Citation:
Pyra M, Anderson P, Haberer JE, Heffron R, Celum C, Asiimwe S, Katabira E, Mugo NR, Bukusi EA and Baeten JM (2019) Tenofovir-Diphosphate as a Marker of HIV Pre-exposure Prophylaxis Use Among East African Men and Women. Front. Pharmacol. 10:401. doi: 10.3389/fphar.2019.00401

Background: Controlled pharmacokinetic (PK) studies in United States populations have defined categories of tenofovir-diphosphate (TFV-DP) in dried blood spots (DBS) for various pre-exposure prophylaxis (PrEP) adherence targets. It is unknown how these categories perform in other populations. Therefore, we evaluated the sensitivity and specificity of these PK-derived categories compared to daily medication electronic adherence monitoring (MEMS) data among East African men and women using daily PrEP.

Methods: Participants were enrolled as members of HIV serodiscordant couples as part of an open-label PrEP study in Kenya and Uganda. Blood samples were taken at quarterly visits and stored as DBS, which were analyzed for TFV-DP concentrations.

Results: Among 150 samples from 103 participants, MEMs data indicated that 87 (58%) took ≥4 doses and 62 (41%) took ≥6 per week consistently over the 4 weeks prior to sample collection. Sensitivities of DBS TFV-DP levels were 62% for the ≥4 doses/week category (≥700 fmol/punch TFV-DP) and 44% for the ≥6 doses/week category (≥1050 fmol/punch TFV-DP); specificities were 86 and 94%, respectively. There were no statistically significant differences in these sensitivities and specificities by gender.

Conclusion: In this sample of East African PrEP users, categories of TFV-DP concentrations developed from directly observed PrEP use among United States populations had high specificity but lower than expected sensitivity. Sensitivity was lowest when MEMS data indicated high adherence (i.e., ≥6 doses/week). PrEP studies and implementation programs should carefully consider the sensitivity and specificity of the TFV-DP levels used for adherence feedback.

Keywords: tenofovir-diphosphate, adherence, pre-exposure prophylaxis, women, Africa, HIV

INTRODUCTION

Clinical trials have shown that pre-exposure prophylaxis (PrEP) is highly effective for preventing HIV (Grant et al., 2010; Baeten et al., 2012; Thigpen et al., 2012). However, effectiveness depends strongly on adherence (Abdool Karim, 2014). Clinical studies and open-label implementation programs have used many methods to assess PrEP adherence. As participants may misreport PrEP use, biomarkers are of particular interest as an objective marker of adherence. Some biomarkers, including concentrations of tenofovir in plasma or emtricitabine-triphosphate in blood cells, detect only recent use and are susceptible to white-coat effects, when individuals take a dose before a visit to appear adherent.

In contrast, the active metabolite tenofovir-diphosphate (TFV-DP) accumulates in blood cells in a dose-proportional manner (Anderson et al., 2017) and is a marker of cumulative use over the prior month. As a biomarker, TFV-DP is increasingly being used to assess adherence in research and implementation projects, with adherence counseling sometimes tailored based on these values (Celum and Delaney-Moretlwe, 2015). Intensive, controlled pharmacokinetic studies in United States populations were conducted with directly observed treatment (DOT) to estimate the expected levels of TFV-DP for specific adherence targets (i.e., ≥2 or ≥4 doses/week) (Anderson et al., 2017); these levels have been associated with HIV protection among men who have sex with men in the iPrEx placebo-controlled trial conducted in the United States, South America, Thailand and South Africa (Grant et al., 2014; Liu et al., 2016). However, the sensitivity and specificity of these levels have not specifically been evaluated in African populations, where PrEP roll-out is actively underway, although biological differences are possible (Anderson et al., 2017). Therefore, our goal was to evaluate the sensitivity and specificity of these categories in African men and women, using electronic adherence monitoring data for comparison.

MATERIALS AND METHODS

Study Sample

These data come from the Partners Demonstration Project, an open-label PrEP demonstration study among HIV serodiscordant couples in East Africa, as previously described (Baeten et al., 2016; Haberer et al., 2017). A total of 1,013 couples were enrolled in Kenya and Uganda. Participants were given electronic monitoring devices (MEMS caps, WestRock, Switzerland), which recorded daily bottle openings. MEMS data were downloaded and other variables collected at quarterly study visits, when PrEP (emtricitabine/tenofovir disoproxil fumarate 200/300 mg, prescribed for daily use) was dispensed. Participants received PrEP adherence counseling at study visits, but neither MEMS data nor TFV-DP concentrations were shared. Blood samples were prepared into dried blood spots (DBS) at quarterly visits and stored at −20°C. The University of Washington Human Subjects Division as well as ethics review committees at each site (either the National HIV/AIDS Research Committee of the Uganda National Council for Science and Technology or the Ethics Review Committee of the Kenya Medical Research Institute approved the protocol). All participants provided written informed consent in their preferred language in accordance with the Declaration of Helsinki.

To ensure variation in adherence patterns, we selected 120 random DBS, stratified by gender and evenly distributed with 0–2, 3–5, or 6–7 recorded openings by MEMS in the week prior to collection. In addition, any samples from the same participant at both the first and third month study visit were included (n = 76 additional samples), to assess changes over time. To optimize accuracy of MEMS data, we excluded any DBS when the participant reported curiosity openings (opening without removing pills, n = 8) or pocket dosing (removing multiple pills, n = 17); we also limited the data to one bottle opening per day. Finally, we excluded any visits during pregnancy (n = 21) (Pyra et al., 2018). TFV-DP was analyzed from DBS by liquid chromatography tandem mass spectrometry (LC-MS/MS) at the University of Colorado (Castillo-Mancilla et al., 2014, 2016; Zheng et al., 2016); values below the lower limit of quantitation of 31.25 fmol/punch were set to half the lower limit.

Statistical Analysis

We considered three adherence targets as recorded by MEMS consistently for the 4 weeks prior to sample collection, and corresponding TFV-DP categories based on previous DOT analyses (Castillo-Mancilla et al., 2012; Anderson et al., 2017): ≥700 fmol/punch for ≥4 doses, ≥1050 fmol/punch for ≥6 doses, and ≥1250 fmol/punch for 7 doses/week. The PK thresholds were established at 25th percentiles from prior studies such that 75% of adherent PrEP users were captured by the category.

We reported concentrations of TFV-DP among consistent users by gender and duration of PrEP use (early use, defined as the first month of PrEP, or later use). We tested gender, duration of PrEP use, total doses over the prior month, and study site in a generalized estimating equation model predicting TFV-DP concentration. We reported sensitivity and specificity, using MEMS data as the standard, with Wald 95% confidence intervals. In a sensitivity analysis, we used average (vs. consistent) doses. We also compared sensitivities and specificities by gender, using an interaction term in generalized estimating equations with a binomial distribution to account for repeated observations. In sensitivity analyses, we excluded samples with unquantifiable TFV-DP and repeated the analyses using doses over only the prior week.

We also calculated positive and negative predictive values over a range of adherence levels. These values indicate how likely an individual's test result correctly predicts adherence and depend not only on sensitivity and specificity but also on the ratio of true positives to false positives, i.e., the prevalence of being adherent. Next, to assess the effect of misclassification, specifically over-reporting, in the MEMS data, we used a multidimensional bias analysis to test a hypothetical scenario where 98% of those truly meeting the adherence targets were captured by MEMS and between 10 and 30% of true low-adherers were misclassified by MEMs as adherent (Lash et al., 2009). Analyses were conducted in SAS 9.4, with bias and predictive value analyses in Excel.

RESULTS

Participant Characteristics

Our analysis includes 150 DBS from 103 participants with 55 samples from 35 women and 95 samples from 68 men. The median age of men was 32.9 years and women was 29.5 years at baseline. Men recorded an average of 4.6 doses/week over the prior 4 weeks and women recorded 4.1 doses/week on average (**Supplementary Table 1**). Twenty-two samples were classified as early PrEP use. Overall, 58% (87) of samples consistently had ≥4 doses, 41% (62) had ≥6 doses, and 14% (21) had 7 doses/week for all of the prior 4 weeks as recorded by MEMS (**Supplementary Figure 1**).

Observed Concentrations

The average concentration among samples with ≥4 doses/week by MEMS was 925 fmol/punch [standard deviation (SD) 509] (**Table 1**). For those with ≥6 doses/week, the average was 994 fmol/punch (SD 517) and for those with 7 doses/week, it was 928 fmol/punch (SD 390). After controlling for site, duration of PrEP use, and total doses by MEMS, the concentration of TFV-DP was 12% higher among women compared to men, which was not statistically significant [adjusted risk difference (aRD) 80.8 fmol/punch (95% CI −78.7, 242.0)]. In the same model, early use (first month of PrEP) was significantly associated with 20% lower concentrations, aRD −129.1 fmol/punch (95% CI −227.8, −30.3).

Sensitivity and Specificity

The sensitivity of the ≥700 fmol/punch category was 62% for the ≥4 doses/week dosing and the specificity was 86% (**Table 2**). The sensitivity of the ≥1050 fmol/punch category was lower, 44%, and the specificity was higher, 93%, for ≥6 doses/week dosing. The ≥1250 fmol/punch category had a sensitivity of 19% and a specificity of 90% for the 7 doses/week dosing. There were no statistical differences by gender, though we were unable to test the difference for the ≥1250 category due to small sample size. When excluding 18 samples with unquantifiable TFV-DP, results were similar (**Table 2**). In addition, results based on doses over the prior 1 week and on average doses were similar to those described (**Supplementary Table 2**).

Using the observed sensitivity and specificity for the ≥700 fmol/punch and ≥1050 fmol/punch categories, we calculated positive and negative predictive values over a range of adherence levels to provide context for interpreting these results. For instance, if more than half of PrEP users are truly meeting the ≥4 doses/week target, there is a >80% probability that a TFV-DP result >700 fmol/punch is identifying a true high-adherent user; however, there is <70% probability that a result <700 fmol/punch is correctly identifying a true low-adherent user (**Supplementary Figure 2**).

Misclassification Bias Analysis

We recognized potential for misclassification of adherence from MEMS data. Both over and under-reporting have been observed with electronic monitoring, but over-reporting would explain the low sensitivities we observed. In the bias analysis, we found there would have to be >20% misclassification among true low-adherers for sensitivities to reach the expected level of 75% (**Supplementary Table 3**).

DISCUSSION

In this analysis, we assessed the sensitivity and specificity of PK-derived categories from United States studies in TFV-DP concentrations from PrEP-taking populations in Africa. We found that these categories have high specificity but relatively low sensitivities, compared to MEMS data. The sensitivity for the ≥700 fmol/punch category for the ≥4 doses/week [62% (95% CI 52%, 72%)] was closest to the expected 75%. The sensitivity declined to 44% and 19% for ≥ 6 and 7 doses/week, indicating unexpectedly low TFV-DP with high MEMS openings. However, the high specificity for all the categories tested (generally ≥ 85%) means that most low-adherent users would have TFV-DP concentrations below the cut-offs.

In planning to use adherence monitoring, especially for counseling, it is important to consider the relative value of correctly identifying true high-adherent versus true low-adherent users, as sensitivity and specificity are trade-offs. While identifying low-adherent users (i.e., true negatives) may be useful to improve adherence, misidentifying participants who are achieving good adherence (i.e., false negatives) could have undesirable consequences (Van der Straten et al., 2018) including demotivation to take PrEP, although additional work is needed in this area. Studies are needed about how best to frame adherence counseling messages using biomarker feedback.

Our results are in line with the existing pharmacokinetic literature. Previous work has found higher TFV-DP levels in women compared to men with similar patterns of pill-taking (Anderson et al., 2017); we found a similar trend by gender, though it did not reach significance. In addition, TFV-DP is not expected to reach steady state within the first month of use (Anderson et al., 2017); in adjusted analyses, we found significantly lower concentrations from early use compared to later use samples.

The low sensitivities we observed were unexpected; several explanations are possible. TFV-DP concentrations have previously been reported to be approximately 14% lower in African-American participants compared to Caucasians, though this finding was not statistically significant in a small study from the United States (Anderson et al., 2017). Potential mechanisms for this difference are not known, but may include differential expression or function of transporters or enzymes that influence TFV and/or TFV-DP cellular pharmacology [including esterases, P-glycoprotein (ABCB1), breast cancer resistance protein (BCRP), adenylate kinase I, pyruvate kinase, nucleoside diphosphate kinase, or factors influencing red blood cell turnover] (Tong et al., 2007; Laizure et al., 2013; Lade et al., 2015). It may be important to conduct additional controlled PK studies in an African population.

However, over-reporting of adherence by MEMS would also bias the results in the direction we observed. To

TABLE 1 | TFV-DP concentrations and detection among consistent users, by gender and by use.

	Consistent≥4 weekly doses			Consistent≥6 weekly doses			Consistent 7 weekly doses		
	Women (*n* = 30)	**Men (*n* = 57)**	**Total (*n* = 87)**	**Women (*n* = 20)**	**Men (*n* = 42)**	**Total (*n* = 62)**	**Women (*n* = 5)**	**Men (*n* = 16)**	**Total (*n* = 21)**
Mean TFV-DP fmol/punch (SD)	898(530)	939(502)	925(509)	931(507)	1024(525)	994(517)	759(233)	981(420)	928(390)
Unquantifiable TFV-DP, % (n)	7%(2)	4%(2)	5%(4)	5%(1)	2.4%(1)	3%(2)	0%(0)	0%(0)	0%(0)
	Early use (*n* = 22)	**Late use (*n* = 65)**	**Total (*n* = 87)**	**Early use (*n* = 18)**	**Late use (*n* = 44)**	**Total (*n* = 62)**	**Early use (*n* = 6)**	**Late use (*n* = 15)**	**Total (*n* = 21)**
Mean TFV-DP fmol/punch (SD)	942(460)	920(528)	925(509)	1011(447)	988(548)	994(517)	1097(537)	860(311)	928(390)
Unquantifiable TFV-DP, % (n)	5%(1)	5%(3)	5%(4)	0%(0)	5%(2)	3%(2)	0%(0)	0%(0)	0%(0)

TABLE 2 | Sensitivity and specificity of TFV-DP categories by consistent doses over prior 4 weeks.

	≥700 fmol/punch (≥4 doses per week)		≥1050 fmol/punch (≥6 doses per week)		≥1250 fmol/punch (7 doses per week)	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
All Samples	62% (52%, 72%)	86% (77%, 94%)	44% (31%, 56%)	93% (88%, 98%)	19% (2%, 36%)	90% (85%, 95%)
Women	60% (42%, 78%)	92% (81%, 100%)	35% (14%, 56%)	94% (87%, 100%)	0% (na)	90% (82%, 98%)
Men	63% (51%, 76%)	82% (69%, 94%)	48% (33%, 63%)	92% (85%, 100%)	25% (4%, 46%)	90% (83%, 97%)
Early use	73% (54%, 91%)	83% (62%, 100%)	33% (12%, 55%)	94% (82%, 100%)	17% (0%, 46%)	89% (78%, 100%)
Quantifiable TFV-DP	65% (55%, 75%)	82% (71%, 92%)	45% (32%, 58%)	92% (85%, 98%)	19% (2%, 36%)	88% (82%, 94%)

address this, we conducted a sensitivity bias analysis and found that a large fraction (>20%) of true low-adherent users would have to be misclassified in order to achieve the expected 75% sensitivity. Finally, while sensitivity and specificity are not dependent on prevalence, in this situation, one group taking exactly 4 doses per week and another group taking exactly 7 doses per week would both meet the dichotomized ≥4 doses/week target – but likely have different proportions ≥700 fmol/punch and thereby different sensitivities. However, this would not explain the very low sensitivity for the 7 doses/week target, where there should be no variation.

The major limitation of this analysis, as already discussed, is the use of MEMS data, which may be subject to misclassification. However, MEMS has been shown to be valid of measure of PrEP and ART use (for example, by AUC compared to other biomarkers) and used as the standard in other studies comparing adherence measures (Arnsten et al., 2001; Musinguzi et al., 2016; Abaasa et al., 2017). Finally, we had small sample sizes for some categories that limited analyses.

These results provide important information regarding the sensitivity and specificity of TFV-DP categories in an African population. In addition, even in United States populations, the PK-derived cut-offs were designed to have only 75% sensitivity, which should be taken into account for studies assessing PrEP adherence. Determining categories of TFV-DP as an objective measure of adherence is important for clinical trials evaluating adherence and for counselors and clinicians using drug levels to provide feedback to patients. Indeed, patients have indicated that biomarker feedback is acceptable and even desired (Koester et al., 2015; Van der Straten et al., 2015). When providing biomarker feedback, it is important to consider the desired sensitivity and specificity in different populations, and to design adherence messaging accordingly.

MEMBERS OF THE PARTNERS DEMONSTRATION PROJECT TEAM

Coordinating Center (University of Washington) and collaborating investigators (Harvard Medical School, Johns Hopkins University, Massachusetts General Hospital): Jared Baeten (protocol chair), CC (protocol co-chair), RH (project director), Deborah Donnell (statistician), Ruanne Barnabas, Jessica Haberer, Harald Haugen, Craig

Hendrix, Lara Kidoguchi, Mark Marzinke, Susan Morrison, Jennifer Morton, Norma Ware, Monique Wyatt. Project sites: Kabwohe, Uganda (Kabwohe Clinical Research Centre): SA, Edna Tindimwebwa. Kampala, Uganda (Makerere University): EK, Nulu Bulya. Kisumu, Kenya (Kenya Medical Research Institute): Elizabeth Bukusi, Josephine Odoyo. Thika, Kenya (Kenya Medical Research Institute, University of Washington): Nelly Rwamba Mugo, Kenneth Ngure. Data Management was provided by DF/Net Research, Inc. (Seattle, WA, United States). PrEP medication was donated by Gilead Sciences.

ETHICS STATEMENT

The study was carried out in accordance with the recommendations of the University of Washington Human Subjects Division and ethics review committees at each of the study sites (for Kabwohe and Kampala Uganda, the National HIV/AIDS Research Committee of the Uganda Council for Science and Technology; for Kisumu and Thika, Kenya, the Ethics Review Committee of the Kenya Medical Research Institute). All participants provided written informed consent in their preferred language in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

MP and JB designed the research question. PA analyzed the samples. SA, EK, NM, and EB collected the data. MP analyzed the data and drafted the manuscript. All authors contributed to the manuscript.

FUNDING

The Partners Demonstration Project was funded by the National Institute of Mental Health of the United States National Institutes of Health (R01 MH095507), the Bill & Melinda Gates Foundation (OPP1056051), and the United States Agency for International Development (AID-OAA-A-12-00023). Research reported in this publication was supported by the UW/Fred Hutch Center for AIDS Research, funded by NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA, NIGMS, and NIDDK of the National Institutes of Health under award number P30 A1027757 and the HIV Prevention Trials Network (UM1 AI068613). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID, NIH, or the United States Government.

ACKNOWLEDGMENTS

We thank the couples who participated in this study and the members of the Colorado Antiviral Pharmacology Laboratory.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00401/full#supplementary-material

REFERENCES

Abaasa, A., Hendrix, C., Gandhi, M., Anderson, P., Kamali, A., Kibengo, F., et al. (2017). Utility of different adherence measures for PrEP: patterns and incremental value. *AIDS Behav.* 22, 1165–1173. doi: 10.1007/s10461-017-1951-y

Abdool Karim, S. S. (2014). *The Potential and Challenges of ARV-Based HIV Prevention: An Overview*. Available at: http://slideplayer.com/slide/5909872/ (accessed May 4, 2016).

Anderson, P. L., Liu, A. Y., Castillo-Mancilla, J. R., Gardner, E. M., Seifert, S. M., McHugh, C., et al. (2017). Intracellular tenofovir-diphosphate and emtricitabine-triphosphate in dried blood spots following directly observed therapy: the DOT-DBS study. *Antimicrob. Agents Chemother.* 62:e01710-17. doi: 10.1128/AAC.01710-17

Arnsten, J. H., Demas, P. A., Farzadegan, H., Grant, R. W., Gourevitch, M. N., Chang, C.-J., et al. (2001). Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 33, 1417–1423. doi: 10.1086/323201

Baeten, J. M., Donnell, D., Ndase, P., Mugo, N. R., Campbell, J. D., Wangisi, J., et al. (2012). Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N. Engl. J. Med.* 367, 399–410. doi: 10.1056/NEJMoa1108524

Baeten, J. M., Heffron, R., Kidoguchi, L., Mugo, N. R., Katabira, E., Bukusi, E. A., et al. (2016). Integrated delivery of antiretroviral treatment and pre-exposure prophylaxis to HIV-1-serodiscordant couples: a prospective implementation study in Kenya and Uganda. *PLoS Med.* 13:e1002099. doi: 10.1371/journal.pmed.1002099

Castillo-Mancilla, J., Seifert, S., Campbell, K., Coleman, S., McAllister, K., Zheng, J.-H., et al. (2016). Emtricitabine-triphosphate in dried blood spots as a marker of recent dosing. *Antimicrob. Agents Chemother.* 60, 6692–6697. doi: 10.1128/AAC.01017-16

Castillo-Mancilla, J. R., Searls, K., Caraway, P., Zheng, J.-H., Gardner, E. M., Predhomme, J., et al. (2014). Short communication: tenofovir diphosphate in dried blood spots as an objective measure of adherence in HIV-infected women. *AIDS Res. Hum. Retroviruses* 31, 428–432. doi: 10.1089/aid.2014.0229

Castillo-Mancilla, J. R., Zheng, J.-H., Rower, J. E., Meditz, A., Gardner, E. M., Predhomme, J., et al. (2012). Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. *AIDS Res. Hum. Retroviruses* 29, 384–390. doi: 10.1089/aid.2012.0089

Celum, C., and Delaney-Moretlwe, S. (2015). *HPTN082 Study Protocol*. doi: 10.1089/aid.2012.0089

Grant, R. M., Anderson, P. L., McMahan, V., Liu, A., Amico, K. R., Mehrotra, M., et al. (2014). Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect. Dis.* 14, 820–829. doi: 10.1016/S1473-3099(14)70847-3

Grant, R. M., Lama, J. R., Anderson, P. L., McMahan, V., Liu, A. Y., Vargas, L., et al. (2010). Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N. Engl. J. Med.* 363, 2587–2599. doi: 10.1056/NEJMoa1011205

Haberer, J. E., Kidoguchi, L., Heffron, R., Mugo, N., Bukusi, E., Katabira, E., et al. (2017). Alignment of adherence and risk for HIV acquisition in a demonstration project of pre-exposure prophylaxis among HIV serodiscordant couples in Kenya and Uganda: a prospective analysis of prevention-effective adherence. *J. Int. AIDS Soc.* 20, 1–9. doi: 10.7448/IAS.20.1.21842

Koester, K. A., Liu, A., Eden, C., Amico, K. R., McMahan, V., Goicochea, P., et al. (2015). Acceptability of drug detection monitoring among participants in an open-label pre-exposure prophylaxis study. *AIDS Care* 27, 1199–1204. doi: 10.1080/09540121.2015.1039958

Lade, J. M., To, E. E., Hendrix, C. W., and Bumpus, N. N. (2015). Discovery of genetic variants of the kinases that activate tenofovir in a compartment-specific manner. *EBioMedicine* 2, 1145–1152. doi: 10.1016/j.ebiom.2015.07.008

Laizure, S. C., Herring, V., Hu, Z., Witbrodt, K., and Parker, R. B. (2013). The role of human carboxylesterases in drug metabolism: have we overlooked their importance? *Pharmacotherapy* 33, 210–222. doi: 10.1002/phar.1194

Lash, T. L., Fink, A. K., and Fox, M. P. (2009). *Applying Quantitative Bias Analysis to Epidemiologic Data (Series: Statistics for Biology & Health)*. New York, NY: Springer.

Liu, A. Y., Cohen, S. E., Vittinghoff, E., Anderson, P. L., Doblecki-Lewis, S., Bacon, O., et al. (2016). Preexposure prophylaxis for HIV infection integrated with municipal- and community-based sexual health services. *JAMA Intern. Med.* 176, 75–84. doi: 10.1001/jamainternmed.2015.4683

Musinguzi, N., Muganzi, C. D., Boum, Y., Ronald, A., Marzinke, M. A., Hendrix, C. W., et al. (2016). Comparison of subjective and objective adherence measures for pre-exposure prophylaxis against HIV infection among serodiscordant couples in East Africa. *AIDS* 30, 1121–1129. doi: 10.1097/QAD.0000000000001024

Pyra, M., Anderson, P., Mugwanya, K., Haberer, J. E., Heffron, R., Asiimwe, S., et al. (2018). "Concentrations of TFV-DP during pregnancy among women using PrEP," in *Proceedings of the 25th Conference on Retroviruses and Opportunistic Infections*, Boston, MA.

Thigpen, M. C., Kebaabetswe, P. M., Paxton, L. A., Smith, D. K., Rose, C. E., Segolodi, T. M., et al. (2012). Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N. Engl. J. Med.* 367, 423–434. doi: 10.1056/NEJMoa1110711

Tong, L., Phan, T. K., Robinson, K. L., Babusis, D., Strab, R., Bhoopathy, S., et al. (2007). Effects of human immunodeficiency virus protease inhibitors on the intestinal absorption of tenofovir disoproxil fumarate *in vitro*. *Antimicrob. Agents Chemother.* 51, 3498–3504. doi: 10.1128/AAC.00671-07

Van der Straten, A., Katz, A., Balan, I. C., Reddy, K., Etima, J., Woeber, K., et al. (2018). *A Qualitative Evaluation of Women's Experience Receiving Drug Feedback in MTN-025/HOPE - An HIV Prevention Open-Label Trial of the Dapivirine Vaginal Ring. in AIDS 2018* (Amsterdam, The Netherlands). doi: 10.1128/aac.00671-07

Van der Straten, A., Montgomery, E. T., Musara, P., Etima, J., Naidoo, S., Laborde, N., et al. (2015). Disclosure of pharmacokinetic drug results to understand nonadherence: results from a qualitative study. *AIDS* 29, 2161–2171. doi: 10.1097/QAD.0000000000000801

Zheng, J.-H., Rower, C., McAllister, K., Castillo-Mancilla, J., Klein, B., Meditz, A., et al. (2016). Application of an intracellular assay for determination of tenofovir-diphosphate and emtricitabine-triphosphate from erythrocytes using dried blood spots. *J. Pharm. Biomed. Anal.* 122, 16–20. doi: 10.1016/j.jpba.2016.01.038

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 24 May 2019
doi: 10.3389/fphar.2019.00578

The Pre-clinical Toolbox of Pharmacokinetics and Pharmacodynamics: *in vitro* and *ex vivo* Models

*Carolina Herrera**

Section of Virology, Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Imperial College London, London, United Kingdom

Prevention strategies against sexual transmission of human immunodeficiency virus (HIV) are essential to curb the rate of new infections. In the absence of a correlate of protection against HIV infection, pre-clinical evaluation is fundamental to facilitate and accelerate prioritization of prevention candidates and their formulations in a rapidly evolving clinical landscape. Characterization of pharmacokinetic (PK) and pharmacodynamic (PD) properties for candidate inhibitors is the main objective of pre-clinical evaluation. *in vitro* and *ex vivo* systems for pharmacological assessment allow experimental flexibility and adaptability at a relatively low cost without raising as significant ethical concerns as *in vivo* models. Applications and limitations of pre-clinical PK/PD models and future alternatives are reviewed in the context of HIV prevention.

Keywords: HIV, antiretrovirals, antibodies, pharmacokinetics and pharmacodynamics, pre-clinical models

OPEN ACCESS

Edited by:
Gerardo Garcia-Lerma, Centers for Disease Control and Prevention (CDC), United States

Reviewed by:
Brad A. Sutherland, University of Tasmania, Australia
Urvi Parikh, University of Pittsburgh, United States

***Correspondence:**
Carolina Herrera
cherrer1@imperial.ac.uk

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *03 January 2019*
Accepted: *06 May 2019*
Published: *24 May 2019*

Citation:
Herrera C (2019) The Pre-clinical Toolbox of Pharmacokinetics and Pharmacodynamics: in vitro and ex vivo Models. Front. Pharmacol. 10:578. doi: 10.3389/fphar.2019.00578

INTRODUCTION

The majority of HIV transmissions currently occur via the genital and the colorectal tracts. Following infection of the initial mucosal founder population (Li et al., 2009), viral amplification is essential for irreversible acquisition of infection and takes place in the first few days (1–3 days) after viral exposure, giving a very short window of opportunity to prevent establishment of infection. In the absence of a vaccine and acknowledging that condoms, male circumcision and behavioral interventions appear insufficient to control the epidemic, the development of mucosal prevention strategies remains an important global public health priority. A prevention method should be safe, acceptable, affordable, and capable of inhibiting viral transmission at the mucosal portals of entry. Effectiveness and adherence of prevention candidates can only be evaluated in clinical trials (Heise et al., 2011); however, phase III clinical trials are expensive, time consuming and require a large number of participants to determine efficacy (Douville et al., 2006; Nuttall et al., 2007). Furthermore, with the introduction of FDA-approved prevention interventions, such as Truvada for oral pre-exposure prophylaxis (PrEP) [Centers for Disease Control and Prevention (CDC), 2012], incidence of infection within communities will decrease, closing the window to perform placebo-controlled trials and causing the trials to become even larger to test later-generation products. Hence, pre-clinical models are increasingly important tools to reduce the risk of late stage failure in clinical trials.

A single model capable of providing all the information to prioritize the best-in-class candidate is not currently available. Furthermore, the drug profile required to prevent mucosal transmission has not been defined, nor has the best pharmacologic measure of efficacy been defined for different dosing routes of candidate inhibitor. Hence, the range of *in vivo*, *ex vivo,* and *in vitro* assays is continuously being expanded to assess parameters such as mechanism of action, potency and selectivity, PK/PD, safety, immune response elicited, stability, formulation, dosing and potential of acceptability, which will facilitate scaling when defining dosing regimens to be tested in humans. Dose-efficacy discrepancies between animal studies and clinical trials have been described (Romano et al., 2013) highlighting the gap in knowledge regarding the concentration-effect relationship in different species and mucosal compartments. This further emphasizes the need to develop models that will allow PK/PD evaluation of candidate inhibitors in the mucosal environment, recapitulate the factors potentially affecting a direct correlation between PK and PD parameters and facilitate appropriate comparisons between animal studies and humans, increasing the predictive capacity of pre-clinical studies.

HIV-inhibitor candidates include antiretrovirals (ARVs) for PrEP and post-exposure prophylaxis (PEP), broadly neutralizing antibodies (bNAbs) delivered by passive immunization (Morris and Mkhize, 2017) and enhancers of the innate mucosal barrier functions (Herrera and Shattock, 2014; Lajoie et al., 2017). The majority of ARVs currently considered for prophylaxis are already used in highly active ARV treatment (HAART); hence, a substantial amount of pharmacological data has been accumulated for these compounds in biological fluids, such as blood plasma and genital secretions (Cohen et al., 2007; Dickinson et al., 2010). However, drug concentration measurements in blood plasma are not representative of mucosal concentrations (Lederman et al., 2004; Cohen et al., 2007; Dumond et al., 2007, 2009; Brown et al., 2011; Trezza and Kashuba, 2014) and mucosal tissues are histologically and immunologically different from blood (Anton et al., 2000), affecting the expected correlation between concentration and efficacy at mucosal sites. In addition, drug accumulation is specific to each mucosal compartment, with differences between the intestinal and the female and male genital tracts (Cohen et al., 2007; Patterson et al., 2011; Louissaint et al., 2013), partially due to tissue-specific expression of drug transporters (Nicol et al., 2014). This review will discuss *in vitro* and *ex vivo* PK and PD models available and in development, their capacity to mimic fundamental aspects of the mucosal environment, their value for different candidates and dosing routes, their limitations and their potential in predicting the outcome of clinical trials (**Table 1**).

PHARMACOLOGICAL PARAMETERS FOR HIV PREVENTION STRATEGIES

PK describes the time course of drug concentration which is affected by absorption, distribution, metabolism and elimination and can be summarized as what the body does to the drug. PD describes the resulting effect of a drug, its intensity, time course and potential toxicity or responses to the drug, i.e., what the drug does to the body. Pharmacological assays aim to define PK and PD measures which include parameters such as Cmin (minimum concentration achieved within a dosing interval); Cmax (maximum concentration achieved within a dosing interval); C*t* (concentration at a certain time point post-dosing); AUC (area under the curve for drug concentration during a period of time); Tmax (time to achieve maximum concentration); $t_{1/2}$ (half-life; time required for concentration to decrease 50%); k_{el} (elimination rate over time); MIC (minimal inhibitory concentration); MEC (minimal effective concentration); *T* (time the concentration remains over the MIC or MEC); IC_{50} (50% inhibitory concentration); EC_{50} (50% effective concentration), extent of viral replication at the last time point of the assay or during a period of time (AUC of viral replication readout between two time points); cytotoxicity and immunological toxicity. The two main read-outs required to calculate these parameters are drug concentration and level of infection after treatment of the model with candidate inhibitors. Drug concentration can be measured as cell-free drug (in culture supernatants, in secretions or in plasma) or intracellularly (in cells or tissues) and new analytical methods are constantly being developed to measure the concentration of candidate inhibitors in these different matrixes. Intracellular measurements are necessary for inhibitors that require metabolization for activation such as some reverse transcriptase inhibitors. Evaluation of viral replication is specific for each pre-clinical model and can be done through measurement of a reporter signal, of gag protein (p24 for HIV and p27 for SIV) by ELISA, or of viral RNA/DNA by PCR or qRT-PCR (Berry et al., 2011).

Assays that provide data to calculate PK/PD will be defined by multiple factors including, among others, the candidate inhibitor, the dosing route, the formulation and the anatomical site of action. ARVs and modulators of mucosal immunity can be formulated for oral or topical dosing, as injectables or as implants; bNAbs can be delivered topically, intravenously or intramuscularly for passive immunization. For mucosal prevention, independently of the dosing route, concentrations measured in the genital and colorectal tracts will need to be sufficient to inhibit viral infection, and these concentrations will be tissue-specific.

CELLULAR MODELS

Inhibitory potency of candidate HIV inhibitors is initially screened in *in vitro* models such as cell lines susceptible to HIV infection that allow calculation of PK parameters and evaluation of cellular toxicity. Compounds or Abs are then tested in *ex vivo* cellular models such as PBMCs, which in addition to PK/PD parameters can provide toxicity and immunological safety information.

Cell Lines

Multiple cell lines are routinely used to screen potential efficacy of compounds and Abs including $CD4^+$T cell lines and non-lymphocytic cells that are transfected to express CD4 and CCR5 and/or CXCR4. Among the $CD4^-$ parental cell lines there are

TABLE 1 | Applications of current *in vitro* and *ex vivo* models for development of HIV-prevention strategies.

Model	Applications	Stage in development pipeline	Advantages	Disadvantages
Cell lines	• PD (*in vitro* or *in vivo* dosing) • PK (drug cellular transport) • Safety (cellular toxicity)	Initial screening	• Short experimental procedure (2–7 days) • Sensitive assays • Low cost • Accessible to most laboratories • Easy-to-use protocol • High throughput • Effect of biological fluids can be assessed	• Low physiological relevance • Over-expression of CD4 and/or HIV co-receptors • Some cell lines are not budding competent (inhibitors of viral maturation cannot be tested) • Do not replicate donor-to-donor variation • Cancerous origin
Primary cells	• PD (*ex vivo* or *in vivo* dosing) • PK (drug cellular transport) • Safety (cellular toxicity, cellular responses)	After initial screening in cell lines	• Increased physiological relevance • Express realistic levels of CD4 and HIV co-receptors • Secrete cytokines • Accessible to most laboratories • Mimic donor-to-donor variability • Effect of biological fluids can be assessed	• Longer procedure (minimum 11 days) • Low mucosal relevance • Ethics approval required and, in some cases, signed informed consent from donor • Medium to high protocol complexity • High cost in some cases
Cellular co-cultures	• PD (*ex vivo* or *in vivo* dosing) • PK (drug cellular transport) • Safety (cellular toxicity, cellular responses)	Following initial screening in cell lines and primary cells	• Mimic *trans* infection • Effect of biological fluids can be assessed	• Longer procedure (minimum 11 days) • Low throughput • Ethics approval required • Medium to high protocol complexity
Tissue explants	• PD (*ex vivo* or *in vivo* dosing) • PK (drug cellular transport) • Safety (toxicity and innate responses)	Before *in vivo* safety and/or efficacy studies	• *in vivo*-like complexity and structure • Mimic *in vivo* viral replication fitness • Innate responses can be measured • Migratory can be isolated in some models • Mimic donor-to-donor variability • Mucosal inflammation can be induced • Good correlation with *in vivo* studies • Effect of biological fluids can be assessed • Versatile model • Good data reproducibility through protocol standardization	• Longer procedure (minimum 11 days) • Progressive decay of structure with culture • Ethics approval and signed informed consent from donor required • Fresh tissue preferable • Cannot replace *in vivo* models yet • Limited specimen size • Low throughput • Medium to high protocol complexity

human glioblastoma cells, U87MG, which stably express human CD4 and CCR5 (U87 $CD4^+$ $CCR5^+$ cells) or CXCR4 (U87 $CD4^+$ $CXCR4^+$ cells) (Bjorndal et al., 1997); indicator cells derived from human osteosarcoma cells, HOS, stably transfected with human CD4, CCR5 and/or CXCR4 [GHOST (3) $CCR5^+$, GHOST (3) $CXCR4^+$ and GHOST (3) $CXCR4^+$ $CCR5^+$ cells] and that express green fluorescent protein (GFP) upon production of the viral trans-activator of transcription (Tat) (Morner et al., 1999); and human cervical epithelial carcinoma reporter cells, TZM-bl, which are HeLa cells expressing CD4, CCR5, CXCR4 and under control Tat, luciferase and β-galactosidase (Platt et al., 1998, 2009; Derdeyn et al., 2000; Wei et al., 2002; Takeuchi et al., 2008). This later cell line is susceptible to HIV-1, HIV-2, simian immunodeficiency virus (SIV), and simian human immunodeficiency virus (SHIV) and is nowadays one of the main cell lines used to screen inhibitory activity of ARVs and neutralization potency of Abs. TZM-bl cells are a single viral cycle assay model that requires 2 days of culture before infectivity is assessed by measurement of luciferase expression in cell lysates as relative light units or by measurement of absorbance with a β-galactosidase colorimetric assay. However, this cellular model does not allow efficient viral budding (Carlson et al., 2008) and therefore the activity of compounds that block viral maturation such as protease inhibitors cannot be evaluated with this assay (Stefanidou et al., 2012). Furthermore, TZM-bl cells are HeLa cells that endogenously express CXCR4 but express artificially high levels of CD4 and CCR5 (Polonis et al., 2008). Another reporter HeLa cell line, Affinofile (Johnston et al., 2009), resolves this issue by expressing variable levels of CD4 and CCR5 or CXCR4 based on the amount of selection antibiotic used in culture.

Drug screening is often completed in this model with evaluation of inhibitory potency in the presence of relevant mucosal fluids. TZM-bl cells have been also used to evaluate anti-viral activity in trials by incubating these cells with mucosal secretions (Keller et al., 2011; Herold et al., 2016) obtained from PrEP trial participants, or serum and plasma (Montefiori et al., 2012) during vaccine trials. However, biological fluids can decrease or enhance the level of infection measured in this model (Ghosh et al., 2010b; Hughes et al., 2016) due to inhibitory, toxic or enhancing factors in the fluid matrix such as innate molecules, secreted metabolites or chemical compounds taken by the donor. Therefore, dilution and/or filtration of the sample is required to avoid cytotoxic effects and contamination of the culture (Fletcher et al., 2009; Ghosh et al., 2010a; Harman et al., 2012; Mukura et al., 2012; Romas et al., 2014; Jais et al., 2016).

No effect has been observed on the susceptibility to infection of TZM-bl cells by the presence of endotoxins in the biological specimens nor with samples obtained at different stages of the menstrual cycle or during pregnancy (Geonnotti et al., 2010; Patel et al., 2014; Hughes et al., 2016); nevertheless, protocols have been developed to avoid artifacts. The innate anti-HIV activity of cationic factors present in cervical secretions can be prevented by selective depletion of cations (Venkataraman et al., 2005). When measuring Ab neutralization potency in serum or plasma, the presence of other HIV-inhibitory factors can be determined by pre-screening the activity of biological specimens in TZM-bl cells against a chimeric HIV-1 virus containing the Env of murine leukemia virus, which will not be recognized by anti-HIV Abs (Sarzotti-Kelsoe et al., 2014a). TZM-bl cells have also been further transfected to develop a model for evaluation of HIV innate responses (Trotard et al., 2016).

Numerous human $CD4^+$T cell lines have been used to determine inhibitory potency parameters. Among them, initial models MT-2 (Harada et al., 1985; Haertle et al., 1988) and MT-4 cells (Harada et al., 1985; Pauwels et al., 1987; Larder et al., 1989) expressing HTLV-1 have been progressively replaced by other T cell lines such as CEM-CCRF cells (Foley et al., 1965), PM-1 cells (Lusso et al., 1995) and C8166 cells (Salahuddin et al., 1983; Lee et al., 1984). $CD4^+CXCR4^+$ A3R5 cells have been transfected with CCR5 as a sensitive T cell model for evaluation of neutralization potency of Abs using luciferase reporter HIV-1 infectious molecular clones (Kim et al., 2003; Montefiori et al., 2012; Sarzotti-Kelsoe et al., 2014b). Assays in $CD4^+$T cells require at least 7 days of culture, allowing multiple rounds of viral replication, and infectivity is determined by measurement of p24 antigen content in culture supernatants with enzyme-linked immunosorbent assay (ELISA) or luciferase expression when using reporter viral plasmids. $CD4^+$T cell lines have also been used to study the mechanism of drug cellular transport and the implications of the PK profile of drug candidates for HIV prevention (Taneva et al., 2015).

Safety of compounds is initially determined in cellular models by assessing the level of potential cytotoxicity via measurement of tetrazolium salt (MTT) cleavage into a blue-colored product (formazan) in viable cells (Slater et al., 1963) or by similar assays of cellular viability. Despite the lack of productive infection, epithelial cell lines represent an important model to study potential toxicity or disruption of epithelium integrity induced by the drug (Dezzutti et al., 2004). To assess the impact of candidates on epithelial permeability, epithelial cells can be cultured on the apical chamber of trans-well systems to measure tight junctions. Drug transporters on mucosal epithelium allow penetration of ARVs in the epithelium to access the submucosal stroma where the initial foci of infection is located (Hu et al., 2015). In colon epithelium drug efflux is mainly mediated by P-glycoprotein (Pgp), multi-drug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP) transporters; and drug uptake is mediated by organic anion transporter OATP2B1 and organic cation transporter OCT1 (Englund et al., 2006; Kis et al., 2010; Drozdzik et al., 2014; Nicol et al., 2014; Mukhopadhya et al., 2016a). In female genital tract expression of efflux [ATP-binding cassette (ABC), BCRP, MRP, and P-gp] and influx [equilibrative nucleoside transporter (ENT), soluble carrier (SLC) and OCT] transporters has been described (Gunawardana et al., 2013; Zhou et al., 2013, 2014; Grammen et al., 2014; Nicol et al., 2014; Hijazi et al., 2015). Hence, this model is also relevant for PK studies to determine the impact of drug transporters in epithelial cells on drug or Ab concentrations when crossing the mucosal epithelial barrier (Konig et al., 2010; Kis et al., 2013; Hoque et al., 2015; Taneva et al., 2015; Swedrowska et al., 2017), to evaluate the potential effect of candidate inhibitors on drug transporters (Reznicek et al., 2017) and to study the safety and efficacy of formulations designed to deliver compounds across the epithelium to the HIV target cells (Kapitza et al., 2007; Zidan et al., 2013). Harvested supernatants from trans-well systems can be used to measure drug concentrations and for PD assays with $CD4^+$ cells. Microscopy has been considered to evaluate absorption/excretion and intracellular distribution of formulated drug candidates (Mandal et al., 2015; Costanzo et al., 2016; Holmstock et al., 2018). Available epithelial cell lines include urogenital epithelial cells [e.g., ME-180 (Sykes et al., 1970), HT-3 (Fogh et al., 1977) and HEC-1-A (Kuramoto, 1972)] and colorectal epithelial cell lines [such as Caco-2 (Fogh et al., 1977) and SW837 (Leibovitz et al., 1976)].

The main drawback of cell lines is their homogeneity, which fails to reproduce the cellular diversity of mucosal tissues and to replicate donor-to-donor variability. Another limitation is that the majority have cancerous cell origins and therefore, do not recapitulate a healthy mucosal environment. However, models such as the TZM-bl assay provide a sensitive and cost-effective tool for quickly assessing activity of candidate inhibitors.

Primary Cells

Primary cells such as lectin-activated peripheral blood mononuclear cells (PBMCs) as well as cells derived from PBMCs, including monocyte-derived macrophages and immature dendritic cells (iDCs), provide more physiologically relevant cellular models for anti-viral activity measurements. These *ex vivo* models involve longer experiments (7 to 14 days) allowing multiple viral replication cycles and therefore, tend to be used after initial assessment in immortalized cell lines. Inhibitory activity, concentration and safety of drugs and Abs have been measured in activated PBMCs following *ex vivo* dosing or in cells obtained from animal studies (Garcia-Lerma et al., 2011; Dobard et al., 2012; Massud et al., 2013; Anderson et al., 2014) and trial participants.

PBMCs provide information about the systemic compartment but are not fully representative of mononuclear cells found in mucosal tissues. Indeed, differences in PK parameters such as Cmax and half-life have been observed between PBMCs and mucosal mononuclear cells isolated from digested mucosal tissues obtained after *in vivo* dosing of NHPs (Garcia-Lerma et al., 2011; Dobard et al., 2012; Massud et al., 2013) and humans (Yang et al., 2014; McGowan et al., 2015). Differences in expression of drug transporter have also been described between circulating and mucosal $CD4^+$T cells (Kis et al., 2010; Mukhopadhya et al., 2016b). Another limitation is that PBMCs exhibit anti-HIV-1 activity in the presence of bacterial lipopolysaccharide (LPS), which will therefore, artefactually enhance the inhibitory

activity of biological specimens if they contain endotoxins (Geonnotti et al., 2010). However, activated PBMCs express more physiologically relevant levels of CD4 and HIV-coreceptors than transfected cell lines such as TZM-bl cells (Polonis et al., 2008), and secrete cytokines upon infection as mucosal tissues. This model is a more stringent tool for PD evaluation than cell lines with values of anti-viral activity closer to those observed in mucosal tissues than those obtained with *in vitro* cellular models. Hence, PBMCs represent an additional filter in the pre-clinical pipeline of modest cost and are accessible to most laboratories.

Primary epithelial cells can also be isolated (Greenhead et al., 2000) and cultured in trans-well systems as described above for epithelial cell lines, to assess safety, concentration and activity of candidate inhibitor after *ex vivo* dosing (Shen et al., 2018, 2019) and safety of excipients used for mucosal dosing (Hu et al., 2016). These cells can be purchased or isolated from primary tissue which involves access to surgical specimens. Furthermore, these cells are difficult to culture and require very specific protocols. These limitations make this model costly and not as accessible as epithelial cell lines.

Cellular Co-cultures

Another important model is based on co-cultures of different cell types. This model can be set up with cell lines and/or primary cells directly in contact to replicate the interaction between DCs and $CD4^+$T cells during the viral amplification of the "founder population" and subsequent viral dissemination to draining lymph nodes. These co-cultures have been used to measure drug and Ab activity against *trans* infection between primary mature DCs (mDCs) and TZM-bl cells or autologous $CD4^+$ T cells (Sagar et al., 2012) and between primary iDCs and PM-1 cells (Hu et al., 2004; Herrera et al., 2016).

Co-cultures of epithelial cells with target cells in a dual-chamber model mimicking trans-epithelial migration of drugs, Abs and virus have been successfully used to assess safety and allow studies of HIV transmission and efficacy of candidate inhibitors. Epithelial cell lines have been co-cultured with target $CD4^+$ cell lines such as TZM-bl cells (Pasetto et al., 2014); or with primary PBMCs (Dezzutti et al., 2004; Guedon et al., 2015) or DC (Van Herrewege et al., 2007). Shen et al. have recently shown with co-cultures of primary epithelial cells and fibroblasts from the female genital tract in trans-well systems that the epithelial barrier can accumulate reverse transcriptase inhibitors, tenofovir and tenofovir alafenamide (TAF), and release them to susceptible $CD4^+$ cells for several days after dosing (Shen et al., 2018).

TISSUE EXPLANTS

The next phase in PK/PD evaluation often utilizes tissue models such as *ex vivo* culture of mucosal tissue explants (Grivel and Margolis, 2009). Explants are obtained as biopsies or as surgically resected tissue which upon arrival at the laboratory are dissected to remove the muscularis and cut into small pieces. Several models have been developed for penile (Fischetti et al., 2009), cervical, vaginal and colorectal tissues including polarized (Collins et al., 2000; Abner et al., 2005; Cummins et al., 2007) and non-polarized systems (Greenhead et al., 2000; Hu et al., 2004; Fletcher et al., 2006; Grivel et al., 2007). In non-polarized models, explants are submerged for *ex vivo* dosing with candidate inhibitor and then with virus for *ex vivo* challenge. After incubation and depending on the type of tissue, explants are transferred either to new plates in submerged conditions for culture of cervicovaginal and penile tissue, or onto gelatin sponge rafts presoaked in media to help maintain the structure of colorectal explants by culturing them at the air-media interface. Non-polarized culture reduces the protective function of the epithelial barrier by exposing target cells on the edges of the explant directly to the virus, and therefore allows PD evaluation in what could be considered the "worst-case scenario," however it also represents the "best-case scenario" of drug or Ab availability to prevent infection of the target cell.

In polarized models the tissue epithelium is oriented upwards on the apical chamber of a trans-well system and the edges are sealed using agarose, Matrigel^{TM} or surgical glue. These models have been prioritized for evaluation of formulated inhibitors to evaluate the ability of the formulation to deliver drugs or Abs to the target cells across the epithelial barrier and to assess the safety profile of the formulation toward the epithelium. Polarized systems require larger tissue explants for an increased surface exposure to the candidate inhibitor, for a correct orientation of the epithelium and to avoid incorrect sealing of the explant edges.

The different mucosal portals of HIV entry have histological and immunological specificities such as epithelium type, abundance of activated HIV-target cells, drug transporter profile, Ab isotype expression, and pH, among others (Fischetti et al., 2009; Hladik and Hope, 2009; Shacklett, 2009; Hijazi et al., 2015; Taneva et al., 2015; Cheeseman et al., 2016; Mukhopadhya et al., 2016a). The known lower level of viral replication in the female genital tract compared to the colorectum *in vivo* is replicated in the tissue explant model with lower read out values of infection in cervicovaginal explants after challenge with a normalized viral input titer (Lapenta et al., 1999; Anton et al., 2000; Poles et al., 2001; Fox et al., 2016). Furthermore, this model recapitulates (Saba et al., 2013) changes in susceptibility to HIV infection in the female genital tract during the menstrual cycle (Rodriguez-Garcia et al., 2013; Thurman et al., 2016; Boily-Larouche et al., 2019) and menopause (Thurman et al., 2017). Gender or location of tissue excision have not been reported to affect the susceptibility to infection of colorectal explants nor the activity of candidate inhibitors (Anton et al., 2011); nevertheless, lower levels of infection are observed in small intestine explants compared to large intestine tissue (Elliott et al., 2018). The predominant transmission of R5-tropic isolates compared with X4-viruses during sexual intercourse (Salazar-Gonzalez et al., 2009; Grivel et al., 2011) is also replicated in the explant model (Herrera et al., 2009). These traits will affect PK and PD profile of candidate inhibitors and therefore are important for the design and evaluation of prevention strategies and their formulation (Trezza and Kashuba, 2014). In fact, tissue drug levels are not only dosing route-dependent but will be distinct for each tissue and distinct from the systemic compartment (Lederman et al., 2004; Cohen et al., 2007; Trezza and Kashuba, 2014), thus affecting the expected correlation between concentration and efficacy at mucosal sites. This can be reflected with the

explant model. Greater concentrations of rilpivirine, a reverse transcriptase inhibitor, are required to inhibit infection in ecto-cervical explants than in colorectal tissue (Dezzutti et al., 2016). The activity of maraviroc, a CCR5-binding entry inhibitor, could only be observed in pre-activated ecto-cervical tissue explants; however, activation of colorectal explants was not necessary although limited inhibition was measured in this tissue. These results reflect the heterogeneity in CCR5 conformation and/or expression in the different mucosal tissues, which cannot be assessed in TZM-bl cells, a model expressing high levels of CCR5 (Fletcher et al., 2016; Herrera et al., 2016). Interestingly, the limited activity of maraviroc observed in tissue explants predicted the lack of efficacy of this drug in oral PrEP NHP studies (Massud et al., 2013) and clinical trials (Fox et al., 2016; Gulick et al., 2017; McGowan et al., 2019) despite accumulation of the drug in the mucosal compartments.

Viability of explants has been questioned. However, despite progressive decay in structure, CD4/CD8 cell ratios remain constant and viral replication is sustained (Fletcher et al., 2006). Tissue explants can be kept in culture for more than 3 weeks although for PK/PD evaluation, cultures are kept for 15 days; except for evaluation of protease inhibitors targeting late stages of the viral replication cycle, when explants are cultured for 21 days. During this period, cultures are fed at different time points by harvesting part of the supernatant and adding fresh media. To mimic pulse exposure to drug or Ab, after incubation explants are washed to remove unbound inhibitor and virus and cultured in media without compound; however, to evaluate sustained release systems such as vaginal rings, implants or injectables, after initial incubation and washes, culture media is supplemented with candidate inhibitor during the entire period of culture (Harman et al., 2012; Stefanidou et al., 2012; Fletcher et al., 2016; Zhang et al., 2017).

In cervical and penile tissue models, cells emigrating from the tissue have been described and these can be cultured separately from the explant and in the presence of $CD4^{+}$T cells, such as PM-1 cells, to assess anti-viral activity of a drug against dissemination by migratory cells (Hu et al., 2004; Fischetti et al., 2009).

To further model the PK/PD profile of a candidate inhibitor in a mucosal compartment and during intercourse, mucosal secretions can be added to the tissue explants. Addition of semen or seminal fluid does not affect the activity of reverse transcriptase inhibitors (Neurath et al., 2006; Fletcher et al., 2009; Dezzutti et al., 2012b). Female genital tract secretions can be obtained as cervicovaginal lavages, which dilute the secretion, or as undiluted fluid with Weck-cel spears, vaginal aspirators or with Instead Cups; however, sparse volumes tend to be obtained. In addition, immune factors in secretions will vary during the menstrual cycle (Birse et al., 2015), other pathologies will modulate the level of inflammation (Roberts et al., 2012; Kaul et al., 2015; Introini et al., 2017a,b; McKinnon et al., 2018), microbial content will not be homogenous among women (Pyles et al., 2014; Klatt et al., 2017; Bayigga et al., 2018; Taneva et al., 2018) and hormonal contraception might increase susceptibility to HIV(Morrison et al., 2015) affecting the PK/PD profile. Hence, modeling the viscosity, pH and osmolarity of the female genital tract secretions, synthetic vaginal fluid (Owen and Katz, 1999) and synthetic cervical fluid (Burruano et al., 2002) have been used in tissue assays as pre-clinical alternatives (Fletcher et al., 2009).

Safety of candidate inhibitors and their formulations can also be pre-clinically evaluated in tissue explants. Clinical trials testing the first generation of topical inhibitors revealed the importance of mucosal safety following enhancement of infection (Honey, 2007; Adams and Kashuba, 2012). Cytotoxicity can be easily measured in tissue explants by the MTT viability assay. Immunological safety biomarkers have been defined for mucosal compartments (Fichorova et al., 2004; Fields et al., 2014). The explant model allows evaluation of mucosal responses to candidate inhibitor exposure by measurement of cytokine modulation (Beer et al., 2006; Gali et al., 2010; Zhang et al., 2017). Pre-clinical safety evaluation has allowed optimization of formulations for different mucosal compartments requiring, for example, modification of the osmolarity of a vaginal gel for rectal application (Rohan et al., 2010; Dezzutti et al., 2012a), which was then found to be safe during clinical trial testing (Anton et al., 2012; McGowan et al., 2013).

Ex vivo modeling of the mucosal compartment provides efficacy, concentration, and safety data. Additionally, tissue explants recapitulate the viral replication fitness of wild type and resistant isolates observed *in vivo* in patients (Abraha et al., 2009; Herrera et al., 2009) strengthening the predictive potential of this model in the context of increasingly prevalent ARV-resistance (Pennings, 2013; Snedecor et al., 2014). It is estimated that in high-income countries, 10–20% of new infections are caused by ARV-resistant isolates harboring mutations that confer resistance to at least one of the three main types of ARV drugs (Salomon et al., 2000; Briones et al., 2001; Duwe et al., 2001; UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance, 2001; Little et al., 2002; Chaix et al., 2003; Mendoza et al., 2003; Weinstock et al., 2004).

Studies in NHPs delivered proof of principle that efficacy of topical dosing with tenofovir against rectal challenge could be replicated by *ex vivo* challenge of tissue resections obtained from NHPs topically dosed *in vivo* (Cranage et al., 2008). In fact, this approach of *ex vivo* challenge of mucosal biopsies is increasingly being used as an endpoint of *ex vivo* efficacy of PrEP (Anton et al., 2012; Harman et al., 2012; Richardson-Harman et al., 2012, 2014; McGowan et al., 2015, 2019; Fox et al., 2016) and vaccine trials (Herrera et al., 2014). This model can be used with cervicovaginal samples frozen at the trial sites and thawed at a centralized facility for *ex vivo* challenge (Gupta et al., 2006; Lackman-Smith et al., 2008); however, it requires the use of fresh tissue when assessing efficacy in the colorectal tract (McGowan et al., 2012).

Despite the variety of explant models, it has been shown that consistent results of anti-viral efficacy can be obtained among different laboratories through protocol standardization for a same model (Richardson-Harman et al., 2009).

The tissue explant model will need to be further developed to assess the PK/PD profiles of new inhibitors and their formulations designed to provide long term efficacy. This will require the model to be adapted physically with protocols that will mimic, for example, mucosal efficacy of injectables; and define new biomarkers of safety and activity. New models should

also be able to evaluate broad spectrum anti-viral drugs and compounds designed to maintain mucosal health.

FUTURE MODELS

The development of engineered human tissues as a model to study physiological functions and pathologies could lead to new systems for safety and PK evaluation of candidate HIV-inhibitors and ideally for efficacy studies. Initial models mimicking the intestinal epithelium were based on the culture of isolated intestinal crypts with human adult stem cells (Sato et al., 2009) embedded in a matrix of Matrigel^{TM} or silk (Chen et al., 2017). Cultures derived from the small intestine are referred to as enteroids and those from colon are known as colonoids; they mimic a three-dimensional functional epithelial barrier capable of eliciting innate immune responses (Chen et al., 2017). Another option is the use of human inducible pluripotent stem cells which differentiate and form spheroid structures that are cultured on a matrix and are known as organoids (Spence et al., 2011; Miura and Suzuki, 2018). However, organoids have fetal and immature phenotypes and therefore, a certain degree of maturity can be obtained during culture as shown with liver organoids (Takebe et al., 2013). To increase the physiological relevance of these models, fluidic devices have been incorporated into models known as "organ-on-a-chip" or "microphysiological systems." Human gut-on-a-chip systems were developed originally using Caco-2 cells (Kim et al., 2012). This represents a structurally oversimplified model lacking immune cells and not achieving fully mature adult phenotypes. However, the field is constantly evolving and new models combining organoid and organ-on-a-chip technologies provide primary gut chips (Kasendra et al., 2018). The limited structural resemblance of these devices with *in vivo* tissue could be resolved using three-dimensional bioprinting techniques (Mittal et al., 2018). Other drawbacks are the microfluidic and chip costs, the complexity of the microengineering and the cytotoxicity induced by defective flow rates. The greater complexity of the female genital tract compared to the gut cannot be modeled with a unique chip but rather by including multiple organs-on-a-chip in one microphysiological system (Loskill et al., 2015; Edington et al., 2018) that can be used to study the biology and pathogenesis of the female genital tract (Young et al., 2017).

These and future models will need to fully recapitulate the cellular diversity of mucosal tissues, the immune responsiveness and the donor-to-donor variation to provide pre-clinical PK and PD information on candidate HIV inhibitors.

CONCLUSIONS

Pre-clinical assays for HIV prevention remain critical to understanding the relative potential of new compounds and combinations and for selecting the best candidates, their formulation and dosing regimen. Furthermore, in an era where HIV cure research has been prioritized, pre-clinical models developed for prevention might be applicable for the evaluation of cure strategies. However, all pre-clinical assays have their limitations and their value in predicting clinical efficacy has yet to be established. Hence, the process of product prioritization needs to be based on a range of criteria that include: *in vitro* drug potency, animal efficacy data, stage of product development, cost of goods, existing safety data, and ability to measure PK/PD parameters in clinical trials. New PK/PD parameters or correlations might need to be defined to pre-clinically predict the outcome of clinical trials. This will require further development of existing models, which have not significantly changed in the last decade, and introduction of new models in the pre-clinical toolbox. Ultimately, validation of *in vitro* and *ex vivo* models will require *in vivo* studies in humans.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

Funding for publication was provided by the Imperial College Open Access Fund.

ACKNOWLEDGMENTS

I am grateful to Dr. Gœury for time and support.

REFERENCES

Abner, S. R., Guenthner, P. C., Guarner, J., Hancock, K. A., Cummins, J. E. Jr., Fink, A., et al. (2005). A human colorectal explant culture to evaluate topical microbicides for the prevention of HIV infection. *J. Infect. Dis.* 192, 1545–1556. doi: 10.1086/462424

Abraha, A., Nankya, I. L., Gibson, R., Demers, K., Tebit, D. M., Johnston, E., et al. (2009). CCR5- and CXCR4-tropic subtype C human immunodeficiency virus type 1 isolates have a lower level of pathogenic fitness than other dominant group M subtypes: implications for the epidemic. *J. Virol.* 83, 5592–5605. doi: 10.1128/JVI.02051-08

Adams, J. L., and Kashuba, A. D. (2012). Formulation, pharmacokinetics and pharmacodynamics of topical microbicides. *Best Pract. Res. Clin. Obstet. Gynaecol.* 26, 451–462. doi: 10.1016/j.bpobgyn.2012.01.004

Anderson, P. L., Glidden, D. V., Bushman, L. R., Heneine, W., and Garcia-Lerma, J. G. (2014). Tenofovir diphosphate concentrations and prophylactic effect in a macaque model of rectal simian HIV transmission. *J. Antimicrob. Chemother.* 69, 2470–2476. doi: 10.1093/jac/dku162

Anton, P. A., Cranston, R. D., Kashuba, A., Hendrix, C. W., Bumpus, N. N., Richardson-Harman, N., et al. (2012). RMP-02/MTN-006: a phase 1 rectal safety, acceptability, pharmacokinetic, and pharmacodynamic study of tenofovir 1% gel compared with oral tenofovir disoproxil fumarate. *AIDS Res. Hum. Retroviruses* 28, 1412–1421. doi: 10.1089/aid.2012.0262

Anton, P. A., Elliott, J., Poles, M. A., Mcgowan, I. M., Matud, J., Hultin, L. E., et al. (2000). Enhanced levels of functional HIV-1 co-receptors on human mucosal T cells demonstrated using intestinal biopsy tissue. *Aids* 14, 1761–1765. doi: 10.1097/00002030-200008180-00011

Anton, P. A., Saunders, T., Elliott, J., Khanukhova, E., Dennis, R., Adler, A., et al. (2011). First phase 1 double-blind, placebo-controlled, randomized rectal microbicide trial using UC781 gel with a novel index of *ex vivo* efficacy. *PLoS ONE* 6:e23243. doi: 10.1371/journal.pone.0023243

Bayigga, L., Kateete, D. P., Anderson, D. J., Sekikubo, M., and Nakanjako, D. (2018). Diversity of vaginal microbiota in sub-Saharan Africa and its effects on HIV transmission and prevention. *Am. J. Obstet. Gynecol.* 220, 155–166 doi: 10.1016/j.ajog.2018.10.014

Beer, B. E., Doncel, G. F., Krebs, F. C., Shattock, R. J., Fletcher, P. S., Buckheit, R. W. Jr., et al. (2006). *In vitro* preclinical testing of nonoxynol-9 as potential anti-human immunodeficiency virus microbicide: a retrospective analysis of results from five laboratories. *Antimicrob. Agents Chemother.* 50, 713–723. doi: 10.1128/AAC.50.2.713-723.2006

Berry, N., Herrera, C., and Cranage, M. (2011). Detection, quantification, and characterisation of HIV/SIV. *Methods Mol. Biol.* 665, 133–160. doi: 10.1007/978-1-60761-817-1_9

Birse, K., Arnold, K. B., Novak, R. M., Mccorrister, S., Shaw, S., Westmacott, G. R., et al. (2015). Molecular signatures of immune activation and epithelial barrier remodeling are enhanced during the luteal phase of the menstrual cycle: implications for HIV susceptibility. *J. Virol.* 89, 8793–8805. doi: 10.1128/JVI.00756-15

Bjorndal, A., Deng, H., Jansson, M., Fiore, J. R., Colognesi, C., Karlsson, A., et al. (1997). Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype. *J. Virol.* 71, 7478–7487.

Boily-Larouche, G., Lajoie, J., Dufault, B., Omollo, K., Cheruiyot, J., Njoki, J., et al. (2019). Characterization of the genital mucosa immune profile to distinguish phases of the menstrual cycle: implications for HIV susceptibility. *J. Infect. Dis.* 219, 856–866. doi: 10.1093/infdis/jiy585

Briones, C., Perez-Olmeda, M., Rodriguez, C., Del Romero, J., Hertogs, K., and Soriano, V. (2001). Primary genotypic and phenotypic HIV-1 drug resistance in recent seroconverters in Madrid. *J. Acquir. Immune Defic. Syndr.* 26, 145–150. doi: 10.1097/00126334-200102010-00006

Brown, K. C., Patterson, K. B., Malone, S. A., Shaheen, N. J., Prince, H. M., Dumond, J. B., et al. (2011). Single and multiple dose pharmacokinetics of maraviroc in saliva, semen, and rectal tissue of healthy HIV-negative men. *J. Infect. Dis.* 203, 1484–1490. doi: 10.1093/infdis/jir059

Burruano, B. T., Schnaare, R. L., and Malamud, D. (2002). Synthetic cervical mucus formulation. *Contraception* 66, 137–140. doi: 10.1016/S0010-7824(02)00336-0

Carlson, L. A., Briggs, J. A., Glass, B., Riches, J. D., Simon, M. N., Johnson, M. C., et al. (2008). Three-dimensional analysis of budding sites and released virus suggests a revised model for HIV-1 morphogenesis. *Cell Host Microbe* 4, 592–599. doi: 10.1016/j.chom.2008.10.013

Centers for Disease Control and Prevention (CDC) (2012). Interim guidance for clinicians considering the use of preexposure prophylaxis for the prevention of HIV infection in heterosexually active adults. *MMWR Morb. Mortal. Wkly. Rep.* 61, 586–589.

Chaix, M., Descamps, D., Mouajjah, S., Deveau, C., André, P., Cottalorda, J., et al. (2003). French national sentinel survey of antiretroviral resistance in patients with HIV-1 primary infection and in antiretroviral-naive chronically infected patients in 2001-2002. *Antiviral Ther.* 8:S137. doi: 10.1097/01.qai.0000155201.51232.2e

Cheeseman, H. M., Carias, A. M., Evans, A. B., Olejniczak, N. J., Ziprin, P., King, D. F., et al. (2016). Expression profile of human Fc receptors in mucosal tissue: implications for antibody-dependent cellular effector functions targeting HIV-1 transmission. *PLoS ONE* 11:e0154656. doi: 10.1371/journal.pone.0154656

Chen, Y., Zhou, W., Roh, T., Estes, M. K., and Kaplan, D. L. (2017). *In vitro* enteroid-derived three-dimensional tissue model of human small intestinal epithelium with innate immune responses. *PLoS ONE* 12:e0187880. doi: 10.1371/journal.pone.0187880

Cohen, M. S., Gay, C., Kashuba, A. D., Blower, S., and Paxton, L. (2007). Narrative review: antiretroviral therapy to prevent the sexual transmission of HIV-1. *Ann. Intern. Med.* 146, 591–601. doi: 10.7326/0003-4819-146-8-200704170-00010

Collins, K. B., Patterson, B. K., Naus, G. J., Landers, D. V., and Gupta, P. (2000). Development of an *in vitro* organ culture model to study transmission of HIV-1 in the female genital tract. *Nat. Med.* 6, 475–479. doi: 10.1038/74743

Costanzo, M., Carton, F., Marengo, A., Berlier, G., Stella, B., Arpicco, S., et al. (2016). Fluorescence and electron microscopy to visualize the intracellular fate of nanoparticles for drug delivery. *Eur. J. Histochem.* 60:2640. doi: 10.4081/ejh.2016.2640

Cranage, M., Sharpe, S., Herrera, C., Cope, A., Dennis, M., Berry, N., et al. (2008). Prevention of SIV rectal transmission and priming of T cell responses in macaques after local pre-exposure application of tenofovir gel. *PLoS Med.* 5:e157; discussion e157. doi: 10.1371/journal.pmed.0050157

Cummins, J. E. Jr., Guarner, J., Flowers, L., Guenthner, P. C., Bartlett, J., Morken, T., et al. (2007). Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. *Antimicrob. Agents Chemother.* 51, 1770–1779. doi: 10.1128/AAC.01129-06

Derdeyn, C. A., Decker, J. M., Sfakianos, J. N., Wu, X., O'brien, W. A., Ratner, L., et al. (2000). Sensitivity of human immunodeficiency virus type 1 to the fusion inhibitor T-20 is modulated by coreceptor specificity defined by the V3 loop of gp120. *J. Virol.* 74, 8358–8367. doi: 10.1128/JVI.74.18.8358-8367.2000

Dezzutti, C. S., Else, L. J., Yandura, S. E., Shetler, C., Russo, J., Back, D. J., et al. (2016). Distinct pharmacodynamic activity of rilpivirine in ectocervical and colonic explant tissue. *Antimicrob. Agents Chemother.* 60, 2765–2770. doi: 10.1128/AAC.00167-16

Dezzutti, C. S., James, V. N., Ramos, A., Sullivan, S. T., Siddig, A., Bush, T. J., et al. (2004). *In vitro* comparison of topical microbicides for prevention of human immunodeficiency virus type 1 transmission. *Antimicrob. Agents Chemother.* 48, 3834–3844. doi: 10.1128/AAC.48.10.3834-3844.2004

Dezzutti, C. S., Rohan, L. C., Wang, L., Uranker, K., Shetler, C., Cost, M., et al. (2012a). Reformulated tenofovir gel for use as a dual compartment microbicide. *J. Antimicrob. Chemother.* 67, 2139–2142. doi: 10.1093/jac/dks173

Dezzutti, C. S., Shetler, C., Mahalingam, A., Ugaonkar, S. R., Gwozdz, G., Buckheit, K. W., et al. (2012b). Safety and efficacy of tenofovir/IQP-0528 combination gels - a dual compartment microbicide for HIV-1 prevention. *Antiviral Res.* 96, 221–225. doi: 10.1016/j.antiviral.2012.08.004

Dickinson, L., Khoo, S., and Back, D. (2010). Pharmacokinetics and drug-drug interactions of antiretrovirals: an update. *Antiviral Res.* 85, 176–189. doi: 10.1016/j.antiviral.2009.07.017

Dobard, C., Sharma, S., Martin, A., Pau, C. P., Holder, A., Kuklenyik, Z., et al. (2012). Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. *J. Virol.* 86, 718–725. doi: 10.1128/JVI.05842-11

Douville, K., Mitchnick, M., Baker, T., and Studebaker, D. (2006). "Modeling phase III microbicide clinical trial costs," in *AIDS* 2006 (Toronto, ON).

Drozdzik, M., Groer, C., Penski, J., Lapczuk, J., Ostrowski, M., Lai, Y., et al. (2014). Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine. *Mol. Pharm.* 11, 3547–3555. doi: 10.1021/mp500330y

Dumond, J. B., Patterson, K. B., Pecha, A. L., Werner, R. E., Andrews, E., Damle, B., et al. (2009). Maraviroc concentrates in the cervicovaginal fluid and vaginal tissue of HIV-negative women. *J. Acquir. Immune Defic. Syndr.* 51, 546–553. doi: 10.1097/QAI.0b013e3181ae69c5

Dumond, J. B., Yeh, R. F., Patterson, K. B., Corbett, A. H., Jung, B. H., Rezk, N. L., et al. (2007). Antiretroviral drug exposure in the female genital tract: implications for oral pre- and post-exposure prophylaxis. *Aids* 21, 1899–1907. doi: 10.1097/QAD.0b013e328270385a

Duwe, S., Brunn, M., Altmann, D., Hamouda, O., Schmidt, B., Walter, H., et al. (2001). Frequency of genotypic and phenotypic drug-resistant HIV-1 among therapy-naive patients of the german seroconverter study. *J. Acquir. Immune Defic. Syndr.* 26, 266–273. doi: 10.1097/00126334-200103010-00010

Edington, C. D., Chen, W. L. K., Geishecker, E., Kassis, T., Soenksen, L. R., Bhushan, B. M., et al. (2018). Interconnected microphysiological systems for quantitative biology and pharmacology studies. *Sci. Rep.* 8:4530. doi: 10.1038/s41598-018-22749-0

Elliott, J., Fulcher, J. A., Ibarrondo, F. J., Tanner, K., Mcgowan, I., and Anton, P. A. (2018). Comparative assessment of small and large intestine biopsies for *ex vivo* HIV-1 pathogenesis studies. *AIDS Res. Hum. Retroviruses* 34, 900–906. doi: 10.1089/aid.2017.0249

Englund, G., Rorsman, F., Ronnblom, A., Karlbom, U., Lazorova, L., Grasjo, J., et al. (2006). Regional levels of drug transporters along the human intestinal

tract: co-expression of ABC and SLC transporters and comparison with Caco-2 cells. *Eur. J. Pharm. Sci.* 29, 269–277. doi: 10.1016/j.ejps.2006.04.010

Fichorova, R. N., Bajpai, M., Chandra, N., Hsiu, J. G., Spangler, M., Ratnam, V., et al. (2004). Interleukin (IL)-1, IL-6, and IL-8 predict mucosal toxicity of vaginal microbicidal contraceptives. *Biol. Reprod.* 71, 761–769. doi: 10.1095/biolreprod.104.029603

Fields, S., Song, B., Rasoul, B., Fong, J., Works, M. G., Shew, K., et al. (2014). New candidate biomarkers in the female genital tract to evaluate microbicide toxicity. *PLoS ONE* 9:e110980. doi: 10.1371/journal.pone.0110980

Fischetti, L., Barry, S. M., Hope, T. J., and Shattock, R. J. (2009). HIV-1 infection of human penile explant tissue and protection by candidate microbicides. *Aids* 23, 319–328. doi: 10.1097/QAD.0b013e328321b778

Fletcher, P., Harman, S., Azijn, H., Armanasco, N., Manlow, P., Perumal, D., et al. (2009). Inhibition of human immunodeficiency virus type 1 infection by the candidate microbicide dapivirine, a nonnucleoside reverse transcriptase inhibitor. *Antimicrob Agents Chemother.* 53, 487–495. doi: 10.1128/AAC.01156-08

Fletcher, P., Herrera, C., Armanasco, N., Nuttall, J., and Shattock, R. J. (2016). Short communication: limited anti-HIV-1 activity of maraviroc in mucosal tissues. *AIDS Res. Hum. Retroviruses* 32, 334–338. doi: 10.1089/aid.2015.0315

Fletcher, P. S., Elliott, J., Grivel, J. C., Margolis, L., Anton, P., Mcgowan, I., et al. (2006). *Ex vivo* culture of human colorectal tissue for the evaluation of candidate microbicides. *Aids* 20, 1237–1245. doi: 10.1097/01.aids.0000232230.96134.80

Fogh, J., Wright, W. C., and Loveless, J. D. (1977). Absence of HeLa cell contamination in 169 cell lines derived from human tumors. *J. Natl. Cancer Inst.* 58, 209–214. doi: 10.1093/jnci/58.2.209

Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., Boone, B. A., and Mccarthy, R. E. (1965). Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* 18, 522–529. doi: 10.1002/1097-0142(196504)18:4<522::AID-CNCR2820180418>3.0.CO;2-J

Fox, J., Tiraboschi, J. M., Herrera, C., Else, L., Egan, D., Dickinson, L., et al. (2016). Brief report: pharmacokinetic/pharmacodynamic investigation of single-dose oral maraviroc in the context of HIV-1 pre-exposure prophylaxis. *J. Acquir. Immune Defic. Syndr.* 73, 252–257. doi: 10.1097/QAI.0000000000001108

Gali, Y., Arien, K. K., Praet, M., Van Den Bergh, R., Temmerman, M., Delezay, O., et al. (2010). Development of an *in vitro* dual-chamber model of the female genital tract as a screening tool for epithelial toxicity. *J. Virol Methods* 165, 186–197. doi: 10.1016/j.jviromet.2010.01.018

Garcia-Lerma, J. G., Aung, W., Cong, M. E., Zheng, Q., Youngpairoj, A. S., Mitchell, J., et al. (2011). Natural substrate concentrations can modulate the prophylactic efficacy of nucleotide HIV reverse transcriptase inhibitors. *J. Virol.* 85, 6610–6617. doi: 10.1128/JVI.00311-11

Geonnotti, A. R., Bilska, M., Yuan, X., Ochsenbauer, C., Edmonds, T. G., Kappes, J. C., et al. (2010). Differential inhibition of human immunodeficiency virus type 1 in peripheral blood mononuclear cells and TZM-bl cells by endotoxin-mediated chemokine and gamma interferon production. *AIDS Res. Hum. Retroviruses* 26, 279–291. doi: 10.1089/aid.2009.0186

Ghosh, M., Fahey, J. V., Shen, Z., Lahey, T., Cu-Uvin, S., Wu, Z., et al. (2010a). Anti-HIV activity in cervical-vaginal secretions from HIV-positive and -negative women correlate with innate antimicrobial levels and IgG antibodies. *PLoS ONE* 5:e11366. doi: 10.1371/journal.pone.0011366

Ghosh, M., Fahey, J. V., and Wira, C. R. (2010b). Differential susceptibility of HIV strains to innate immune factors in human cervical-vaginal secretions. *Virus Adaptation Treat.* 2, 63–71. doi: 10.2147/VAAT.S8033

Grammen, C., Baes, M., Haenen, S., Verguts, J., Augustyns, K., Zydowsky, T., et al. (2014). Vaginal expression of efflux transporters and the potential impact on the disposition of microbicides *in vitro* and in rabbits. *Mol. Pharm.* 11, 4405–4414. doi: 10.1021/mp5005004

Greenhead, P., Hayes, P., Watts, P. S., Laing, K. G., Griffin, G. E., and Shattock, R. J. (2000). Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. *J. Virol.* 74, 5577–5586. doi: 10.1128/JVI.74.12.5577-5586.2000

Grivel, J. C., Elliott, J., Lisco, A., Biancotto, A., Condack, C., Shattock, R. J., et al. (2007). HIV-1 pathogenesis differs in rectosigmoid and tonsillar tissues infected *ex vivo* with CCR5- and CXCR4-tropic HIV-1. *Aids* 21, 1263–1272. doi: 10.1097/QAD.0b013e3281864667

Grivel, J. C., and Margolis, L. (2009). Use of human tissue explants to study human infectious agents. *Nat. Protoc.* 4, 256–269. doi: 10.1038/nprot.2008.245

Grivel, J. C., Shattock, R. J., and Margolis, L. B. (2011). Selective transmission of R5 HIV-1 variants: where is the gatekeeper? *J. Transl. Med.* 9(Suppl. 1):S6. doi: 10.1186/1479-5876-9-S1-S6

Guedon, J. T., Luo, K., Zhang, H., and Markham, R. B. (2015). Monoclonal and single domain antibodies targeting beta-integrin subunits block sexual transmission of HIV-1 in *in vitro* and *in vivo* model systems. *J. Acquir. Immune Defic. Syndr.* 69, 278–285. doi: 10.1097/QAI.0000000000000609

Gulick, R. M., Wilkin, T. J., Chen, Y. Q., Landovitz, R. J., Amico, K. R., Young, A. M., et al. (2017). Phase 2 study of the safety and tolerability of maraviroc-containing regimens to prevent HIV infection in men who have sex with men (HPTN 069/ACTG A5305). *J. Infect. Dis.* 215, 238–246. doi: 10.1093/infdis/jiw525

Gunawardana, M., Mullen, M., Moss, J. A., Pyles, R. B., Nusbaum, R. J., Patel, J., et al. (2013). Global expression of molecular transporters in the human vaginal tract: implications for HIV chemoprophylaxis. *PLoS ONE.* 8:e77340. doi: 10.1371/journal.pone.0077340

Gupta, P., Ratner, D., Patterson, B. K., Kulka, K., Rohan, L. C., Parniak, M. A., et al. (2006). Use of frozen-thawed cervical tissues in the organ culture system to measure anti-HIV activities of candidate microbicides. *AIDS Res. Hum. Retroviruses* 22, 419–424. doi: 10.1089/aid.2006.22.419

Haertle, T., Carrera, C. J., Wasson, D. B., Sowers, L. C., Richman, D. D., and Carson, D. A. (1988). Metabolism and anti-human immunodeficiency virus-1 activity of 2-halo-2',3'-dideoxyadenosine derivatives. *J. Biol. Chem.* 263, 5870–5875.

Harada, S., Koyanagi, Y., and Yamamoto, N. (1985). Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. *Science* 229, 563–566. doi: 10.1126/science.2992081

Harman, S., Herrera, C., Armanasco, N., Nuttall, J., and Shattock, R. J. (2012). Preclinical evaluation of the HIV-1 fusion inhibitor L'644 as a potential candidate microbicide. *Antimicrob. Agents Chemother.* 56, 2347–2356. doi: 10.1128/AAC.06108-11

Heise, L. L., Watts, C., Foss, A., Trussell, J., Vickerman, P., Hayes, R., et al. (2011). Apples and oranges? Interpreting success in HIV prevention trials. *Contraception* 83, 10–15. doi: 10.1016/j.contraception.2010.06.009

Herold, B. C., Chen, B. A., Salata, R. A., Marzinke, M. A., Kelly, C. W., Dezzutti, C. S., et al. (2016). Impact of sex on the pharmacokinetics and pharmacodynamics of 1% tenofovir gel. *Clin. Infect. Dis.* 62, 375–382. doi: 10.1093/cid/civ913

Herrera, C., Armanasco, N., Garcia-Perez, J., Ziprin, P., Olejniczak, N., Alcami, J., et al. (2016). Maraviroc and reverse transcriptase inhibitors combinations as potential preexposure prophylaxis candidates. *AIDS* 30, 1015–1025. doi: 10.1097/QAD.0000000000001043

Herrera, C., Cranage, M., Mcgowan, I., Anton, P., and Shattock, R. J. (2009). Reverse transcriptase inhibitors as potential colorectal microbicides. *Antimicrob. Agents Chemother.* 53, 1797–1807. doi: 10.1128/AAC.01096-08

Herrera, C., and Shattock, R. J. (2014). Candidate microbicides and their mechanisms of action. *Curr. Top. Microbiol. Immunol.* 383, 1–25. doi: 10.1007/82_2013_326

Herrera, C., Veazey, R., Schuetz, A., Olejniczak, N., Chenine, A.-L., Nitayaphan, S., et al. (2014). Valuation of mucosal tissue explants as *ex vivo* surrogates of *in vivo* vaccination of non-human primates (NHPs) and humans. *AIDS Res. Hum. Retroviruses* 30:A24. doi: 10.1089/aid.2014.5033.abstract

Hijazi, K., Cuppone, A. M., Smith, K., Stincarelli, M. A., Ekeruche-Makinde, J., De Falco, G., et al. (2015). Expression of genes for drug transporters in the human female genital tract and modulatory effect of antiretroviral drugs. *PLoS ONE* 10:e0131405. doi: 10.1371/journal.pone.0131405

Hladik, F., and Hope, T. J. (2009). HIV infection of the genital mucosa in women. *Curr. HIV/AIDS Rep.* 6, 20–28. doi: 10.1007/s11904-009-0004-1

Holmstock, N., Oorts, M., Snoeys, J., and Annaert, P. (2018). MRP2 inhibition by HIV protease inhibitors in rat and human hepatocytes: a quantitative confocal microscopy study. *Drug Metab. Dispos.* 46, 697–703. doi: 10.1124/dmd.117.079467

Honey, K. (2007). Microbicide trial screeches to a halt. *J. Clin. Invest.* 117:1116. doi: 10.1172/JCI32291

Hoque, M. T., Kis, O., De Rosa, M. F., and Bendayan, R. (2015). Raltegravir permeability across blood-tissue barriers and the potential role of

drug efflux transporters. *Antimicrob. Agents Chemother.* 59, 2572–2582. doi: 10.1128/AAC.04594-14

Hu, M., Patel, S. K., Zhou, T., and Rohan, L. C. (2015). Drug transporters in tissues and cells relevant to sexual transmission of HIV: Implications for drug delivery. *J. Control Release* 219, 681–696. doi: 10.1016/j.jconrel.2015.08.018

Hu, M., Zhou, T., Dezzutti, C. S., and Rohan, L. C. (2016). The effect of commonly used excipients on the epithelial integrity of human cervicovaginal tissue. *AIDS Res. Hum. Retroviruses* 32, 992–1004. doi: 10.1089/aid.2016.0014

Hu, Q., Frank, I., Williams, V., Santos, J. J., Watts, P., Griffin, G. E., et al. (2004). Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J. Exp. Med.* 199, 1065–1075. doi: 10.1084/jem.20022212

Hughes, B. L., Dutt, R., Raker, C., Barthelemy, M., Rossoll, R. M., Ramratnam, B., et al. (2016). The impact of pregnancy on anti-HIV activity of cervicovaginal secretions. *Am. J. Obstet. Gynecol.* 215, 748e741–748 e712. doi: 10.1016/j.ajog.2016.06.057

Introini, A., Bostrom, S., Bradley, F., Gibbs, A., Glaessgen, A., Tjernlund, A., et al. (2017a). Correction: seminal plasma induces inflammation and enhances HIV-1 replication in human cervical tissue explants. *PLoS Pathog.* 13:e1006492. doi: 10.1371/journal.ppat.1006492

Introini, A., Bostrom, S., Bradley, F., Gibbs, A., Glaessgen, A., Tjernlund, A., et al. (2017b). Seminal plasma induces inflammation and enhances HIV-1 replication in human cervical tissue explants. *PLoS Pathog.* 13:e1006402. doi: 10.1371/journal.ppat.1006402

Jais, M., Younes, N., Chapman, S., Cu-Uvin, S., and Ghosh, M. (2016). Reduced levels of genital tract immune biomarkers in postmenopausal women: implications for HIV acquisition. *Am. J. Obstet. Gynecol.* 215, 324.e1–324.e10. doi: 10.1016/j.ajog.2016.03.041

Johnston, S. H., Lobritz, M. A., Nguyen, S., Lassen, K., Delair, S., Posta, F., et al. (2009). A quantitative affinity-profiling system that reveals distinct CD4/CCR5 usage patterns among human immunodeficiency virus type 1 and simian immunodeficiency virus strains. *J. Virol.* 83, 11016–11026. doi: 10.1128/JVI.01242-09

Kapitza, S. B., Michel, B. R., Van Hoogevest, P., Leigh, M. L., and Imanidis, G. (2007). Absorption of poorly water soluble drugs subject to apical efflux using phospholipids as solubilizers in the Caco-2 cell model. *Eur. J. Pharm. Biopharm.* 66, 146–158. doi: 10.1016/j.ejpb.2006.08.010

Kasendra, M., Tovaglieri, A., Sontheimer-Phelps, A., Jalili-Firoozinezhad, S., Bein, A., Chalkiadaki, A., et al. (2018). Development of a primary human small intestine-on-a-chip using biopsy-derived organoids. *Sci. Rep.* 8:2871. doi: 10.1038/s41598-018-21201-7

Kaul, R., Prodger, J., Joag, V., Shannon, B., Yegorov, S., Galiwango, R., et al. (2015). Inflammation and HIV transmission in sub-Saharan Africa. *Curr. HIV/AIDS Rep.* 12, 216–222. doi: 10.1007/s11904-015-0269-5

Keller, M. J., Madan, R. P., Torres, N. M., Fazzari, M. J., Cho, S., Kalyoussef, S., et al. (2011). A randomized trial to assess anti-HIV activity in female genital tract secretions and soluble mucosal immunity following application of 1% tenofovir gel. *PLoS ONE* 6:e16475. doi: 10.1371/journal.pone.0016475

Kim, H. J., Huh, D., Hamilton, G., and Ingber, D. E. (2012). Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab. Chip* 12, 2165–2174. doi: 10.1039/c2lc40074j

Kim, J. H., Pitisuttithum, P., Kamboonruang, C., Chuenchitra, T., Mascola, J., Frankel, S. S., et al. (2003). Specific antibody responses to vaccination with bivalent CM235/SF2 gp120: detection of homologous and heterologous neutralizing antibody to subtype E (CRF01.AE) HIV type 1. *AIDS Res. Hum. Retroviruses* 19, 807–816. doi: 10.1089/088922203769232601

Kis, O., Robillard, K., Chan, G. N., and Bendayan, R. (2010). The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol. Sci.* 31, 22–35. doi: 10.1016/j.tips.2009.10.001

Kis, O., Zastre, J. A., Hoque, M. T., Walmsley, S. L., and Bendayan, R. (2013). Role of drug efflux and uptake transporters in atazanavir intestinal permeability and drug-drug interactions. *Pharm. Res.* 30, 1050–1064. doi: 10.1007/s11095-012-0942-y

Klatt, N. R., Cheu, R., Birse, K., Zevin, A. S., Perner, M., Noel-Romas, L., et al. (2017). Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women. *Science* 356, 938–945. doi: 10.1126/science.aai9383

Konig, S. K., Herzog, M., Theile, D., Zembruski, N., Haefeli, W. E., and Weiss, J. (2010). Impact of drug transporters on cellular resistance towards saquinavir and darunavir. *J. Antimicrob. Chemother.* 65, 2319–2328. doi: 10.1093/jac/dkq324

Kuramoto, H. (1972). Studies of the growth and cytogenetic properties of human endometrial adenocarcinoma in culture and its development into an established line. *Acta Obstet. Gynaecol. Jpn.* 19, 47–58.

Lackman-Smith, C., Osterling, C., Luckenbaugh, K., Mankowski, M., Snyder, B., Lewis, G., et al. (2008). Development of a comprehensive human immunodeficiency virus type 1 screening algorithm for discovery and preclinical testing of topical microbicides. *Antimicrob. Agents Chemother.* 52, 1768–1781. doi: 10.1128/AAC.01328-07

Lajoie, J., Mwangi, L., and Fowke, K. R. (2017). Preventing HIV infection without targeting the virus: how reducing HIV target cells at the genital tract is a new approach to HIV prevention. *AIDS Res. Ther.* 14:46. doi: 10.1186/s12981-017-0166-7

Lapenta, C., Boirivant, M., Marini, M., Santini, S. M., Logozzi, M., Viora, M., et al. (1999). Human intestinal lamina propria lymphocytes are naturally permissive to HIV-1 infection. *Eur. J. Immunol.* 29, 1202–1208. doi: 10.1002/(SICI)1521-4141(199904)29:04<1202::AID-IMMU1202>3.0.CO;2-O

Larder, B. A., Darby, G., and Richman, D. D. (1989). HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* 243, 1731–1734. doi: 10.1126/science.2467383

Lederman, M. M., Veazey, R. S., Offord, R., Mosier, D. E., Dufour, J., Mefford, M., et al. (2004). Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5. *Science* 306, 485–487. doi: 10.1126/science.1099288

Lee, T. H., Coligan, J. E., Sodroski, J. G., Haseltine, W. A., Salahuddin, S. Z., Wong-Staal, F., et al. (1984). Antigens encoded by the 3'-terminal region of human T-cell leukemia virus: evidence for a functional gene. *Science* 226, 57–61. doi: 10.1126/science.6089350

Leibovitz, A., Stinson, J. C., Mccombs Iii, W. B., Mccoy, C. E., Mazur, K. C., and Mabry, N. D. (1976). Classification of human colorectal adenocarcinoma cell lines. *Cancer Res.* 36, 4562–4569.

Li, Q., Estes, J. D., Schlievert, P. M., Duan, L., Brosnahan, A. J., Southern, P. J., et al. (2009). Glycerol monolaurate prevents mucosal SIV transmission. *Nature* 458, 1034–1038. doi: 10.1038/nature07831

Little, S. J., Holte, S., Routy, J. P., Daar, E. S., Markowitz, M., Collier, A. C., et al. (2002). Antiretroviral-drug resistance among patients recently infected with HIV. *N. Engl. J. Med.* 347, 385–394. doi: 10.1056/NEJMoa013552

Loskill, P., Marcus, S. G., Mathur, A., Reese, W. M., and Healy, K. E. (2015). muOrgano: a Lego(R)-Like plug & play system for modular multi-organ-chips. *PLoS ONE* 10:e0139587. doi: 10.1371/journal.pone.0139587

Louissaint, N. A., Cao, Y. J., Skipper, P. L., Liberman, R. G., Tannenbaum, S. R., Nimmagadda, S., et al. (2013). Single dose pharmacokinetics of oral tenofovir in plasma, peripheral blood mononuclear cells, colonic tissue, and vaginal tissue. *AIDS Res. Hum. Retroviruses* 29, 1443–1450. doi: 10.1089/aid.2013.0044

Lusso, P., Cocchi, F., Balotta, C., Markham, P. D., Louie, A., Farci, P., et al. (1995). Growth of macrophage-tropic and primary human immunodeficiency virus type 1 (HIV-1) isolates in a unique CD4+ T-cell clone (PM1): failure to downregulate CD4 and to interfere with cell-line-tropic HIV-1. *J. Virol.* 69, 3712–3720.

Mandal, S., Zhou, Y., Shibata, A., and Destache, C. J. (2015). Confocal fluorescence microscopy: an ultra-sensitive tool used to evaluate intracellular antiretroviral nano-drug delivery in HeLa cells. *AIP Adv.* 5:084803. doi: 10.1063/1.4926584

Massud, I., Aung, W., Martin, A., Bachman, S., Mitchell, J., Aubert, R., et al. (2013). Lack of prophylactic efficacy of oral maraviroc in macaques despite high drug concentrations in rectal tissues. *J. Virol.* 87, 8952–8961. doi: 10.1128/JVI.01204-13

McGowan, I., Cranston, R. D., Duffill, K., Siegel, A., Engstrom, J. C., Nikiforov, A., et al. (2015). A phase 1 randomized, open label, rectal safety, acceptability, pharmacokinetic, and pharmacodynamic study of three formulations of tenofovir 1% gel (the CHARM-01 study). *PLoS ONE* 10:e0125363. doi: 10.1371/journal.pone.0125363

McGowan, I., Hoesley, C., Cranston, R. D., Andrew, P., Janocko, L., Dai, J. Y., et al. (2013). A phase 1 randomized, double blind, placebo controlled rectal safety and acceptability study of tenofovir 1% gel (MTN-007). *PLoS ONE* 8:e60147. doi: 10.1371/journal.pone.0060147

McGowan, I., Tanner, K., Elliott, J., Ibarrondo, J., Khanukhova, E., Mcdonald, C., et al. (2012). Nonreproducibility of "snap-frozen" rectal biopsies for later use in *ex vivo* explant infectibility studies. *AIDS Res. Hum. Retroviruses* 28, 1509–1512. doi: 10.1089/aid.2012.0062

McGowan, I., Wilkin, T., Landovitz, R. J., Wu, C., Chen, Y., Marzinke, M. A., et al. (2019). The pharmacokinetics, pharmacodynamics, and mucosal responses to maraviroc-containing pre-exposure prophylaxis regimens in MSM. *AIDS* 33, 237–246. doi: 10.1097/QAD.0000000000002038

McKinnon, L. R., Liebenberg, L. J., Yende-Zuma, N., Archary, D., Ngcapu, S., Sivro, A., et al. (2018). Genital inflammation undermines the effectiveness of tenofovir gel in preventing HIV acquisition in women. *Nat. Med.* 24, 491–496. doi: 10.1038/nm.4506

Mendoza, C., Rodriguez, C., Corral, A., Gallego, O., Romero, J., and Soriano, V. (2003). Evidence for a different transmission efficiency of viruses with distinct drug-resistance genotypes. *Antiviral Ther.* 8:S144. doi: 10.1086/424668

Mittal, R., Woo, F. W., Castro, C. S., Cohen, M. A., Karanxha, J., Mittal, J., et al. (2018). Organ-on-chip models: implications in drug discovery and clinical applications. *J. Cell Physiol.* 234, 8352–8380 doi: 10.1002/jcp.27729

Miura, S., and Suzuki, A. (2018). Brief summary of the current protocols for generating intestinal organoids. *Dev. Growth Differ.* 60, 387–392. doi: 10.1111/dgd.12559

Montefiori, D. C., Karnasuta, C., Huang, Y., Ahmed, H., Gilbert, P., De Souza, M. S., et al. (2012). Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. *J. Infect. Dis.* 206, 431–441. doi: 10.1093/infdis/jis367

Morner, A., Bjorndal, A., Albert, J., Kewalramani, V. N., Littman, D. R., Inoue, R., et al. (1999). Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J. Virol.* 73, 2343–2349.

Morris, L., and Mkhize, N. N. (2017). Prospects for passive immunity to prevent HIV infection. *PLoS Med.* 14:e1002436. doi: 10.1371/journal.pmed.1002436

Morrison, C. S., Chen, P. L., Kwok, C., Baeten, J. M., Brown, J., Crook, A. M., et al. (2015). Hormonal contraception and the risk of HIV acquisition: an individual participant data meta-analysis. *PLoS Med.* 12:e1001778. doi: 10.1371/journal.pmed.1001778

Mukhopadhya, I., Murray, G. I., Berry, S., Thomson, J., Frank, B., Gwozdz, G., et al. (2016a). Drug transporter gene expression in human colorectal tissue and cell lines: modulation with antiretrovirals for microbicide optimization. *J. Antimicrob. Chemother.* 71, 372–386. doi: 10.1093/jac/dkv335

Mukhopadhya, I., Murray, G. I., Duncan, L., Yuecel, R., Shattock, R., Kelly, C., et al. (2016b). Transporters for antiretroviral drugs in colorectal CD4+ T cells and circulating alpha4beta7 integrin CD4+ T cells: implications for HIV microbicides. *Mol. Pharm.* 13, 3334–3340. doi: 10.1021/acs.molpharmaceut.6b00351

Mukura, L. R., Ghosh, M., Fahey, J. V., Cu-Uvin, S., and Wira, C. R. (2012). Genital tract viral load in HIV Type 1-positive women correlates with specific cytokine levels in cervical-vaginal secretions but is not a determinant of infectious virus or anti-HIV activity. *AIDS Res. Hum. Retroviruses* 28, 1533–1539. doi: 10.1089/aid.2011.0394

Neurath, A. R., Strick, N., and Li, Y. Y. (2006). Role of seminal plasma in the anti-HIV-1 activity of candidate microbicides. *BMC Infect. Dis.* 6:150. doi: 10.1186/1471-2334-6-150

Nicol, M. R., Fedoriw, Y., Mathews, M., Prince, H. M., Patterson, K. B., Geller, E., et al. (2014). Expression of six drug transporters in vaginal, cervical, and colorectal tissues: Implications for drug disposition in HIV prevention. *J. Clin. Pharmacol.* 54, 574–583. doi: 10.1002/jcph.248

Nuttall, J., Romano, J., Douville, K., Galbreath, C., Nel, A., Heyward, W., et al. (2007). The future of HIV prevention: prospects for an effective anti-HIV microbicide. *Infect. Dis. Clin. North. Am.* 21, 219–239, x. doi: 10.1016/j.idc.2007.01.009

Owen, D. H., and Katz, D. F. (1999). A vaginal fluid simulant. *Contraception* 59, 91–95. doi: 10.1016/S0010-7824(99)00010-4

Pasetto, S., Pardi, V., and Murata, R. M. (2014). Anti-HIV-1 activity of flavonoid myricetin on HIV-1 infection in a dual-chamber in vitro model. *PLoS ONE* 9:e115323. doi: 10.1371/journal.pone.0115323

Patel, M. V., Ghosh, M., Fahey, J. V., Ochsenbauer, C., Rossoll, R. M., and Wira, C. R. (2014). Innate immunity in the vagina (Part II): Anti-HIV activity and antiviral content of human vaginal secretions. *Am. J. Reprod. Immunol.* 72, 22–33. doi: 10.1111/aji.12218

Patterson, K. B., Prince, H. A., Kraft, E., Jenkins, A. J., Shaheen, N. J., Rooney, J. F., et al. (2011). Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. *Sci. Transl. Med.* 3:112re114. doi: 10.1126/scitranslmed.3003174

Pauwels, R., De Clercq, E., Desmyter, J., Balzarini, J., Goubau, P., Herdewijn, P., et al. (1987). Sensitive and rapid assay on MT-4 cells for detection of antiviral compounds against the AIDS virus. *J. Virol. Methods* 16, 171–185. doi: 10.1016/0166-0934(87)90002-4

Pennings, P. S. (2013). HIV drug resistance: problems and perspectives. *Infect. Dis. Rep.* 5:e5. doi: 10.4081/idr.2013.s1.e5

Platt, E. J., Bilska, M., Kozak, S. L., Kabat, D., and Montefiori, D. C. (2009). Evidence that ecotropic murine leukemia virus contamination in TZM-bl cells does not affect the outcome of neutralizing antibody assays with human immunodeficiency virus type 1. *J. Virol.* 83, 8289–8292. doi: 10.1128/JVI.00709-09

Platt, E. J., Wehrly, K., Kuhmann, S. E., Chesebro, B., and Kabat, D. (1998). Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1. *J. Virol.* 72, 2855–2864.

Poles, M. A., Elliott, J., Taing, P., Anton, P. A., and Chen, I. S. (2001). A preponderance of CCR5(+) CXCR4(+) mononuclear cells enhances gastrointestinal mucosal susceptibility to human immunodeficiency virus type 1 infection. *J. Virol.* 75, 8390–8399. doi: 10.1128/JVI.75.18.8390-8399.2001

Polonis, V. R., Brown, B. K., Rosa Borges, A., Zolla-Pazner, S., Dimitrov, D. S., Zhang, M. Y., et al. (2008). Recent advances in the characterization of HIV-1 neutralization assays for standardized evaluation of the antibody response to infection and vaccination. *Virology* 375, 315–320. doi: 10.1016/j.virol.2008.02.007

Pyles, R. B., Vincent, K. L., Baum, M. M., Elsom, B., Miller, A. L., Maxwell, C., et al. (2014). Cultivated vaginal microbiomes alter HIV-1 infection and antiretroviral efficacy in colonized epithelial multilayer cultures. *PLoS ONE* 9:e93419. doi: 10.1371/journal.pone.0093419

Reznicek, J., Ceckova, M., Ptackova, Z., Martinec, O., Tupova, L., Cerveny, L., et al. (2017). MDR1 and BCRP transporter-mediated drug-drug Interaction between rilpivirine and abacavir and effect on intestinal absorption. *Antimicrob. Agents Chemother.* 61. doi: 10.1128/AAC.00837-17

Richardson-Harman, N., Hendrix, C. W., Bumpus, N. N., Mauck, C., Cranston, R. D., Yang, K., et al. (2014). Correlation between compartmental tenofovir concentrations and an *ex vivo* rectal biopsy model of tissue infectibility in the RMP-02/MTN-006 phase 1 study. *PLoS ONE* 9:e111507. doi: 10.1371/journal.pone.0111507

Richardson-Harman, N., Lackman-Smith, C., Fletcher, P. S., Anton, P. A., Bremer, J. W., Dezzutti, C. S., et al. (2009). Multisite comparison of anti-human immunodeficiency virus microbicide activity in explant assays using a novel endpoint analysis. *J. Clin. Microbiol.* 47, 3530–3539. doi: 10.1128/JCM.00673-09

Richardson-Harman, N., Mauck, C., Mcgowan, I., and Anton, P. (2012). Dose-response relationship between tissue concentrations of UC781 and explant infectibility with HIV type 1 in the RMP-01 rectal safety study. *AIDS Res. Hum. Retroviruses* 28, 1422–1433. doi: 10.1089/aid.2012.0073

Roberts, L., Liebenberg, L., Barnabas, S., and Passmore, J. A. (2012). Vaginal microbicides to prevent human immunodeficiency virus infection in women: perspectives on the female genital tract, sexual maturity and mucosal inflammation. *Best Pract. Res. Clin. Obstet. Gynaecol.* 26, 441–449. doi: 10.1016/j.bpobgyn.2012.02.002

Rodriguez-Garcia, M., Patel, M. V., and Wira, C. R. (2013). Innate and adaptive anti-HIV immune responses in the female reproductive tract. *J. Reprod. Immunol.* 97, 74–84. doi: 10.1016/j.jri.2012.10.010

Rohan, L. C., Moncla, B. J., Kunjara Na Ayudhya, R. P., Cost, M., Huang, Y., Gai, F., et al. (2010). *In vitro* and *ex vivo* testing of tenofovir shows it is effective

as an HIV-1 microbicide. *PLoS ONE* 5:e9310. doi: 10.1371/journal.pone.0009310

Romano, J., Kashuba, A., Becker, S., Cummins, J., Turpin, J., and Veronese, F. (2013). Pharmacokinetics and pharmacodynamics in HIV prevention; current status and future directions: a summary of the DAIDS and BMGF sponsored think tank on pharmacokinetics (PK)/pharmacodynamics (PD) in HIV prevention. *AIDS Res. Hum. Retroviruses* 29, 1418–1427. doi: 10.1089/aid.2013.0122

Romas, L. M., Hasselrot, K., Aboud, L. G., Birse, K. D., Ball, T. B., Broliden, K., et al. (2014). A comparative proteomic analysis of the soluble immune factor environment of rectal and oral mucosa. *PLoS ONE* 9:e100820. doi: 10.1371/journal.pone.0100820

Saba, E., Origoni, M., Taccagni, G., Ferrari, D., Doglioni, C., Nava, A., et al. (2013). Productive HIV-1 infection of human cervical tissue *ex vivo* is associated with the secretory phase of the menstrual cycle. *Mucosal. Immunol.* 6, 1081–1090. doi: 10.1038/mi.2013.2

Sagar, M., Akiyama, H., Etemad, B., Ramirez, N., Freitas, I., and Gummuluru, S. (2012). Transmembrane domain membrane proximal external region but not surface unit-directed broadly neutralizing HIV-1 antibodies can restrict dendritic cell-mediated HIV-1 trans-infection. *J. Infect. Dis.* 205, 1248–1257. doi: 10.1093/infdis/jis183

Salahuddin, S. Z., Markham, P. D., Wong-Staal, F., Franchini, G., Kalyanaraman, V. S., and Gallo, R. C. (1983). Restricted expression of human T-cell leukemia–lymphoma virus (HTLV) in transformed human umbilical cord blood lymphocytes. *Virology* 129, 51–64. doi: 10.1016/0042-6822(83)90395-1

Salazar-Gonzalez, J. F., Salazar, M. G., Keele, B. F., Learn, G. H., Giorgi, E. E., Li, H., et al. (2009). Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. *J. Exp. Med.* 206, 1273–1289. doi: 10.1084/jem.20090378

Salomon, H., Wainberg, M. A., Brenner, B., Quan, Y., Rouleau, D., Cote, P., et al. (2000). Prevalence of HIV-1 resistant to antiretroviral drugs in 81 individuals newly infected by sexual contact or injecting drug use. Investigators of the Quebec primary infection study. *Aids* 14, F17–F23. doi: 10.1097/00002030-200001280-00003

Sarzotti-Kelsoe, M., Bailer, R. T., Turk, E., Lin, C. L., Bilska, M., Greene, K. M., et al. (2014a). Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. *J. Immunol. Methods* 409, 131–146. doi: 10.1016/j.jim.2013.11.022

Sarzotti-Kelsoe, M., Daniell, X., Todd, C. A., Bilska, M., Martelli, A., Labranche, C., et al. (2014b). Optimization and validation of a neutralizing antibody assay for HIV-1 in A3R5 cells. *J. Immunol. Methods* 409, 147–160. doi: 10.1016/j.jim.2014.02.013

Sato, T., Vries, R. G., Snippert, H. J., Van De Wetering, M., Barker, N., Stange, D. E., et al. (2009). Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 459, 262–265. doi: 10.1038/nature07935

Shacklett, B. L. (2009). Cell-mediated immunity to HIV in the female reproductive tract. *J. Reprod. Immunol.* 83, 190–195. doi: 10.1016/j.jri.2009.07.012

Shen, Z., Rodriguez-Garcia, M., Patel, M., Bodwell, J., and Wira, C. (2018). Role of epithelial cells and fibroblasts from the female reproductive tract in TFV and TAF protection of CD4+ T cells from HIV infection. *AIDS Res. Hum. Retroviruses* 34:13. doi: 10.1089/aid.2018.5000.abstracts

Shen, Z., Rodriguez-Garcia, M., Patel, M. V., Bodwell, J., and Wira, C. R. (2019). Epithelial cells and fibroblasts from the human female reproductive tract accumulate and release TFV and TAF to sustain inhibition of HIV infection of CD4+ T cells. *Sci. Rep.* 9:1864. doi: 10.1038/s41598-018-38205-y

Slater, T. F., Sawyer, B., and Straeuli, U. (1963). Studies on succinate-tetrazolium reductase systems. III. Points of coupling of four different tetrazolium salts. *Biochim. Biophys. Acta* 77, 383–393. doi: 10.1016/0006-3002(63)90513-4

Snedecor, S. J., Sudharshan, L., Nedrow, K., Bhanegaonkar, A., Simpson, K. N., Haider, S., et al. (2014). Burden of nonnucleoside reverse transcriptase inhibitor resistance in HIV-1-infected patients: a systematic review and meta-analysis. *AIDS Res. Hum. Retroviruses* 30, 753–768. doi: 10.1089/aid.2013.0262

Spence, J. R., Mayhew, C. N., Rankin, S. A., Kuhar, M. F., Vallance, J. E., Tolle, K., et al. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. *Nature* 470, 105–109. doi: 10.1038/nature09691

Stefanidou, M., Herrera, C., Armanasco, N., and Shattock, R. J. (2012). Saquinavir inhibits early events associated with establishment of HIV-1 infection: potential role for protease inhibitors in prevention. *Antimicrob. Agents Chemother.* 56, 4381–4390. doi: 10.1128/AAC.00399-12

Swedrowska, M., Jamshidi, S., Kumar, A., Kelly, C., Rahman, K. M., and Forbes, B. (2017). *In silico* and *in vitro* screening for P-glycoprotein interaction with tenofovir, darunavir, and dapivirine: an antiretroviral drug combination for topical prevention of colorectal HIV transmission. *Mol. Pharm.* 14, 2660–2669. doi: 10.1021/acs.molpharmaceut.7b00133

Sykes, J. A., Whitescarver, J., Jernstrom, P., Nolan, J. F., and Byatt, P. (1970). Some properties of a new epithelial cell line of human origin. *J. Natl. Cancer Inst.* 45, 107–122.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499, 481–484. doi: 10.1038/nature12271

Takeuchi, Y., Mcclure, M. O., and Pizzato, M. (2008). Identification of gammaretroviruses constitutively released from cell lines used for human immunodeficiency virus research. *J. Virol.* 82, 12585–12588. doi: 10.1128/JVI.01726-08

Taneva, E., Crooker, K., Park, S. H., Su, J. T., Ott, A., Cheshenko, N., et al. (2015). Differential mechanisms of tenofovir and tenofovir disoproxil fumarate cellular transport and implications for topical preexposure prophylaxis. *Antimicrob. Agents Chemother.* 60, 1667–1675. doi: 10.1128/AAC.02793-15

Taneva, E., Sinclair, S., Mesquita, P. M., Weinrick, B., Cameron, S. A., Cheshenko, N., et al. (2018). Vaginal microbiome modulates topical antiretroviral drug pharmacokinetics. *JCI Insight.* 3, e99545 doi: 10.1172/jci.insight.99545

Thurman, A. R., Chandra, N., Yousefieh, N., Zalenskaya, I., Kimble, T., Asin, S., et al. (2016). Comparison of follicular and luteal phase mucosal markers of HIV susceptibility in healthy women. *AIDS Res. Hum. Retroviruses* 32, 547–560. doi: 10.1089/aid.2015.0264

Thurman, A. R., Yousefieh, N., Chandra, N., Kimble, T., Asin, S., Rollenhagen, C., et al. (2017). Comparison of mucosal markers of human immunodeficiency virus susceptibility in healthy premenopausal versus postmenopausal women. *AIDS Res. Hum. Retroviruses* 33, 807–819. doi: 10.1089/aid.2016.0320

Trezza, C. R., and Kashuba, A. D. (2014). Pharmacokinetics of antiretrovirals in genital secretions and anatomic sites of HIV transmission: implications for HIV prevention. *Clin. Pharmacokinet.* 53, 611–624. doi: 10.1007/s40262-014-0148-z

Trotard, M., Tsopoulidis, N., Tibroni, N., Willemsen, J., Binder, M., Ruggieri, A., et al. (2016). Sensing of HIV-1 infection in Tzm-bl cells with reconstituted expression of STING. *J. Virol.* 90, 2064–2076. doi: 10.1128/JVI.02966-15

UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance (2001). Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. *BMJ* 322, 1087–1088. doi: 10.1136/bmj.322.7294.1087

Van Herrewege, Y., Michiels, J., Waeytens, A., De Boeck, G., Salden, E., Heyndrickx, L., et al. (2007). A dual chamber model of female cervical mucosa for the study of HIV transmission and for the evaluation of candidate HIV microbicides. *Antiviral Res.* 74, 111–124. doi: 10.1016/j.antiviral.2006.10.010

Venkataraman, N., Cole, A. L., Svoboda, P., Pohl, J., and Cole, A. M. (2005). Cationic polypeptides are required for anti-HIV-1 activity of human vaginal fluid. *J. Immunol.* 175, 7560–7567. doi: 10.4049/jimmunol.175.11.7560

Wei, X., Decker, J. M., Liu, H., Zhang, Z., Arani, R. B., Kilby, J. M., et al. (2002). Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. *Antimicrob. Agents Chemother.* 46, 1896–1905. doi: 10.1128/AAC.46.6.1896-1905.2002

Weinstock, H. S., Zaidi, I., Heneine, W., Bennett, D., Garcia-Lerma, J. G., Douglas, J. M. Jr., et al. (2004). The epidemiology of antiretroviral drug resistance among drug-naive HIV-1-infected persons in 10 US cities. *J. Infect Dis.* 189, 2174–2180. doi: 10.1086/420789

Yang, K. H., Hendrix, C., Bumpus, N., Elliott, J., Tanner, K., Mauck, C., et al. (2014). A multi-compartment single and multiple dose pharmacokinetic comparison of rectally applied tenofovir 1% gel and oral tenofovir disoproxil fumarate. *PLoS ONE* 9:e106196. doi: 10.1371/journal.pone.0106196

Young, A. N., Moyle-Heyrman, G., Kim, J. J., and Burdette, J. E. (2017). Microphysiologic systems in female reproductive biology. *Exp. Biol. Med.* 242, 1690–1700. doi: 10.1177/1535370217697386

Zhang, L., Herrera, C., Coburn, J., Olejniczak, N., Ziprin, Z., Kaplan, D. L., et al. (2017). Stabilization and sustained release of HIV inhibitors by encapsulation in silk fibroin disks. *ACS Biomaterials Sci. Eng.* 3, 1654–1665. doi: 10.1021/acsbiomaterials.7b00167

Zhou, T., Hu, M., Cost, M., Poloyac, S., and Rohan, L. (2013). Short communication: expression of transporters and metabolizing enzymes in the female lower genital tract: implications for microbicide research. *AIDS Res. Hum. Retroviruses* 29, 1496–1503. doi: 10.1089/aid.2013.0032

Zhou, T., Hu, M., Pearlman, A., Patton, D., and Rohan, L. (2014). Expression and localization of p-glycoprotein, multidrug resistance protein 4, and breast cancer resistance protein in the female lower genital tract ofhuman and pigtailed macaque. *AIDS Res. Hum. Retroviruses* 30, 1106–1116. doi: 10.1089/aid.2013.0281

Zidan, A. S., Spinks, C. B., Habib, M. J., and Khan, M. A. (2013). Formulation and transport properties of tenofovir loaded liposomes through Caco-2 cell model. *J. Liposome Res.* 23, 318–326. doi: 10.3109/08982104.2013.810645

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 05 July 2019
doi: 10.3389/fphar.2019.00721

Comparing Self-Report Pre-Exposure Prophylaxis Adherence Questions to Pharmacologic Measures of Recent and Cumulative Pre-Exposure Prophylaxis Exposure

Jill Blumenthal[1], Elizabeth C. Pasipanodya[2], Sonia Jain[3], Shelly Sun[3], Eric Ellorin[1], Sheldon Morris[1] and David J. Moore[2]*

[1] Department of Medicine, University of California San Diego, La Jolla, CA, United States, [2] Department of Psychiatry, University of California San Diego, La Jolla, CA, United States, [3] Family Medicine and Public Health, University of California San Diego, La Jolla, CA, United States

OPEN ACCESS

Edited by:
Peter L. Anderson, University of Colorado Anschutz Medical Campus, United States

Reviewed by:
Jose R. Castillo-Mancilla, University of Colorado Denver, United States
Jenna Yager, University of Colorado Anschutz Medical Campus, United States

***Correspondence:**
Jill Blumenthal
jblumenthal@ucsd.edu

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *01 January 2019*
Accepted: *05 June 2019*
Published: *05 July 2019*

Citation:
Blumenthal J, Pasipanodya EC, Jain S, Sun S, Ellorin E, Morris S and Moore DJ (2019) Comparing Self-Report Pre-Exposure Prophylaxis Adherence Questions to Pharmacologic Measures of Recent and Cumulative Pre-Exposure Prophylaxis Exposure. Front. Pharmacol. 10:721. doi: 10.3389/fphar.2019.00721

As pre-exposure prophylaxis (PrEP) effectiveness is strongly linked to adherence, we sought to determine if certain self-report measures could be used to inform objective PrEP adherence. We studied participants from the TAPIR study (a multicenter randomized study of daily text messages to support adherence to PrEP In At-Risk), a 48-week randomized controlled trial of HIV-uninfected men who have sex with men (MSM) randomized to receive text message to support adherence versus standard of care. Self-reported medication adherence was assessed using several validated measures modified for PrEP. Objective PrEP adherence was determined through dried blood spot (DBS) measurement of intracellular tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP). A summary of adherence was estimated using responses to the seven adherence items at weeks 12 and 48 using confirmatory factor analysis. Correlations between self-report questions and drug concentrations were estimated with Pearson's correlations for continuous outcomes and point-biserial correlations for dichotomous outcomes. Receiver operating characteristic (ROC) analyses were conducted to assess the performance of self-report measures in predicting protective or perfect TFV-DP concentrations. Of the 369 participants who completed week 12 or 48 visits, the mean age was 35 (standard deviation 9 years), with 79% White, 12% Black, and 29% Hispanic. Correlations between self-report measures of adherence (both individual items and the adherence factor) and quantifiable FTC-TP and continuous TFV-DP concentrations showed that all self-report measures were significantly associated with these objective measures. Compared to a summary measure of self-reported adherence, the 4-week percent taken question medication recall was the only self-report item similarly or more strongly associated with recent adherence and long-term protective and perfect adherence at weeks 12 and 48. ROC analysis also showed that 4-week percent taken question had a reasonable AUC (0.798 at week 12 and 0.758 at week 48) in predicting protective TFV-DP concentrations. All single-item self-report questions assessing PrEP adherence were significantly associated with biomarker quantification, with the 4-week percent taken question performing best.

Therefore, in the absence of drug concentration measurements, a 4-week self-report percent taken question may be a good single-item measure of PrEP adherence.

Keywords: adherence, men who have sex with men (MSM), self-report, pre-exposure (PrEP) prophylaxis, pharmacologic measures

BACKGROUND

Over the last several years, HIV prevention has increasingly included biomedical strategies using pre-exposure prophylaxis (PrEP). Once daily tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) has been shown to greatly reduce the risk of HIV infection in populations at risk for HIV acquisition (Grant et al., 2010; Baeten et al., 2012; Thigpen et al., 2012; Grant et al., 2014; Liu et al., 2016; McCormack et al., 2016). Sub-optimal adherence severely undercuts its effectiveness as an HIV prevention strategy. In men who have sex with men (MSM), four doses or more of PrEP per week has been shown to confer upwards of 99% risk reduction (Anderson et al., 2012; Desai et al., 2017). Taking less than four doses per week is considered inadequate to provide sufficient protection against HIV infection (Grant et al., 2014; Liu et al., 2016; Hojilla et al., 2018). As PrEP becomes increasingly available in clinical settings, one of the challenges for providers becomes how to accurately assess PrEP adherence outside of a research setting.

Presently, no single "gold standard" has been ubiquitously adopted to assess adherence. Research studies have employed biomarker quantification as an objective adherence measure. At present, these pharmacological assays are costly, require specialized laboratory equipment and personnel, and take time to yield results; however, there is research underway to develop point of care immunoassays in urine that could be used as clinical tools (Koenig et al., 2017; Gandhi et al., 2018). Until real-time drug concentration testing is available, biomarker quantification can be used to gauge the practicality and accuracy of alternative methods, including self-report measures. Several clinical trials have determined concentration thresholds for TFV, FTC, and their metabolites [tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP)] in various biologic mediums that correspond to recent and long-term PrEP dosing, respectively (Anderson et al., 2012; Baxi et al., 2015; Wahl et al., 2017). Unfortunately, studies analyzing the concordance between biomarker concentrations and other adherence methodologies have returned with mixed results. Device-assisted medication event monitoring system (MEMS)-caps (Musinguzi et al., 2016) is moderately correlated with drug concentrations, but they present unique technological challenges and are costly. Pill counting and medication possession ratios (i.e., pharmacy refills) have weaker concordance to electronic monitoring, as they assume all unaccounted doses were ingested and can be manipulated by patients. (Haberer et al., 2015).

Self-report adherence measures generally have the lowest concordance with drug concentrations (Amico et al., 2014; Baxi et al., 2015; Musinguzi et al., 2016; van der Straten et al., 2016). Clinical trials have often adapted self-report adherence questions from antiretroviral therapy (ART) research for use in PrEP, but the lack of a standardized method results in large variability of collecting adherence outcomes (Musinguzi et al., 2016). Moreover, subjective adherence reporting is fundamentally flawed. In particular, overestimation of adherence either as a result of social desirability or recall bias is common across medical disciplines and has been observed in many PrEP efficacy trials and demonstration projects (Amico et al., 2014; Musinguzi et al., 2016; van der Straten et al., 2016; Baker et al., 2018). Despite these limitations, self-report is a non-invasive, ecologically valid, low-burden method that has already been implemented in clinical settings. Further investigation into developing, adapting, and refining accurate self-report PrEP adherence measures is warranted (Haberer, 2016).

In this current analysis, we analyze the concordance between several self-report adherence questions with two pharmacologic drug level measures used in a PrEP demonstration project of MSM to identify the most accurate PrEP-appropriate self-report adherence measures.

METHODS

Participants and Procedures

We employed a well-characterized high-risk cohort of MSM enrolled in the California Collaborative Treatment Group (CCTG) 595 TAPIR study (A Multicenter Randomized Study of Daily **T**ext Messages to Support **A**dherence to **P**rEP **I**n At-**R**isk for HIV Individuals; NCT01761643) (Moore et al., 2017). Participants from four Southern California medical centers [University of California, San Diego (UCSD); University of Southern California; LA Biomed at Harbor-University of California Los Angeles; and Long Beach Department of Health and Human Services] were randomly assigned to a daily text-messaging intervention (individualized Texting for Adherence Building—iTAB) versus standard of care to determine the efficacy of iTAB on PrEP adherence.

TAPIR participants were MSM, 18 years or older, and HIV-negative as confirmed by an antigen/antibody (Ag/Ab) assay or Ab assay plus HIV nucleic acid test. Additional eligibility criteria included having persistent elevated risk of HIV acquisition through condomless anal intercourse with HIV-positive men and/or partners of unknown status or having a recent sexually transmitted infection (STI) diagnosis. Over a 48-week study period, all participants received once-daily PrEP with TDF/FTC, risk reduction and adherence counseling, safety monitoring, and HIV/STI testing every 3 months. Data were collected at baseline, weeks 4, 12, 24, 36, and 48 by both confidential in-person interviews and computer assisted self-interview (CASI) instruments, which included a survey with self-report adherence measures. All CASI questions were answered and

recorded by the participant. At weeks 12 and 48, biologic markers of adherence were measured, described in the next section. In the main study, iTAB participants were more likely to have tenofovir drug concentrations corresponding to near-perfect adherence (~7 doses per week). This analysis includes the 369 TAPIR participants with completed study visits at weeks 12 or 48 after initiating PrEP.

Measures

Pre-exposure prophylaxis as a biological marker of adherence was determined through dried blood spot (DBS) measurement of intracellular TFV-DP and FTC-TP (measuring predominately intra-erythrocytic concentrations). Both TFV-DP and FTC-TP were quantified at weeks 12 and 48 using a liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay previously validated (Castillo-Mancilla et al., 2013). Protective TFV-DP drug concentrations were defined as >700 fmol/punch (~4 doses per week) and perfect TFV-DP drug concentrations were defined as >1,250 fmol/punch (~7 doses per week). FTC-TP concentrations were considered consistent with dosing in the last 48 h if they had quantifiable values.

Self-reported medication adherence was measured at all study visits after baseline using seven CASI questions, some of which were taken from the ACTG Adherence Instrument (Chesney et al., 2000) and other commonly used adherence questions (Berg et al., 2012; Wilson et al., 2014), modified to assess PrEP (**Table 1**). Each question was examined individually. We used CASI self-reported adherence data from weeks 12 and 48.

Statistical Analysis

A summary of adherence was additionally estimated using participant responses to the seven adherence items at weeks 12 and 48 using confirmatory factor analysis (CFA). CFA is a structural equation modeling technique of examining the relationship between observed variables and their underlying latent constructs. Similar to other clustering methods, CFA summarizes data and estimates the amount of shared variance between a set of variables (Jackson et al., 2009). A one-factor model, accounting for the correlations among all seven PrEP questions and capturing a single latent construct of adherence to PrEP that could be considered a "purer" measure of adherence purged of measurement error, was estimated using robust maximum likelihood estimation (MLR) that is robust to non-normally distributed data (Muthén and Muthén, 1998–2015). Several indices of model fit were used to evaluate the fit of the one-factor model to the data; in particular, the Comparative Fit Index (CFI), the Root Mean Square Error of Approximation (RMSEA), and the Standardized Root Mean Square Residual (SRMR) were examined and guidelines for model evaluation were used to judge fit (i.e., CFI > 0.90, RMSEA < 0.06, and SRMR < 0.08 are generally regarded as indicating good-fitting models) (Hu and Bentler, 1999).

Associations between individual self-report questions assessing adherence and objective measures of adherence (FTC-TP and TFV-DP) were assessed using correlation analyses. The adherence factor and objective adherence measures were also correlated to obtain a measure of association between a summary measure of self-reported adherence and DBS measurements. Correlations between continuous variables were estimated as Pearson's correlations while correlations between continuous self-report adherence measures and dichotomous DBS measurements were estimated as point-biserial correlations using robust maximum likelihood estimation (MLR). To account for the non-independence of observations due to participants' repeated assessment, standard errors were adjusted using a Huber–White sandwich estimator (Huber, 1967; Muthén and Muthén, 1998–2015).

To identify the self-report item best assessing PrEP adherence, the relative strength of associations between individual self-report items and objective adherence were compared to the strength of association between the summary adherence factor and objective measures of adherence. To do so, correlation coefficients were first converted into z-scores using Fisher Z transformations and tests of the equality of correlation

TABLE 1 | Self-reported adherence questions and responses.

Type	Questions with Possible Responses (number)
4-week ability	Thinking about the past 4 weeks, how would you rate your ability to take all of your PrEP medications? Very poor, Poor, Fair, Good, Very Good, Excellent (6)
4-week frequency	Thinking about the past 4 weeks, how often did you take all of your PrEP medications? None, A Little, Sometimes, Most of the time, All of the time (5)
4-week percent taken	Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications? 0–100%
3-month recall	When was the last time you were not able to take your PrEP medication? Never, >3 months ago, 1–3 months, 2–4 weeks, 1–2 weeks, past week (6)
1-month good job	In the last 30 days, how GOOD A JOB did you do at taking your PrEP medication in the way you are supposed to? Very poor, Poor, Fair, Good, Very Good, Excellent (6)
1-month frequency	In the last 30 days, how OFTEN did you take PrEP medication in the way you are supposed to? Never, Rarely, Sometimes, Usually, Most always, Always (6)
1-month difficulty	In the last 30 days, how HARD was it for you to take your PrEP medication in the way you are supposed to? Never, Rarely, Sometimes, Usually, Most always, Always (6)

coefficients (i.e., comparisons associations between individual self-report items and biologically-quantified adherence versus the association between the summary adherence factor and biologically-quantified adherence) were carried out using asymptotic *z*-tests (Steiger, 1980; Lee and Preacher, 2013). Corrections for multiple comparisons were made using the Benjamini–Hochberg procedure at a false discovery rate of 0.25 (Benjamini and Hochberg, 1995).

Receiver operating characteristic (ROC) analyses were also conducted to assess the performance of these self-report measures in predicting protective or perfect TFV-DP concentrations (Fawcett, 2006). Area under the ROC curve (AUC) was calculated with a 95% confidence interval. An AUC of 0.5 indicates no discrimination and an AUC of 1.0 indicates a perfect diagnostic test. Therefore, consistent with current statistical consensus, an AUC of < 0.7 was considered poor, 0.7 to 0.8 adequate, and 0.8 to 0.9 very good. Factor analyses and correlational associations were carried out in Mplus v7.4 while ROC analyses were carried out using Rv3.5.1 (Muthén and Muthén, 1998–2015).

RESULTS

Of the 369 participants contributing data to these analyses, the mean age was 35 with a standard deviation of 9 years. The majority of participants were white (79%), with 12% Black and 29% Hispanic. More than half of the participants held a bachelor's or advanced degree and nearly two-thirds had a monthly income of $2,000 or greater. Half were in the intervention arm of TAPIR (**Table 2**).

The fit of a one-factor CFA composed of all self-report adherence was adequate (CFI = .946, RMSEA = .078, and SRMR = .024), and all adherence items had large and significant factor loadings, suggesting good associations with the latent adherence construct (**Table 3**) (Hu and Bentler, 1999). Correlation analyses showed significant associations between all self-report adherence questions and pharmacologic measures. Specifically, individual self-report items and the adherence factor were correlated with quantifiable FTC-TP concentrations, continuous TFV-DP concentrations, and TFV-DP dichotomized at protective (>700 fmol/punch) and perfect (>1,250 fmol/punch) concentrations. Furthermore, the largest correlations were consistently between the various self-report measures and protective levels of TFV-DP (**Table 4**). We then compared 1) the associations between self-report items and biologically quantified adherence versus 2) the association between the summary adherence factor and biologically quantified adherence. Results of asymptotic *z*-tests suggested that 4-week percent taken was more significantly associated with recent adherence (quantifiable FTC) while 4-week ability, 4-week frequency, 3-month recall, and 1-month difficulty were associated with recent adherence to the same degree as the summary measure. The remaining self-report items had significantly weaker correlations. In terms of longer-term adherence, 4-week percent taken was associated with protective TFV-DP concentrations (>700 fmol/punch) to the same degree as the summary measure; all other measures were significantly less correlated than the summary measure. With regards to perfect TFV-DP adherence (>1,250 fmol/punch), compared with the summary measure, 4-week percent taken, 4-week ability, 4-week frequency, and 3-month recall had a similar correlations. The remaining measures were marginally or significantly less correlated with perfect TFV-DP concentrations than the summary measure (**Supplementary Table 1**). When comparing the adherence measures with continuous concentrations of TFV-DP, only 4-week percent taken and 3-month recall were similarly associated to TFV-DP concentrations as the summary adherence measure. Thus, compared with a summary measure of self-reported adherence, the 4-week percent taken medication recall was the only self-report item to consistently be similarly or more strongly associated with recent adherence and long-term protective and perfect PrEP adherence at weeks 12 and 48.

Finally, we evaluated the performance of the self-report questions in predicting protective or perfect TFV-DP concentrations using ROC analyses. AUC values were calculated at weeks 12 and 48. Similar to findings above, ROC analyses also showed that 4-week percent taken question had a fairly good AUC (0.798 at week 12 and 0.758 at week 48) in predicting protective TFV-DP concentrations. However, all self-reported measures were not particularly good for predicting perfect TFV-DP (AUC all below 0.7). Results from week 48 are displayed in **Figure 1**.

DISCUSSION

We found that all single-item self-report questions assessing PrEP adherence were significantly associated with biomarker quantification with the 4-week percent taken question performing best. In addition, the 4-week percent taken question had a reasonable AUC value in predicting protective TFV-DP concentrations at both weeks 12 and 48. Therefore, in the absence of drug level measurements, our findings suggest that a 4-week self-report percent taken question may be a good single-item measure of both recent and cumulative adherence.

Several recent studies have similarly examined different combinations of subjective and objective PrEP adherence

TABLE 2 | Participant demographics and sample characteristics.

Characteristic	Descriptive statistic
Age, mean (SD)	35 (9)
Education, n (%)	
High school or Less	24 (7%)
Some college	137 (37%)
Bachelors	130 (35%)
Some post-graduate	18 (5%)
Advanced degree	60 (16%)
Race and ethnicity, n (%)	
White	292 (79%)
Black	44 (12%)
Hispanic	105 (289%)
Income, n (%)	
<$2,000 per month	76 (21%)
≥$2,000 per month	238 (65%)
Intervention arm, n (%)	182 (49%)

TABLE 3 | Factor indicators and loadings in a one-factor model of adherence.

Indicator	Standardized loading	Standard error
4-week ability	0.908***	0.027
4-week frequency	0.869***	0.027
4-week percent taken	0.842***	0.021
3-month recall (reversed)	0.551***	0.033
1-month good job	0.910***	0.022
1-month frequency	0.893***	0.027
1-month difficulty	0.651***	0.050

measures and have found varying degrees of concordance among them. In the TDF2 clinical trial of men and women in Botswana, self-report adherence *via* an interview question assessing missed doses over the last 3 days was only modestly associated with quantifiable drug concentrations of tenofovir (TFV) and FTC quantified in plasma (Kebaabetswe et al., 2015). Similar results were observed in serodiscordant couples in East Africa where three types of self-reported adherence questions were not able to discriminate between steady-state daily dosing and less than steady-state daily dosing plasma TVF concentrations (Musinguzi et al., 2016). Within the preexposure prophylaxis initiative (iPrEx) trial, there were differences in consistency between self-report and PrEP drug detection by study site with good concordance in the US but large discrepancies in subjective and objective measures in non-US study sites. Self-reported recent PrEP dosing using neutral interviewing was a strong predictor of TFV quantitation in plasma in the iPrEX open-label extension (OLE) (Amico et al., 2016). Our study is an important addition to this literature because it 1) examines several self-report items assessing different aspects of adherence behavior and duration of recall, 2) employs unique statistical methods using latent constructs to develop a theoretical adherence measure without measurement errors, 3) evaluates recent and cumulative PrEP adherence using DBS quantification, and 4) includes continuous and dichotomous outcomes of objective adherence.

Because PrEP efficacy highly depends on medication adherence, it is frequently studied in HIV prevention research and evaluated in PrEP clinical care. Subjective adherence reporting is the most commonly used method to quantify adherence behavior in real time (Agot et al., 2015). As is true for self-report of medication adherence in general (Stirratt et al., 2015), there is no standard self-report adherence measure of PrEP used across research studies or clinical practice. A large number and wide variety of self-report adherence measures have already been adapted or developed to quantify PrEP adherence (Muchomba et al., 2012). As a result, it may be difficult to evaluate or compare PrEP adherence self-report in a systematic way due to lack of inconsistency in measures used. Across several large PrEP clinical trials, there was minimal overlap of self-report questions used to evaluate adherence (Amico et al., 2014; Agot et al., 2015; Kebaabetswe et al., 2015; Amico et al., 2016; Musinguzi et al., 2016). Differences in how questions are framed, what period is used, and which response options are offered may affect how adherence is reported and thus measured. Having a least one self-report measure of adherence that is widely utilized and perhaps recommended in guidelines or expert options could improve research findings and clinical outcomes through harmonization of subjective adherence assessment.

PrEP delivery in limited resource locations necessitates inexpensive and easy approaches to offer and monitor PrEP. As self-report may be the sole method to measure PrEP adherence, a question that is both sensitive and specific could offer an accurate appraisal of adherence and help direct resources to those needing additional adherence support in well-resourced but high-volume PrEP clinics. Using a single question that elicits a response best reflecting real adherence behavior is an efficient way to gauge adherence. In addition, one adherence question could be included in clinic intake forms that patients can answer more privately, potentially reducing social desirability bias (Bowling, 2005).

While our study had several strengths including a large sample size and assessments performed at multiple time points, there are some limitations. Since our study included only MSM in a resource-rich setting, our results may not be generalizable to other at-risk populations or those in resource-limited settings taking PrEP. Study timing may have significantly influenced our findings, as it was conducted soon after the FDA approval

TABLE 4 | Correlations between pharmacological and self-report adherence measures.

Adherence measures	[a]FTC-TP	[b]TFV-DP	[a]TFV-DP ≥ 700 fm/p	[a]TFV-DP ≥ 1250 fm/p
Summary self-report (adherence factor)	0.252***	0.360***	0.465***	0.262***
4-week ability	0.239***	0.317***	0.424***	0.237***
4-week frequency	0.215***	0.309***	0.384***	0.234***
4-week percent taken	0.324***	0.372***	0.439***	0.251***
3-month recall (reversed)	0.169**	0.283***	0.296***	0.239***
1-month good job	0.177**	0.305***	0.338***	0.235***
1-month frequency	0.187**	0.299***	0.380***	0.189***
1-month difficulty	0.173**	0.209***	0.272***	0.168***

***0.05>p>0.001; ***p<0.001;*

FTC-TP, emtricitabine triphosphate; TFV-DP, tenofovir diphosphate; fm/p, fmol/punch.

[a]Correlations using dichotomous pharmacological measures of adherence are point-biserial correlations.

[b]Correlations using continuous pharmacological measures of adherence are Pearson correlations.

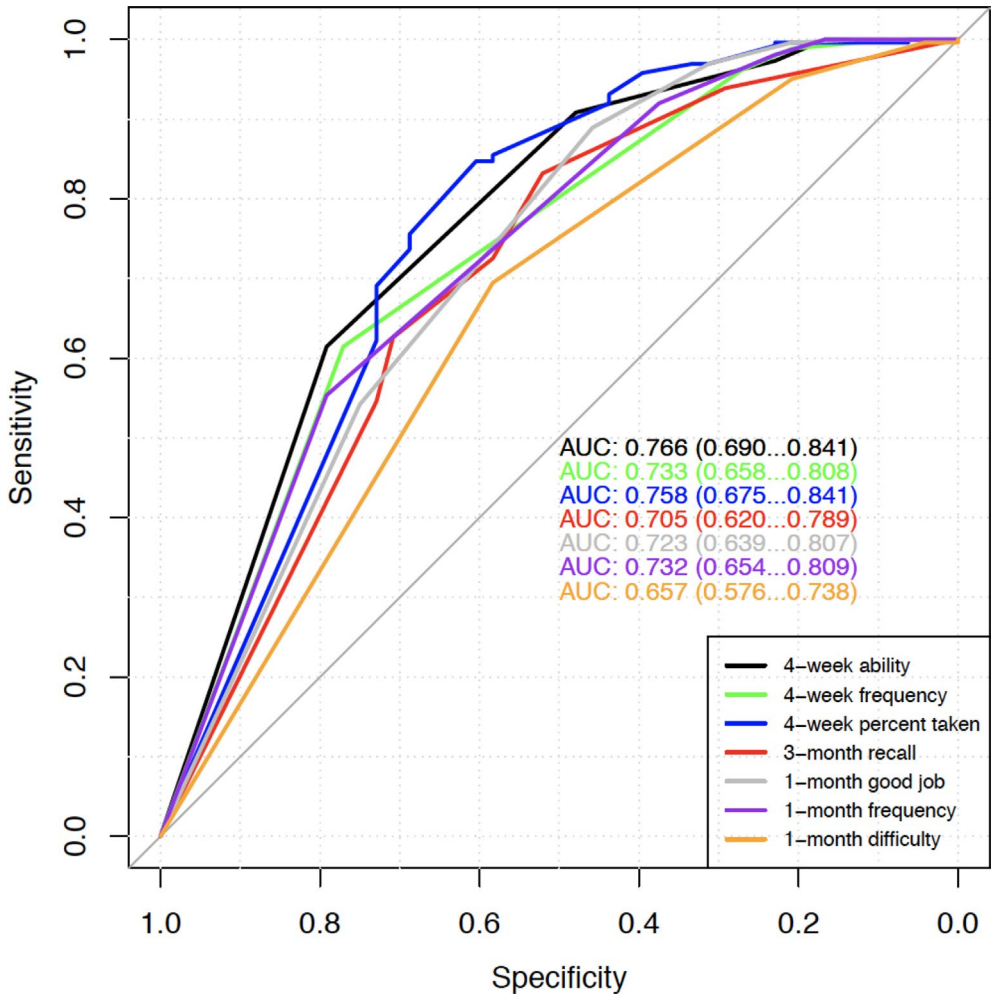

FIGURE 1 | Week 48 ROC for predicting protective TFV-DP concentrations (>700 versus ≤700). AUC values with 95% confidence intervals of the seven self-report adherence questions are shown.

of PrEP. We had many early adopters who had high overall adherence based on DBS concentrations and were less likely to overestimate adherence. In addition, we only used single-item adherence questions to evaluate associations with PrEP drug concentrations. Some questions were designed to be asked as part of a group so alone they may have less intrinsic value. Finally, there are other commonly used self-report questions, which may be similarly or better correlated with biological measurements, not included in our study.

CONCLUSIONS

Self-report questions to measure PrEP adherence are commonly utilized in clinical research and may be the only method deployed to assess medication adherence. It is essential to better understand which subjective measures are most accurate and how they can effectively be integrated into PrEP research and clinical care. Our findings demonstrate that a 4-week percent taken question of medication recall may best reflect true recent and longer-term adherence behavior. In the future, we will explore combinations of different self-report adherence questions that may yield even stronger associations with PrEP drug concentrations.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the UCSD Human Research Protections Program with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the UCSD Human Research Protections Program.

AUTHOR CONTRIBUTIONS

JB, DM, and SM contributed to the conception and design of the study; EE organized the database; SJ and SS performed the

statistical analysis; JB wrote the first draft of the manuscript; EP and EE wrote sections of the manuscript. All authors contributed to manuscript revision, and read and approved the submitted version.

FUNDING

The work was supported by award EI11-SD-005 from the California HIV/AIDS Research Program, award IN-US-276-D036 from Gilead and 1KL2TR0001444 to Dr. Blumenthal.

ACKNOWLEDGMENTS

The authors would like to thank the participants for volunteering for this study. The authors would also like to acknowledge the efforts of our excellent study staff at across all the CCTG.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00721/full#supplementary-material

REFERENCES

Agot, K., Taylor, D., Corneli, A. L., Wang, M., Ambia, J., Kashuba, A. D., et al. (2015). Accuracy of self-report and pill-count measures of adherence in the FEM-PrEP clinical trial: implications for future HIV-prevention trials. *AIDS Behav.* 19, 743–751. doi: 10.1007/s10461-014-0859-z

Amico, K. R., Marcus, J. L., McMahan, V., Liu, A., Koester, K. A., Goicochea, P., et al. (2014). Study product adherence measurement in the iPrEx placebo-controlled trial: concordance with drug detection. *J. Acquir. Immune Defic. Syndr.* 66, 530–537. doi: 10.1097/QAI.0000000000000216

Amico, K. R., Mehrotra, M., Avelino-Silva, V. I., McMahan, V., Veloso, V. G., Anderson, P., et al. (2016). Self-reported recent PrEP dosing and drug detection in an open label PrEP study. *AIDS Behav.* 20, 1535–1540. doi: 10.1007/s10461-016-1360-7

Anderson, P. L., Glidden, D. V., Liu, A., Buchbinder, S., Lama, J. R., Guanira, J. V., et al. (2012). Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. *Sci. Transl. Med.* 4, 151ra25. doi: 10.1126/scitranslmed.3004006

Baeten, J. M., Donnell, D., Ndase, P., Mugo, N. R., Campbell, J. D., Wangisi, J., et al. (2012). Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N. Engl. J. Med.* 367, 399–410. doi: 10.1056/NEJMoa1108524

Baker, Z., Javanbakht, M., Mierzwa, S., Pavel, C., Lally, M., Zimet, G., et al. (2018). Predictors of over-reporting HIV pre-exposure prophylaxis (PrEP) adherence among young men who have sex with men (YMSM) in self-reported versus biomarker data. *AIDS Behav.* 22, 1174–1183. doi: 10.1007/s10461-017-1958-4

Baxi, S. M., Liu, A., Bacchetti, P., Mutua, G., Sanders, E. J., Kibengo, F. M., et al. (2015). Comparing the novel method of assessing PrEP adherence/exposure using hair samples to other pharmacologic and traditional measures. *J. Acquir. Immune Defic. Syndr.* 68, 13–20. doi: 10.1097/QAI.0000000000000386

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B.* 57, 289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x

Berg, K. M., Wilson, I. B., Li, X., and Arnsten, J. H. (2012). Comparison of antiretroviral adherence questions. *AIDS Behav.* 16, 461–468. doi: 10.1007/s10461-010-9864-z

Bowling, A. (2005). Mode of questionnaire administration can have serious effects on data quality. *J. Public Health. (Oxf)* 27, 281–291. doi: 10.1093/pubmed/fdi031

Castillo-Mancilla, J. R., Zheng, J. H., Rower, J. E., Meditz, A., Gardner, E. M., Predhomme, J., et al. (2013). Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. *AIDS Res. Hum. Retroviruses* 29, 384–390. doi: 10.1089/aid.2012.0089

Chesney, M. A., Ickovics, J. R., Chambers, D. B., Gifford, A. L., Neidig, J., Zwickl, B., et al. (2000). Self-reported adherence to antiretroviral medications among participants in HIV clinical trials: the AACTG adherence instruments. Patient Care Committee & Adherence Working Group of the Outcomes Committee of the Adult AIDS Clinical Trials Group (AACTG). *AIDS Care* 12, 255–266. doi: 10.1080/09540120050042891

Desai, M., Field, N., Grant, R., and McCormack, S. (2017). Recent advances in pre-exposure prophylaxis for HIV. *BMJ* 359, j5011. doi: 10.1136/bmj.j5011

Fawcett, T. (2006). An introduction to ROC analysis. *Pattern Recognit. Lett.* 27, 861–874. doi: 10.1016/j.patrec.2005.10.010

Gandhi, M., Bacchetti, P., Rodrigues, W. C., Spinelli, M., Koss, C. A., and Drain, P. K. (2018). Development and validation of an immunoassay for tenofovir in urine as a real-time metric of antiretroviral adherence. *ECLinicalMedicine* 2–3, 23–30. doi: 10.1016/j.eclinm.2018.08.004

Grant, R. M., Anderson, P. L., McMahan, V., Liu, A., Amico, K. R., Mehrotra, M., et al. (2014). Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect. Dis.* 14, 820–829. doi: 10.1016/S1473-3099(14)70847-3

Grant, R. M., Lama, J. R., Anderson, P. L., McMahan, V., Liu, A. Y., Vargas, L., et al. (2010). Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N. Engl. J. Med.* 363, 2587–2599. doi: 10.1056/NEJMoa1011205

Haberer, J. E. (2016). Current concepts for PrEP adherence in the PrEP revolution: from clinical trials to routine practice. *Curr. Opin. HIV AIDS* 11, 10–17. doi: 10.1097/COH.0000000000000220

Haberer, J. E., Bangsberg, D. R., Baeten, J. M., Curran, K., Koechlin, F., Amico, K. R., et al. (2015). Defining success with HIV pre-exposure prophylaxis: a prevention-effective adherence paradigm. *AIDS* 29, 1277–1285. doi: 10.1097/QAD.0000000000000647

Hojilla, J. C., Vlahov, D., Glidden, D. V., Amico, K. R., Mehrotra, M., Hance, R., et al. (2018). Skating on thin ice: stimulant use and sub-optimal adherence to HIV pre-exposure prophylaxis. *J. Int. AIDS Soc.* 21, e25103. doi: 10.1002/jia2.25103

Hu, L.-t., and Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Struct. Equation Model Multidiscip. J.* 6, 1–55. doi: 10.1080/10705519909540118

Huber, P. J. (1967). "The behavior of maximum likelihood estimates under nonstandard conditions," in *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability, Volume 1: Statistics* (Berkeley, Calif: University of California Press), 221–233.

Jackson, D. L., Gillaspy, J. A. Jr., and Purc-Stephenson, R. (2009). Reporting practices in confirmatory factor analysis: an overview and some recommendations. *Psychol. Methods* 14, 6–23. doi: 10.1037/a0014694

Kebaabetswe, P. M., Stirratt, M. J., McLellan-Lemal, E., Henderson, F. L., Gray, S. C., Rose, C. E., et al. (2015). Factors associated with adherence and concordance between measurement strategies in an HIV Daily Oral Tenofovir/Emtricitibine as pre-exposure Prophylaxis (Prep) clinical trial, botswana, 2007-2010. *AIDS Behav.* 19, 758–769. doi: 10.1007/s10461-014-0891-z

Koenig, H. C., Mounzer, K., Daughtridge, G. W., Sloan, C. E., Lalley-Chareczko, L., Moorthy, G. S., et al. (2017). Urine assay for tenofovir to monitor adherence in real time to tenofovir disoproxil fumarate/emtricitabine as pre-exposure prophylaxis. *HIV Med.* 18, 412–418. doi: 10.1111/hiv.12518

Lee, I. A., and Preacher, K. J. (2013). Calculation for the test of the difference between two dependent correlations with one variable in common. [Computer software]. Available from http://quantpsy.org.z

Liu, A. Y., Cohen, S. E., Vittinghoff, E., Anderson, P. L., Doblecki-Lewis, S., Bacon, O., et al. (2016). Preexposure prophylaxis for HIV infection integrated with municipal- and community-based sexual health services. *JAMA Intern. Med.* 176, 75–84. doi: 10.1001/jamainternmed.2015.4683

McCormack, S., Dunn, D. T., Desai, M., Dolling, D. I., Gafos, M., Gilson, R., et al. (2016). Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. *Lancet* 387, 53–60. doi: 10.1016/S0140-6736(15)00056-2

Moore, D. J., Jain, S., Dube, M. P., Daar, E. S., Sun, X., Young, J., et al. (2017). Randomized controlled trial of daily text messages to support adherence to PrEP in at-risk for HIV individuals: the tapir study. *Clin. Infect. Dis.* 66 (10), 1566–1572. doi: 10.1093/cid/cix1055

Muchomba, F. M., Gearing, R. E., Simoni, J. M., and El-Bassel, N. (2012). State of the science of adherence in pre-exposure prophylaxis and microbicide trials. *J. Acquir. Immune Defic. Syndr.* 61, 490–498. doi: 10.1097/QAI.0b013e31826f9962

Musinguzi, N., Muganzi, C. D., Boum, Y., 2nd, Ronald, A., Marzinke, M. A., Hendrix, C. W., et al. (2016). Comparison of subjective and objective adherence measures for preexposure prophylaxis against HIV infection among serodiscordant couples in East Africa. *AIDS* 30, 1121–1129. doi: 10.1097/QAD.0000000000001024

Muthén, B., and Muthén, L. (1998–2015). *Mplus User's Guide*. 7th Ed. Edited by Muthén & Muthén. Los Angeles: Muthén & Muthén.

Steiger, J. H. (1980). Tests for comparing elements of a correlation matrix. *Psychol. Bull.* 87, 245–251. doi: 10.1037/0033-2909.87.2.245

Stirratt, M. J., Dunbar-Jacob, J., Crane, H. M., Simoni, J. M., Czajkowski, S., Hilliard, M. E., et al. (2015). Self-report measures of medication adherence behavior: recommendations on optimal use. *Transl Behav Med.* 5, 470–482. doi: 10.1007/s13142-015-0315-2

Thigpen, M. C., Kebaabetswe, P. M., Paxton, L. A., Smith, D. K., Rose, C. E., Segolodi, T. M., et al. (2012). Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N. Engl. J. Med.* 367, 423–434. doi: 10.1056/NEJMoa1110711

van der Straten, A., Brown, E. R., Marrazzo, J. M., Chirenje, M. Z., Liu, K., Gomez, K., et al. (2016). Divergent adherence estimates with pharmacokinetic and behavioural measures in the MTN-003 (VOICE) study. *J. Int. AIDS Soc.* 19, 20642. doi: 10.7448/IAS.19.1.20642

Wahl, A., Ho, P. T., Denton, P. W., Garrett, K. L., Hudgens, M. G., Swartz, G., et al. (2017). Predicting HIV pre-exposure prophylaxis efficacy for women using a preclinical pharmacokinetic-pharmacodynamic in vivo model. *Sci. Rep.* 7, 41098. doi: 10.1038/srep41098

Wilson, I. B., Fowler, F. J., Jr., Cosenza, C. A., Michaud, J., Bentkover, J., Rana, A., et al. (2014). Cognitive and field testing of a new set of medication adherence self-report items for HIV care. *AIDS Behav.* 18, 2349–2358. doi: 10.1007/s10461-013-0610-1

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 12 July 2019
doi: 10.3389/fphar.2019.00785

Assessment of Demographic and Socio-Behavioral Factors on Adherence to HIV Pre-Exposure Prophylaxis Using a Markov Modeling Approach

Surulivelrajan Mallayasamy[1], Ayyappa Chaturvedula[1], Michael J. Fossler[1,2], Mark E. Sale[1,3], Craig W. Hendrix[4], Jessica E. Haberer[5] on behalf of the Partners Demonstration Project Team*

[1] UNT System College of Pharmacy, UNTHSC, Fort Worth, TX, United States, [2] Trevena Inc, King of Prussia, PA, United States, [3] Nuventra, Raleigh, NC, United States, [4] Johns Hopkins University School of Medicine, Baltimore, MD, United States, [5] Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States

OPEN ACCESS

Edited by:
Albert Liu, San Francisco Department of Public Health, United States

Reviewed by:
David Glidden, University of California, San Francisco, United States
Max Von Kleist, Robert Koch Institute, Germany
Kate M. Mitchell, Imperial College London, United Kingdom

***Correspondence:**
Jessica E. Haberer
jhaberer@mgh.harvard.edu

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *29 December 2018*
Accepted: *17 June 2019*
Published: *12 July 2019*

Citation:
Mallayasamy S, Chaturvedula A, Fossler MJ, Sale ME, Hendrix CW and Haberer JE (2019) Assessment of Demographic and Socio-Behavioral Factors on Adherence to HIV Pre-Exposure Prophylaxis Using a Markov Modeling Approach. Front. Pharmacol. 10:785. doi: 10.3389/fphar.2019.00785

Purpose: Adherence is important for the effectiveness of human immunodeficiency virus (HIV) preexposure prophylaxis (PrEP). The objective of the current work is to assess the impact of multiple demographic and socio-behavioral factors on the adherence to tenofovir-based PrEP among HIV serodiscordant couples in East Africa using Markov mixed-effects modeling approach.

Methods: The Partners Demonstration Project was a prospective, open-label, implementation science-driven study of HIV PrEP among heterosexual HIV serodiscordant couples in Kenya and Uganda. The uninfected partner received oral PrEP according to the "bridge to antiretroviral therapy [ART]" strategy (i.e., until the infected partner had been on ART for ≥6 months). Adherence was monitored electronically; demographic and socio-behavioral data were collected during study visits. Analyzed data reflect 12 months of follow-up per participant. A two-state, first-order, discrete time Markov model was developed with longitudinal adherence data characterized by "dose taking (1)" and "dose missing (0)." Covariate effects were linearly added in the logit domain of transition probability parameters (P01 and P10) in the model. The full covariate model was initially developed, followed by backward elimination process to reduce the model. All significant covariates reported by a prior primary statistical analysis of the same data were included in the full covariate model.

Results: The model included data from 920 participants, who were predominantly male (65%). Significant covariates associated with higher adherence were 25 years or older [odds ratio (OR) for P10, 0.61], female sex (OR for P10, 0.67), participant wanting the relationship with the partner to succeed (OR for P10, 0.79; OR for P01, 1.45), and sex with partner either with 100% or <100% condom use compared to those reported no sex (OR for P10, 0.84; OR for P01, 1.21). Significant covariates associated with lower adherence were partner on ART >6 months (OR for P01, 0.86; OR for P10, 1.34), subject

in the study for >6 months (OR for P01, 0.8; OR for P10, 1.25), and problematic alcohol use (OR for P01, 0.63; OR for P10, 1.16).

Conclusion: The developed Markov model provides a mechanistic understanding of relationship between demographic, socio-behavioral covariates, and PrEP adherence, by indicating the pattern of adherence influenced by each factor over time. Such data can be used for further intervention development to promote PrEP adherence.

Keywords: adherence, Markov model, HIV, preexposure prophylaxis, covariates

INTRODUCTION

The oral formulation of tenofovir disoproxil fumarate (TDF) in a fixed-dose combination with emtricitabine (FTC) was approved by the U.S. FDA in 2012 for preexposure prophylaxis (PrEP) to reduce the risk of sexually acquired HIV-1 infections. Adherence is highly correlated to the prophylactic efficacy in various clinical trials (Haberer, 2016). Adherence is defined as the extent to which a person's behavior corresponds with medications, diet, and lifestyle as recommended by a health care provider (World Health Organization, 2003). Adherence involves three distinct components known as initiation, execution, and persistence of prescribed therapy (Blaschke et al., 2012; Vrijens and Urquhart, 2014), and non-adherence can be seen in any one or all of these components (Vrijens et al., 2012). Non-adherence to medications is complex and may be influenced by various domains, including socio-economic, health system, disease condition, treatment, or patient-related factors. Understanding factors associated with these domains is the key to understanding adherence-related problems in a holistic manner and designing interventions to suitably address them (World Health Organization, 2003).

Monitoring adherence in clinical trials and routine patient care is a difficult task that requires resources and staffing. Direct and indirect monitoring methods have been employed to monitor adherence; each method has its own advantages and shortcomings (Farmer 1999; Lam and Fresco, 2015). Directly observed therapy (DOT), which verifies adherence, is the most reliable method and has been a mainstay in many tuberculosis treatment protocols, but is highly resource-intensive (Chaulk and Kazandjian, 1998). Self-report by patients and pharmacy refill are easy-to-implement indirect methods, but they tend to overestimate adherence (El Alili et al., 2016). Electronic monitoring methods, such as the Medication Event Monitoring System (MEMS®), involve containers that record each opening as a proxy for medication-taking behavior; this approach has been found to be more reliable than patient self-reports and has been used in numerous clinical trials (Van Onzenoort et al., 2010; Riekert and Rand, 2002). Pharmacological measures of treatment adherence in PrEP are gaining prominence in clinical practice (Brooks and Anderson, 2018; Hendrix, 2018). TDF is a prodrug that rapidly hydrolyzed to tenofovir (TFV) in plasma and further phosphorylated to an active intracellular metabolite, TFV diphosphate (TFV-DP). Establishing adherence benchmarks of TFV and TFV-DP concentrations for HIV PrEP was recently conducted through a DOT study design (Hendrix et al., 2016). This approach has a limited view into past dosing information and does not provide high resolution of patterns of adherence. Importantly, electronic monitoring is the only method that provides day-to-day records of longitudinal data, thus providing an opportunity to understand the adherence patterns of the population in a detailed manner (Osterberg and Blaschke, 2005).

Adherence is commonly expressed as a summary measure, such as percentage adherence, which does not take into account variations in adherence patterns over time (Brown and Bussell, 2011). Non-therapeutic time (NTT) is an alternative to summary measures of adherence and may be calculated using electronic adherence data; it is expressed as a sum of all non-therapeutic intervals during a course of therapy and reflects cumulative non-adherence (Girard et al., 1998).

Electronic adherence monitoring provides longitudinal data on dose-taking behavior. Adherence can be described as sequence of two discrete states, such as "dose-taking" and "dose-missing." Because prior states may influence future states, analytic methods should account for within subject correlation. In addition to the within subject correlation, there could be a dependence between the successive outcomes. If the future evolution of a system depends only on the current state, but not on the history, then the system can be considered to exhibit the Markov property (Stewart, 2009). It has been shown that ignoring Markovian tendencies in the data could lead to elevated type 1 error rates in covariate selection (Silber et al., 2009). Assessing transitions between or within the two states is the basis for a Markov modeling approach. Adherence can be quantified and modeled by deriving probability parameters for transition between the discrete states that explain an individual's dose-taking pattern over a period. A Markov mixed-effects modeling approach has been implemented for analyzing adherence, describing drug holiday patterns, and identifying influential covariates of PrEP adherence from the MEMS-documented adherence data sets (Girard et al., 1998; Fellows et al., 2015; Madrasi et al., 2017). The Markov modeling approach has also been used to analyze data in diverse studies, such as those dealing with adverse effects of drugs, seizures counts, and patterns of sleep stages (Karlsson et al., 2000; Zingmark et al., 2005; Ito et al., 2008; Henin et al., 2009; Bisaso et al., 2015).

Adherence is particularly important for the effectiveness of HIV PrEP (Haberer, 2016). With high adherence, TDF and emtricitabine (FTC) in combination have been successfully used as a PrEP regimen in high-risk groups. A number of demonstration projects are currently underway globally using this regimen (Global Advocacy for HIV prevention, 2017). Understanding the

covariates that influence adherence patterns is vital in designing appropriate patient counselling and interventions that will lead to better adherence. The Partners Demonstration Project involved delivery of TDF-FTC as PrEP to the HIV-uninfected members of heterosexual HIV serodiscordant couples in East Africa. PrEP adherence was measured electronically. The objective of this analysis was to assess the impact of demographic, social, and behavioral attributes on PrEP adherence using Markov mixed-effects modeling approach.

METHODS

Study Participants and Enrollment

The Partners Demonstration Project was a prospective, open-label, implementation science-driven study of HIV PrEP among heterosexual HIV serodiscordant couples in Kenya and Uganda. Ethical statements, subject enrollment, and follow-up details are described in the primary publication on this study (Baeten et al., 2016). Briefly, serodiscordant couples were enrolled into the study, and the HIV-uninfected partner of the couple was encouraged to take PrEP (combination of emtricitabine 200 mg/TFV disoproxil fumarate 300 mg once daily) until the partner living with HIV had been on antiretroviral therapy (ART) for at least 6 months, when viral suppression was assumed (the "bridge" strategy), and if there were no concerns about ART adherence and/or the HIV status of additional partners. Medication was provided in a MEMS container (AARDEX Group, Switzerland). Study participants' adherence records were downloaded from the containers during their follow-up study visits (1 month after enrollment, then quarterly for up to 2 years).

Demographic and socio-behavioral data were collected during study visits as well; details on the measures used are published in the primary analysis of these data (Haberer et al., 2017a).

Data Set Preparation

Electronic adherence data consisted of the dates and times of the medication container openings, which are considered as a surrogate for dose-taking events. Multiple openings in a day were considered as only one dose-taking event on that day. Since the active metabolite, TFV-DP, has a long half-life (48 to 125 h) (Duwal et al., 2012; Louissaint et al., 2013), dose-time errors in a single day were not expected to significantly impact the therapeutic efficacy. Covariates based on the results of the primary analysis of this study were incorporated into the data set (Haberer et al., 2017a).

Analysis of Adherence Transition States

The transition between dose-missing and dose-taking events in each of the two subsequent, adjacent days was assessed for the adherence data. Four transitions are possible in the data: [01], [11], [10], and [00], as per the first-order Markov model. For generating this transition for the first day in an individual, the previous state was considered as 1, which signifies a "dose taken" state. The first dosing state was assumed to be 1 based on the high overall adherence; moreover, prior modeling showed limited impact of the initial state assumption on outcomes (Madrasi et al., 2017). Transitions [01] and [11] signify transition from a "dose-missing" state to a "dose-taken state" and staying in a "dose-taking" state, respectively. Transitions [10] and [00] signify transition from a "dose-taking" state to a "dose-missing" state and continuing in a "dose-missing" state, respectively. The total number of each of these four transitions was calculated for each covariate.

Model Development

Electronic adherence data available for the entire duration of observation (up to 24 months of follow-up) was used for base model selection. To match the primary statistical analysis (Haberer et al., 2017a), covariate analysis was conducted on the data until 12 months of follow-up. Adherence data were modeled using a logistic model, as well as a Markov model to identify the suitable approach (base model) to describe the data. The logistic model assumes adherence as series of coin flips and this model can be described as follows:

$$\text{LOGIT} = Ln\frac{P1}{1-P1} = \Theta_1 + \eta i \tag{1}$$

where Θ_1 is the population parameter of probability in the logit domain and ηi is the between subject variability with a mean of zero and variance of ω^2.

The probability (P1) of dose-taking was modeled in the logit domain and then transformed back to the probability space (between 0 and 1) as follows:

$$P1 = \left(\frac{e^{\Theta 1}}{1+e^{\Theta 1}}\right) \tag{2}$$

The probability of missing dose (P0) is defined as follows:

$$P0 = 1 - P1 \tag{3}$$

The Markov model describes adherence as a series of transitions from the dose-taking state and dose-missing state defined by transition probabilities (**Figure 1**). The probabilities of transition between states were parameterized using logit functions. The model can be described as follows:

$$\text{LOGIT1} = Ln\frac{P01}{1-P01} = \Theta_1 + \eta i \tag{4}$$

$$\text{LOGIT2} = Ln\frac{P10}{1-P10} = \Theta_2 + \eta i \tag{5}$$

where $\Theta 1$ and $\Theta 2$ are the population parameters of the transition probability in the logit domain, and ηi is the between-subject variability with a mean of zero and variance of ω^2.

The transition probabilities between the discrete states of dose taking (1) and dose missing (0) was modeled in the logit domain

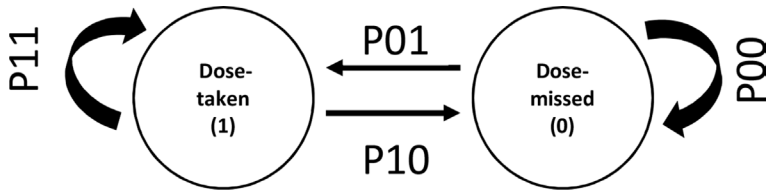

FIGURE 1 | Discrete states and transitions representing adherence behavior in a Markov model. P01 and P10 represent transition probabilities between two states of adherence viz. "dose taken" and "dose missed." P11 and P00 represent the transition probabilities for continuing same states.

and then transformed back to the probability space (between 0 and 1) as follows:

$$P01 = \left(\frac{e^{\Theta 1}}{1 + e^{\Theta 1}} \right) \tag{6}$$

$$P10 = \left(\frac{e^{\Theta 2}}{1 + e^{\Theta 2}} \right) \tag{7}$$

$$P00 = 1 - P01 \tag{8}$$

$$P11 = 1 - P10 \tag{9}$$

where P01, P10, P11, and P00 are transition probabilities between dose-taking and dose-missing states. When testing for covariate effects, covariates were linearly added in the logit domain of transition probability parameters.

Logistic and Markov models were developed without adding covariates into the model to compare between these two approaches. Based on the empirical Bayesian estimates of transition probabilities derived from these two approaches, adherence patterns were simulated using the 'Markov chain' package within the R software platform version 3.4.2 (R Core Team, 2017). The NTT was calculated from the simulated adherence patterns as an indicator of the cumulative index of non-adherence (Girard et al., 1998). Duration of action was assumed to be 24 h based on the nominal dosing frequency of daily dosing. The NTT of an individual was measured as the sum of all the days where dose was missed for two or more consecutive instances. Each individual transition matrix was used to simulate 100 Markov chains to account for stochastic noise. The NTT calculation was repeated for all 100 Markov chains, and the mean for each participant was used for plotting. The correlation between the observed NTT and the mean NTT predicted from 100 realizations of the Markov model was used as diagnostic plot for model development.

To evaluate the demographic and socio-behavioral data, a full covariate model approach was implemented (Gastonguay, 2011). First, all the covariates of interest were selected based on a prior statistical analysis from the Partners Demonstration Project using standard, multivariable generalized estimating equation modeling (Haberer et al., 2017a); all the significant covariates in that analysis were included in this analysis to form a full model. During subsequent steps, model reduction was carried out by dropping non-significant covariates from the model based on 95% confidence intervals (calculated from the standard error of the parameter estimate for each covariate). Any covariate that included unity in its confidence interval of the odds ratio (OR) was dropped from the model. With rest of the covariates, the model run process was continued until none of the covariates dropped out of the model. Covariates that had <5% of transitions in any one of the four transitions ([01], [11], [10] and [00]) were also dropped from the model. Data formatting and plotting was carried out using the software package R version 3.4.2 (R Core Team, 2017). Modeling of the data was performed using NONMEM® (ICON, Ellicott City, Maryland, version 7.3) software package, with Intel/GFortran compilers with Perl-speaks-NONMEM as the interface (Parke et al., 1999; Lindbom et al., 2004). The Laplacian method was used for parameter estimation.

Simulation of the Pharmacokinetic Profile

For visually illustrating the impact of covariate effects on PrEP adherence patterns and consequent pharmacokinetic (PK) profiles of TFV, a simulation exercise was performed. One typical subject was simulated for five of the significant covariates of the final model depending on the order of the size of their effect (higher ORs). The model estimated the typical probability values of each transition probability parameter (i.e., P01, P00, P10, and P11), which were used for simulating adherence patterns using the Markov chain package within R software (R Core Team, 2017). These adherence patterns were incorporated into a NONMEM data set to simulate a one-month dosing period. A population PK model of TFV reported by our group (Lu et al., 2017) in a subset of patients from this study was used to simulate the PK profiles. Comparative PK profiles for each of the significant covariates were plotted for each covariate.

RESULTS

Demographic Data and Adherence Profiles

A total of 985 participants were enrolled into the study. Data of subjects with missing visits and missing data due to a broken or lost device were dropped from analysis. Data for 920 participants were available for inclusion into the analysis. The majority were men (n = 601, 65%), and ≥25 years of age (n = 737, 80%).

TABLE 1 | Demographic characteristics of study participants.

S No	Variables	Number (%)
	Total	**920 (100)**
1	Sex	
	Male	601 (65)
	Female	319 (35)
2	**Age**	
	Age >25 years	737 (80)
	Age <25 years	187 (20)
3	**Age difference**	
	Male partner > 5 years older than female	409 (44)
	Male partner NOT > 5 years older than female	511 (56)
4	**Sex risk**	
	No sex	40 (4)
	Sex with partner with 100% condom use	311 (34)
	Sex with partner less than 100% condom use	569 (62)
5	**ART status**	
	Partner on ART for 6 months	481 (52)
	Partner on ART for less than 6 months	439 (48)
6	**Concerns for taking PrEP**	
	No concerns	828 (90)
	Have concerns	92 (10)
7	**Relationship Desire**	
	Wants relation to succeed	809 (88)
	Not concerned with relation	111 (12)
8	**Pregnancy status and intentions**	
	Not pregnant and not trying	739 (80)
	Trying for pregnancy	63 (7)
	Pregnant	118 (13)
9	**Follow-up status**	
	More than 6 months follow-up	523 (57)
	Less than 6 months follow-up	397 (43)
10	**Relationship status**	
	Couple with study partner	914 (99)
	No longer couple	6 (1)
11	**Alcohol problem**	
	Problem alcohol use	184 (20)
	No alcohol problem	736 (80)
12	**PrEP initiation time**	
	PrEP started on enrollment	900 (98)
	PrEP started in 1 month	16 (1.5)
	PrEP started in 3+ months	4 (0.5)

All the demographic characteristics the data was captured at the base line. For time sensitive items like participant in the follow-up for 6 months, Partner in ART for 6 months, PrEP initiation time were captured at the end of follow-up period.

Most of the participants (n = 900, 98%) started PrEP upon enrollment, with the exception of 16 (1.5%) subjects who started 1 month after enrollment and 4 (0.5%) who started more than 3 months after enrollment. A demographic summary of subjects is provided in **Table 1**, and a summary of adherence data is provided in **Supplementary Table 1**. The time course of adherence patterns in a few representative subjects is shown in **Supplementary Figure 1**.

Analysis of Transitions

A total of 258,714 transitions between dose-missing (0) and dose-taking (1) states were observed in the data. The transition [11] was the most commonly observed transition, accounting for 65% of the total transitions, whereas the transitions [01] and [10] were the least observed transitions, accounting for around 7.5%. Several covariates, i.e., concern for taking PrEP, continuing to be in a relationship with the study partner, and PrEP initiation time, had < 5% of the state transitions in some of their sub-categories. The summary of all four state transitions for all covariates is presented in **Supplementary Table 2**. A summary of transitions at an individual level are presented in **Supplementary Table 3**.

Model Development

The Markov model resulted in a ~50,000 point drop in the NONMEM objective function compared to the logistic model. The observed and the predicted numbers of transitions between dose-missing (0) and dose-taking (1) states are shown in **Table 2**. Individual probability parameters from the logistic and Markov models were used to generate the predicted NTT. The comparison between observed and predicted NTT between the logistic and Markov models is shown in **Figure 2**. This plot shows that the Markov model better predicts NTT than the logistic model. Thus, the Markov model was selected as the base model for further covariate analysis.

Seven significant covariates remained in the final model after the iterative model reduction process. The full list of covariates and results of iterative stages of model reduction steps are presented in **Supplementary Table 4**. The significant covariates were age, female sex, partner on ART for ≥6 months, desire for the

TABLE 2 | Comparison of the predictability of state transitions in the adherence data between the logistic and Markov models: Base model development.

State Transition	Observed	Simulation 1	Simulation 2	Simulation 3	Simulation 4	Simulation 5
Markov model predictions						
[11]	**201119**	200090	199090	200327	200385	200241
[10]	**24431**	24652	24885	24725	24678	24515
[01]	**23986**	24392	24617	24459	24407	24237
[00]	**62747**	63149	63691	62772	62813	63290
Logistic model predictions						
[11]	**201119**	183053	183100	183203	183705	183190
[10]	**24431**	42070	42115	42099	42006	42043
[01]	**23986**	41809	41833	41831	41725	41735
[00]	**62747**	45351	45235	45150	44847	45315

Predicted transitions (counts) from both Markov and Logistic models. Four transitions ([11], [10], [01], [00]) of the predicted adherence data were compared against transitions in the observed data. A total of five simulations were carried out for both models to show the stochastic noise. Transitions predicted by the Markov model were closer to the observed values than the logistic model in all instances.

TABLE 3 | Final model with significant covariates and their impact on adherence.

S. No	Covariates and impact on transitions	OR	95% CI	Interpretation
1.	Age >25 years on P10	0.61	0.49–0.77	Reduced P10 leading to increased adherence
2.	Female sex on P10	0.67	0.55–0.80	Reduced P10 leading to increased adherence
3.	Partner on ART for 6 months on P01	0.86	0.84–0.88	Reduced P01 leading to reduced adherence
4.	Partner on ART for 6 months on P10	1.34	1.30–1.38	Increased P10 leading to reduced adherence
5.	Wants relationship to succeed on P01	1.45	1.35–1.56	Increased P01 leading to increased adherence
6.	Wants relationship to succeed on P10	0.79	0.73–0.85	Reduced P10 leading to increased adherence
7.	In follow-up for 6 months on P01	0.80	0.79–0.82	Reduces P01 leading to reduced adherence
8.	In follow-up for 6 months on P10	1.25	1.22–1.28	Increased P10 leading to reduced adherence
9.	Problem alcohol use on P01	0.63	0.59–0.66	Reduced P01 leading to reduced adherence
10.	Problem alcohol use on P10	1.16	1.10–1.24	Increased P10 leading to reduced adherence
11.	Group with 100% condom use vs no sex group on P10	0.84	0.81–0.87	Reduced P10 leading to increased adherence
12.	Group with less than 100% condom use vs no sex group on P01	1.21	1.18–1.24	Increased P01 leading to increased adherence
13.	Group with less than 100% condom use vs no sex group on P10	0.69	0.67–0.72	Reduced P10 leading to increased adherence

The 95% confidence intervals were calculated with NONMEM asymptotic standard errors on covariate parameters using the following formula: CI = $e^{\theta \pm 1.96(SE)}$. Any covariate, which contained '1' in its confidence interval was considered as non-significant and dropped from the model.

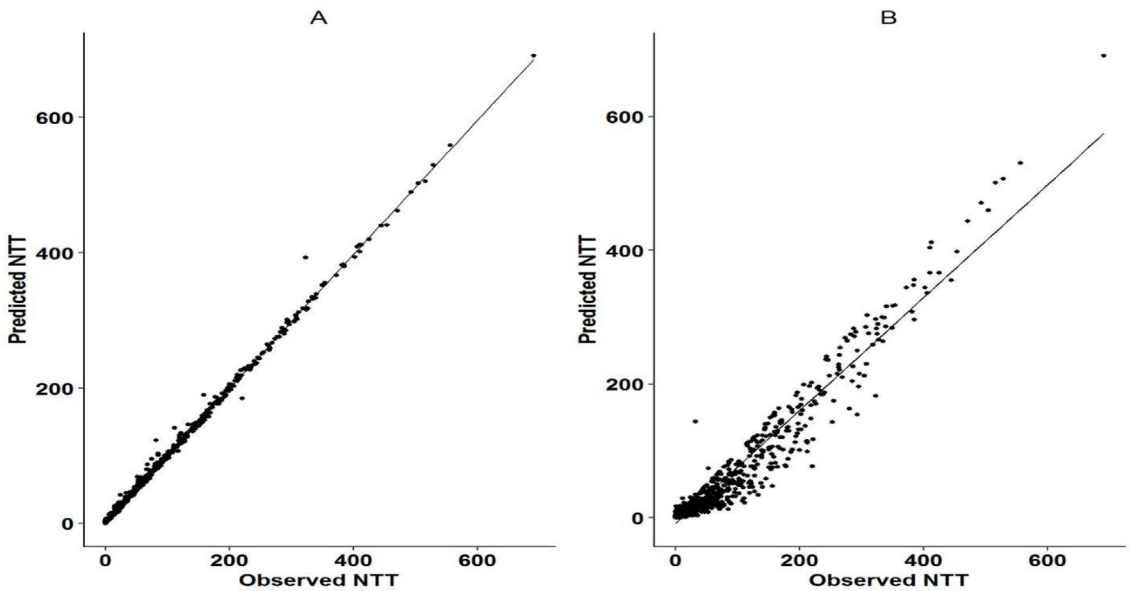

FIGURE 2 | Observed vs predicted NTT for Markov model and logistic regression mode. Panel **A** shows the correlation between the observed and predicted NTT as per the Markov model. Panel **B** shows the correlation between the observed and predicted NTT as per the logistic regression model. NTT was calculated as the cumulative time (in days) that sequential doses were missed in a Markov chain. It was assumed that 24 h was the duration of action of each administered dose and two sequential missed doses were considered as one NTT. Cumulative sums were calculated for subsequent missing doses.

relationship to succeed, study follow-up for ≥6 months, problem alcoholic use, and sex risk with partner with respect to condom use. When looking at the mechanisms of impact on adherence, the covariates acted in two different ways. First, certain covariates positively impacted adherence, when the participant re-initiated medication (transition [01]). Second, certain covariates negatively influenced adherence, when participants discontinued taking medications (transition [10]). For example, female participants and 25 years or older had lower odds of discontinuation. Those whose partners were on ART therapy for ≥6 months were more likely to discontinue PrEP and less likely to re-initiate.

Participants who desired the relationship with their partners to succeed were more likely to re-initiate PrEP and less likely to discontinue. Those who were in the follow-up for 6 months were more likely to discontinue PrEP and less likely to re-initiate.

Participants, who had an alcohol use problem, were more likely to discontinue PrEP and less likely to re-initiate. Those who reported sex with their partner and 100% condom use had lower odds of discontinuation, whereas those who reported less than 100% condom use had lower odds of discontinuation and higher odds of re-initiation of PrEP compared with those who reported no sex with their partners.

The impact of all significant covariates associated with the respective transition probability parameters is presented in **Table 3**. The mechanisms responsible for the impact of covariates on the adherence are presented in **Table 4**.

TABLE 4 | Key mechanisms of covariates on PrEP adherence.

S.No.	Covariate	Impact on adherence	Key mechanism
1.	Age > 25 years	Increase	Reduced discontinuation
2.	Female sex	Increase	Reduced discontinuation
3.	Partner is on ART for 6 months	Reduce	Increased discontinuation and reduced re-initiation
4.	Wants relationship to succeed	Increase	Increased re-initiation and reduced discontinuation
5.	In the study for 6 months	Reduce	Increased discontinuation and reduced re-initiation
6.	Problem alcohol use	Reduce	Increased discontinuation and reduced re-initiation
7.	100% condom use in sex with partner compared to no sex group	Increase	Reduced discontinuation
8.	<100% condom use in sex with partner compared to no sex group	Increase	Increased re-initiation and reduced discontinuation

Simulation of the PK profile

The comparative PK profiles from the simulation are presented in **Figure 3.** These profiles illustrate the potential impact of non-persistence due to covariate effects on the average PK profile of TFV. For TFV, the estimated protective effect against HIV at plasma concentrations of >40/mL was 91% (Donnell et al., 2014). Non-persistence resulted in higher sequential drug omissions and caused the average PK concentrations to decrease below 40 ng/mL in participants with no desire for the relationship to succeed, with problem alcohol use, younger than 25 years, participants that with partners on ART for more than 6 months and male participants.

DISCUSSION

Our analysis identified several participant characteristics that are associated with non-adherence to PrEP in a Markov mixed-effects model framework. The covariate effects found to be significant are in general agreement with prior reports (Haberer et al 2013; Koenig et al., 2013; Amico and Stirratt, 2014; Corneli et al., 2014; Gengiah et al., 2014; Psaros et al., 2014; Kebaabetswe et al., 2015; Haberer et al., 2017a; Haberer et al., 2017b). The distinction of our approach was to utilize a parametric Markov model that provided insights into mechanisms of non-adherence (correlation to either P01 or P10) and predictive capabilities of adherence patterns in addition to the inferential analysis compared to the primary statistical analysis (Haberer et al, 2017a). The dependence of future state on the current state (persistence, execution) in the electronic adherence measurements was evident by the better fit of the Markov model compared with the logistic model. A similar observation was made in previous reports on the analysis of electronic adherence data (Girard et al., 1998; Madrasi et al., 2017). Some of the issues associated with ignoring the Markov element in the data when present include increased type I error rates on covariate inclusion, overestimation of information content in the data, and unrealistic simluation of individual time course of the outcomes (Silber et al., 2009).

In the present analysis, the Markov model predicted that NTT values were closer to the observed NTT in the data set than those of the logistic model, indicating a better description of adherence pattern. The logistic model predicted a much higher number of transitions between dose missing (0) and dose taking (1) compared with the Markov model and thus provides less predictive capability of adherence patterns. These findings support the use of the Markov modeling approach to describe adherence data compared to logistic modeling approach.

Age, sex, marriage or relationship status with partner, risk perception, concerns about taking PrEP, problematic alcohol use, age difference between partners, and sex with the partner or abstinence have been found to be important factors that influence adherence behaviors in other PrEP studies (Haberer et al 2013; Koenig et al., 2013; Amico and Stirratt, 2014; Corneli et al., 2014; Gengiah et al., 2014; Psaros et al., 2014; Kebaabetswe et al., 2015; Haberer et al., 2017a; Haberer et al., 2017b). In the present study, we found that many of these reported factors influencing PrEP adherence of study participants as a validation to the Markov modeling approach. Moreover, the assessment of transition states in the Markov model allow important insights into the mechanism by which these factors influence PrEP adherence.

For instance, participants older than 25 years and/or female sex had lower odds of transitioning from the dose-taking state to the dose-missing state and thus had higher persistence. Younger age has been reported to negatively influence PrEP adherence in other studies (Amico and Stirratt, 2014; Haberer et al., 2017a; Madrasi et al., 2017). This finding might be related to the level of maturity associated with age. The role of sex has been qualitatively explored in serodiscordant couples in Kenya. Carroll et al explored the sex power dynamics within households, which influenced adherence behaviors. They noted that in many instances, women were expected to be responsible for daily health care management tasks for them and their spouses, even though decisions were taken by their husbands. It was also noted that many seronegative men, but not women, found PrEP burdensome (Carroll et al., 2016).

Study participants who desired their relationship to succeed with their partners had higher odds of transitioning from dose-missing state to dose-taking state and lower odds of transitioning from dose-taking to dose-missing state compared with those without such desire. This covariate had the highest odds compared with other covariates in improving adherence. Married participants tend to have better PrEP adherence compared with those who were single (Amico and Stirratt, 2014; Corneli et al., 2014). The desire to continue a relationship with the partner could be a motivation for married subjects to continue PrEP. In the current study, couples had mutually disclosed their HIV serostatus; therefore, the uninfected partner had a more accurate perception of risk for HIV transmission, which may have resulted in better adherence.

Participants who were in the study for ≥ 6 months were more likely to transition from dose-taking state to dose-missing state and less likely to transition from dose-missing state to dose-taking state compared to those who were in the study for <6

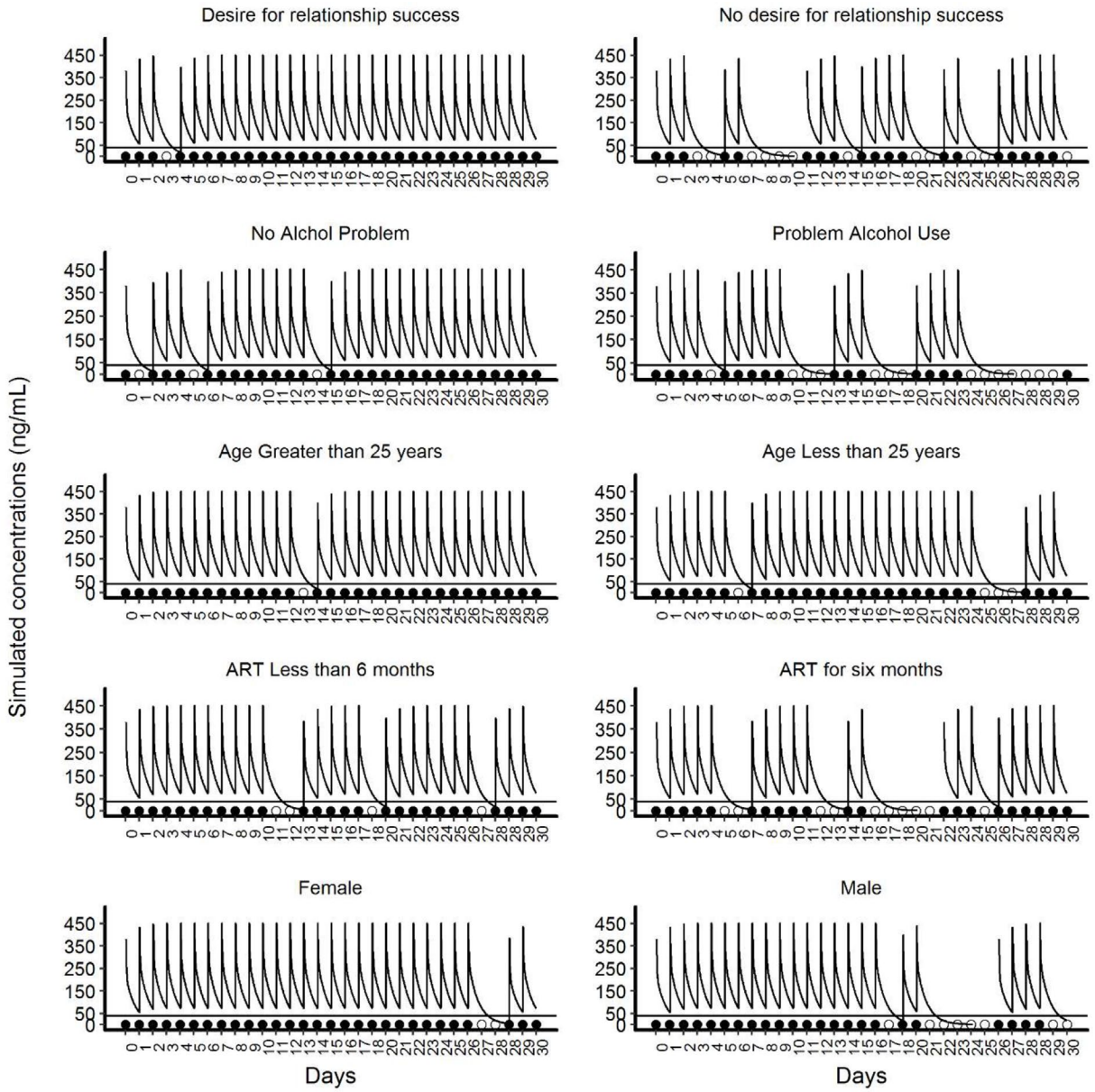

FIGURE 3 | Illustrative simulated pharmacokinetic profiles for the categories of significant covariates of the final Markov model. This panel of plots presents illustrative simulated pharmacokinetic profiles based on the categories of five significant covariates. The panels on the left side show the categories of covariates that increase adherence and the panels on the right side show the categories of the covariates that reduce adherence. In each panel, the line that intercepts at 40ng/mL is the threshold level of protection for TFV. The filled circles near the x-axis represent "doses taken" and unfilled circles indicate 'doses missed'.

months. This finding may reflect fatigue with taking PrEP for a relatively long period. Additionally, longer time of follow-up could reflect the bridge strategy for PrEP in this study (i.e., PrEP should be taken until the partner living with HIV had taken ART for >6 months). Indeed, those whose partners were on ART rfor >6 months had a tendency to miss medication. Risk perception by uninfected partners could have changed if they perceived their partners as being less infectious after six months on ART (assuming they were not concerned about the partner's ART adherence and/or presence of outside sexual partnerships). It should be kept in mind, however, that duration of follow-up and partner time on ART were not necessarily equivalent, as many partners did not start ART until well into follow-up; these variables were thus tested independently in our model.

In the current analysis, it was observed that participants with problematic alcohol use were more likely to miss doses and continue in the same dose-missing state. Heavy use of alcohol has been similarly associated with poor adherence to PrEP (Amico and Stirratt, 2014; Haberer et al., 2017a).

Sex with the study partner or with others also influenced adherence to PrEP in this and other studies. Those who had sex with the study partner known to be living with HIV tended to have higher adherence compared to those who reported no sex with the study partner (Haberer et al., 2013; Kebaabetswe et al., 2015; Haberer et al., 2017a). In the current analysis, we observed that the participants who had sex with the partner, either with or without condoms, had higher adherence than those who reported no sex. These groups also had higher odds for persisting on PrEP; this finding might have been due to the higher level of risk perception by the uninfected partners of the serodiscordant couples. Those who abstained from sex with the study partner had lower adherence to PrEP possibly because of lower risk, although risk from any potential outside partnerships was unknown.

The current report agrees with our prior report (Madrasi et al., 2017) on Markov modeling of MEMS based adherence data on the approach and covariate effects. There are two important differences between these two analyses. First, the input data in the current analysis is from the Partners Demonstration Project (Haberer et al., 2017a) and the prior analysis used input data from the Partners PrEP Ancillary Adherence Sub-study (Haberer et al., 2013). Both studies evaluated a different set of covariates with common variables being age, sex, and problematic alcohol use. Thus, a direct comparison of results is not possible on all covariate effects. Second, a full covariate modeling approach was used in the current analysis compared to step-wise approach in the previous report. Findings on the common covariates between studies agreed well. Madrasi et al found that female sex and older age had a positive impact on adherence, which is similar to the current report. In addition, the current analysis identified problematic alcohol use as a significant covariate negatively associated with adherence, whereas it was not significant in prior report. The primary statistical analysis of both studies using regression modeling found similar results. The lack of significance in the Partners PrEP sub-study may reflect the lower prevalence of problematic alcohol use (10.6% versus 20% in current study).

The simulated PK profiles illustrated the impact of covariates on PrEP adherence and the subsequent effect on TFV levels. We do not imply a causative link of TFV levels to prophylactic efficacy. Rather, intracellular TFV-DP and FTC-TP levels are responsible for viral suppression and efficacy. Our Markov model can be linked to mechanistic HIV viral dynamic models (Duwal et al, 2016) to understand the onset and offset of prophylactic efficacy. The covariates that had negative impact on adherence may result in a PK profile with substantial length of time below the threshold value for protection. This situation may reduce PrEP effectiveness and may leave the participant unprotected in case of viral exposure during this period.

When interventions are planned to enhance adherence to therapy, it is important to understand associated issues and mechanisms of non-adherence. The Markov modeling approach identified significant covariates that impact adherence, along with mechanisms by which they act on adherence. Adherence interventions can be tailored based on the type and number of risky covariates in a subject. Intervention designs should consider the mechanisms of non-adherence, whether the subject has problems in initiation or with persistence to therapy.

There are several limitations to this study. Electronic adherence records may not be able to differentiate true dosing events and false-positive openings of MEMS containers, thus adding a certain level of uncertainty to the data. Unfortunately, the false-positive rate from the electronic adherence monitoring system cannot be known; however, it is generally low (Musinguzi et al., 2016). Additionally, some covariates used in the data reflect subjective information provided by participants, which cannot be verified independently. The results of the present study also have to be interpreted in the context of the socio-demographic and cultural background of the study participants. Finally, given the observational design of the study, there could be some unaccounted factors affecting adherence. Thus, the significant covariates identified in this analysis represent correlations rather than causality.

CONCLUSIONS

The Markov mixed-effects modeling approach was used to study the impact of various factors on adherence to PrEP medications in serodiscordant couples. Female sex, older than 25 years, desire for the relationship with the partner to succeed, and use of condoms during sex with the partner were positively associated with PrEP adherence. Problematic alcohol use negatively associated with PrEP adherence. Although participation in the study for 6 months and the partner having taken ART for 6 months or more were found to be negatively associated with PrEP adherence, this finding is consistent with the bridge strategy and indicates potential for adherence to the bridge strategy to work well with this population. The developed Markov model provides insight into the stages of PrEP adherence (i.e., initiation, execution, and/or persistence) and can be used to develop further interventions to promote PrEP adherence.

PARTNERS DEMONSTRATION PROJECT TEAM

Coordinating Center (University of Washington) and collaborating investigators (Harvard Medical School, Johns Hopkins University, Massachusetts General Hospital): Jared Baeten (protocol chair), Connie Celum (protocol co-chair), Renee Heffron (project director), Deborah Donnell (statistician), Ruanne Barnabas, Jessica Haberer, Harald Haugen, Craig Hendrix, Lara Kidoguchi, Mark Marzinke, Susan Morrison, Jennifer Morton, Norma Ware, Monique Wyatt.

PROJECT SITES

Kabwohe, Uganda (Kabwohe Clinical Research Centre): Stephen Asiimwe, Edna Tindimwebwa

Kampala, Uganda (Makerere University): Elly Katabira, Nulu Bulya.

Kisumu, Kenya (Kenya Medical Research Institute): Elizabeth Bukusi, Josephine Odoyo.

Thika, Kenya (Kenya Medical Research Institute, University of Washington): Nelly Rwamba Mugo, Kenneth Ngure.

Data management: DF/Net Research.

ETHICS STATEMENT

The study protocol was approved by the University of Washington Human Subjects Division (STUDY00001674) and ethics review committees at each study site (Kabwohe: UNCST HS1410, NARC 135; Kampala: UNCST HS1289, NARC 126; Kisumu: KEMRI SSC NO 2441; Thika: KEMRI P286/05/2012). Participants provided written informed consent.

AUTHOR CONTRIBUTIONS

SM, AC, and JH developed the analysis. SM and AC conducted the modeling with significant input from MF, MS, CH, and JH. SM wrote the first draft of the paper, which was edited and approved by all authors.

FUNDING

The Partners Demonstration Project was funded by the Bill and Melinda Gates Foundation (OPP1056051), the National Institute of Mental Health of the US National Institutes of Health (R01MH095507 and R01MH098744) and the United States Agency for International Development (AID-OAA-A-12-00023); the study also supported by the University of Washington/Fred Hutch Center for AIDS Research (P30 AI027757), supported by NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA, NIGMS, NIDDK of the National Institutes of Health. This work is made possible by the generous support of the American people through USAID; the contents are the responsibility of the authors and do not necessarily reflect the views of USAID, NIH, or the United States Government. PrEP medication was donated by Gilead Sciences.

ACKNOWLEDGMENTS

We thank the couples who participated in this study for their motivation and dedication and the referral partners, community advisory groups, institutions and communities that supported this work.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00785/full#supplementary-material

REFERENCES

Amico, K. R., and Stirratt, M. J. (2014). Adherence to preexposure prophylaxis: current, emerging, and anticipated bases of evidence. *Clin. Infect. Dis.* 59 Suppl 1, S55–60. doi: 10.1093/cid/ciu266

Baeten, J., Heffron, R., Kidoguchi, L., Mugo, N. R., Katabira, E., Bukusi, E. A., et al. (2016). Integrated delivery of antiretroviral treatment and pre-exposure prophylaxis to HIV-1–serodiscordant couples: a prospective implementation study in Kenya and Uganda. *PLoS Med.* 13 (8), e1002099. doi: 10.1371/journal.pmed.1002099

Bisaso, K. R., Mukonzo, J. K., and Ette, E. I. (2015). Markov model for characterizing neuropsychologic impairment and Monte Carlo simulation for optimizing efavirenz therapy. *J. Clin. Pharmacol.* 55 (11), 1229–1235. doi: 10.1002/jcph.533

Blaschke, T. F., Osterberg, L., Vrijens, B., and Urquhart, J. (2012). Adherence to medications: insights arising from studies on the unreliable link between prescribed and actual drug dosing histories. *Annu. Rev. Pharmacol. Toxicol.* 52, 275–301. doi: 10.1146/annurev-pharmtox-011711-113247

Brooks, K. M., and Anderson, P. L. (2018). Pharmacologic-based methods of adherence assessment in HIV prevention. *Clin. Pharmacol. Ther.* 104, 1056–1059. doi: 10.1002/cpt.1201

Brown, M. T., and Bussell, J. K. (2011). Medication adherence: WHO cares? *Mayo Clin. Proc.* 86 (4), 304–314. doi: 10.4065/mcp.2010.0575

Carroll, J. J., Ngure, K., Heffron, R., Curran, K., Mugo, N. R., and Baeten, J. M. (2016). Gendered differences in the perceived risks and benefits of oral PrEP among HIV-serodiscordant couples in Kenya. *AIDS Care* 28 (8), 1000–1006. doi: 10.1080/09540121.2015.1131972

Chaulk, C. P., and Kazandjian, V. A. (1998). Directly observed therapy for treatment completion of pulmonary tuberculosis: consensus statement of the public health tuberculosis guidelines panel. *Jama* 279 (12), 943–948. doi: 10.1001/jama.279.12.943

Corneli, A. L., Deese, J., Wang, M., Taylor, D., Ahmed, K., Agot, K., et al., (2014). FEM-PrEP: adherence patterns and factors associated with adherence to a daily oral study product for pre-exposure prophylaxis. *J. Acquir. Immune Defic. Syndr.* 66, 3, 324–331. doi: 10.1097/QAI.0000000000000158

Donnell, D., Baeten, J. M., Bumpus, N. N., Brantley, J., Bangsberg, D. R., Haberer, J. E., et al. (2014). HIV protective efficacy and correlates of tenofovir blood concentrations in a clinical trial of PrEP for HIV prevention. *J. Acquir. Immune Defic. Syndr.* 66 (3), 340–348. doi: 10.1097/QAI.0000000000000172

Duwal, S., Schutte, C., and von Kleist, M. (2012). Pharmacokinetics and pharmacodynamics of the reverse transcriptase inhibitor tenofovir and prophylactic efficacy against HIV-1 infection. *PloS One* 7 (7), e40382. doi: 10.1371/journal.pone.0040382

Duwal, S., Sunkara, V., and von Kleist, M. (2016). Multiscale systems-pharmacology pipeline to assess the prophylactic efficacy of NRTIs against HIV-1. *CPT Pharmacometrics Syst. Pharmacol.* 5 (7), 377–387. doi: 10.1002/psp4.12095

El Alili, M., Vrijens, B., Demonceau, J., Evers, S. M., and Hiligsmann, M. (2016). A scoping review of studies comparing the medication event monitoring system (MEMS) with alternative methods for measuring medication adherence. *Br. J. Clin. Pharmacol.* 82 (1), 268–279. doi: 10.1111/bcp.12942

Farmer, C. K. (1999). Methods for measuring and monitoring medication adherence in clinical trial and clinical practice. *Clin. Ther.* 21 (6), 1074–1090. doi: 10.1016/S0149-2918(99)80026-5

Fellows, K., Rodriguez-Cruz, V., Covelli, J., Droopad, A., Alexander, S., and Ramanathan, M. (2015). A hybrid Markov chain-von Mises density model for the drug-dosing interval and drug holiday distributions. *AAPS J* 17 (2), 427–437. doi: 10.1208/s12248-014-9713-5

Gastonguay, M. R. (2011). "Full Covariate Models as an Alternative to Methods Relying on tatistical Significance for Inferences about Covariate Effects: A Review of Methodology and 42 Case Studies", in: *Annual meeting of Population Approach Group in Europe*. (Athens, Greece: PAGE).

Gengiah, T. N., Moosa, A., Naidoo, A., and Mansoor, L. E. (2014). Adherence challenges with drugs for pre-exposure prophylaxis to prevent HIV infection. *Int. J. Clin. Pharm.* 36 (1), 70–85. doi: 10.1007/s11096-013-9861-1

Girard, P., Blaschke, T. F., Kastrissios, H., and Sheiner, L. B. (1998). A Markov mixed effect regression model for drug compliance. *Stat. Med.* 17 (20), 2313–2333. doi: 10.1002/(SICI)1097-0258(19981030)17:20<2313::AID-SIM935>3.0.CO;2-V

Global Advocacy for HIV prevention. (2017). Ongoing and planned PrEP demonstration and implementation studies. Retrieved from https://www.avac.

org/resource/ongoing-and-planned-prep-demonstration-and-implementation-studies [Accessed 05/07/2018 2018].

Haberer, J. E. (2016). Current concepts for PrEP adherence in the PrEP revolution: from clinical trials to routine practice. *Curr. Opin. HIV AIDS* 11, 10–17. doi: 10.1097/COH.0000000000000220

Haberer, J. E., Baeten, J. M., Campbell, J., Wangisi, J., Katabira, E., Ronald, A., et al. (2013). Adherence to antiretroviral prophylaxis for HIV prevention: a substudy cohort within a clinical trial of serodiscordant couples in East Africa. *PLoS Med.* 10 (9), e1001511. doi: 10.1371/journal.pmed.1001511

Haberer, J. E., Kidoguchi, L., Heffron, R., Mugo, N., Bukusi, E., Katabira, E., et al. (2017a). Alignment of adherence and risk for HIV acquisition in a demonstration project of pre-exposure prophylaxis among HIV serodiscordant couples in Kenya and Uganda: a prospective analysis of prevention-effective adherence. *J. Int. AIDS Soc.* 20 (1), 21842. doi: 10.7448/IAS.20.1.21842

Haberer, J. E., Ngure, K., Muwonge, T., Mugo, N., Katabira, E., Heffron, R., et al. (2017b). Brief Report: Context Matters: PrEP adherence is associated with sexual behavior among HIV serodiscordant couples in East Africa. *J. Acquir. Immune Defic. Syndr.* 76 (5), 488–492. doi: 10.1097/QAI.0000000000001548

Hendrix, C. W., Andrade, A., Bumpus, N. N., Kashuba, A. D., Marzinke, M. A., Moore, A., et al. (2016). Dose frequency ranging pharmacokinetic study of tenofovir-emtricitabine after directly observed dosing in healthy volunteers to establish adherence benchmarks (HPTN 066). *AIDS Res. Hum. Retroviruses* 32, 32–43. doi: 10.1089/aid.2015.0182

Hendrix, C. W. (2018). HIV antiretroviral pre-exposure prophylaxis: development challenges and pipeline promise. *Clin. Pharmacol. Ther.* 104, 1082–1097. doi: 10.1002/cpt.1227

Henin, E., You, B., VanCutsem, E., Hoff, P. M., Cassidy, J., Twelves, C., et al. (2009). A dynamic model of hand-and-foot syndrome in patients receiving capecitabine. *Clin. Pharmacol. Ther.* 85 (4), 418–425. doi: 10.1038/clpt.2008.220

Ito, K., Hutmacher, M., Liu, J., Qiu, R., Frame, B., and Miller, R. (2008). Exposure-response analysis for spontaneously reported dizziness in pregabalin-treated patient with generalized anxiety disorder. *Clin. Pharmacol. Ther.* 84 (1), 127–135. doi: 10.1038/sj.clpt.6100491

Karlsson, M. O., Schoemaker, R. C., Kemp, B., Cohen, A. F., van Gerven, J. M., Tuk, B., et al. (2000). A pharmacodynamic Markov mixed-effects model for the effect of temazepam on sleep. *Clin. Pharmacol. Ther.* 68 (2), 175–188. doi: 10.1067/mcp.2000.108669

Kebaabetswe, P. M., Stirratt, M. J., McLellan-Lemal, E., Henderson, F. L., Gray, S. C., Rose, C. E., et al. (2015). Factors associated with adherence and concordance between measurement strategies in an HIV daily oral tenofovir/emtricitibine as pre-exposure prophylaxis (Prep) clinical trial, Botswana, 2007-2010. *AIDS Behav.* 19 (5), 758–769. doi: 10.1007/s10461-014-0891-z

Koenig, L. J., Lyles, C., and Smith, D. K. (2013). Adherence to antiretroviral medications for HIV pre-exposure prophylaxis: lessons learned from trials and treatment studies. *Am. J. Prev. Med.* 44 (1 Suppl 2), S91–98. doi: 10.1016/j.amepre.2012.09.047

Lam, W. Y., and Fresco, P. (2015). Medication adherence measures: an overview. *Biomed. Res. Int.* 2015, 217047. doi: 10.1155/2015/217047

Lindbom, L., Ribbing, J., and Jonsson, E. N. (2004). Perl-speaks-NONMEM (PsN)–a Perl module for NONMEM related programming. *Comput. Methods Prog. Biomed.* 75 (2), 85–94. doi: 10.1016/j.cmpb.2003.11.003

Lu, Y., Goti, V., Chaturvedula, A., Haberer, J. E., Fossler, M. J., Sale, M. E., et al. (2016). Population pharmacokinetics of tenofovir in HIV-1-Uninfected members of serodiscordant couples and effect of dose reporting methods. *Antimicrob. Agents Chemother.* 60, 5379–5386. doi: 10.1128/AAC.00559-16

Louissaint, N. A., Cao, Y. J., Skipper, P. L., Liberman, R. G., Tannenbaum, S. R., Nimmagadda, S., et al. (2013). Single dose pharmacokinetics of oral tenofovir in plasma, peripheral blood mononuclear cells, colonic tissue, and vaginal tissue. *AIDS Res. Hum. Retroviruses* 29 (11), 1443–1450. doi: 10.1089/aid.2013.0044

Madrasi, K., Chaturvedula, A., Haberer, J. E., Sale, M., Fossler, M. J., Bangsberg, D., et al. (2017). Markov mixed effects modeling using electronic adherence monitoring records identifies influential covariates to HIV preexposure prophylaxis. *J. Clin. Pharmacol.* 57 (5), 606–615. doi: 10.1002/jcph.843

Musinguzi, N., Muganzi, C. D., Boum, Y., 2nd, Ronald, A., Marzinke, M. A., Hendrix, C. W., et al. (2016). Comparison of subjective and objective adherence measures for preexposure prophylaxis against HIV infection among serodiscordant couples in East Africa. *AIDS* Apr 24; 30 (7), 1121–9. doi: 10.1097/QAD.0000000000001024

Osterberg, L., and Blaschke, T. (2005). Adherence to medication. *N. Engl. J. Med.* 353 (5), 487–497. doi: 10.1056/NEJMra050100

Parke, J., Holford, N. H. G., and Charles, B. G. (1999). A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. *Comput. Methods Prog. Biomed.* 59 (1), 19–29. doi: 10.1016/S0169-2607(98)00098-4

Psaros, C., Haberer, J. E., Katabira, E., Ronald, A., Tumwesigye, E., Campbell, J. D., et al. (2014). An intervention to support HIV preexposure prophylaxis adherence in HIV-serodiscordant couples in Uganda. *J. Acquir. Immune Defic. Syndr.* 66 (5), 522–529. doi: 10.1097/QAI.0000000000000212

R Core Team. (2017). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for statistical computing. Retrieved from https://www.R-project.org/.

Riekert, K. A., and Rand, C. S. (2002). Electronic monitoring of medication adherence: when is high-tech best? *J. Clin. Psychol. Med. Settings* 9 (1), 25–34. doi: 10.1023/A:1014131928789

Silber, H. E., Kjellsson, M. C., and Karlsson, M. O. (2009). The impact of misspecification of residual error or correlation structure on the type I error rate for covariate inclusion. *J. Pharmacokinet. Pharmacodyn.* 36, 81–99. doi: 10.1007/s10928-009-9112-1

Stewart, J. W. (2009). *Probability, Markov chains, Queues, and Simulation*. Princeton University Press: New Jersey. doi: 10.2307/j.ctvcm4gtc

Van Onzenoort, H.a.W., Verberk, W. J., Kessels, A. G. H., Kroon, A. A., Neef, C., Van Der Kuy, P.-H. M., et al. (2010). Assessing medication adherence simultaneously by electronic monitoring and pill count in patients with mild-to-moderate hypertension. *Am. J. Hypertens.* 23, 149–154. doi: 10.1038/ajh.2009.207

Vrijens, B., and Urquhart, J. (2014). Methods for measuring, enhancing, and accounting for medication adherence in clinical trials. *Clin. Pharmacol. Ther.* 95 (6), 617–626. doi: 10.1038/clpt.2014.59

Vrijens, B., De Geest, S., Hughes, D. A., Przemyslaw, K., Demonceau, J., Ruppar, T., et al. (2012). A new taxonomy for describing and defining adherence to medications. *Br. J. Clin. Pharmacol.* 73 (5), 691–705. doi: 10.1111/j.1365-2125.2012.04167.x

World Health Organization. (2003). *Adherence to Long-Term Therapies: Evidence for Action.* Retrieved from http://www.who.int/chp/knowledge/publications/adherence_ introduction.pdf?ua = 1.

Zingmark, P. H., Kagedal, M., and Karlsson, M. O. (2005). Modelling a spontaneously reported side effect by use of a Markov mixed-effects model. *J. Pharmacokinet. Pharmacodyn.* 32 (2), 261–281. doi: 10.1007/s10928-005-0021-7

Conflict of Interest Statement: MF was employed by Trevena Inc, and MS was employed by Nuventra. JH has served as a consultant for Merck.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor and reviewer MVK declared their involvement as co-editors in the Research Topic, and confirm the absence of any other collaboration.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 07 November 2019
doi: 10.3389/fphar.2019.01269

Transformation of Australian Community Pharmacies Into Good Clinical Practice Compliant Trial Pharmacies for HIV Pre-Exposure Prophylaxis

Luxi Lal[1,2,3], Kathleen Ryan[1,2], Iris Yi Liu[1], Brian Price[1], Timmy Lockwood[1], Ivette Aguirre[1], Peter Slobodian[4], Ada Lam[4], Manoj Vassan[5], Kie Lim[5], John Silverii[6], Joseph Tesoriero[7], Johnny Phu[8], Wan Lim[8], Bharathy Naidoo[9], Nick Russell[10], Matthew Rundle[11], Rowan Sewell[11], Craig Cooper[11], Alexander Hardman[12], Martin Quinn[12], Anne Mak[1†] and Edwina J. Wright[1,2,3,13†] on behalf of the PrePX Study Team[‡]*

[1] Alfred Health, Melbourne, VIC, Australia, [2] Burnet Institute, HIV Elimination Program, Melbourne, VIC, Australia, [3] Department of Infectious Diseases, Alfred Health, Monash University, Melbourne, VIC, Australia, [4] Clinical Trials Pharmacy, Royal Adelaide Hospital, Adelaide, SA, Australia, [5] Prahran Central Pharmacy, South Yarra, VIC, Australia, [6] John Silverii's Pharmacy, Fitzroy North, VIC, Australia, [7] HealthSmart Pharmacy Alfred, Melbourne, VIC, Australia, [8] Newton & Leung Pharmacy, Collingwood, VIC, Australia, [9] Bain & Co. Pharmacy, St Kilda, VIC, Australia, [10] Centre Pharmacy, Central Market Arcade, Adelaide, SA, Australia, [11] Epic Pharmacy New Town, Hobart, TAS, Australia, [12] Epic Pharmacy Kings Meadows, Launceston, TAS, Australia, [13] The Peter Doherty Institute of Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia

Background: In Australia, clinical trial drugs are conventionally dispensed through clinical trial pharmacies only, while community pharmacies dispense drugs approved by Australia's regulatory body. A large HIV pre-exposure prophylaxis study aimed to deliver clinical trial drug through community pharmacies to improve convenience and mimic real world prescribing. This paper describes the process of making community trials compliant with good clinical practice and reports outcomes of delivering clinical trial drug through community pharmacies.

Methods: Eight community and four clinical trial pharmacies across three Australian states were approached to participate. A good clinical practice checklist was generated and pharmacies underwent a number of changes to meet clinical trial pharmacy requirements prior to study opening. Changes were made to community pharmacies to make them compliant with good clinical trial practice including; staff training, structural changes, and implementing monitoring of study drug and prescribing practices. Study drug was ordered through standard clinical trial processes and dispensed from study pharmacies by accredited pharmacists. Throughout the trial, record logs for training, prescriber signature and delegation, temperature, participant, and drug accountability were maintained at each pharmacy. The study team monitored each log and delivered on-site training to correct protocol variations.

Results: Each pharmacy that was approached agreed to participate. All community pharmacies achieved good clinical practice compliance prior to dispensing study drug.

OPEN ACCESS

Edited by:
Albert Liu, San Francisco Department of Public Health, United States

Reviewed by:
Chin Moi Chow, University of Sydney, Australia
Nathalie K. Zgheib, American University of Beirut, Lebanon

***Correspondence:**
Luxi Lal
luxi.lal@burnet.edu.au

[†]*These authors share senior authorship*

[‡]Group information:
The PrEPX study team members appear at the end of the article

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *04 April 2019*
Accepted: *04 October 2019*
Published: *07 November 2019*

Citation:
Lal L, Ryan K, Liu IY, Price B, Lockwood T, Aguirre I, Slobodian P, Lam A, Vassan M, Lim K, Silverii J, Tesoriero J, Phu J, Lim W, Naidoo B, Russell N, Rundle M, Sewell R, Cooper C, Hardman A, Quinn M, Mak A and Wright EJ (2019) Transformation of Australian Community Pharmacies Into Good Clinical Practice Compliant Trial Pharmacies for HIV Pre-Exposure Prophylaxis. Front. Pharmacol. 10:1269. doi: 10.3389/fphar.2019.01269

Over the course of the study, 20,152 dispensations of study drug occurred, 83% of these occurred at community pharmacies. Only 2.0% of dispensations had an error, and errors were predominantly minor. On five occasions a pharmacist who was not accredited dispensed study drug.

Conclusions: Community based pharmacies can undergo training and modifications to achieve good clinical practice compliance and dispense clinical trial study drug. Community based pharmacies recorded few variations from study protocol. Community based pharmacies offer a useful alternative to clinical trial pharmacies to increase convenience for study participants and expanded use of these pharmacies should be considered for large clinical trials, including HIV prevention trials.

Keywords: pre-exposure prophylaxis, pre-exposure prophylaxis expanded, pharmacy, clinical trials, human immunodeficiency virus prevention, prophylaxis

INTRODUCTION

Clinical trials are essential to the evaluation and approval of novel therapeutic agents, however a number of barriers limit clinical trial participation (Bower et al., 2014). Population groups including people who are culturally and linguistically diverse, people living in regional/remote areas, youth, and indigenous populations are underrepresented in clinical trials (Schmotzer, 2012; Bower et al., 2014; Curran et al., 2015; Ernst and Young, 2016). In Australia, many trials struggle to meet recruitment and retention targets (Ernst and Young, 2016).

In Australia, clinical trials are conventionally conducted through tertiary hospitals with study drug dispensed by the hospitals' clinical trial pharmacies. Hospital-based clinical trial pharmacies dispense investigational drugs that have not been approved by the Therapeutic Goods Administration (TGA) and are required to adhere to state-based legislation and the relevant research governance framework (Australian Government Department of Health TGA, 2018). Clinical trial drugs are typically dispensed at no cost to the study participant. Conversely, community pharmacies (also known as retail pharmacies) are only permitted to dispense drugs that have been approved by the TGA, must adhere to federal legislation in relation to pharmacy dispensing, and individuals are required to pay for each drug dispensing event [for drugs listed on Australia's Pharmaceutical Benefits Scheme (PBS) in 2018: $39.50 Australian dollars (AUD) or AUD$6.40 for concession (Australian Government Department of Health, 2018)].

HIV pre-exposure prophylaxis (PrEP) is the use of co-formulated tenofovir and emtricitabine by HIV-negative people to prevent HIV acquisition (Grant et al., 2010). Randomized clinical trials and demonstration projects have demonstrated that PrEP is highly efficacious and effective at preventing the sexual and injection transmission of HIV when high medication adherence is achieved (Grant et al., 2010; Baeten et al., 2012; Thigpen et al., 2012; Choopanya et al., 2013; Grant et al., 2014; Molina et al., 2015; McCormack et al., 2016). HIV PrEP was registered on Australia's TGA in 2016 (Australian Government Department of Health TGA, and biologicals, 2016), however it was not listed on the PBS until April 2018 (Health AGD of. Pharmaceutical Benefits Scheme (PBS) | For PBS Prescribers [Internet], 2018). Prior to the PBS listing of PrEP, access to PrEP was primarily through participation in local clinical trials that aimed to demonstrate the clinical effectiveness (Lal et al., 2017; Rodriguez et al., 2018; Zablotska et al., 2018) and the population level prevention benefit of PrEP (AVAC, 2015; Grulich et al., 2018; Rodriguez et al., 2018; Ryan et al., 2018; Western Australia AIDS Council, 2018).

The pre-exposure prophylaxis expanded (PrEPX) study was a multi-site, one-armed, open-label, population-level PrEP intervention study undertaken in Australia. The primary objective of the PrEPX study was to measure the change in HIV incidence at a population-level (Ryan et al., 2018). The PrEPX study originated in Victoria providing 3,800 study places, and expanded to include South Australia (650 study places) and Tasmania (100 study places). PrEPX was designed to mimic the anticipated real world conditions of PrEP prescribing that would be in place if PrEP were to receive PBS approval. Real world conditions included: attending primary care and sexual health services for study visits, having study drug dispensed at community pharmacies which included paying conventional clinical service and PBS co-payments for drug dispensing.

The PrEPX study was the first clinical trial undertaken in Australia whose protocol design permitted community pharmacists to dispense clinical trial medications in accordance with International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines (ICH-GCP ICOHGCP, 1996; Abraham, 2009; Australian Government Department of Health TGA, 2018). A smaller Victorian PrEP demonstration study (Lal et al., 2017) and PrEP demonstration studies in other Australian jurisdictions did not utilize community pharmacies for prescribing, nor require PBS co-payments (Grulich et al., 2018; Rodriguez et al., 2018; Western Australia AIDS Council, 2018; Zablotska et al., 2018). In this paper we describe how clinical trial dispensing was implemented through community pharmacies across three Australia states, Victoria, South Australia, and Tasmania. We describe the consultation with community pharmacists, steps required to meet clinical trial pharmacy requirements, ongoing monitoring, and outcomes of utilizing community pharmacies to dispense clinical trial drug (See **Figure 1**).

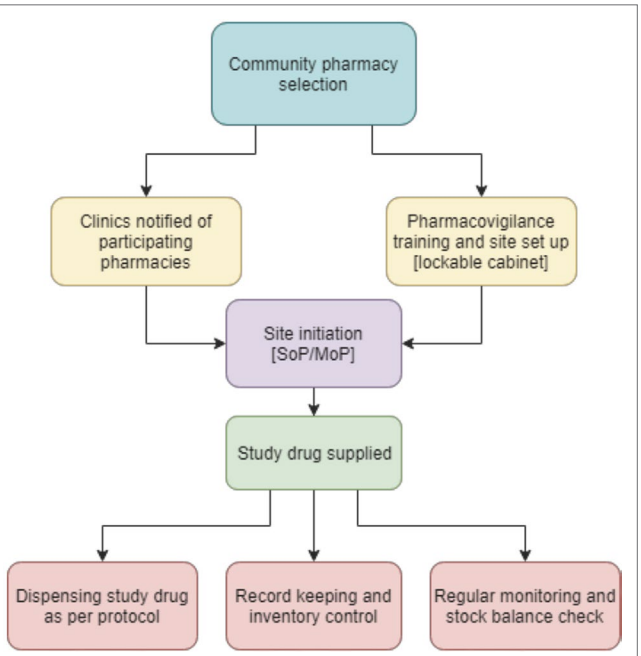

FIGURE 1 | Flow chart of the set up process for involvement of community pharmacies (SoP—Standard Operating Procedure; MoP—Manual of Operations).

METHODS

Study Overview

The PrEPX protocol has been described elsewhere (Ryan et al., 2018). Here, we detail the implementation and outcomes of the study's community pharmacy dispensing of study drug. The PrEPX study was a population level demonstration study aimed to recruit 2,600 individuals at risk of HIV, in order to show a decline in HIV incidence (Traeger et al., 2019).

The study design required participants to attend study clinics, including hospital, primary care, and/or sexual health services, for quarterly HIV and sexually transmitted infection testing and provision of a study drug prescriptions. Participants then attended participating study pharmacies to have study-specific prescriptions dispensed. Participants could attend any of the participating PrEPX pharmacies to have study drug dispensed. Community pharmacies participating in PrEPX underwent specific modifications and training to meet clinical trial pharmacy requirements to participate as a PrEPX clinical trial pharmacy.

The PrEPX pharmacy team included three key team members—lead pharmacist, pharmacy manager, and pharmacy monitor. The PrEPX lead pharmacist was registered with the Pharmacy Board of Australia, and appointed from the Alfred Clinical Trials Pharmacy. The PrEPX lead pharmacist held a bachelor's degree in pharmacy with more than 30 years experience of working in clinical trials. The PrEPX pharmacy manager had completed a bachelor of pharmacy degree and the pharmacy monitor held a life sciences degree. Alfred Health Clinical Trials Pharmacy provided pharmacy oversight, in line with ICH-GCP guidelines and relevant federal and state-based legislation. Registered, board certified community pharmacy owner(s) were trained and delegated PrEPX dispensing duties as per the approved study protocol.

Funding for PrEPX was announced in January 2016. The PrEPX study opened for enrolment on 26 July 2016 in Victoria, and was funded to enroll 2,600 participants; subsequently, further funding permitted the study to enroll 3,800 participants however the study went on to enroll 4,275 participants in Victoria. The PrEPX study closed to enrolment in Victoria when PrEP was subsidized on the PBS on April 1st 2018 in Victoria. The study expanded to South Australia on 15 May 2017 where 650 participants were enrolled and to Tasmania where 100 participants were enrolled until the study closed to enrolment on 30 June 2018 in South Australia and Tasmania, in accordance with the approved protocol.

Pre-Study Set Up

A pharmacy compliance group was established at Alfred Health to oversee the pharmacy component of the PrEPX study. The PrEPX pharmacy team was responsible for engaging with, training, and supervising PrEPX dispensing over the course of the study, in all participating states.

The PrEPX pharmacy team, created a checklist for GCP compliance and regulatory dispensing requirements for a community pharmacy acting as a clinical trial pharmacy. Key features of a clinical trial pharmacy are 1) drug storage, 2) record keeping, 3) monitoring of stock, 4) destruction of drugs if permitted by the protocol, 5) assistance with adherence to study protocol, 6) counseling of participants and monitoring medication adherence, 7) and provision of information to participants (Abraham, 2009; Australian Government Department of Health TGA, 2018).

Community pharmacies in close proximity to PrEPX study clinics were approached and invited to participate as a clinical trial pharmacy in the study. A brief survey was sent out to selected pharmacies to determine their current practices, and to identify gaps in clinical trial pharmacy requirements. The PrEPX pharmacy team worked with individual pharmacies to address each identified gap so that pharmacies could dispense clinical trial study drug, in accordance with ICH-GCP requirements (ICH-GCP ICOHGCP, 1996; Abraham, 2009), as per **Supplementary Figure 1**.

Structural Factors Implemented

Ambient temperature conditions are not routinely monitored or recorded within community pharmacies in Australia. Pharmacies that did not have temperature monitoring were provided with a testo automated temperature monitoring system (Testo Pty Ltd., 2015) at no cost to the pharmacy. This device was selected as it had been used successfully within Alfred Health Hospital Pharmacies. An active Wi-Fi connection was required to activate individual temperature monitors at each pharmacy. Temperatures were continuously recorded during the study, sending automatic hourly notifications to the secure PrEPX Cloud account. The minimum and maximum temperatures (15°C, 30°C) were set on each device, in line with the study drug storage conditions recommend by the drug manufacturer (Mylan Health Pty Ltd., 2014). In the event of temperatures exceeding the minimum and

maximum, an alarm was automatically sent *via* text message to the PrEPX pharmacy manager and the site pharmacist. Hospital-based clinical trial pharmacies are conventionally connected to back up generators, however back-up generators were not purchased for this study as it was determined that pharmacy ambient temperature could be corrected in a timely manner.

The PrEPX study supplied individual, lockable cabinets for study drug storage to participating community pharmacies as needed. The lockable cabinets were placed in the dispensary to comply with GCP requirements that study drug be stored in a restricted access area, under lock and key (Abraham, 2009; Australian Government Department of Health TGA, 2018).

Training

GCP training was provided to all study pharmacists who did not have current GCP certification (Whitehall Training, 2016). The TransCelerate online training course (Whitehall Training, 2016) was used and all study pharmacists were required to achieve at least 85% correct responses and provide a certificate of competency to PrEPX pharmacy manager prior to dispensing PrEPX study medications. Pharmacists were reimbursed $350AUD and provided six (Australian Government Department of Health, 2018) Continuing Professional Development points for successfully completing the GCP course (Pharmacy Board of Australia, 2018). Pharmacists who successfully completed GCP training were required to attend a site initiation visit, at which the study protocol and implementation were described. Site pharmacists who were not GCP certified were not permitted to dispense PrEPX study drug, in accordance with Australian Clinical Trial Regulations (Australian Government Department of Health TGA, 2018).

Study Prescription

A PrEPX study prescription form was designed by The PrEPX pharmacy team. The prescription form collects all data required in a clinical trial prescription and is consistent with standard practice at the Australian Clinical Trial pharmacies. The prescription form was printed on bright yellow paper to distinguish it from regular PBS prescriptions (Health AGD of. Pharmaceutical Benefits Scheme (PBS) | For PBS Prescribers [Internet], 2018; Australian Government Department of Health, 2018). The PrEPX prescription form included participant name, date of birth, address, participant study number, study visit number or visit month, allergies, consent yes/no, Medicare number, concession number (if applicable), name of prescriber, prescribing date, treatment date, and signature of prescriber (**Supplementary Figure 1**). All PrEPX prescriptions were required to be filled out by hand, by the PrEPX prescriber, using only black ink.

Study Protocol

Ordering Study Drug

The PrEPX study purchased generic co-formulated tenofovir disoproxil fumarate and emtricitabine from Mylan Pharmaceuticals. PrEPX study drug was delivered from Mylan to a centralized, TGA approved distribution and warehousing service for controlled substances, pharmaceutical packaging professionals (PPP), then onto pharmacies as required. PrEPX study drug was supplied to the study pharmacies at no cost to the pharmacy.

Site pharmacists emailed orders for PrEPX study drug to the pharmacy manager in batches of orders consisting of 300 bottles, (3 month supply for 100 prescriptions). The pharmacy manager reviewed all PrEPX study drug orders and emailed approved orders to PPP. Orders were dispatched directly from PPP to participating pharmacies and upon receipt of the PrEPX pharmacy manager approval. Site pharmacists were required to complete a receipt form, confirming the number of bottles received, batch number, and expiration date. The pharmacy manager maintained a log of all orders to generate estimates of dispensing and stock.

Dispensing

PrEPX participants were prescribed 3 months (90 days) of study drug at each study visit. Pharmacists were permitted to charge participants the current PBS co-payment fee at each dispensing event (Australian Government Department of Health, 2018). Dispensing fees served as remuneration for the pharmacies' work in dispensing the study drug, including the required clinical trial record keeping. Dispensing fees could be waived at the pharmacists' discretion.

The baseline study prescriptions had to be dispensed and collected within 7 days of the treatment date written on the prescription. This design was requisite to prevent a participant from filling the prescription beyond 7 days after the treatment date in case they acquired HIV between the clinic visit and collecting their script; in which case they could have remained undiagnosed with HIV and commenced two-drug treatment for HIV, which is inadequate. For the same reason the participants' follow-up prescriptions had to be dispensed and collected within 21 days (conventional prescriptions in Australia are valid for 12 months). In addition the limited time between prescribing and filling PrEPX prescriptions was designed to ensure participants had adequate supply of study drug for daily dosing, as per the approved study protocol. In the event of a participant attending a pharmacy outside of these date ranges, the site pharmacist was instructed to contact the PrEPX pharmacy manager to request permission to dispense on a case-by-case basis.

Monitoring During the Study

Monitoring Prescribing

A check box was printed at the bottom of each PrEPX study prescription, to be completed by the dispensing pharmacist to ensure each step of the dispensing process was executed (**Supplementary Figure 1**). The check boxes were 1) dispensing record checked, 2) prescribers' name and signature listed in delegation log, 3) dispensed medications checked by, 4) handed out by, 5) received by patient. The check boxes required initialing and dating by the authorized delegated pharmacist for compliance (**Supplementary Figure 1**).

The PrEPX pharmacy monitor reviewed PrEPX prescriptions on site at all participating PrEPX pharmacies. The pharmacy monitor completed prescription data entry and data maintenance

in accordance with the approved study protocol. Every dispensed PrEPX prescription was checked by either the pharmacy monitor, or pharmacy manager for completion and accuracy in accordance with the key performance indicator checklist. Refer to the sub-section pharmacy monitoring for full details (**Supplementary Figure 2**). Any discrepancies were discussed with the pharmacist and rectified accordingly.

Record Logs

Record logs were provided to PrEPX study pharmacies to record practices in line with clinical trial pharmacy practice (**Table 1**). Each log was printed in color to improve ease of use. The PrEPX pharmacy manager was on call to answer issues related to dispensing and completion of record logs over the course of the study. The signed training, signature, and delegation logs were authorized by the principal investigator for GCP compliance.

PrEPX-pharmacy training logs, signature, and delegation logs were produced in line with the Australian clinical trial handbook (Australian Government Department of Health TGA, 2018). The training log included data of site initiation visit and was signed and dated by pharmacists who attended the initiation. The signature and delegation logs included the name and signature of each pharmacist at each pharmacy that was authorized to dispense PrEPX study drug. Copies of the training and delegation logs were stored electronically by the PrEPX pharmacy team, and in hard copy format at each PrEPX participating pharmacy, as per GCP requirements (**Table 1**).

The prescriber signature and delegation logs were completed by all PrEPX prescribers and authorized by the study's principal investigator. Prescriber signature logs included names and signatures of approved prescribers and the duration for which they were permitted to prescribe study drug for the purpose of the trial. Prescriber signature logs ensured study prescriptions were written and signed only by authorized PrEPX prescribers. Prescriber logs were provided to all pharmacies in hard copy format and electronically, *via* email. Prescriber signature and delegation logs were updated and supplied to study pharmacies when new PrEPX prescribers joined the study (**Supplementary Figure 3**).

Template temperature logs were supplied to study pharmacies to manually record daily temperatures as a back-up, in the event of temperature monitoring failure with the testo system. Temperature logs included date of entry and both the minimum and maximum temperatures displayed within a 24-h period. There were no temperature monitoring failures at any of the PrEPX pharmacy sites over the course of the study. The PrEPX pharmacy team provided PrEPX pharmacies written advice on how to manage any potential temperature excursion. All temperature excursions were to be reported to the PrEPX pharmacy manager for investigation. All PrEPX stock that was subject to investigation due to a temperature excursion was required to be placed in quarantine and not dispensed to participants until disposition was determined by the PrEPX study management team.

Participant logs were completed by PrEPX pharmacists at a participant's first prescription dispensation at that pharmacy. Participant logs included dispensing date, participant name, and date of birth (DOB) or medical record number (MRN), participant study number, and consent (**Supplementary Figure 4**).

Drug accountability logs were completed by PrEPX pharmacists at each drug dispensing event. The drug accountability log recorded receipt of study drug, dispensing details, study drug returns, and balance of study drug product (**Supplementary Figure 5**). Date, visit number or month of study visit, participant ID, quantity (bottles), and initials of an authorized GCP trained pharmacist, confirming dispensing details were required to be completed for every PrEPX prescription dispensed at each participating pharmacy. The number of bottles of study drug remaining was recorded in the balance column after the dispensing details. The returns column, recorded the date, quantity of bottles, or number of tablets returned, initials of study pharmacist.

Study Team Monitoring

Regular monitoring by the pharmacy monitor or pharmacy manager, using key performance indicators for procedural deviations, drug storage, temperature excursions, and drug accountability accuracy were conducted monthly for the first 6 months of the study, then every 2–3 months thereafter. However, pharmacies that dispensed greater than 300 study prescriptions per month continued to be monitored monthly, or fortnightly for those pharmacies dispensing more than 500 prescriptions per month.

TABLE 1 | Characteristics of pre-exposure prophylaxis expanded study pharmacies.

		Total	Community/retail pharmacy	Hospital-based clinical trials pharmacy
Total number		12	8	4
Number per state	Victoria	8	5	3
	South Australia	2	1	1
	Tasmania	2	2	0
Open after 5 pm on weekdays	Yes	8	8	0
	No	4	0	4
Open on weekend	Yes	8	8	0
	No	4	0	4
Distance from closest PrEPX study clinic	Meters (range)	0.05–2.0 kms	0.05–2.0 kms	0.05 km
Number of PrEPX prescriptions filled	N	20,152	16,724	3,428
Number of study pharmacists onsite	N (range)	2–6	2	3
Charged PrEPX participants PBS co-payment		12	8	4

Pharmacy monitors undertook monitoring using a checklist, which was devised by the PrEPX pharmacy team. The monitoring checklist included indicators about a prescribing event: consent checked, study ID recorded, prescription signed by an authorized PrEPX prescriber, date study drug collected (for baseline prescriptions within 7 days, and for follow-up prescriptions within 21 days). The monitor also recorded completion of dispensing check boxes; all entries initialed and dated correctly in the drug accountability log, stock count balance, batch numbers, and page numbers filled out correctly on each page of the accountability logs, single line entries on each log, logs in date order, prescriptions filed in chronological order for review. Structural elements that were monitored were study drug stored in the designated, locked cabinet/drawer, and temperature records, which were checked for excursions using the PrEPX pharmacy monitoring checklist (**Supplementary Figure 2**).

Every dispensed PrEPX prescription was checked for completion and accuracy in line with clinical trial pharmacy requirements and the key performance indicator checklist (**Supplementary Figure 2**) Following study monitoring, the participating pharmacy was emailed items to action and/or issues to be resolved. A report from each monitoring visit was presented to the monitored pharmacy and the PrEPX pharmacy team.

Data Collection and Reporting

Data in this paper include description of participating pharmacies, outcomes of training, and structural improvements required to meet GCP guidelines. Dispensing log data (date range) reports the number of prescriptions dispensed by state and pharmacy type (community/hospital). Monitoring data from the drug accountability logs and participant logs (July 2016–June 2018) reported the number of errors that required follow-up and described the types of issued rectified.

Ethics

This project was approved by the Alfred Hospital Health Research Ethics Committee for the study to be conducted in Victoria and South Australia (AH/HREC16/100). Separate ethics approval was sought and granted from the University of Tasmania for the PrEPX study to be conducted in Tasmania (H0016607). The study was registered on the Australian and New Zealand Clinical Trials Registry (ACTRN12616001215415). All study participants provided signed consent at enrollment.

RESULTS

Pre-Study Set Up

Outcome of Engagement With Community Pharmacies

All 12 pharmacies across the three states that were approached agreed to participate and to undertake training and structural changes to meet clinical trial pharmacy requirements. Study pharmacies across the three states comprised five community pharmacies and three hospital based clinical trials pharmacies in Victoria, one community pharmacy, and one hospital based clinical trials pharmacy in South Australia, and two community pharmacies in Tasmania. At baseline, all hospital based clinical trial pharmacies met GCP requirements. The mean distance from the community pharmacies to the closest PrEPX study clinic was 325 meters (range: 50 m–2 km) (**Table 2** and **Supplementary Figures 6** and **7**).

Structural Improvements

None of the eight community pharmacies had temperature monitors. Eight testo temperature monitors were installed in each pharmacy. During the course of the study, there were 26 temperature alarms, most of which occurred during winter, over the weekend, or when pharmacies were closed. All temperature alarms were promptly rectified within 1 h. The 1-h

TABLE 2 | Summary of pharmacy study logs used in the pre-exposure prophylaxis expanded study.

	Prescriber training log	Pharmacy training log	Prescriber delegation log	Pharmacy delegation log	Drug accountability log	Participant log
Completed by	Clinic staff and study physicians/prescribers	Pharmacists	Study physicians/ prescribers	Pharmacists	Pharmacists	Pharmacists
Completed when	Clinic site initiation	Pharmacy site initiation	Clinic site initiation	Pharmacy site initiation	Every time drug is delivered and for each dispensing	The first time a participant attends PrEPX dispensing pharmacy
Site name	X	X	X	X	X	X
Date	X	X	X	X	X	X
Name	X	X	X	X		X (Participant and pharmacist)
Title	X	X	X	X	–	–
Signature of	Prescriber(s)	Pharmacist(s)	Prescriber(s)	Pharmacist(s)	Dispensing pharmacist	Dispensing pharmacist
Study role	X	X	X	X	–	–
PI Authorization	X	–	X	X	–	–
Participant ID	–	–	–	–	X	X
Participant DOB/MRN	–	–	–	–	–	X
Consent checked	–	–	–	–	–	X
Study visit no.	–	–	–	–	X	X
Drug quantity (bottle)	–	–	–	–	X	–

time frame was insufficient for study drug to equilibrate to the alarmed temperature.

Two of the eight (17%) community pharmacies had lockable storage. Lockable storage was purchased for the remaining six community pharmacies and installed in the restricted access dispensing area, in accordance with GCP study drug storage procedures.

Training

None of the eight (0%) community pharmacies had pharmacists with current GCP licenses. All of the hospital based clinical trial pharmacists (100%) had current GCP licenses. As a result, 54 GCP licenses were completed by community pharmacists across eight PrEPX study pharmacies.

Dispensing

A total of 60,456 bottles of co-formulated tenofovir disoproxil fumarate and emtricitabine, containing 30 tablets were purchased from Mylan over the course of the PrEPX study. In total, 20,152 prescriptions were dispensed across the twelve PrEPX dispensing pharmacies during the study periods: (Victoria: 18,188; South Australia: 1576; Tasmania: 361). Of those, 16,724 prescriptions (83%) were dispensed by community pharmacies (**Figure 2**). The number of participants seen at each site is not known as participants were free to attend more than one study pharmacy for dispensation.

Monitoring Outcomes

Dispensing Errors Identified Through Monitoring

Monitoring visits revealed that the number of action items for follow-up at each participating community pharmacy were greater at the beginning of the study and declined during the first 12 months but rebounded during the following 10 months. The number of action items in a 1-month period ranged from 0 (0%) to 22 (2%), and the greatest number of action items was recorded in study month 18 (**Figure 3**). Action items during the study period included verification of a prescriber code or signature (n = 119) and participant details documented incorrectly, or missing from prescription e.g. DOB and verification of study participant details e.g. study number, confirmation of consent (n = 291). A total of 410 dispensing errors [91.5% (n = 375) community; 8.5% (n = 35) hospital] were identified out of 20,152 (2.0%) of dispensed study prescriptions. Study logs were checked for accuracy and a stock balance check was performed at each monitoring visit. During the course of the study, there were 19 incorrect balance checks observed on the drug accountability logs, which were all attributable to recorded documentation errors. There were no errors noted with the participant logs, or any of the site signature or delegation logs. The number of errors increased when additional study places were released and when new study sites were established (**Figure 3**).

Over the study period the chief items that required follow-up were dispensing signature box not completed (n = 119), prescriptions dispensed outside of the 7/21 day window period (n = 345) and consent confirmation not documented on the prescription (n = 291). Most items that required follow-up occurred at the PrEPX community pharmacies and were able to be rectified in a timely manner (see **Supplementary Figure 2** detailed list of monitoring check items).

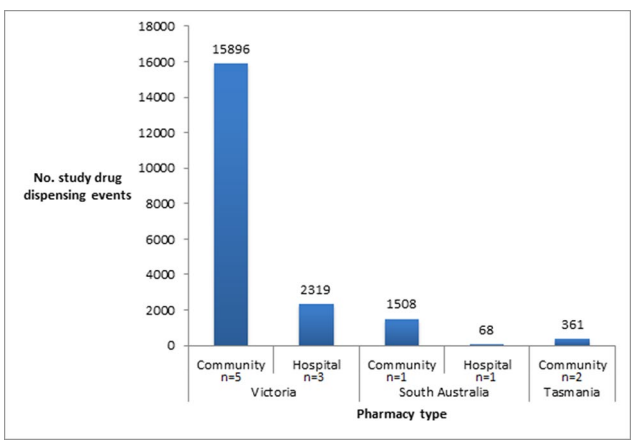

FIGURE 2 | Number of prescriptions dispensed in participating PrEPX Community and Hospital pharmacies.

Dispensing Without Good Clinical Practice Certificate

There were five occasions in which locum or relief pharmacists working in community pharmacies dispensed study drug despite not having the GCP certification. The pharmacy manager provided additional training and discussed in detail any events arising from locum or relief pharmacists with the PrEPX authorized site pharmacist.

DISCUSSION

In this large clinical PrEP trial involving over 5,000 participants we showed that it is highly feasible to engage community pharmacies to dispense clinical trial study drug. In this study, over 80% of medications were dispensed by community pharmacies with a low dispensing error rate, which is in accordance with similar published studies (McCormack et al., 2016; AVAC, 2018; Grulich et al., 2018). These data suggest that community pharmacies can be utilized in large clinical trials.

Dispensing of trial medication by community pharmacies for PrEPX was integral to the success of the roll-out of the study whereupon community pharmacies dispensed over 80% of all prescriptions in the PrEPX study. The community pharmacies provided convenient access to participants, which may have improved timely collection of the study drug, augmented study retention (Testo Pty Ltd., 2015), and contributed to a smooth transition for clinicians, participants, and community pharmacies when PrEP was listed on the PBS in April 2018. Furthermore community pharmacy dispensing is likely to have contributed to the recruitment into the PrEPX study wherein 1,000 participants were enrolled in 3 weeks and 2,000 participants were enrolled in 10 weeks (Ryan et al., 2017) which is the fastest recruitment known to have occurred into any PrEP trials to date. In an Australian modeling study, the rapid scale-up of PrEP use was shown to be necessary for PrEP to have a population level impact

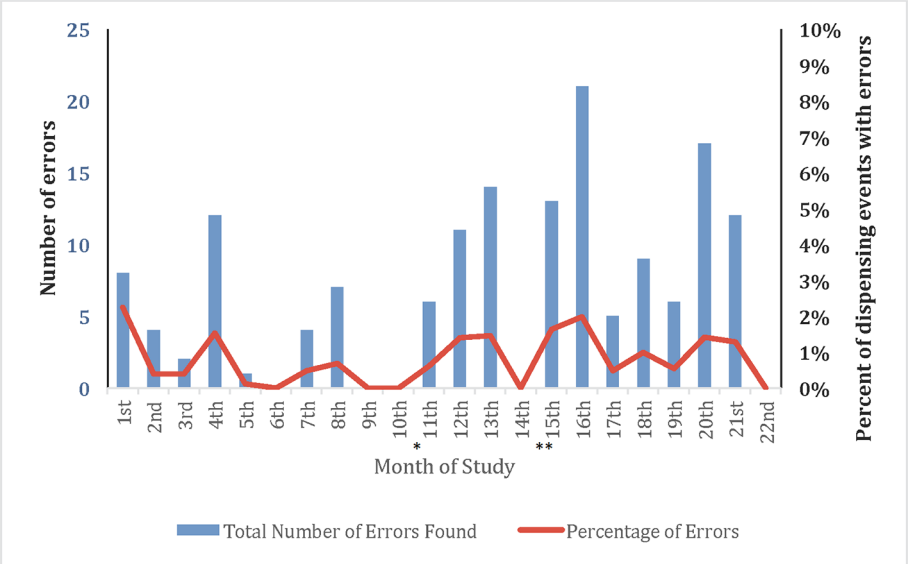

FIGURE 3 | Number of pharmacy errors across all PrPEX study pharmacies in relation to the number of prescriptions dispensed (26 July 2016–30 June 2018). Community 91.5% (n = 375); Hospital 8.5% (n = 35). *South Australian sites open **Tasmanian sites open.

on reducing HIV transmission (Kirby Institute and the Centre for Social Research in Health, 2017). The United Nations General Assembly has endorsed a declaration to end AIDS by 2030 by scaling up HIV prevention, treatment, and care programs (WHO, 2016). Hence jurisdictions planning to meet this goal will need to offer rapid access to PrEP and if they do so in the setting of a clinical trial, this study's findings, where over 20,000 dispensing events occurred with only 2.0% of errors suggests that they could plausibly adopt this model to dispense *via* community pharmacies.

Significant investment was needed to achieve successful implementation of clinical trial drug dispensing at community pharmacies for the PrEPX study. Study pharmacies required extensive pre-study phase engagement and ongoing monitoring and support throughout the study. We sought to offset the impost on community pharmacies for their participation in PrEPX by paying for pharmacists to undertake GCP training and accreditation, providing study drug at no cost and allowing retention of dispensing fees for study prescriptions.

The successful implementation of clinical trial drug dispensing through community pharmacies may signal the potential for further community pharmacy dispensing in future clinical drug trials. A recent review reported that only 20% of Australian Clinical Trials met their recruitment deadline, and only 50% of Australian clinical sites met their recruitment requirements (Ernst and Young, 2016). Hence all efforts to increase clinical trial participation should be made including enhancing access to clinical trial drug dispensing. This study demonstrates that community pharmacy dispensing is feasible and it should be further evaluated for its potential to enhance clinical trial recruitment and participant retention.

There are a number of limitations in this paper. We did not collect data from community pharmacists about their experiences with participating in the PrEPX study. However, all pharmacies that were approached agreed to participate, no pharmacies withdrew during the study and high accuracy in dispensing was observed across all sites suggesting participation was acceptable. Additionally, we have not reported on participants' experiences using the study's community pharmacies. Furthermore, the findings reported here are specific to the implementation of clinical trial dispensing in Australian community pharmacies and may not be transferrable to international settings with different regulatory and health systems. Finally this was not a randomized study and we did not have a second study arm where participants were randomized to only collect study drug from a hospital clinical trials pharmacy. Therefore we could not evaluate whether clinical trial enrolment and participant retention were different between community and hospital trial pharmacies.

Conclusions

Clinical trial drug dispensing of PrEP at community pharmacies for a large, rapidly enrolling population level study was highly feasible and practicable. These findings suggest that community pharmacies could be used in large future clinical trials including those delivering HIV prevention strategies.

THE PREPX STUDY TEAM

Edwina Wright, Brian Price, Mark Stoové, Simon Ruth, Colin Batrouney, Michael West, Dean Murphy, John de Wit, Luxi Lal, Jennifer Audsley, Christina Chang, Carol El-Hayek, Anne Mak, Alison Duncan, Joe Sasadeusz, Brent Allan, Michael Whelan, Daniel McPhail, David Wilson, Olga Vujovic, Martin Holt, Chris Williams, Steve Wesselingh, James Ward, Danny Gallant, Alison Ward, Jason Asselin, Tim Spelman, John Timothy Lockwood, Alistair Chong, Katharine McKinnon, Kathleen Ryan, Michael Traeger, Christopher Fairley, Ivette Aguirre, Ban Kiem Tee, Norman Roth, Vincent Cornelisse, Timothy Read, Richard Moore, Jeff Willcox, George Forgan-Smith, John Gall, Matthew Penn, Helen Lau, Danielle

Collins, Sian Edwards, Susan Boyd, Claire Pickett, Emma Paige, Pauline Cundill, Amanda Wade, Charlotte Bell, William Donohue, Samuel Elliot, Helen Calabretto, Louise Owen.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This project was approved by The Alfred Hospital Health Research Ethics Committee for the study to be conducted in Victoria and South Australia (AH/HREC16/100). Separate ethics approval was sought and granted from The University of Tasmania for The PrEPX Study to be conducted in Tasmania (H0016607). The study was registered on the Australian and New Zealand Clinical Trials Registry (ACTRN12616001215415).

AUTHOR CONTRIBUTIONS

EW, AM and LL contributed conception and design of the study; IL, TL and LL organized the database; LL and KR performed the statistical analysis; LL, KR, AM and EW wrote the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

FUNDING

This study was funded by the Victorian Department of Health, Victoria, Australia; Alfred Health, Melbourne, Australia; The Victorian AIDS Council, Melbourne, Australia; The Government of South Australia, Australia; The Department of Health and Human Services, Tasmania, Australia.

ACKNOWLEDGMENTS

The authors wish to acknowledge PrEPX participants, the participating study clinics and pharmacies, the HIV-affected community of Victoria, and all human and animal participants of previous preexposure prophylaxis (PrEP) studies.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01269/full#supplementary-material

REFERENCES

Abraham, J. (2009). "Handbook of Transnational Economic Governance Regimes," in *International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use*. Martinus Nijhoff Publishers. doi: 10.1163/ej.9789004163300.i-1081.897

Australian Government Department of Health TGA, and biologicals: [Internet]. 2016. Available from: https://www.tga.gov.au/sites/default/files/prescription-medicines-registration-new-chemical-entities-australia-2016.pdf.

Australian Government Department of Health TGA. (2018). *Australian clinical trial handbook Guidance on conducting clinical trials in Australia using 'unapproved' therapeutic goods [Internet]*, Australian Department of Health, [cited 2018 Nov 28]. Available from: https://www.tga.gov.au/sites/default/files/australian-clinical-trial-handbook.pdf.

Australian Government Department of Health. (2018). *Education guide - Writing PBS and RPBS prescriptions - Australian Government Department of Human Services*, [cited 2018 Nov 28]. Australian Department of Health. Available from: https://www.humanservices.gov.au/organisations/health-professionals/enablers/education-guide-writing-pbs-and-rpbs-prescriptions/45511.

Australian Government Department of Health. *Pharmaceutical Benefits Scheme (PBS) | About the PBS [Internet]*, 2018, [cited 2018 Jun 8]. Available from: http://www.pbs.gov.au/info/about-the-pbs.

AVAC. *Queensland PrEP Demonstration Project [Internet]*, 2015, Global Advocacy for HIV Prevention. Available from: https://www.avac.org/trial/queensland-prep-demonstration-project-qprep.

AVAC. *Ongoing and Planned PrEP Open Label, Demonstration and Implementation Projects*, Vol. 2018. pp. 1–5, 2018, Global Advocacy for HIV Prevention. Available from: https://www.avac.org/sites/default/files/resource-files/ongoing_planned_oralPrEP_studies_aug2018.pdf.

Baeten, J. M., Donnell, D., Ndase, P., Mugo, N. R., Campbell, J. D., Wangisi, J., et al. (2012). Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N. Engl. J. Med*. 367, 399–410.

Bower, P., Brueton, V., Gamble, C., Treweek, S., Smith, C. T., Young, B., et al. (2014). Interventions to improve recruitment and retention in clinical trials: a survey and workshop to assess current practice and future priorities. *Trials* 15 (1), 1–9. doi: 10.1186/1745-6215-15-399

Choopanya, K., Martin, M., Suntharasamai, P., Sangkum, U., Mock, P. A., Leethochawalit, M., et al. (2013). Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): A randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*. 381, 2083–2090. doi: 10.1016/S0140-6736(13)61127-7

Curran, W., Bruner, J., Yeager, K. A., Bruner, D. W., and Pugh, S. L. (2015). Cartographic Mapping and Travel Burden to Assess and Develop Strategies to Improve Minority Access to National Cancer Clinical Trials. *Int. J. Radiat. Oncol*. 93 (3), 702–709. doi: 10.1016/j.ijrobp.2015.06.041

Ernst and Young. (2016). Scoping and analysis of recruitment and retention in Australian clinical trials - Final report. *Dep. Heal.* 1–50. Available from: http://www.health.gov.au/internet/main/publishing.nsf/content/ee207d978a44e4b8ca257fa90081b212/$file/ey final report - recruitment and retention in australian clinical trials 30 june 2016.pdf.

Grant, R. M., Lama, J. R., Anderson, P. L., Mcmahan, V., Liu, A. Y., Vargas, L., et al. (2010). Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men for the iPrEx Study Team* Abstract. *N. Engl. J. Med*. 363, 2587–2599.

Grant, R. M., Anderson, P. L., McMahan, V., Liu, A., Amico, K. R., Mehrotra, M., et al. (2014). Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect. Dis.* 14(9), 820–829. doi: 10.1016/S1473-3099(14)70847-3

Grulich, A. E., Guy, R., Amin, J., Jin, F., Selvey, C., Holden, J., et al. (2018). Population-level effectiveness of rapid, targeted, high-coverage roll-out of HIV pre-exposure prophylaxis in men who have sex with men: the EPIC-NSW prospective cohort study. *Lancet HIV* 5 (11), e629–e637. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2352301818302157. doi: 10.1016/S2352-3018(18)30215-7

Health AGD of. Pharmaceutical Benefits Scheme (PBS) | For PBS Prescribers [Internet]. (2018) [cited 2018 Nov 28]. Available from: http://www.pbs.gov.au/info/healthpro/explanatory-notes/section1/Section_1_2_Explanatory_Notes#PBS-Prescription-forms

ICH-GCP ICOHGCP., *E6_R1_GuidelineInternational Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use*,

Vol. 1996, 1996, [cited 2018 Jun 8]. International Conference on Harmonisation. Available from: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf.

Kirby Institute and the Centre for Social Research in Health. (2017). *Discussion paper: Estimates of the number of people eligible for PrEP in Australia, and related cost-effectiveness*. Sydney. Australian Federation of AIDS Organizations. Available from: https://www.afao.org.au/wp-content/uploads/2018/04/KI-CSRH-PrEP-discussion-paper-formatted-sent-to-AFAO.pdf.

Lal, L., Audsley, J., Murphy, D. A., Fairley, C. K., Stoove, M., Roth, N., et al. (2017). Medication adherence, condom use and sexually transmitted infections in Australian preexposure prophylaxis users. *AIDS* 31 (12), 1709–1714. doi: 10.1097/QAD.0000000000001519

McCormack, S., Dunn, D. T., Desai, M., Dolling, D. I., Gafos, M., Gilson, R., et al. (2016). Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): Effectiveness results from the pilot phase of a pragmatic open-label randomised trial. *Lancet*. 387, 53–60. doi: 10.1016/S0140-6736(15)00056-2

Molina, J.-M., Capitant, C., Spire, B., Pialoux, G., Cotte, L., Charreau, I., et al. (2015). On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. *N. Engl. J. Med*. 373, 2237–2246. doi: 10.1056/NEJMoa1506273

Mylan Health Pty Ltd., (2014). *Emtricitabine / Tenofovir Disoproxil Fumarate 200 mg / 300 mg Tablets*, Mylan Laboratories

Pharmacy Board of Australia, (2018). *Pharmacy Board of Australia - Continuing Professional Development (CPD) FAQ*, Pharmacy Board of Australia, [cited 2018 Nov 28]. Available from: https://www.pharmacyboard.gov.au/codes-guidelines/faq/cpd-faq.aspx.

Rodriguez, Michael, Research Data Manager C and HH and HS. Queensland Preexposure {rophylaxis Demonstration Project QPrEPd. Queensland AIDS Council (QuAC), University of Queensland (UQ). 2018.

Ryan, K., Lockwood, T., Asselin, J., Mak, A., Price, B., Murphy, D., et al. (2017). "9th IAS Conference on HIV Science," in *Health systems and study design features permitting rapid enrolment of individuals at high risk of HIV acquisition into a pre-exposure prophylaxis study in Melbourne, Victoria, Australia* (Paris, France: IAS;), 387. Available from: http://www.ias2017.org/Portals/1/Files/IAS2017_LO.compressed.pdf?ver=2017-07-27-211231-197.

Ryan, K. E., Mak, A., Stoove, M., Price, B., Fairley, C. K., Ruth, S., et al. (2018). Protocol for an HIV Pre-exposure Prophylaxis (PrEP) Population Level Intervention Study in Victoria Australia: The PrEPX Study. *Front. Public Heal.* 6, 151. doi: 10.3389/fpubh.2018.00151

Schmotzer, G. L. (2012). Barriers and facilitators to participation of minorities in clinical trials. *Ethnicity Dis*. 22 (2), 226–230.

Testo Pty Ltd. (2015). *The testo Saveris data monitoring system | Testo Australia*, Testo SE and Co. KGaA, [cited 2018 Nov 28]. Available from: https://www.testo.com/en-AU/saveris/datamonitoring.

Thigpen, M. C., Kebaabetswe, P. M., Paxton, L., Smith, D. K., Rose, C. E., Segolodi, T. M., et al. (2012). Antiretroviral Preexposure Prophylaxis for Heterosexual HIV Transmission in Botswana. *N. Engl. J. Med*. 367 (5), 423–434. doi: 10.1056/NEJMoa1110711

Traeger, M. W., Cornelisse, V. J., Asselin, J., Price, B., Roth, N. J., Willcox, J., et al. (2019). Association of HIV Preexposure Prophylaxis With Incidence of Sexually Transmitted Infections Among Individuals at High Risk of HIV Infection. *JAMA*. 321 (14), 1380–1390. doi: 10.1001/jama.2019.2947

Western Australia AIDS Council., *PREP: HOW TO ACCESS IT IN WESTERN AUSTRALIA [Internet]*, 2018, [cited 2018 Nov 28]. Western Australia AIDS Council. Available from: https://waaids.com/hiv/where-to-get-prep.html.

Whitehall Training. (2016). *Pharmaceutical Courses & Training Online - Whitehall Training*, Infonetica, [cited 2018 Nov 28]. Available from: https://www.whitehalltraining.com/.

WHO. *WHO General Assembly- Political Declaration on HIV and AIDS: On the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030*, 2016, World Health Organization. Available from: https://www.un.org/en/ga/search/view_doc.asp?symbol=A/RES/70/266.

Zablotska, I. B., Selvey, C., Guy, R., Price, K., Holden, J., Schmidt, H. M., et al. (2018). Expanded HIV pre-exposure prophylaxis (PrEP) implementation in communities in New South Wales, Australia (EPIC-NSW): Design of an open label, single arm implementation trial. *BMC Public Health* 18 (1). doi: 10.1186/s12889-018-5173-7

Zablotska, I. B., Vaccher, S. J., Bloch, M., Carr, A., Foster, R., Grulich, A. E., et al. (2018). High Adherence to HIV Pre-exposure Prophylaxis and No HIV Seroconversions Despite High Levels of Risk Behaviour and STIs: The Australian Demonstration Study PrELUDE. *AIDS Behav*. 18 (1), 210. doi: 10.1007/s10461-018-2290-3

Conflict of Interest: EW has received financial support from Gilead Sciences; Abbott Laboratories; Janssen-Cilag; Boehringer Ingelheim; ViiV Healthcare; and Merck Sharp & Dohme. Gilead Sciences donated study drug to the VicPrEP study (precursor to the PrEPX study). Authors MV and KL were employed by the company Prahran Central Pharmacy, South Yarra, Victoria, Australia. Author JS was employed by the company John Silverii's Pharmacy, Fitzroy North, Victoria, Australia. Author JT was employed by the company HealthSmart Pharmacy at The Alfred, Melbourne, Victoria, Australia. Authors JP and WL were employed by the company Newton and Leung Pharmacy, Collingwood, Victoria, Australia. Author BN was employed by the company Bain and Co. Pharmacy, St Kilda, Victoria, Australia. Author NR was employed by the company Centre Pharmacy, Adelaide, South Australia. Authors MR, RS and CC were employed by the company Epic Pharmacy, New Town, Hobart, Tasmania. Authors AH and MQ were employed by the company Epic Pharmacy Kings Meadows, Launceston, Tasmania.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 31 January 2020
doi: 10.3389/fphar.2019.01514

Modeling HIV Pre-Exposure Prophylaxis

Thomas Straubinger[1], *Katherine Kay*[2] *and Robert Bies*[1]*

[1] Department of Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, NY, United States,
[2] Metrum Research Group, Tariffville, CT, United States

Pre-exposure prophylaxis (PrEP) has emerged as a promising strategy for preventing the transmission of HIV. Although only one formulation is currently approved for PrEP, research into both new compounds and new delivery systems for PrEP regimens offer intriguing challenges from the perspective of pharmacokinetic and pharmacodynamic modeling. This review aims to provide an overview the current modeling landscape for HIV PrEP, focused on PK/PD and QSP models relating to antiretroviral agents. Both current PrEP treatments and new compounds that show promise as PrEP agents are highlighted, as well as models of uncommon administration routes, predictions based on models of mechanism of action and viral dynamics, and issues related to adherence to therapy. The spread of human immunodeficiency virus (HIV) remains one of the foremost global health concerns. In the absence of a vaccine, other prophylactic strategies have been developed to prevent HIV transmission. One approach, known as pre-exposure prophylaxis (PrEP), allows HIV-negative individuals who are at high risk of exposure to the virus, be it through an HIV-positive sexual partner or through the shared use of drug injection equipment, to substantially reduce the risk of developing an HIV infection. PrEP is a relatively recent approach to combating the HIV epidemic, with the only currently approved treatment being Truvada, a daily oral antiretroviral (ARV) therapy initially indicated in the treatment of active HIV-1 infections, but approved for HIV PrEP in 2012. Although PrEP therapy has consistently demonstrated high efficacy in preventing HIV infection, this efficacy is dependent on patient adherence to the prescribed treatment regimen. This can present a significant problem in low- and middle-income countries, which may lack the infrastructure to provide sufficient access to PrEP medication to maintain daily dosing regimens. Furthermore, while the conventional approach has generally been to advocate for continuous administration akin to regimens used for viral suppression in infected patients, there has been some discussion of whether a better treatment paradigm might be to push for PrEP therapy primarily during those known periods of heightened exposure risk, while relying on post-exposure prophylaxis regimens to prevent infection after unanticipated exposures during low-risk periods. These considerations have led to a push for the development of long-duration and on-demand PrEP formulations, including subdermal and subcutaneous implants, slow-release intramuscular depot injections, vaginal and rectal antimicrobial gels, and intravaginal rings and dissolving films. PrEP

OPEN ACCESS

Edited by:
Max Von Kleist,
Robert Koch Institute, Germany

Reviewed by:
Abdallah El-Sayed Allam,
Tanta University, Egypt
Kumpal Madrasi,
Applied BioMath, United States

***Correspondence:**
Robert Bies
robertbi@buffalo.edu

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *12 March 2019*
Accepted: *21 November 2019*
Published: *31 January 2020*

Citation:
Straubinger T, Kay K and Bies R (2020) Modeling HIV Pre-Exposure Prophylaxis. Front. Pharmacol. 10:1514. doi: 10.3389/fphar.2019.01514

therapy is a quickly evolving field, with a variety of antiretroviral compounds and formulations under investigation. This review aims to report on notable drugs and formulations from a pharmacokinetic/pharmacodynamic (PK/PD) modeling perspective. Given the nature of PrEP as a preventive therapy designed for long-term use, clinical trials for PrEP therapies can last for months or even years, particularly in the case of long-duration formulations. Furthermore, in contrast to antiretroviral trials in infected patients, pharmacodynamic endpoints in PrEP therapies are difficult to quantify, as the primary endpoint for efficacy is generally the rate of seroconversion. Computational modeling approaches offer flexible and powerful tools to provide insight into drug behavior in clinical settings, and can ultimately reduce the time, expense, and patient burden incurred in the development of PrEP therapies.

Keywords: pharmacokinetics, pharmacodynamics, HIV, PrEP, Truvada, tenofovir, emtricitabine, maraviroc

CURRENT AND POTENTIAL PREP THERAPIES

Tenofovir Disoproxil

Tenofovir (TFV) is a nucleotide reverse transcriptase inhibitor (NRTI), a nucleoside phosphonate analogue of the endogenous nucleoside monophosphate, or nucleotide, adenosine 5'-monophosphate, and was one of the first compounds identified as a potential candidate for HIV prophylaxis. A 1995 study demonstrated that subcutaneous injections of TFV could protect macaques from simian immunodeficiency virus (SIV). (Tsai et al., 1995; Kearney et al., 2004) Tenofovir disoproxil fumarate (TDF) is a prodrug of TFV and has been in use for HIV treatment in the US since 2001. (Chapman et al., 2003) Studies have demonstrated the efficacy of TDF with and without emtricitabine (FTC) in preventing HIV infection in a variety of populations, including men who have sex with men (MSM), transgender women, heterosexual men and women, and people who inject drugs. (Grant et al., 2010; Baeten et al., 2012; Thigpen et al., 2012; Choopanya et al., 2013) Two major studies were terminated due to a lack of efficacy, however in both studies blood samples revealed that despite high self-reported adherence rates among patients in the treatment arms, actual adherence rates were low, with the fraction of patients with detectable plasma levels of drug ranging from 23-40%. (Van Damme et al., 2012; Marrazzo et al., 2015)

Preclinical testing revealed that TFV has low oral bioavailability due primarily to the ionic charges on its phosphonate group. (Cundy et al., 1998) The structure of TDF masks these charges, improving intestinal absorption and making an oral formulation feasible. (Shaw et al., 1997) After absorption in the intestine, TDF is converted into TFV through hydrolysis of its two ester groups. TFV is therefore the primary circulating compound in TDF-based treatments. (Kearney et al., 2004)

After uptake into cells, TFV undergoes sequential phosphorylation by adenylate kinase and nucleoside diphosphate kinase into its active form, tenofovir diphosphate (TFV-DP). TFV-DP inhibits HIV-1 replication by competing with endogenous deoxyadenosine 5'-triphosphate (dATP), inhibiting HIV-1 activity and halting strand elongation when incorporated into viral DNA.

Several pharmacokinetic models of TFV have been developed, but relatively few have focused specifically on PrEP therapy. Duwal et al. developed a pharmacokinetic model linking plasma concentrations of orally-administered TDF to intracellular concentrations of TFV-DP, which is used to drive a viral dynamics model. (Duwal et al., 2012) This model allows for the estimation of prophylactic efficacy while taking into account variable dosing of TDF, a necessity given that variability in adherence to the prescribed dosing regimen has been observed as a determinant of the efficacy of PrEP therapy. A two-compartment model was used to describe the PK of TFV, with a first-order rate constant describing the absorption of TDF and its conversion to TFV. A third compartment is used to depict the intracellular concentration of TFV-DP, with a Vmax model describing the saturable processes of cellular uptake of TFV and its phosphorylation to TFV-DP. A diagram of the compartmental model is included in **Supplementary Figure 1**. The group chose to ignore inter-individual variability in the plasma pharmacokinetics of TFV, as it is arguably negligible relative to the degree of variability in the intracellular pharmacokinetics of TFV-DP. The pharmacodynamic model borrowed a hybrid stochastic-deterministic model of viral dynamics described by von Kleist et al. (von Kleist et al., 2011) Briefly, the model incorporated free infectious and non-infectious virus, as well as uninfected, early infection, and late-stage infection T-cells and macrophages. For each possible event in the infection process, such as infection of a cell, integration of the viral genome, or the production of new virus particles, the rate constant is determined by both the quantity of the species involved and a propensity function describing the likelihood of the event occurring. If either the propensity function or the quantity of any of the species involved in a given reaction are below a pre-specified threshold, that reaction is modeled as a stochastic process. Otherwise each reaction is treated as a deterministic process. Simulations of HIV challenges suggested that variability in adherence had little effect on the efficacy of

TDF PrEP therapy for adherence above 60%, but the effect became significant when adherence dropped below 40%. However, the size of the viral inoculum had a significant impact on efficacy regardless of adherence rates. This leads von Kleist et al. to suggest that TDF-based PrEP may be most effective when used in the prevention of sexual transmission of HIV, as this route generally involves smaller inoculum sizes than transmission *via* shared needles or blood transfusions.

Prophylactic therapies against HIV require sufficient drug concentrations at the site of exposure. As sexual contact is the most common route of transmission, characterizing the distribution of antiretrovirals in anogenital tissues is of particular importance in the development of HIV PrEP therapies. (Centers for Disease Control and Prevention, 2018) Collins et al. recently published a population PK model relating plasma and rectal tissue concentrations of TFV, demonstrating that non-linear mixed-effects (NLME) modeling is a viable approach for predicting TFV tissue exposures using a sparse tissue and rich plasma sampling scheme. (Collins et al., 2017) A diagram of the compartmental model used by Collins et al. can be found in **Supplementary Figure 2**.

Various long-duration formulations of TFV are being investigated for PrEP. Vaginal gel, ring, and film formulations have been developed with the goal of providing women in high-risk populations with multiple options for prophylaxis in an effort to improve adherence. More recently, there have been efforts to develop rectal topical TFV formulations, as receptive anal intercourse is a common route of exposure to HIV.

Gao and Katz created a multicompartment physiological model for the pharmacokinetics of TFV administered *via* a vaginal gel. (Gao and Katz, 2013) The model allows for the simulation of concentrations across the vaginal mucosa, with dedicated compartments for the gel, vaginal epithelium, stroma, and uptake into the blood and lymphatic systems. This model offered insights into the spatial distribution of TFV throughout the layers of the vaginal mucosa, which is important for assessing whether prophylactic concentrations of TFV are being achieved in the vaginal stroma. Additionally, it suggested that variations over the course of a menstrual cycle, such as changes in the thickness of the epithelium, could have a significant impact TFV transport into the stroma.

More recently, Gao and Katz published a physiological model for TFV administration *via* an enema delivery vehicle. (Gao and Katz, 2017) Compared to the vaginal delivery model, the geometry of the colorectal canal is fairly complex, with both macroscopic folds and creases and microscopic, columnar, fluid-filled crypts in the rectal wall. As a result, modeling rectal drug delivery requires a more detailed mathematical description of the movement of the delivery vehicle itself. Given the larger overall surface area and thinner epithelium of the rectal mucosa, the model predicts much more rapid delivery of TFV *via* rectal administration than *via* vaginal.

An important aspect of PK/PD studies of topically-administered microbicides is accurately and reliably characterizing drug concentration profiles in tissues. This can be difficult due to both inherent variabilities in drug concentrations in mucosal tissues and luminal fluid, and limitations in the frequency with which tissue biopsies can be performed. In contrast, acquiring pharmacokinetic data from blood samples is relatively simple and can be carried out more frequently to provide a richer depiction of the pharmacokinetic profile than might be possible from fluid or tissue samples. Recently, Govil and Katz published a proof of concept study of a modelling approach utilizing feedforward neural networks to link plasma pharmacokinetic models of TFV to vaginal tissue PK and PD endpoints. (Govil and Katz, 2019)

Emtricitabine

Emtricitabine (FTC) is a nucleoside reverse transcriptase inhibitor effective against HIV-1. In the context of PrEP, FTC is administered as a combination oral therapy with the NRTI tenofovir disoproxil fumarate. Like tenofovir, FTC undergoes intracellular phosphorylation to its active form, emtricitabine 5'-triphosphate (FTC-TP), an analogue of deoxycytidine 5'-triphosphate (dCTP). Incorporation of FTC-TP into HIV-1 DNA during viral DNA replication terminates chain elongation. (Modrzejewski and Herman, 2004)

A recent model published by Garrett et al. found that FTC plasma concentrations were best described by a two-compartment PK model with first-order absorption and saturable metabolite formation, similar to the previously described model for TDF. (Garrett et al., 2018) The metabolite FTC-TP is described by a one-compartment model representing concentration within peripheral blood monocytes (PBMCs), the main site of action, with movement from the intracellular space to plasma represented by a first-order process.

FTC has not been investigated as a monotherapy for HIV PrEP. However, Valade et al. have published a population model for FTC in HIV-1 infected patients with varying degrees of renal impairment, as renal elimination appears to be a primary determinant of FTC pharmacokinetics. (Valade et al., 2014) This model was later expanded to include seminal plasma FTC concentrations in MSM, as a measure of both viral suppression and to characterize concentrations in male genital tissues. (Valade et al., 2015) The parameter estimates from these models are shown in **Table 1**. In addition, non-compartmental PK parameters for FTC are included in **Supplementary Table 1**.

TABLE 1 | Model-Specific Values for Emtricitabine[a].

Parameter	Value in Male and Female Adult Patients (%RSE)	Value in MSM (%RSE)
CL/F (L/h)	15.1 (6)	14.8 (4)
V_c/F (L)	42.3 (12)	51.6 (11)
Q/F (L/h)	5.75 (38)	8.19 (26)
V_p/F (L)	55.4 (28)	106 (44)
k_a (h^{-1})	0.53 (Fixed)	0.53 (Fixed)
Effect of creatinine clearance on CL/F	0.278 (23)	0.178 (35)
Inter-Individual Variability of CL/F	0.174 (14)	0.255 (12)
Residual Variability (Proportional Error)	0.422 (7)	0.339 (6)

[a]*Parameter values taken from (Valade et al., 2014; Valade et al., 2015).*

Although not necessarily directly applicable to PrEP therapies, these models may provide initial values for future models of FTC.

Tenofovir Disoproxil and Emtricitabine

Originally approved in 2004 for the treatment of HIV infection, a fixed-dose, oral, combination TDF-FTC therapy, Truvada received approval in 2012 for use as a PrEP therapy in individuals at high risk of contracting HIV and was the first therapy approved for HIV PrEP. (U.S. Food and Drug Administration, 2012) The use of a combination therapy incorporating two different nucleotide analogues provides a synergistic effect and reduces the impact of resistance to either of the two drugs individually. Additionally, the incorporation of nucleoside analogues during reverse transcription is a saturable process. Each viral DNA sequence contains a finite number of each nucleoside, so by targeting multiple nucleoside, the overall probability of incorporating an inhibitory nucleoside analog is increased.

Although the pharmacokinetics of the two drugs can be modeled independently, a model published by Cottrell et al. attempts to capture the distribution of both TDF and FTC in vaginal, cervical, and rectal tissue in order to connect tissue concentrations to protective effect against HIV infection. (Cottrell et al., 2016) A diagram of the model can be found in **Supplementary Figure 3**. Their study suggested that TFV has a propensity to distribute to colorectal tissue while FTC is more prone to accumulate in the female genital tract. Furthermore, by including endogenous nucleotide concentrations, the ratios of TFV-DP to dATP and FTC-TP to dCTP can be used as PD endpoints. The distribution of endogenous nucleotides also shows tissue specificity, with significantly higher nucleotide concentrations in female genital tract tissues. Based on these tissue distribution characteristics, it was predicted that adherence to 2 of 7 weekly doses of oral TDF with or without FTC was sufficient to provide protection in colorectal tissue, while adherence to a minimum of 6 out of 7 weekly doses was necessary to protect the female genital tract from HIV infection. These predictions are consistent with the results of the iPrEX trial, in which two doses of TDF-FTC per week were sufficient to significantly decrease the risk of rectal HIV acquisition in MSM, as well as the FEM-PrEP and VOICE studies, which found that similarly low levels of adherence did not confer any reduction in the rate of vaginal HIV acquisition. (Van Damme et al., 2012; Grant et al., 2014; Marrazzo et al., 2015)

Tenofovir Alafenamide

Tenofovir alafenamide fumarate (TAF) is a novel prodrug of tenofovir, and shows potential as areplacement for TDF in PrEP therapy. (De Clercq, 2016) In October 2019, a combination therapy of TAF and FTC became the second approved PrEP medication in the US, though it was only approved for use in men and transgender women. While TDF is an ester prodrug that undergoes rapid metabolism in plasma to TFV, TAF is primarily metabolized intracellularly by the enzyme cathepsin A. (Birkus et al., 2007) In clinical studies TAF been shown dramatically increase TFV-DP exposure in PBMCs, with a 8 mg of TAF being approximately equivalent to a 300 mg dose of TDF. (Ruane et al., 2013) An overview of the parameters of TAF and TDF is presented in **Table 2**. The fact that TAF is metabolized intracellularly reduces systemic concentrations of TFV. Unlike TFV, TAF is not a substrate for the renal organic anion transporters OAT1 and OAT3 which both reduces its rate of renal elimination and the risk of nephrotoxicity associated with TFV. (De Clercq, 2018) However, a recent meta-analysis of clinical trials comparing the efficacy and safety of TAF and TDF monotherapies with and without the pharmacokinetic enhancers ritonavir (RTV) and cobicistat (COBI) found that TAF reduced the incidence of bone mineral density depletion and had slightly better viral suppression than TDF, but only when administered with RTV and COBI. (Hill et al., 2018).

In addition to TDF and FTC, Garrett et al. included a model of TAF in their 2018 publication. (Garrett et al., 2018) Unlike TDF and FTC, they depict TAF using a single plasma compartment, likely owing to the fact that the TAF prodrug is

TABLE 2 | Comparison of TAF and TDF Pharmacokinetics[a].

Parameter	TAF			TDF
	8 mg	25 mg	40 mg	300 mg
TAF Multiple Dose PK, Day 10				
AUC_{last} (h ng/mL), mean (CV%)	54.7 (92.6)	115.2 (33.4)	308.9 (33.6)	
C_{max} (ng/mL), mean (CV%)	85.8 (116.3)	223.6 (58.8)	629.5 (57.0)	
T_{max} (h), median (Q1, Q3)	0.50 (0.50, 0.50)	0.50 (0.50, 0.75)	0.50 (0.38, 0.50)	
$t_{1/2}$ (h), median (Q1, Q3)	0.38 (0.26, 0.50)	0.39 (0.34, 0.54)	0.42 (0.32, 0.49)	
TFV Multiple Dose PK, Day 10				
AUC_{tau} (h ng/mL), mean (CV%)	65.5 (23.5)	267.7 (26.7)	405.8 (12.7)	1918.0 (39.4)
C_{max} (ng/mL), mean (CV%)	4.2 (24.7)	15.7 (22.1)	28.3 (8.7)	252.1 (36.6)
C_{tau} (ng/mL), mean (CV%)	2.1 (33.8)	9.2 (26.1)	13.3 (16.0)	38.7 (44.7)
T_{max} (h), median (Q1, Q3)	1.50 (1.00, 1.98)	1.50 (1.25, 1.75)	1.29 (1.04, 1.50)	1.25 (0.58, 2.00)
$t_{1/2}$ (h), median (Q1, Q3)	30.77 (26.90, 55.61)	40.19 (28.98, 44.84)	35.95 (26.38, 42.90)	14.86 (12.18, 16.81)
TFV-DP in PBMC, Multiple Dose PK, Day 10				
AUC_{tau} (μM h), mean (CV%)	3.5 (77.1)	21.4 (76.9)	74.5 (92.7)	3.0 (119.6)

[a]*Table reproduced from (Ruane et al., 2013).*

TABLE 3 | Model-Specific PK Parameters for Maraviroc[a].

Parameter	Description	Final Model Estimate (%SE)	1000 Bootstrap Run Statistics Mean (± SD)	CV%
Sturctural Model				
CL (L/h)	Systemic clearance	51.45[b]		
E_H	Hepatic extraction ratio	0.662 (1.5)	0.66 (0.01)	1.52
$E_{H,\ Race}$	Effect of race on E_H	-0.0948 (15.0)	-0.09 (0.01)	13.77
V_c (L)	Central compartment volume	132 (2.7)	131.6 (3.08)	2.34
CL_{ic} (L/h)	Inter-compartment clearance	16.4 (3.9)	16.27 (0.57)	3.52
$CL_{ic,\ Race}$	Effect of race on CL_{ic}	-0.298 (17.7)	-0.29 (0.05)	15.82
$CL_{ic,\ Age}$	Effect of age on CL_{ic}	0.349 (28.3)	0.35 (0.09)	26.21
V_p (L)	Peripheral compartment volume	277 (4.2)	276.07 (10.13)	3.67
$V_{p,\ Race}$	Effect of race on V_p	-0.637 (8.5)	-0.63 (0.05)	7.35
k_a (h^{-1})	Absorption rate constant	0.277 (21.0)	0.28 (0.06)	20.64
θ_{ka}	Exponential change in k_a relative to 1 mg dose	0.173 (22.9)	0.18 (0.04)	22.82
$\theta_{ka,\ 1\ mg}$	Fed-state fractional change in fasted k_a	0.547 (18.6)	0.56 (0.10)	17.95
ABS_{Emax}	Maximum fraction absorbed	1 (Fixed)		
$\theta_{ABS\ Emax}$	Fed-state exponential change in E_{max}	-0.258 (27.3)	-0.24 (0.09)	36.23
ED_{50}	Dose producing half-maximal absorption	51.2 (12.8)	50.82 (8.06)	15.87
θ_{ED50}	Fed-state exponential change in ED_{50}	0.594 (34.5)	0.66 (0.41)	62.46
γ	Sigmoidicity	1.39 (15.3)	1.38 (0.30)	21.9
T_{lag} (h)	Absorption Lag time	0.198 (4.1)	0.20 (0.01)	4.13
FQ (L/h)	Hepatic blood flow	59.59 (Fixed)		
Residual Error				
P_{max} (%)	Maximum change in residual variability from baseline	74.2 (3.9)	74.59 (2.43)	3.26
T_{max} (h)	Time of maximum residual variability	0.950 (8.8)	0.95 (0.08)	8.35
K	Exponential rate constant for residual variability	0.403 (6.4)	0.40 (0.02)	5.99
Base (%)	Baseline residual variability	20.2 (3.3)	20.0 (0.66)	3.28
Inter-subject Variability				
$\omega[ED_{50}]$ (%)	ISV of ED_{50}	58.9 (32.6)	61.17 (15.03)	24.57
$\omega[E_H]$ (%)	ISV of E_H	8.3 (11.0)	8.27 (0.43)	5.22
$\omega[V_c]$ (%)	ISV of V_c	11.5 (77.3)	8.92 (5.64)	63.21
$\omega[CL_{ic}]$ (%)	ISV of CL_{ic}	30.5 (22.7)	29.8 (3.41)	11.45
$\omega[k_a]$ (%)	ISV of k_a	40.0 (13.9)	39.92 (2.59)	6.48
$\omega[V_p]$ (%)	ISV of V_p	27.8 (21.2)	27.23 (2.89)	10.63

[a]*Table reproduced from (Chan et al., 2008).*

[b]*Calculated from $CL_H + CL_R$, where $CL_H = FQ \cdot E_H$, and CL_R is fixed to 12 L/h.*

metabolized to TFV intracellularly, drastically reducing the circulating concentrations of TFV, which is usually modeled with two-compartment disposition. A transit compartment and first order input are used to model the uptake into PBMCs, conversion into TFV, and subsequent phosphorylation into TFV-DP. Elimination from PBMCs is described as a first-order process.

Maraviroc

Maraviroc (MVC) is a small-molecule antagonist of the chemokine co-receptor CCR5. (Dorr et al., 2005) HIV-1 infection begins with a gp120 glycoprotein trimer on the virion binding to three CD4 proteins on the target cell. This causes a conformational change in gp120 that exposes additional binding sites that must interact with a co-receptor on the cell surface, with CXCR4 and CCR5 being the two primary co-receptors used by HIV-1. Interaction with the correct co-receptor allows a second protein, gp41, to undergo a conformational change and penetrate the cell membrane of the target cell, which in turn allows membrane fusion between the HIV-1 virion and target cell, followed by the release of HIV-1 RNA into the cytoplasm of the host cell. (Panos and Watson, 2015) HIV-1 strains can display an affinity, or tropism, toward utilizing either CXCR4 or CCR5, in which case they are referred to as the X4 or R5 variants, respectively. Interestingly, the relative prevalence of these variants shifts over the course of the disease, with the R5 variant being far more prevalent during the initial infection, with the X4 variant gradually increasing as the disease progresses toward AIDS. (Berger et al., 1999) The reason for this shift in tropism has not been definitively established, but what is clear is that the R5 variant plays a key role in HIV-1 transmission, so much so that two individuals with homozygous mutations in the CCR5 gene proved extremely resistant to HIV-1 infection, despite repeated exposures. (Liu et al., 1996) This makes CCR5 an attractive target for PrEP therapy, as it appears to be integral to the establishment of the initial HIV infection.

Dose escalation studies of MVC in healthy volunteers found it was well absorbed after oral absorption, reaching T_{max} within 30 min to 4 h post dose. (Abel et al., 2008b) MVC exhibits non-dose proportional pharmacokinetics, with higher dose levels leading to proportionally smaller increases in AUC and C_{max}. The absolute oral bioavailability of MVC was estimated at 23% for an oral dose of 100 mg, increasing to 33% for a dose of 300 mg. (Abel et al., 2008a) Mass-balance analysis suggested 60% of orally administered MVC is lost to first pass metabolism. (Abel et al., 2009) MVC is a substrate for both the metabolizing enzyme cytochrome P450 (CYP) 3A4 and the efflux transporter

P-glycoprotein, which likely accounts for the non-proportional pharmacokinetics. (Abel et al., 2001) Approximately 23% of MVC clearance is renal, the remaining 77% is believed to be metabolic, and overall clearance does not appear to be affected by dose. (Abel et al., 2008a). A table of non-compartmental parameters from the FDA clinical pharmacology and biopharmaceutics review of MVC can be found in **Supplementary Table 2**.

Chan et al. developed a population pharmacokinetic model of MVC based on a meta-analysis of 17 phase 1 and 2 studies in both healthy and HIV-infected subjects, and the resulting parameter estimates are presented in **Table 3**, and a diagram of the model can be found in **Supplementary Figure 4**. (Chan et al., 2008) MVC disposition was characterized by a two-compartment model, with drug input from oral dosing described by a first-order absorption rate constant with a time delay. The model incorporated a sigmoidal E_{max} model to describe the nonlinearity of the extent of absorption (F_{abs}), with F_{abs} expressed as a function of ABS_{Emax}, the maximum fraction absorbed, and ED50, the dose producing 50% of maximal absorption. A power function was used to describe the relationship between the absorption rate constant (ka) and dose. The effect of food emerged as a significant covariate, with a fed state causing a linear reduction in ka and an exponential reduction in ABS_{Emax} and ED50. Interpatient variability was included on ED50, hepatic extraction ratio (E_H), intercompartmental clearance (CL_{ic}), absorption rate constant, and central and peripheral volumes of distribution (V_c and V_p). Both race and age also emerged as statistically significant covariates, with race affecting V_p and CL_{ic} and age influencing CL_{ic}. In the final model, race was implemented as a binary variable of Asian vs. non-Asian. In Asian subjects, estimates for E_H were reduced by approximately 14%, which translated into a 17.7% increase in F due to a reduction in first-pass hepatic elimination. Asian patients were also estimated to have a 1.8% decrease in CL_{ic} and only a 0.23% decrease in V_p. Age emerged as a covariate for CL_{ic}, with an increase of 0.349 L/h for each year of age over 30. Despite being statistically significant, the differences due to race and age were deemed to be clinically insignificant, requiring no dose adjustment. Weight, sex, and HIV status were also included in the covariate modeling process, but had no significant impact on model parameters. The majority of residual error occurred during the absorption phase, so the error model was fit as a function of time after dose.

Dapivirine

Dapivirine (DPV) is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI). Initially intended for use in highly active antiretroviral therapy (HAART) against HIV strains resistant to first generation NNRTIs, evidence of poor oral absorption early in development led to the investigation of DPV as a topical microbicide. (de Béthune, 2010) A monthly intravaginal DPV ring under development by the International Partnership for Microbicides (IPM), which currently holds exclusive rights to DPV, has multiple formulations in development, including vaginal and rectal gels, intravaginal films, and intravaginal rings. A monthly DPV intravaginal ring being developed by IPM has been through Phase III and Phase IIIb testing, and a regulatory decision is anticipated at some point in 2019. (Baeten et al., 2016; Nel et al., 2016; Baeten et al., 2018; Nel et al., 2018)

Given the poor performance of DPV as an oral PrEP compound, and its repurposing for topical delivery, there have been relatively few modeling studies performed. Hawles et al. developed a pharmacokinetic model for intravaginal delivery *via* a DPV gel, based on the TFV gel model developed by Gao and Katz. (Gao and Katz, 2013; Halwes et al., 2016) More recently, Kay et al. published a physiologically-based pharmacokinetic model DPV delivered *via* either an intravaginal ring or film. (Kay et al., 2018b) The model captures physiological determinants of DPV absorption in the cervicovaginal tract, including compartments for the device itself, vaginal luminal fluid, vaginal epithelium, vaginal stromal tissue, and stromal blood. A diagram of the model is available in **Supplementary Figure 5**. This level of granularity in depicting the characteristics of individual tissue types is necessary given the level of variability in vaginal drug delivery.

Long-Acting Injectable Formulations: Rilpivirine and Cabotegravir

Long-acting injectable (LAI) formulations have recently been the subject of research interest for PrEP therapy. This administration route avoids the problems with topical or enteral absorption, while allowing for long-term sustained release of drug into systemic circulation. The drugs rilpivirine (RPV) and cabotegravir (CAB) have recently shown promise as a combination LAI treatment for HIV-1 infected adults. (Spreen et al., 2013)

Like dapivirine, rilpivirine is a second-generation NNRTI currently being investigated for the treatment of HIV variants resistant to common NNRTIs such as efavirenz (EFV) and nevirapine (NVP). (Ripamonti et al., 2014) Currently prescribed as an oral formulation for treatment-naïve HIV-1 patients, RPV is being investigated as a long-acting intramuscular injectable for HIV PrEP. Cabotegravir is an integrase strand transfer inhibitor (INSTI) being investigated for use in both HIV treatment and prophylaxis. Although an oral formulation is being tested, the low solubility and slow metabolism of CAB make it suitable for use as a long-acting injectable (LAI). (Cattaneo and Gervasoni, 2018) To date, CAB and RPV have undergone separate clinical trials for HIV PrEP in the ECLAIR and MWRI-01 studies, respectively. (McGowan et al., 2016; Markowitz et al., 2017) Clinical trials assessing CAB and RPV combination LAI formulations have yet to be undertaken.

Rajoli et al. developed a general physiologically-based PK model for LAI formulations. (Rajoli et al., 2015) Although the model was initially validated using oral drug formulations, it was able to simulate the pharmacokinetics of LA RPV administered *via* intramuscular injection. Unfortunately, the model does not include tissue compartments that are relevant to PrEP, such as rectal and female genital tract tissues. Despite this, it may serve as a useful starting point for future physiologically-based models of LAI formulations.

VIRAL DYNAMICS AND PHARMACOLOGY

Ultimately the goal of PK/PD modeling is to connect drug exposure to clinical response. In the case of modeling antiretroviral therapies for HIV, this requires some description of HIV viral dynamics. Pharmacodynamic parameters can be derived from *in vitro* and ex vivo assays, but caution must be exercised when attempting to translate these results to *in vivo* efficacy. Tissue explant models, for example, can demonstrate high levels of inter-patient variability in infectivity. (Kay et al., 2018a) Furthermore, a large viral inoculum is required to establish an infection in ex vivo systems, far in excess of what would be required *in vivo*. While HIV dynamics in an active infection can generally be modeled as a deterministic process, the underlying behavior of individual virions is inherently stochastic. A very small number of initial virions serve as progenitors during the initial infection, which is best described as a stochastic process. (Carlson et al., 2014)

Duwal et al. have described a multiscale modeling approach for predicting the efficacy of HIV PrEP candidates. (Duwal et al., 2016) Their modular framework incorporates models for pharmacokinetics, viral transmission, and long term efficacy, but key to the estimation of efficacy are the viral replication and molecular mechanism of action (MMOA) models. The MMOA model was developed by Von Kleist et al, and attempts to mechanistically describe the mechanism of action of NRTIs. (von Kleist et al., 2012) Briefly, the model depicts the process of DNA polymerization using a Markov jump process, where each state in the model represents the incorporation of an additional nucleoside. From each state, the chain can either shorten through pyrophosphorolysis, extend by incorporation of a nucleoside through polymerization, or be terminated *via* incorporation of a nucleoside analog. The reaction rates for each of these process are specific to the each nucleoside and nucleoside analog. Nucleoside analogs achieve inhibition of viral replication by increasing the amount of time required to complete polymerization of viral DNA, as sequences incorporating a nucleoside analog cannot continue the polymerization process until the analog has been removed. If the virus cannot replicate its DNA quickly enough, it is cleared intracellularly. By computing the mean time to complete the polymerization of the full viral DNA sequence and comparing it to the mean time required for intracellular clearance, it is possible to estimate the probability of a virus successfully replicating itself. Based on the binding affinity and polymerization rate constant of both endogenous nucleosides and their analogs, it is then possible to estimate the effect of a given concentration of nucleoside analog on viral proliferation.

The effects of NRTIs on viral replication are then incorporated into a model of HIV viral dynamics. This model represents the process of infection by describing the viral replication cycle as a Markov jump process with five possible states: free virus, early infected T-cell, late infected T-cell, infected T-cell producing viral progeny, and virus cleared from the system before reaching the productive infection state. The effects of NRTIs are incorporated into the model in two ways. First, they reduce the rate of transition from the free-virus state to the early infected T-cell state, by increasing the time required for the virus to enter the cell and successfully transcribe its genome. Second, they increase the rate of clearance of the virus due to failed attempts to infect a cell. Though the study focused on the effects of NRTIs, the viral dynamics model can easily incorporate the mechanisms of other classes of antiretroviral compounds. (Duwal et al., 2019) The effects of co-receptor antagonists can be modeled as inhibition of transition from the free virus to early infection as well as inhibition of clearance due to a failed attempted infection, integrase inhibitors can be described by inhibiting the rate of transition from early to late stage infected T-cells, and protease inhibitors can impede the transition from productive infected cells to free virus.

The primary goal of this level of mechanistic detail is the prediction and identification of compounds likely to be well-suited to PrEP. The widespread use of pharmacokinetic modeling has significantly reduced the rates of drug failures due to pharmacokinetics in the later stages of development, as compounds with poor PK properties are relatively easy to screen for. Screening compounds based on their pharmacodynamics is significantly more involved, particularly in a paradigm like PrEP, where adherence and transmission rates can have a significant impact on efficacy. In their analyses, Duwal et al. identified several antiretrovirals that appear to have favorable pharmacodynamic properties. Efavirenz, nevirapine, etravirine, and rilpivirine were all found to be highly potent PrEP agents, with prophylactic efficacy maintained even after a three-day gap in administration. The group also found that maraviroc and rilpivirine maintain 50% and 72% efficacy, respectively, at low concentrations, and noted that simulations suggested that after three days of missed doses, the efficacies of raltegravir and maraviroc dropped to 8% and 50%, respectively, while rilpivirine maintained 100% prophylactic efficacy.

ADHERENCE AND TRANSMISSION

Given that the efficacy of PrEP is highly dependent on patient adherence, it may be important to incorporate models of adherence when modeling PrEP at a population level. (Haberer et al., 2015; Fonner et al., 2016) To date, there are few published models describing HIV transmission in a population utilizing PrEP, and of those very few incorporate PK/PD. One exception is the previously described PK/PD model of FTC, TDF, and TAF created by Garrett et al., based on earlier studies by Cottrell et al. (Cottrell et al., 2016; Cottrell et al., 2017; Garrett et al., 2018) Using Monte Carlo simulations of 1000 patients each, a variety of treatment scenarios were investigated. In addition to the standard treatment doses (300 mg TDF, 200 mg FTC, or 25 mg TAF), dosing regimens included double the standard dose, steady-state dosing with one to seven doses per week, and on-demand dosing involving a double dose either 2 or 24 h pre-exposure, followed by standard treatment doses at 24 and 48 h post-exposure. All three monotherapies and both TDF + FTC and TAF + FTC combination therapies were simulated for all dosing scenarios, with protective effect estimated based on the ratio of endogenous

nucleosides to nucleoside analogues. However this assumption has been criticized for failing to account for the nonlinear, saturable nature of the polymerization process. (Duwal et al., 2016)

A second notable example of a model incorporating transmission, adherence, and PK/PD is the previously mentioned multiscale modeling framework described by Duwal et al. (Duwal et al., 2016) The group incorporated a model of viral exposure to quantify the relationship between donor viral load and the number of transmitted viral particles. Briefly, they assumed a linear relationship between the log of the viral load in the donor and the log of the probability of infection, which lead to the derivation of a power function relating viral load to the number of transmitted viral particles per sexual encounter. The viral content of infected individuals was assumed to be log-normally distributed, based on observed data from individuals shortly following seroconversion. By combining estimates of viral load, the corresponding estimate of number of transmitted proteins, and the estimates from the viral dynamics model described in the previous section, an overall per-encounter probability of infection can be calculated. Finally, a population-level model incorporating the number of infected individuals and the probability of unprotected sex acts can be used with the outputs of the viral exposure model to simulate clinical trials and estimate an overall trial efficacy.

The majority of models of adherence in HIV PrEP therapy are epidemiological models of HIV transmission in a population. Although these models generally do not incorporate pharmacokinetics or pharmacodynamics, they may be informative to population PK modelers looking to capture the effects of non-adherence. One major caveat to the use of these models is their potential lack of generalizability, as the behavioral and societal factors influencing adherence rates vary with geography and culture. Even within the same geographic region, different subpopulations may exhibit different rates of adherence to PrEP, which may make it difficult to develop a generalized model of adherence.

A 2008 paper by Vissers et al. details a simulation study of various PrEP therapy scenarios in Botswana, Nyanza Province in Kenya, and Southern India. (Vissers et al., 2008) This study focuses on HIV transmission in the sex industry, with sex workers and their clients considered high risk relative to the rest of the population. The group used a compartmental model adapted from earlier models of antiretroviral therapy and male circumcision interventions.(Nagelkerke et al., 2002; Nagelkerke et al., 2007) Briefly, the model population is stratified high- and low-risk groups, with compartments for uninfected, uninfected on PrEP, early HIV infection, early infection on PrEP, and late-stage infection. Male and female populations are modeled separately within each compartment. Only heterosexual transmission is modeled, with three distinct types of sexual relationships able to spread HIV: client and sex worker, marriage-like relationships, and nonpaid casual relationships. It is assumed that HIV transmission through the latter two relationships only occurs in the low-risk population. In other words, it is assumed that the only relationships engaged in by the high-risk population are client-sex worker relationships. Additionally, the model assumes that condoms are only used during client-sex worker relationships. A certain percentage of each risk group is assumed to move to the other group annually, at which point it is assumed they will discontinue PrEP, should they be in the PrEP group. In the event that a member of the PrEP group becomes infected, be it through failure of the treatment or lack of adherence, it is assumed that individuals will continue to take PrEP for an average of one year. Simulations suggested that PrEP would lead to a significant decrease in HIV infections in Africa. However, the study found that under certain circumstances PrEP could actually lead to an increase in HIV cases in southern India, primarily due to high rates of condom use in the sex industry. If the adoption of PrEP were to lead to a fairly small decrease in condom use, roughly 15%, the model predicts that the number of new HIV cases would increase. The authors assert that any implementation strategy must emphasize that PrEP is a supplement to condom use, not a substitution.

One concern raised with the introduction of PrEP therapy was its potential impact on the prevalence of drug resistance. Van de Vijver et al. performed a model comparison study in order to investigate this issue. (Van De Vijver et al., 2013) Three models of HIV transmission and disease progression were investigated. The first was the Synthesis Transmission Model, a stochastic model for heterosexual HIV transmission in sub-Saharan Africa beginning in the 1980s, with demographic information primarily incorporated from the HIV epidemic in South Africa. (Phillips et al., 2011) This model simulates individual-level HIV transmission based on age, gender, viral load, sexual risk behavior, presence of antiretroviral drugs, presence of specific drug-resistance mutations, and adherence to drug regimens. Sexual risk behavior was based on the number of short-term unprotected sex partners and presence of a long-term unprotected sex partner within a given three month period. In the adaptation by Van de Vijver et al, PrEP was introduced *via* a campaign targeting serodiscordant couples in long-term partnerships. Adherence was incorporated with both a fixed inherent tendency to adhere and a period-to-period variability in adherence. Adherence is further modified by drug toxicity, probability of a patient voluntarily interrupting clinic visits, and probability of interruptions in the drug supply. All of these parameters are assumed to vary by geographic region.

The second model in the comparison by Van de Vijver et al. is the South African Transmission Model, initially developed by Abbas et al. and based on PrEP trials in South and Sub-Saharan Africa. (Abbas et al., 2013) Like the previous model, it was calibrated based on the progression of the South African HIV-1 epidemic, and exclusively models heterosexual transmission. While the first model was entirely stochastic, this model provides a more deterministic framework by incorporating disease progression and viral dynamics. Briefly, the model stratifies the population based on gender, PrEP/ARV treatment status, infection status, stage of disease, and HIV-1 drug susceptibility, with susceptibility classified as either drug-sensitive or drug-resistant, and drug-resistance further classified as acquired or transmitted resistance. Inappropriate PrEP use, which is described as PrEP use subsequent to acute

HIV infection, is modeled based on whether the individual taking PrEP is in a pre- or postseroconversion stage of the infection. After seroconversion it is assumed that PrEP use continues for a length of time corresponding to the HIV testing interval, the default being six months. In order to model sexual transmission, individuals of both genders are stratified into four sexual activity levels. These levels are used to construct a sexual activity matrix that describes, for any individual of gender g and activity level k, denoted g_k, and a prospective partner of opposite gender g' and activity level l, denoted g_l', the probability of forming a sexual partnership denoted g_{kl}. (Garnett and Anderson, 1993) The probability is derived from the total population of g_l', the tendency of g_k to engage in assortative versus random mixing, and the rate at which g_k individuals change partners when in a partnership with g_l' individuals. The probability of HIV transmission for a single sex act within a sexual partnership is represented as a function of the partner's ARV treatment status, disease stage, and HIV-1 variant. The total probability of HIV transmission for a partnership is then the per-sex act probability multiplied by the total number of sex acts for a partnership between two individuals g_k and g_l'. The protective effect of PrEP on an individual is modeled as a reduction in the susceptibility of that individual to the transmission of a given HIV variant, multiplied by the average adherence of the individual, which is itself determined by the individual's adherence stratum.

The third and final model included in the comparison was the Macha Transmission Model. (Nichols et al., 2013) While the other studies included in the comparison focused on the South African HIV epidemic, the model's namesake is a rural hospital in Southern Zambia, roughly 80 kilometers from the nearest town, and serves as the only major HIV clinic for roughly 90,000 people. Despite being calibrated to a different population, the Macha model shares a number of features with the South African model. The Macha model is a deterministic, compartmental model incorporating HIV disease progression. Once again the population is stratified based on sexual activity level, with higher activity levels corresponding to a greater number of sexual partners per year. The disease progression model depicts the stages of infection as acute HIV, chronic HIV, early AIDS, and late AIDS, with the AIDS compartment subdivided primarily to reflect changes in sexual activity associated with progression to AIDS, assuming that early stage AIDS is characterized by a reduction in sexual activity, and therefore transmission, while sexual activity halts entirely in the late stage of the disease. Just as in the South African model, the Macha model adapts the mixing matrix described by Garnett and Anderson in order to model transmission in a heterosexual population stratified by sexual activity level. (Garnett and Anderson, 1993) However, the Macha model differs from the South African model in that it stratifies the infected population into individuals who have undergone HIV testing and are aware of their infection, and those who are unaware. The model assumes that individuals who are aware of their seropositive status may make some effort to reduce their acquisition rate of new sexual partners. It assumes this effect is not uniform across all sexual activity levels, with the two lowest levels reducing acquisition rates by up to 40% while the two highest levels show no change in behavior. This stratification leads to two mixing matrices; one is identical to the previously described matrix and applies to individuals who are unaware of their HIV infections, while a second matrix incorporates the reduction in the rate of partner acquisition for individuals who are aware of their infection.

CONCLUSION

PrEP therapy for HIV remains an active and growing field of research. In addition to the currently approved PrEP therapies, several alternatives are in the mid to late stages of development. Many of these therapies are long-acting or on-demand approaches that aim to address problems of adherence and availability. The primary aim of this review was to provide an overview of the available pharmacokinetic models of both current PrEP regimens and antiretrovirals currently under investigation as PrEP agents, while highlighting some of the challenges associated with modeling more complex formulations and delivery systems. In addition, it is important to note the challenges involved in translating *in vitro* and ex vivo estimates of antiretroviral efficacy into estimates of clinical outcomes. Finally, an overview of some of the disease progression and viral transmission models that have been used to investigate HIV PrEP has been included, as population-level variables such as the frequency and routes of HIV exposure, propensity to modify high-risk behavior, and crucially, patient adherence to PrEP regimens, must be taken into account when modeling HIV PrEP at the population level. The diverse array of administration routes, compounds and dosing regimens presents novel challenges to drug development. In silico modeling and simulation approaches offer powerful tools to inform clinical trials, and allow for rapid investigation of pharmacokinetic and pharmacodynamic questions that arise during the drug development process. Moreover, modeling and simulation approaches provide investigators with the ability to examine scenarios related to changes in transmission, treatment adherence, and sexual behavior that might otherwise be precluded from clinical studies due to practical or ethical concerns. Ultimately, there are still many aspects of the HIV PrEP problem space that have yet to be explored through computational modeling.

AUTHOR CONTRIBUTIONS

TS drafted the review article. TS, RB, and KK revised and edited the article for clarity and content.

FUNDING

RB is the recipient of grant funding from the US National Institutes of Health grant 1U19AI120249.

ACKNOWLEDGMENTS

We gratefully acknowledge funding support from the US National Institute of Allergy and Infectious Diseases Division of AIDS.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01514/full#supplementary-material

REFERENCES

Abbas, U. L., Glaubius, R., Mubayi, A., Hood, G., and Mellors, J. W. (2013). Antiretroviral therapy and pre-exposure prophylaxis: combined impact on HIV transmission and drug resistance in South Africa. *J. Infect. Dis.* 208 (2), 224–234. doi: 10.1093/infdis/jit150

Abel, S., Beaumont, K. C., Crespi, C. L., Eve, M. D., Fox, L., Hyland, R., et al. (2001). Potential role for P-glycoprotein in the non-proportional pharmacokinetics of UK-343,664 in man. *Xenobiotica* 31 (8-9), 665–676. doi: 10.1080/00498250110052779

Abel, S., Russell, D., Whitlock, L. A., Ridgway, C. E., Nedderman, A. N. R., and Walker, D. K. (2008a). Assessment of the absorption, metabolism and absolute bioavailability of maraviroc in healthy male subjects. *Br. J. Clin. Pharmacol.* 65 (1), 60–67. doi: 10.1111/j.1365-2125.2008.03137.x

Abel, S., Van Der Ryst, E., Rosario, M. C., Ridgway, C. E., Medhurst, C. G., Taylor-Worth, R. J., et al. (2008b). Assessment of the pharmacokinetics, safety and tolerability of maraviroc, a novel CCR5 antagonist, in healthy volunteers. *Br. J. Clin. Pharmacol.* 65 (1), 5–18. doi: 10.1111/j.1365-2125.2008.03130.x

Abel, S., Back, D. J., and Vourvahis, M. (2009). Maraviroc: pharmacokinetics and drug interactions. *Antiviral Ther.* 14 (5), 607–618. Retrieved from: https://www.intmedpress.com/serveFile.cfm?sUID=a4fc50f1-3a90-4964-ba26-0b20c0e93539

Baeten, J. M., Donnell, D., Ndase, P., Mugo, N. R., Campbell, J. D., Wangisi, J., et al. (2012). Antiretroviral Prophylaxis for HIV Prevention in Heterosexual Men and Women. *New Engl. J. Med.* 367 (5), 399–410. doi: 10.1056/NEJMoa1108524

Baeten, J. M., Palanee-Phillips, T., Brown, E. R., Schwartz, K., Soto-Torres, L. E., Govender, V., et al. (2016). Use of a vaginal ring containing dapivirine for HIV-1 prevention in women. *New Engl. J. Med.* 375 (22), 2121–2132. doi: 10.1056/NEJMoa1506110

Baeten, J., Palanee-Phillips, T., Mgodi, N., Mayo, A., Nel, A., Rosenberg, Z., et al. (2018). "High Uptake and Reduced HIV-1 Incidence in an Open-Label Trial of the Dapivirine Ring," in *25th Conference on Retroviruses and Opportunistic Infections (2018 CROI Foundation/International Antiviral Society-USA)* (Boston, Massachusetts). Abstract 143LB. Retrieved from http://www.croiconference.org/sessions/high-uptake-and-reduced-hiv-1-incidence-open-label-trial-dapivirine-ring

Berger, E. A., Murphy, P. M., and Farber, J. M. (1999). CHEMOKINE RECEPTORS AS HIV-1 CORECEPTORS: roles in viral entry, tropism, and disease. *Annu. Rev. Immunol.* 17 (1), 657–700. doi: 10.1146/annurev.immunol.17.1.657

Birkus, G., Wang, R., Liu, X., Kutty, N., MacArthur, H., Cihlar, T., et al.(2007). cathepsin a is the major hydrolase catalyzing the intracellularhydrolysis of the antiretroviral nucleotide phosphonoamidate prodrugs GS-7340 and GS-9131. *Antimicrob. Agents Chemother.* 51 (2), 543–550. doi: 10.1128/AAC.00968-06

Carlson, J. M., Schaefer, M., Monaco, D. C., Batorsky, R., Claiborne, D. T., Prince, J., et al. (2014). Selection bias at the heterosexual HIV-1 transmission bottleneck. *Science* 345 (6193), 1254031. doi: 10.1126/science.1254031

Cattaneo, D., and Gervasoni, C.(2018). Pharmacokinetics and Pharmacodynamics of Cabotegravir, a Long-Acting HIV Integrase Strand Transfer Inhibitor. *Eur. J. Drug Metab.Pharmacokinet.* 44, 319–327. doi: 10.1007/s13318-018-0526-2

Centers for Disease Control and Prevention. (2018). HIV Surveillance Report, 2017. 29, 17–18. Retrieved from http://www.cdc.gov/hiv/library/reports/hiv-surveillance.html

Chan, P. L. S., Weatherley, B., and McFadyen, L. (2008). A population pharmacokinetic meta-analysis of maraviroc in healthy volunteers and asymptomatic HIV-infected subjects. *Br. J. Clin. Pharmacol.* 65 (1), 76–85. doi: 10.1111/j.1365-2125.2008.03139.x

Chapman, T. M., McGavin, J. K., and Noble, S. (2003). Tenofovir Disoproxil Fumarate. *Drugs* 63 (15), 1597–1608. doi: 10.2165/00003495-200363150-00006

Choopanya, K. M. D., Martin, M. D., Suntharasamai, P. M. D., Sangkum, U. M. D., Mock, P. A. M., Leethochawalit, M. M. D., et al. (2013). Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 381 (9883), 2083–2090. doi: 10.1016/S0140-6736(13)61127-7

Collins, J. W., Heyward Hull, J., and Dumond, J. B. (2017). Comparison of tenofovir plasma and tissue exposure using a population pharmacokinetic model and bootstrap: a simulation study from observed data. *J. Pharmacokinet. Pharmacodyn.* 44 (6), 631–640. doi: 10.1007/s10928-017-9554-9

Cottrell, M. L., Yang, K. H., Prince, H. M. A., Sykes, C., White, N., Malone, S., et al. (2016). A translational pharmacology approach to predicting outcomes of preexposure prophylaxis against HIV in men and women using tenofovir disoproxil fumarate with or without emtricitabine. *J. Infect. Dis.* 214 (1), 55–64. doi: 10.1093/infdis/jiw077

Cottrell, M. L., Garrett, K. L., Prince, H. M. A., Sykes, C., Schauer, A., Emerson, C. W., et al. (2017). Single-dose pharmacokinetics of tenofovir alafenamide and itsactive metabolite in the mucosal tissues. *J. Antimicrob. Chemother.* 72 (6), 1731–1740. doi: 10.1093/jac/dkx064

Cundy, K. C., Sueoka, C., Lynch, G. R., Griffin, L., Lee, W. A., and Shaw, J. P.(1998). Pharmacokinetics and bioavailability of the anti-humanimmunodeficiency virus nucleotide analog 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) indogs. *Antimicrob. Agents Chemother.* 42 (3), 687–690. doi: 10.1128/AAC.42.3.687

de Béthune, M.-P. (2010). Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989–2009). *Antiviral Res.* 85 (1), 75–90. doi: 10.1016/j.antiviral.2009.09.008

De Clercq, E. (2016). Tenofovir alafenamide (TAF) as the successor of tenofovir disoproxil fumarate (TDF). *Biochem. Pharmacol.* 119, 1–7. doi: 10.1016/j.bcp.2016.04.015

De Clercq, E. (2018). Role of tenofovir alafenamide (TAF) in the treatment and prophylaxis of HIV and HBV infections. *Biochem. Pharmacol.* 153, 2–11. doi: 10.1016/j.bcp.2017.11.023

Dorr, P., Westby, M., Dobbs, S., Griffin, P., Irvine, B., Macartney, M., et al. (2005). Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human-immunodeficiency virus type 1 activity. *Antimicrob. Agents Chemother.* 49 (11), 4721–4732. doi: 10.1128/AAC.49.11.4721-4732.2005

Duwal, S., Schütte, C., and von Kleist, M. (2012). Pharmacokinetics and pharmacodynamics of the reverse transcriptase inhibitor tenofovir and prophylactic efficacy against HIV-1 infection. *PloS One* 7 (7), e40382. doi: 10.1371/journal.pone.0040382

Duwal, S., Sunkara, V., and Von Kleist, M.(2016). Multiscale Systems-Pharmacology Pipeline to Assess the Prophylactic Efficacy of NRTIs Against HIV-1. *CPT: Pharmacometrics Syst. Pharmacol.* 5 (7), 377–387. doi: 10.1002/psp4.12095

Duwal, S., Dickinson, L., Khoo, S., and von Kleist, M. (2019). Mechanistic framework predicts drug-class specific utility of antiretrovirals for HIV prophylaxis. *PloS Comput. Biol.* 15 (1), e1006740. doi: 10.1371/journal.pcbi.1006740

Fonner, V. A., Dalglish, S. L., Kennedy, C. E., Baggaley, R., O'Reilly, K. R., Koechlin, F. M., et al. (2016). Effectiveness and safety of oral HIV preexposure prophylaxis for all populations. *AIDS* 30 (12), 1973–1983. doi: 10.1097/QAD.0000000000001145

Gao, Y., and Katz, D. F. (2013). Multicompartmental pharmacokinetic model of tenofovir delivery by a vaginal gel. *PloS One* 8 (9), e74404. doi: 10.1371/journal.pone.0074404

Gao, Y., and Katz, D. F. (2017). Multicompartmental pharmacokinetic model of tenofovir delivery to the rectal mucosa by an enema. *PloS One* 12 (1), e0167696. doi: 10.1371/journal.pone.0167696

Garnett, G. P., and Anderson, R. M. (1993). Factors controlling the spread of HIV in heterosexual communities in developing countries: patterns of mixing between different age and sexual activity classes. *Philos. Trans. R. Soc. London. Ser. B: Biol. Sci.* 342 (1300), 137–159. doi: 10.1098/rstb.1993.0143

Garrett, K. L., Chen, J., Maas, B. M., Cottrell, M. L., Prince, H. A., Sykes, C., et al. (2018). A pharmacokinetic/pharmacodynamic model to predict effective HIV prophylaxis dosing strategies for people who inject drugs. *J. Pharmacol. Exp. Ther.* 367 (2), 245–251. doi: 10.1124/jpet.118.251009

Govil, S., and Katz, D. F. (2019). Deducing mucosal pharmacokinetics and pharmacodynamics of the Anti-HIV molecule tenofovir from measurements in blood. *Sci. Rep.* 9 (1), 82–10. doi: 10.1038/s41598-018-36004-z

Grant, R. M., Lama, J. R., Anderson, P. L., McMahan, V., Liu, A. Y., Vargas, L., et al. (2010). Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *New Engl. J. Med.* 363 (27), 2587–2599. doi: 10.1056/NEJMoa1011205

Grant, R. M. P., Anderson, P. L. P., McMahan, V. B., Liu, A. M. D., Amico, K. R. P., Mehrotra, M. M. P.H., et al. (2014). Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect. Dis.* 14 (9), 820–829. doi: 10.1016/S1473-3099(14)70847-3

Haberer, J. E., Bangsberg, D. R., Baeten, J. M., Curran, K., Koechlin, F., Amico, K. R., et al. (2015). Defining success with HIV pre-exposure prophylaxis: a prevention-effective adherence paradigm. *AIDS* 29 (11), 1277–1285. doi: 10.1097/QAD.0000000000000647

Halwes, M. E., Steinbach-Rankins, J. M., and Frieboes, H. B. (2016). Pharmacokinetic modeling of a gel-delivered dapivirine microbicide in humans. *Eur. J. Pharm Sci.* 93, 410–418. doi: 10.1016/j.ejps.2016.08.037

Hill, A., Hughes, S. L., Gotham, D., and Pozniak, A. L. (2018). Tenofovir alafenamide versus tenofovir disoproxil fumarate: isthere a true difference in efficacy and safety? *J. Virus Erad.* 4 (2), 72. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5892670/

Kay, K., Cook, S., Dezzutti, C., Anderson, P., Bushman, L., Shetler, C., et al. (2018a). Pharmacokinetic-pharmacodynamic modelling of MK-2048 in ex vivo cervical tissue. *Abstr. Annu. Meet. Popul. Ap. Group Europe*. 2018, 27. Abstract 8363. Retrieved from www.page-meeting.org/?abstract=8636

Kay, K., Shah, D. K., Rohan, L., and Bies, R. (2018b). Physiologically-based pharmacokinetic model of vaginally administered dapivirine ring and film formulations. *Br. J. Clin. Pharmacol.* 84 (9), 1950–1969. doi: 10.1111/bcp.13625

Kearney, B. P., Flaherty, J. F., and Shah, J. (2004). Tenofovir disoproxil fumarate: clinical pharmacology and pharmacokinetics 43, Generic, 595–612. doi: 10.2165/00003088-200443090-00003

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., et al. (1996). homozygous defect in HIV-1 Coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86 (3), 367–377. doi: 10.1016/S0092-8674(00)80110-5

Markowitz, M. P., Frank, I. P., Grant, R. M. P., Mayer, K. H. P., Elion, R. M. D., Goldstein, D. M. D., et al. (2017). Safety and tolerability of long-acting cabotegravir injections in HIV-uninfected men (ECLAIR): a multicentre, double-blind, randomised, placebo-controlled, phase 2a trial. *Lancet HIV* 4 (8), e331–e340. doi: 10.1016/S2352-3018(17)30068-1

Marrazzo, J. M., Ramjee, G., Richardson, B. A., Gomez, K., Mgodi, N., Nair, G., et al. (2015). Tenofovir-based preexposure prophylaxis for HIV infection among African women. *New Engl. J. Med.* 372 (6), 509–518. doi: 10.1056/NEJMoa1402269

McGowan, I. P., Dezzutti, C. S. P., Siegel, A. B. S., Engstrom, J. M. B. A., Nikiforov, A. B. S., Duffill, K. B. S., et al. (2016). Long-acting rilpivirine as potential pre-exposure prophylaxis for HIV-1 prevention (the MWRI-01 study): an open-label, phase 1, compartmental, pharmacokinetic and pharmacodynamic assessment. *Lancet HIV* 3 (12), e569–e578. doi: 10.1016/S2352-3018(16)30113-8

Modrzejewski, K. A., and Herman, R. A. (2004). Emtricitabine: a once-daily nucleoside reverse transcriptase inhibitor. *Ann. Pharmacother.* 38 (6), 1006–1014. doi: 10.1345/aph.1D302

Nagelkerke, N. J., Jha, P., Vlas, S.J.d., Korenromp, E. L., Moses, S., Blanchard, J. F., et al. (2002). Modelling HIV/AIDS epidemics in Botswana and India: impact of interventions to prevent transmission. *Bull. World Health Organ.* 80, 89–96. Retrieved from https://www.scielosp.org/article/bwho/2002.v80n2/89-96/

Nagelkerke, N. J., Moses, S., de Vlas, S. J., and Bailey, R. C. (2007). Modelling the public health impact of male circumcision for HIV prevention in high prevalence areas in Africa. *BMC Infect. Dis.* 7 (1), 16. doi: 10.1186/1471-2334-7-16

Nel, A., van Niekerk, N., Kapiga, S., Bekker, L.-G., Gama, C., Gill, K., et al. (2016). Safety and efficacy of a dapivirine vaginal ring for HIV prevention in women. *New Engl. J. Med.* 375 (22), 2133–2143. doi: 10.1056/NEJMoa1602046

Nel, A., Niekerk, N. V., Baelen, B. V., and Rosenberg, Z. (2018). "HIV incidence and adherence in DREAM: an open-label trial of dapivirine vaginal ring," in *25th Conference on Retroviruses and Opportunistic Infections (2018 CROI Foundation/International Antiviral Society-USA)* (Boston, Massachusetts). Abstract 144LB. Retrieved from http://www.croiconference.org/sessions/hiv-incidence-and-adherence-dream-open-label-trial-dapivirine-vaginal-ring

Nichols, B. E., Boucher, C. A., van Dijk, J. H., Thuma, P. E., Nouwen, J. L., Baltussen, R., et al. (2013). Cost-effectiveness of pre-exposure prophylaxis (PrEP) in preventing HIV-1 infections in rural Zambia: a modeling study. *PloS One* 8 (3), e59549. doi: 10.1371/journal.pone.0059549

Panos, G., and Watson, D. C. (2015). Effect of HIV-1 subtype and tropism on treatment with chemokine coreceptor entry inhibitors; Overview of viral entry inhibition. *Crit. Rev. in Microbiol.* 41 (4), 473–487. doi: 10.3109/1040841X.2013.867829

Phillips, A. N., Pillay, D., Garnett, G., Bennett, D., Vitoria, M., Cambiano, V., et al. (2011). Effect on transmission of HIV-1 resistance of timing of implementation of viral load monitoring to determine switches from first to second-line antiretroviral regimens in resource-limited settings. *Aids* 25 (6), 843–850. doi: 10.1097/QAD.0b013e328344037a

Rajoli, R. K. R., Back, D. J., Rannard, S., Freel Meyers, C. L., Flexner, C., Owen, A., et al. (2015). Physiologically based pharmacokinetic modelling to inform development of intramuscular long-acting nanoformulations for HIV. *Clin. Pharmacokinet.* 54 (6), 639–650. doi: 10.1007/s40262-014-0227-1

Ripamonti, D., Bombana, E., and Rizzi, M. (2014). Rilpivirine: drug profile of a second-generation non-nucleoside reverse transcriptase HIV-inhibitor. *Expert Rev. Anti-Infective Ther.* 12 (1), 13–29. doi: 10.1586/14787210.2014.863708

Ruane, P. J., DeJesus, E., Berger, D., Markowitz, M., Bredeek, U. F., Callebaut, C., et al. (2013). Antiviral Activity, Safety, and Pharmacokinetics/Pharmacodynamics of Tenofovir Alafenamide as 10-Day Monotherapy in HIV-1–Positive Adults. *JAIDS J. Acquir. Immune Defic. Syndr.* 63 (4), 449–455. doi: 10.1097/QAI.0b013e3182965d45

Shaw, J.-P., Sueoka, C. M., Oliyai, R., Lee, W. A., Arimilli, M. N., Kim, C. U., et al. (1997). Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) in Dogs. *Pharm. Res.* 14 (12), 1824–1829. doi: 10.1023/a:1012108719462

Spreen, W. R., Margolis, D. A., and Pottage, J. J. C. (2013). Long-acting injectable antiretrovirals for HIV treatment and prevention. *Curr. Opin. HIV AIDS* 8 (6), 565–571. doi: 10.1097/COH.0000000000000002

Thigpen, M. C., Kebaabetswe, P. M., Paxton, L. A., Smith, D. K., Rose, C. E., Segolodi, T. M., et al. (2012). Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *New Engl. J. Med.* 367 (5), 423–434. doi: 10.1056/NEJMoa1110711

Tsai, C.-C., Follis, K. E., Sabo, A., Beck, T. W., Grant, R. F., Bischofberger, N., et al. (1995). Prevention of SIV infection in Macaques by (R)-9-(2-Phosphonylmethoxypropyl)adenine. *Science* 270 (5239), 1197–1199. doi: 10.1126/science.270.5239.1197

U.S. Food and Drug Administration. (2012). Truvada NDA 21752/S-030 Supplement Approval Letter. Application number: 021752Orig1s30. Available: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/021752Orig1s030Approv.pdf

Valade, E., Tréluyer, J.-M., Bouazza, N., Ghosn, J., Foissac, F., Benaboud, S., et al. (2014). Population pharmacokinetics of emtricitabine in HIV-1-infectedadult patients. *Antimicrob. Agents Chemother.* 58 (4), 2256–2261. doi: 10.1128/AAC.02058-13

Valade, E., Tréluyer, J.-M., Illamola, S. M., Bouazza, N., Foissac, F., De Sousa Mendes, M., et al. (2015). Emtricitabine seminal plasma and blood plasma population pharmacokinetics in HIV-infected men in the EVARIST ANRS-EP 49 study. *Antimicrob. Agents Chemother.* 59 (11), 6800–6806. doi: 10.1128/AAC.01517-15

Van Damme, L., Corneli, A., Ahmed, K., Agot, K., Lombaard, J., Kapiga, S., et al. (2012). Preexposure Prophylaxis for HIV Infection among African Women. *New Engl. J. Med.* 367 (5), 411–422. doi: 10.1056/NEJMoa1202614

Van De Vijver, D. A., Nichols, B. E., Abbas, U. L., Boucher, C. A., Cambiano, V., Eaton, J. W., et al. (2013). Preexposure prophylaxis will have a limited impact on HIV-1 drug resistance in sub-Saharan Africa: a comparison of mathematical models. *Aids* 27 (18), 2943–2951. doi: 10.1097/01.aids.0000433237.63560.20

Vissers, D. C., Voeten, H. A., Nagelkerke, N. J., Habbema, J. D. F., and de Vlas, S. J. (2008). The impact of pre-exposure prophylaxis (PrEP) on HIV epidemics in Africa and India: a simulation study. *PloS One* 3 (5), e2077. doi: 10.1371/journal.pone.0002077

von Kleist, M., Menz, S., Stocker, H., Arasteh, K., Schütte, C., and Huisinga, W. (2011). HIV Quasispecies dynamics during pro-active treatment switching: impact on multi-drug resistance and resistance archiving in latent reservoirs. *PloS One* 6 (3), e18204. doi: 10.1371/journal.pone.0018204

von Kleist, M., Metzner, P., Marquet, R., and Schütte, C. (2012). HIV-1 polymerase inhibition by nucleoside analogs: cellular-and kinetic parameters of efficacy, susceptibility and resistance selection. *PloS Comput. Biol.* 8 (1), e1002359. doi: 10.1371/journal.pcbi.1002359

Conflict of Interest: RB has received consulting fees from Janssen Pharmaceuticals and grant funding from Takeda Pharmaceuticals, the US Department of Defense, and the US National Institutes of Health. KK was employed by Metrum Research Group.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 19 March 2020
doi: 10.3389/fphar.2020.00286

Urine Assay to Measure Tenofovir Concentrations in Patients Taking Tenofovir Alafenamide

Linden Lalley-Chareczko[1], Emily Hiserodt[1], Ganesh Moorthy[2], Athena Zuppa[2], Karam Mounzer[1] and Helen Koenig[1,3]*

[1] Philadelphia FIGHT Community Health Centers, Philadelphia, PA, United States, [2] Department of Anesthesiology and Critical Care Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA, United States, [3] The Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, United States

Background: HIV pre-exposure prophylaxis (PrEP) with tenofovir/emtricitabine is effective when taken daily. Previously, we developed a urine assay capable of detecting the prodrug tenofovir (TFV) in patients taking tenofovir disoproxil fumarate (TDF)-based PrEP. However, tenofovir alafenamide (TAF) has replaced TDF due to its different safety profile for HIV treatment and was recently approved as PrEP. Given the need to ensure the aforementioned assay remains available for the purpose of objective adherence monitoring, it is critical to ensure its accuracy for detecting TFV in patients taking TAF.

Methods: Blood and urine samples were collected from 3 cohorts of patients: (1) 10 participants living with HIV (PLWH) with suppressed virus on a TAF-based regimen, (2) 10 HIV-participants administered 1 dose of TAF/FTC followed by urine and plasma sampling for 7 days starting 1–3 h post-dose, and (3) 10 HIV-participants administered 7 doses of TAF/FTC followed by urine and plasma sampling for 10 days starting 1–3 h after the last dose. Samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with high sensitivity and specificity for TFV. HIV-samples were compared to a historical cohort administered one dose of TDF/FTC.

Results: PLWH were 90% male, 40% African American, and 10% Hispanic (mean age = 57 y; SD 8.88 y). HIV-participants were 55% male and 70% Caucasian (mean age = 31.6 y; SD 7.70 y). Samples from PLWH demonstrated TFV concentrations 2 logs higher in urine than plasma (1,000 ng/mL vs ±10 ng/mL) at the time of collection. Urine samples following a single dose of TAF in HIV-participants yielded TFV concentrations ranging from 100 to 1,000 ng/mL 1–3 h post-dose and remained >100 ng/mL for 6 days in 8 of 10 participants. Urine samples collected after 7 consecutive doses of TAF yielded TFV concentrations >1,000 ng/mL 1–3 h after dosing discontinuation, with TFV concentrations >1,00 ng/mL 7 days post discontinuation in 8 of 10 participants. Urine TFV concentrations following TAF administration were comparable to those from a historical cohort administered TDF/FTC. Plasma TFV concentrations were low(±10 ng/mL) in both HIV-cohorts at all time points.

OPEN ACCESS

Edited by:
Albert Liu,
San Francisco Department of Public Health, United States

Reviewed by:
Jose R. Castillo-Mancilla, University of Colorado Denver, United States
Jill Blumenthal, University of California, San Diego, United States

****Correspondence:***
Linden Lalley-Chareczko
lchareczko@fight.org

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *04 June 2019*
Accepted: *27 February 2020*
Published: *19 March 2020*

Citation:
Lalley-Chareczko L, Hiserodt E, Moorthy G, Zuppa A, Mounzer K and Koenig H (2020) Urine Assay to Measure Tenofovir Concentrations in Patients Taking Tenofovir Alafenamide. Front. Pharmacol. 11:286. doi: 10.3389/fphar.2020.00286

Conclusions: TFV persists in urine at detectable concentrations in participants taking TAF/FTC for at least 7 days despite largely undetectable plasma concentrations, with urine TFV concentrations comparable to patients taking TDF/FTC. This study demonstrates the ability of a urine TFV assay to measure recent TAF adherence.

Keywords: PrEP, HIV - human immunodeficiency virus, tenofovir alafenamide (TAF), tenofovir, therapaeutic drug monitoring

INTRODUCTION

Pre-exposure prophylaxis (PrEP) with emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) is highly effective in preventing HIV when taken daily (Garcia-Lerma et al., 2010; Grant et al., 2010; Myers and Mayer, 2011; Prejean et al., 2011; Baeten et al., 2012; Thigpen et al., 2012; Choopanya et al., 2013; Centers for disease control and prevention, 2014; Van Laarhoven et al., 2017), but patient self-report and pill counts are unreliable methods for monitoring adherence (Mimiaga et al., 2009; Poynten et al., 2010). Young men of color who have sex with men (yMSMc) and transgender women (TGW) often struggle to maintain adherence to PrEP (Mimiaga et al., 2009; Poynten et al., 2010; Brinker et al., 2014; Grant et al., 2014) despite high levels of self-reported adherence to PrEP (Hosek et al., 2012), and are heavily impacted by new HIV infections (Brunen et al., 2011; CDC, 2017; Hiemke, 2017).

How to accurately identify suboptimal adherence and develop targeted, strategic interventions to maintain necessary adherence levels for PrEP effectiveness represents a key gap in implementing this otherwise highly effective prevention therapy. Tenofovir (TFV) measurement in urine using liquid chromatography and tandem mass-spectrometry (LC-MS/MS) is a non-invasive and commercially available tool that can be used currently for objective monitoring of people taking FTC/TDF-based PrEP. In patients taking TDF-based regimens, it has been demonstrated that TFV concentrations can be reliably measured in urine, that urine TFV concentrations correlate well with plasma concentrations, and TFV detection in urine reflects medication usage over a window of 1 to at least 7 days after oral FTC/TDF ingestion (Koenig et al., 2017). In a 24-week study of 10 HIV-negative subjects receiving daily FTC/TDF for PrEP, urine TFV concentration >1,000 ng/mL was highly predictive of presence of TFV in plasma (>10 ng/mL) (PPV 0.95, 95%CI, 0.82–0.99; NPV 0.79, 95%CI, 0.49–0.95), suggesting that the urine assay could clearly identify patients who had not taken medication within the previous 48 h (i.e., recent adherence) as their urine TFV concentrations were <1,000 ng/mL, patients who had not taken any medication in the previous 7 days as their urine TFV concentrations were 0 ng/mL, as well as provide some information about intermittent/suboptimal dosing within the previous 7–10 days (>10 to >100 ng/mL; Koenig et al., 2017). Urine TFV testing in this context has also been shown to closely correlate with the research gold standard for objective adherence monitoring, dried blood spot (DBS; Patel et al., 2017), and is preliminarily highly acceptable to yMSMc, particularly among adolescents and young adults (Wertheimer et al., 2006; Liu et al., 2014; Koenig et al., 2017; Hunt et al., 2019). Additionally, urine TFV assessment fills a gap left by plasma, DBS, and hair assessments by providing information about medication adherence over at least a 7 day period: single plasma concentrations only reflect a small window of exposure (2–3 days; Clevenbergh et al., 2002; Nettles et al., 2006; Castillo-Mancilla et al., 2013), and hair analysis and DBS reflect average drug exposures over 1–3 months (Garrett et al., 2019; Hare et al., 2019). In yMSMc, a population known to struggle with adherence (Mimiaga et al., 2009; Poynten et al., 2010), current (previous week) non-adherence data may have greater value than average non-adherence over the prior 3 months given increased vulnerability to HIV exposure, and may create a greater number of opportunities for clinicians to reinforce PrEP adherence behaviors (Koenig et al., 2017; Hunt et al., 2019).

Tenofovir alafenamide (TAF) is replacing TDF as an equally effective tenofovir prodrug in HIV treatment regimens, i.e., TAF/FTC/EVG/COBI, TAF/RPV/FTC, and FTC/TAF and has recently been FDA approved as an alternative oral PrEP agent (Garrett et al., 2019; Hare et al., 2019). When compared to standard dose TDF (300 mg/daily), TAF, at a dose of 25 mg/day, has a 7-fold higher peripheral blood mononuclear cell intracellular tenofovir diphosphate concentration, with only∼10% of the plasma tenofovir exposure. At steady state, 25 mg of TAF yielded mean TFV plasma exposures [area under the plasma concentration-time curve (AUCtau)] of 86% lower as compared with the TFV exposures observed with 300 mg of TDF. Increased intracellular concentrations may translate into FTC/TAF's greater antiviral efficacy, a higher barrier to resistance, and an improved safety profile relative to TDF (Ray et al., 2016). Recent findings from the DISCOVER trial, in which 5,387 at-risk adults were randomized to daily FTC/TAF vs. daily FTC/TDF, indicate non-inferiority of FTC/TAF as PrEP relative to FTC/TDF, with a significantly lower overall seroconversion rate than anticipated by investigators (0.26/100PY; Hare et al., 2019).

Given these data, the primary objective of the present study was to determine how long TFV is excreted in the urine of participants who have taken one dose or seven daily doses of FTC/TAF. Based on the pharmacokinetics of TAF, we hypothesized cut-offs indicative of no/intermittent/recent adherence in patients on TAF-based regimens would be approximately 1 log (or 10-fold) lower than those in patients taking TDF-based regimens. Accordingly, we hypothesized the TFV concentration in urine associated with concentrations in plasma for patients on TAF-based regimens would be 100 ng/mL instead of 1,000 ng/mL (i.e., 1 log lower). We also hypothesized

that TFV detectability would persist at least 1–2 days longer in the urine of participants dosing at steady state vs. those with limited FTC/TAF exposure.

METHODOLOGY

Study Setting

Participant recruitment/enrollment and sample collection were conducted at Philadelphia FIGHT Community Health Centers, an urban community-based federally qualified health center. Urine and plasma TFV analyses were performed by the Children's Hospital of Philadelphia (CHOP) Pharmacology Research Unit. Urine samples were sent to a local, commercial laboratory for assessment of specific gravity, urine creatinine, and pH to control for inter-subject variability.

Study Design and Participant Recruitment

This study employed a sequential, 3-cohort design with a sample size of 10 for each cohort (total $n = 30$). All participants were 18 years of age or older and able to provide written informed consent in English. PLWH were recruited via electronic medical record prescreening and face-to-face requests during clinic visits to determine interest in the study. HIV-negative participants were recruited by flyers and word of mouth from FIGHT associated clinics and the surrounding community. This study was approved by the Institutional Review Board at Philadelphia FIGHT.

The first cohort employed both a qualitative and semi-quantitative evaluation of the relationship between urine and plasma TFV in 10 PLWH with undetectable viral loads for greater than 12 weeks prior to consent per available medical records and a recent undetectable viral load in the previous 4 weeks on an antiretroviral regimen containing FTC/TAF (i.e., Genvoya™, Odefsey™, or Descovy™ in combination with another HIV medication or medications). Participants returned to the clinic at their convenience and underwent a one-time, pre-dose urine and plasma collection for TFV concentration analyses, reflecting drug concentrations ∼24 h after last medication ingestion. Participants also kept a daily diary of FTC/TAF dosing for the 3 days prior to sample collection.

The second and third cohorts enrolled HIV negative participants who were given either a single dose (cohort 2) or 7 daily doses (cohort 3) of FTC/TAF, with the study design of the 7 dose cohort based on data showing that 7 daily doses of FTC/TDF achieves therapeutic drug concentrations consistent with protection from HIV (Wertheimer et al., 2006). These cohorts underwent additional laboratory screenings for acute or chronic hepatitis B infection, renal dysfunction (Creatinine Clearance < 50 mL/min by Cockroft-Gault equation), and/or DAIDS grade 3 laboratory abnormality at screening, and were asked to report any history of severe infections requiring treatment such as tuberculosis, bone fractures not explained by trauma and/or a known allergy/sensitivity to the study FTC/TAF or its components in accordance with standard of care practices when prescribing FTC/TAF. Concurrent participation in an HIV vaccine study or concurrent use of any other antiretroviral agent were also assessed by participant self-report. Morning urine and plasma samples were then collected starting 1 h post-dose and for 6 (cohort 2) or 9 (cohort 3) consecutive days thereafter.

TABLE 1 | Participant demographics.

	Cohort 1	Cohort 2	Cohort 3
Mean age (range)	57 (51–79) years	33 (23–47) years	30 (23–43) years
Race % (*n*)			
Caucasian	60% (6)	70% (7)	60% (6)
Black/African American	40% (4)	20% (2)	20% (2)
Asian	0	10% (1)	10% (1)
Other	0	0	10% (1)
Ethnicity % (*n*)			
Hispanic	10% (1)	0	0
Non-hispanic	90% (9)	100% (10)	100% (10)
Gender % (*n*) No participants identified as transgender			
Male	90% (9)	60% (6)	50% (5)
Female	10% (1)	30 (3)	40% (4)
Gender Non-conforming	0	10% (1)	10% (1)

Sample Processing

Urine and plasma samples were stored at −78°C until analysis. TFV concentrations are stable at least for 48 weeks at −78°C (Lalley-Chareczko et al., 2018). Urine samples were diluted 50-fold in blank plasma, extracted and then analyzed by LC-MS/MS. Pre-dilution of urine samples with blank human plasma was utilized as an approach to minimize the impact of urine characteristics in causing variability in TFV concentration measurements. Plasma samples were analyzed without dilution. The range of concentration in diluted urine samples were below lower limit of quantitation (10 ng/mL) to 500 ng/mL (assay range: 10–10,000 ng/mL; Koenig et al., 2017).

RESULTS

Cohort 1 (Participants living with HIV): PLWH were 90% male, 10% female, 40% African American, 60% Caucasian, and 10% Hispanic. The median age was 53.5 years, with a range of 51 to 79 years (**Table 1**). Recorded HIV treatment regimens in this cohort included FTC/TAF plus one of the following: dolutegravir (3), boosted elvitegravir (3), boosted darunavir (2), raltegravir (1), or rilpivirine (1). Urinalysis laboratory results were collected for future studies and are listed in **Appendix A** in Supplementary Material. Urine and plasma samples from PLWH were collected between 13.5 and 28.3 h after the last TAF containing medication dose as per participant report (m = 20.2 h); urine and plasma samples were collected, on average, 4.4 min apart (min = 1 min; max = 14 min). Urine samples from PLWH demonstrated TFV concentrations 2 logs higher than plasma (>1,000 ng/mL vs. ±10 ng/mL, respectively); plasma TFV concentrations were low (70% >10 ng/mL; 30% <10 ng/mL) for all PLWH (**Figure 1**).

Cohorts 2 & 3: HIV-negative participants, collectively, were 55% male, 45% female, 65% Caucasian, 20% Black/African American, 10% Asian, and 5% endorsing other racial backgrounds. The median age for HIV-negative patients

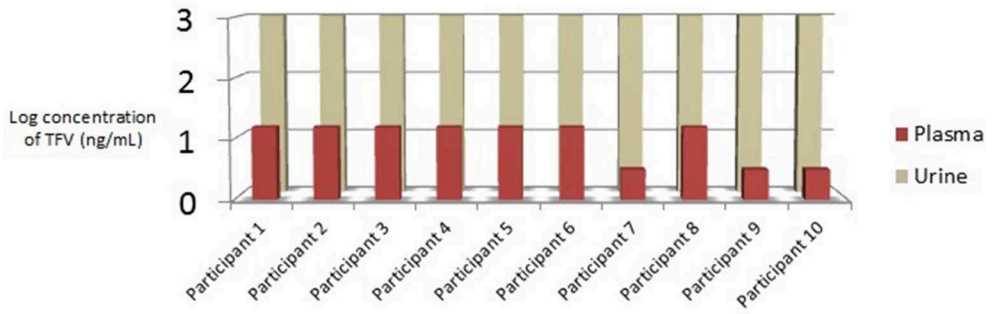

FIGURE 1 | Cohort 1: Relationship between urine and plasma TFV concentrations in PLWH taking daily TAF. TFV, tenofovir; PLWH, Participants Living with HIV; TAF, tenofovir alafenamide; ng/mL, nanograms per millimeter.

TABLE 2 | Observed urine concentrations from the single dose cohort.

Days post TAF dosing	% Urine TFV >10,000 ng/mL	% Urine TFV >1,000 ng/mL	% Urine TFV >100 ng/mL	% Urine TFV >10 ng/mL	% Urine TFV <10 ng/mL
0	–	60	40	–	–
1	–	60	40	–	–
2	–	30	70	–	–
3	–	20	80	–	–
4	–	–	100	–	–
5	–	–	90	10	–
6	10*	–	70	10	10

TFV, tenofovir; HIV+, Human Immunodeficiency Virus-Positive; TAF, tenofovir alafenamide; ng/mL, nanograms per milliliter.
**Verified upon repeat analysis.*

was 30.5 years with a range of 23–47 years (**Table 1**). As in Cohort 1, urinalysis results are listed in **Appendix A** in Supplementary Material.

Cohort 2: Urine samples collected following a single dose of FTC/TAF in all 10 HIV-negative participants yielded TFV semi-quantitative concentrations ranging from 100 to >1,000 ng/mL 1–3 h post-dose, with 6 of 10 participant samples (60%) demonstrating TFV concentrations >1,000 ng/mL at that time. At 24 h post-dose, 6 of 10 participant samples had urine TFV concentrations >1,000 ng/mL; however, samples did not necessarily come from the same participants as those who had TFV concentrations >1,000 ng/mL 1–3 h post-dose. For example, participant #1 had TFV concentrations >100 ng/mL 1–3 h post-dose and 24 h post-dose, whereas participants 2, 7, and 10 had TFV concentrations increase from >100 ng/mL 1–3 h post-dose to >1,000 ng/mL 24 h post-dose. Participants 3, 4, and 9 had urine TFV concentrations >1,000 ng/mL 1–3 h post-dose that fell to >100 ng/mL 24 h post-dose. Participants 5, 6, and 8 had urine TFV concentrations that remained consistent at >1,000 ng/mL from 1–3 to 24 h post-dose. Urine TFV concentrations observed over the remaining 5 days of collection displayed a downward trend with the same variability described above (**Table 2**; **Figure 2**).

These concentrations were lower than those from a historical cohort administered FTC/TDF (Koenig et al., 2017); urine TFV concentrations rose more rapidly after medication ingestion in subjects receiving FTC/TDF and were, on average, higher for the first 4 days after discontinuation of medication compared to those receiving FTC/TAF (**Figure 3**). Sixty percent of samples from participants dosed with FTC/TDF displayed urine TFV concentrations >10,000 ng/mL 1–3 and 24 h after dosing, whereas 60% of participants reached the >1,000 ng/mL concentration 1–3 and 24 h after a single FTC/TAF dose. One hundred percent of patients dosed with FTC/TDF displayed urine TFV concentrations >1,000 ng/mL through the second day post dose, and 90% continued to display this concentration on the 3rd sampling day. However, after a single FTC/TAF dose, TFV concentrations dropped below 1,000 ng/mL in 70% of samples 2 days after dosing and 80% of samples 3 days after dosing (**Figure 3**).

Plasma concentrations from the single-dose cohort remained low throughout sample collection, as expected given the plasma penetration of TAF. Plasma TFV concentrations 1–3 h post-dose were undetectable in 1 subject (10%), detectable but <10 ng/mL in 5 (50%) subjects, and <100 ng/mL in 4 (40%) of subjects, with plasma TFV concentrations falling quickly over subsequent collections (**Figure 3**). These concentrations are low in comparison to the historical TDF cohort where 70% of samples displayed >100 ng/mL 1–3 h after dosing and 100% maintained low-level detectability 24 h after dosing.

Cohort 3: Urine samples collected after 7 consecutive doses of FTC/TAF (steady state dosing) yielded TFV concentrations

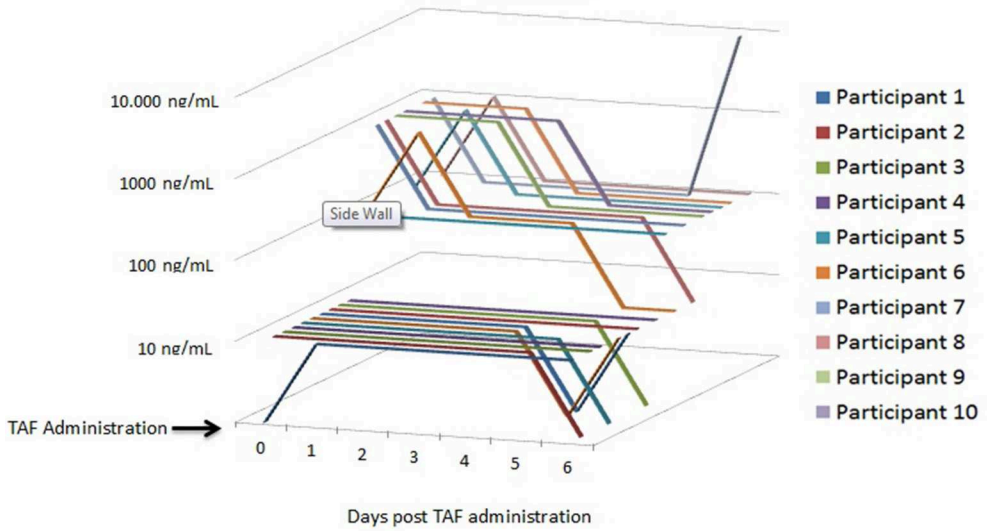

FIGURE 2 | Cohor t 2: Urine/Plasma TFV Concentrations following a Single Dose of FTC/TAF in 10 HIV-Participants. TFV, tenofovir; FTC/TAF, emtricitabine/tenofovir alafenamide; TAF, tenofovir alafenamide; ng/mL, nanograms per millimeter.

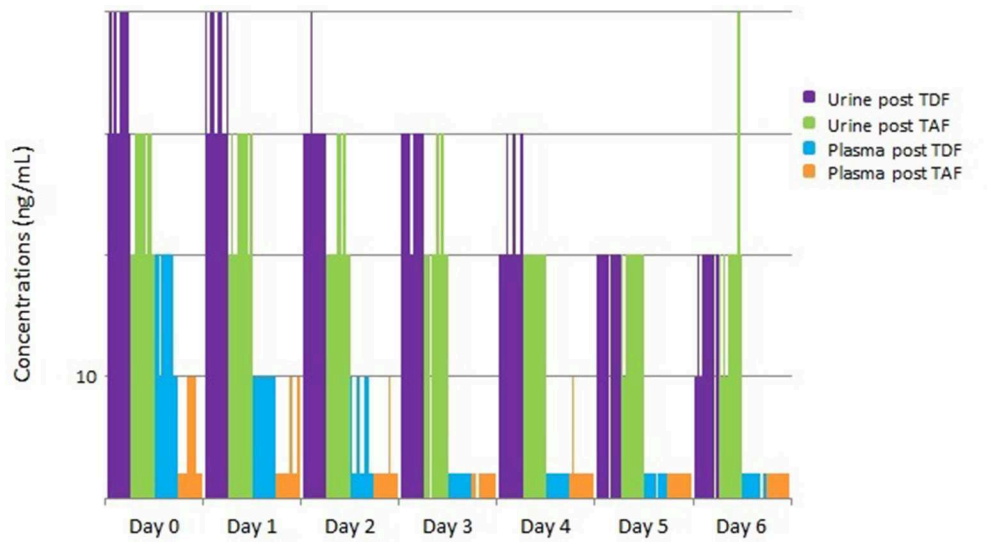

FIGURE 3 | Urine/Plasma TFV concentrations after a single dose of FTC/TAF in 10 HIV-subjects, with comparison to historical cohort of subjects given a single dose of FTC/TDF. TFV, tenofovir; FTC/TAF, emtricitabine/tenofovir alafenamide; HIV–, Human Immunodeficiency Virus-Negative; FTC/TDF: emtricitabine/tenofovir disoproxil fumarate; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; ng/mL, nanograms per millimeter.

>1,000 ng/mL 1–3 h after discontinuation of dosing in all 10 participants with TFV concentrations remaining >1,000 ng/mL in 80% samples collected 24 after dosing discontinuation. As in the single dose cohort, urine TFV concentrations remained detectable, with 80% samples yielding TFV concentrations >1,000 ng/mL at 2 and 3 days post dosing discontinuation; however, these were not necessarily the samples coming from the same participant, as described above. As in the single-dose cohort, participant samples displayed a downward trend with variability similar to the single-dose cohort over the remaining 6 days of sample collection (**Table 3**; **Figure 4**). In comparison to subjects who took a single dose of FTC/TAF, participants at steady state demonstrated urine TFV concentrations that started higher immediately post-dose, and remained higher at all measured time points, reflecting a longer "look-back period" (period of time from when sample is collected that a clinician may have insight into recent dosing, i.e., in this case 7–10 days) in participants taking daily FTC/TAF. Plasma samples collected after 7 consecutive doses of FTC/TAF

TABLE 3 | Observed urine concentrations from the seven dose cohort.

Days post TAF dosing	% Urine TFV >10,000 ng/mL	% Urine TFV >1,000 ng/mL	% Urine TFV >100 ng/mL	% Urine TFV >10 ng/mL	% Urine TFV <10 ng/mL
0	–	100	–	–	–
1	–	80	20	–	–
2	–	80	20	–	–
3	–	80	20	–	–
4	–	30	70	–	–
5	–	30	70	–	–
6	–	20	80	–	–
7	–	20	70	10	–
8	–	–	90	10	–
9	–	–	90	10	–

TFV, tenofovir; HIV+, Human Immunodeficiency Virus-Positive; TAF, tenofovir alafenamide; ng/mL, nanograms per milliliter.

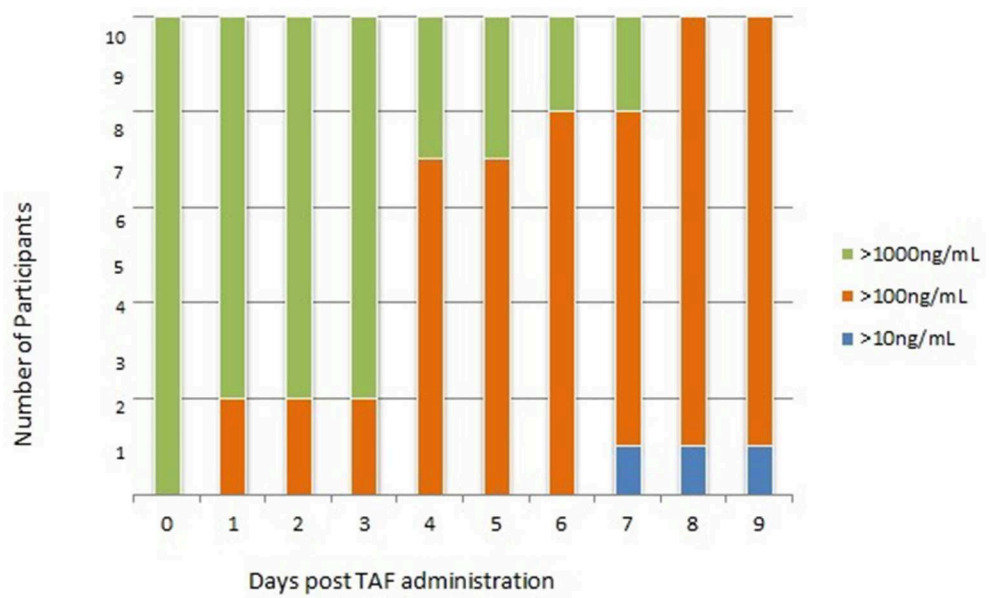

FIGURE 4 | Cohort 3: Urine TFV Concentrations following 7 consecutive doses of FTC/TAF in 10 HIV-subjects. TFV, tenofovir; FTC/TAF, emtricitabine/tenofovir alafenamide; HIV–, Human Immunodeficiency Virus-Negative; TAF, tenofovir alafenamide; ng/mL, nanograms per millimeter.

yielded TFV concentrations similar to those observed in the single-dose cohort.

DISCUSSION

The primary objective of this study was to determine the pattern of TFV excretion in the urine and plasma of participants taking FTC/TAF. Based on the published pharmacokinetics of TAF, we hypothesized TFV concentrations in the urine and plasma of participants taking FTC/TAF, whether living with HIV or not, would be approximately 1 log (or 10-fold) lower than those observed in a historical cohort of participants taking FTC/TDF, and the TFV concentration in urine associated with concentrations in plasma for participants on TAF-based regimens would be 100 ng/mL instead of 1,000 ng/mL (i.e., 1 log lower).

The results from this study demonstrate that TFV concentrations in urine and plasma samples were indeed approximately 10-fold (1 log) lower in patients taking FTC/TAF relative to those taking FTC/TDF, with only 60% of participants in the (comparable) single-dose study exceeding the previously established clinical cut-off for recent adherence of 1,000 ng/mL within 24 h after the last FTC/TAF dose. However, in the steady state cohort (cohort 3), urine TFV concentrations were >1,000 ng/mL within 24 h after the last FTC/TAF dose in the majority (80%) of patients. The observed 1 log differential (both in urine and plasma) between TFV from TDF and TAF dosing is expected given the known pharmacokinetics of both drugs. Additionally, both formulations of tenofovir (TDF and TAF) are

renally cleared, thus generating higher urine TFV concentrations than plasma.

The present study also compared the excretion pattern of TFV in patients taking daily FTC/TAF for 7 days (i.e., steady state dosing) to the TFV excretion patterns in those who have taken FTC/TAF for extremely short periods of time (i.e., a single dose). TFV remained detectable in the urine of participants exposed to 7 consecutive doses of FTC/TAF for 9 days after the discontinuation of daily dosing, extending the period of detectability from that observed after a single FTC/TAF dose (6 days). Urine TFV concentrations >1,000 ng/mL, suggestive of recent adherence, persisted for 48–96 h for the majority (80%) of participants, similar to TFV concentrations observed in a historical cohorts of patients taking FTC/TDF (Koenig et al., 2017); in comparison, only 30% of urine samples contained concentrations >1,000 ng/mL 48 h after a single dose of FTC/TAF. None of the 7-dose cohort samples reached "undetectability" (<10 ng/mL) in urine during the 10-day observation period. The longer period in which TFV was detected post-dosing in the 7-day cohort relative to the single-dose cohort is likely due to higher drug concentrations achieved through steady state dosing and thus a longer time to clear completely from the urine.

Exploring TFV excretion patterns using a cohort of patients dosing at steady state may provide a more robust understanding of urinary TFV cut-offs indicative of recent PrEP dosing once more information is available about duration of protection after stopping FTC/TAF. For example, MSM taking daily FTC/TDF are considered to have protective TFV concentrations for rectal exposure to HIV until 7 days after last dose (Anderson et al., 2016). If the same is shown to be true for patients taking FTC/TAF, and with FDA approval of FTC/TAF for the purpose of PrEP, then the data from this study may provide a basis for the formulation of standard urinary TFV concentrations consistent with protection against HIV in this population. Furthermore, establishing urine TFV norms for persons taking FTC/TAF for PrEP is an important step for prescribers and providers who order urine TFV testing as a measure of adherence and tailor supportive counseling based on those results.

The present study is limited by a fair degree of subject-to-subject variability in concentrations over the washout period. As a result, it was more difficult to identify indications at time points that would be clinically useful for physicians, compared to those previously established for patients taking FTC/TDF. A next step would include determination of the best way to correct urine TFV values for inter-subject variability by assessing which measure (specific gravity, urine creatinine, pH) will maximize the correlation between urine TFV concentrations and an ideal line of elimination. Secondly, as all 10 participants in the "steady state" cohort still had detectable tenofovir in their urine at the end of the study period (10 days after final dose of FTC/TAF) using uncorrected urine TFV concentrations, we are not able to establish definitive thresholds for imperfect recent adherence (more than 24–48 days post-dose) and non-adherence.

However, at the time of this writing, a fully quantitative assay is being developed and validated by UrSure, Inc, which will be able to provide more specific thresholds for these categories of adherence and may also reveal more nuanced variation between cut-off values for the urine TFV assay in patients taking FTC/TAF vs. those taking FTC/TDF.

This study demonstrates the feasibility of using a urine TFV assay to assess recent adherence to TAF using similar cutoffs to those in patients taking TDF-based regimens and provides proof of concept to further develop this assay for use in patients taking TAF-based regimens. Future efforts will focus on refining these cut-offs for patients taking TDF- and TAF-based regimens using a fully quantitative assay, as well as better addressing the differences in urinary TFV clearance patterns between TDF and TAF-based regimens.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Philadelphia FIGHT Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HK and KM were responsible for protocol conceptualization and oversight. LL-C and EH were responsible for participant recruitment, sample collection, and data management. GM and AZ ran sample analyses via liquid chromatography-tandem mass spectrometry. LL-C and HK were primarily responsible for drafting the manuscript, however, all authors participated in revisions and edits.

FUNDING

This research was funded by Gilead Sciences.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00286/full#supplementary-material

REFERENCES

Anderson, P. L., García-Lerma, J. G., and Heneine, W. (2016). Nondaily preexposure prophylaxis for HIV prevention. *Curr. Opin. HIV AIDS* 11, 94–101. doi: 10.1097/COH.0000000000000213

Baeten, J. M., Donnell, D., Ndase, P., Mugo, N. R., Campbell, J. D., and Wangisi, J. (2012). Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N. Engl. J. Med.* 367, 399–410. doi: 10.1056/NEJMoa1108524

Brinker, S., Pandey, A., Ayers, C., Price, A., Raheja, P., Arbique, D., et al. (2014). Therapeutic drug monitoring facilitates blood pressure control in resistant

hypertension. *J. Am. Coll. Cardiol.* 63, 834–835. doi: 10.1016/j.jacc.2013.10.067

Brunen, S., Vincent, P. D., Baumann, P., Hiemke, C., and Havemann-Reinecke, U. (2011). Therapeutic drug monitoring for drugs used in the treatment of substance-related disorders: literature review using a therapeutic drug monitoring appropriateness rating scale. *Ther. Drug Monit.* 33, 561–572. doi: 10.1097/FTD.0b013e31822fbf7c

Castillo-Mancilla, J. R., Zheng, J. H., Rower, J. E., Meditz, A., Gardner, E. M., Predhomme, J., et al. (2013). Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. *AIDS Res. Hum. Retroviruses* 29, 384–390. doi: 10.1089/aid.2012.0089

CDC (2017). *HIV Surveillance Report 2016*. Available online at: https://www.cdc.gov/hiv/pdf/library/reports/surveillance/cdc-hiv-surveillance-report-2015-vol-27.pdf (accessed June 27, 2017).

Centers for disease control and prevention (2014). *Preexposure Prophylaxis for the Prevention of HIV Infection in the United States – 2014 Clinical Practice Guideline [Homepage on the Internet]*. Available online at: http://www.cdc.gov/hiv/pdf/prepguidelines2014.pdf

Choopanya, K., Martin, M., Suntharasamai, P., Sangkum, U., Mock, P. A., Leethochawalit, M., et al. (2013). Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok tenofovir study): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 381, 2083–2090. doi: 10.1016/S0140-6736(13)61127-7

Clevenbergh, P., Garraffo, R., Durant, J., and Dellamonica, P. (2002). PharmAdapt: a randomized prospective study to evaluate the benefit of therapeutic monitoring of protease inhibitors: 12 week results. *AIDS* 16, 2311–2315. doi: 10.1097/00002030-200211220-00011

Garcia-Lerma, J. G., Paxton, L., Kilmarx, P. H., and Heneine, W. (2010). Oral pre-exposure prophylaxis for HIV prevention. *Trends Pharmacol. Sci.* 31,74–81. doi: 10.1016/j.tips.2009.10.009

Garrett, K. L., Cottrell, M. L., Prince, H. M., Sykes, C., Schauer, A., and Peery, A. (2019). "Concentrations of TFV and TFVdp in Female Mucosal Tissues After a Single Dose of TAF," in *26th Conference on Retroviruses and Opportunistic Infections* (Seattle, WA).

Grant, R. M., Anderson, P. L., McMahan, V., Liu, A., Amico, K. R., Mehrotra, M., et al. (2014). Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet* 14, 820–829. doi: 10.1016/S1473-3099(14)70847-3

Grant, R. M., Lama, J. R., Anderson, P. L., McMahan, V., Liu, A. Y., Vargas, L., et al. (2010). Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N. Engl. J. Med.* 363, 2587–2599. doi: 10.1056/NEJMoa1011205

Hare, C. B., Josep, C., Ruane, P., Molina, J. M., Mayer, K. H., Jessen, H., et al. (2019). "The phase 3 Discover Study: daily F/TAF or F/TDF for HIV preexposure prophylaxis," in *23rd Conference on Retroviruses and Opportunistic Infections* (Boston, MA).

Hiemke, C. (2017). Clinical utility of drug measurement and pharmacokinetics: therapeutic drug monitoring in psychiatry. *Eur. J. Clin. Pharmacol.* 64, 159–166. doi: 10.1007/s00228-007-0430-1

Hosek, S., Siberry, G., Bell, M., Lally, M., Kapogiannis, B., Green, K., et al. (2012). The Adolescent Trials Network for HIVAIDS Interventions (ATN). The acceptability and feasibility of an HIV pre-exposure prophylaxis (PrEP) trial with young men who have sex with men (YMSM). *J. Acquir. Immune Defic. Syndr.* 62, 447–456. doi: 10.1097/QAI.0b013e3182801081

Hunt, T., Lalley-Chareczko, L., Daughtridge, G., Swyryn, M., and Koenig, H. (2019). Challenges to PrEP use and perceptions of urine tenofovir adherence monitoring reported by individuals on PrEP. *AIDS CARE* 31, 1203–1206. doi: 10.1080/09540121.2019.1587369

Koenig, H. C., Mounzer, K., Daughtridge, G. W., Sloan, C. E., Lalley-Chareczko, L., Moorthy, G. S., et al. (2017). Urine assay for tenofovir to monitor adherence in real time to tenofovir disoproxil fumarate/emtricitabine as pre-exposure prophylaxis. *HIV Med.* 18, 412–418. doi: 10.1111/hiv.12518

Lalley-Chareczko, L., Clark, D., Conyngham, C., Zuppa, A., Moorthy, G., Mounzer, K., et al. (2018). Delivery of TDF/FTC for pre-exposure prophylaxis to prevent HIV-1 Acquisition in young adult men who have sex with men and transgender women of color using a urine adherence assay. *J. AIDS* 79, 173–178. doi: 10.1097/QAI.0000000000001772

Liu, A. Y., Yang, Q., Huang, Y., Bacchetti, P., Anderson, P. L., Jin, C., et al. (2014). Strong relationship between oral dose and tenofovir hair levels in a randomized trial: hair as a potential adherence measure for pre-exposure prophylaxis (PrEP). *PLoS ONE* 9:e83736. doi: 10.1371/journal.pone.0083736

Mimiaga, M. J., Case, P., Johnson, C. V., Safren, S. A., and Mayer, K. H. (2009). Preexposure antiretroviral prophylaxis attitudes in high-risk Boston area men who report having sex with men: limited knowledge and experience but potential for increased utilization after education. *J. Acquir. Immune Defic. Syndr.* 50, 77–83. doi: 10.1097/QAI.0b013e31818d5a27

Myers, G. M., and Mayer, K. H. (2011). Oral preexposure anti-HIV prophylaxis for high-risk U.S. populations: current considerations in light of new findings. *AIDS Patient Care STDS* 25, 63–71. doi: 10.1089/apc.2010.0222

Nettles, R. E., Kieffer, T. L., Parsons, T., Johnson, J., Cofrancesco, J. Jr., Gallant, J. E., et al. (2006). Marked intraindividual variability in antiretroviral concentrations may limit the utility of therapeutic drug monitoring. *Clin. Infect. Dis.* 42, 1189–1196. doi: 10.1086/501458

Patel, R. R., Amico, R., Harrison, L. C., Lalley-Chareczko, L., Anderson, P., Presti, R., et al. (2017). "Comparing Urine with Dried Blood Spots to Assess Tenofovir Levels for HIV Pre-Exposure Prophylaxis Adherence (Abstract 976)" *Poster Presentation at the International AIDS Society Conference* (Paris).

Poynten, I. M., Jin, F., Prestage, G. P., Kaldor, J. M., Imrie, J., and Grulich, A. E. (2010). Attitudes towards new HIV biomedical prevention technologies among a cohort of HIV-negative gay men in Sydney, Australia. *HIV Med.* 11, 282–288. doi: 10.1111/j.1468-1293.2009.00777.x

Prejean, J., Song, R., Hernandez, A., Ziebell, R., Green, T., Walker, D., et al. (2011). Estimated HIV Incidence in the United States, 2006–2009. *PLoS ONE* 6:e17502. doi: 10.1371/journal.pone.0017502

Ray, A. S., Fordyce, M. W., and Hitchcock, M. J. (2016). Tenofovir alafenamide: a novel prodrug of tenofovir for the treatment of human immunodeficiency virus. *Antiviral Res.* 125, 63–70. doi: 10.1016/j.antiviral.2015.11.009

Thigpen, M. C., Kebaabetswe, P. M., Paxton, L. A., Smith, D. K., Rose, C. E., Segolodi, T. M., et al. (2012). Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N. Engl. J. Med.* 367, 423–434. doi: 10.1056/NEJMoa1110711

Van Laarhoven, A., Hoornenborg, E., Achterbergh, R., De Vries, H., Prins, M., and Van Der Loeff, M. S. (2017). Changes in sexual risk behavior among daily PrEP users after 6 months of use in the Amsterdam PrEP project. *Sex. Transm. Infect.* 93:A20. doi: 10.1136/sextrans-2017-053264.50

Wertheimer, B. Z., Freedberg, K. A., Walensky, R. P., Yazdanapah, Y., and Losina, E. (2006). Therapeutic drug monitoring in HIV treatment: a literature review. *HIV Clin. Trials* 7, 59–69. doi: 10.1310/4CCJ-KT1T-H6PV-NLFC

Conflict of Interest: Philadelphia FIGHT received research funds from Gilead Sciences for the conduct of this research. Funds provided covered the cost of laboratory analyses, patient stipends, and travel/publication costs. As a funder, Gilead Sciences approved our independently drafted study design, but had no further role in study implementation, data collection and analysis, decision to publish, or preparation of the manuscript. HK, and KM are both paid consultants for Gilead Sciences. HK is also co-founder of UrSure, Inc., developers and manufacturers of urine assays to monitor medication adherence (http://www.ursureinc.com) based in Boston, Massachusetts, USA.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership