CLINICAL PHARMACOLOGY CHALLENGES IN THE DEVELOPMENT OF HIV PRE-EXPOSURE PROPHYLAXIS IN MEN WHO HAVE SEX WITH MEN AND TRANSGENDER WOMEN

by
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ABSTRACT

Men who have sex with men (MSM) and transgender women (TGW) are vulnerable populations at high risk for HIV infection. Existing HIV prevention methods including oral preexposure prophylaxis (PrEP) do not adequately accommodate the diverse practices and needs of these vulnerable populations. The overarching theme of the thesis is developing PrEP that takes into account the lifestyle and preferences of MSM and TGW. Specifically, the thesis responds to the desire by MSM for a medicated lubricant as PrEP and the value that TGW place on gender affirming hormone therapy (GAHT) when considering PrEP.

Chapter 2 is an open-label study of the colorectal distribution and retention of a rectal gel applied with manual dosing as a sexual lubricant compared with applicator dosing. SPECT/CT was used to measure distribution and retention 4 hours after application of radiolabeled gel. Compared to applicator dosing, manual dosing resulted in delivery of a smaller and more variable dose with more variable colonic distribution.

Chapter 3 is a pharmacokinetic study evaluating the effects of GAHT on the plasma and tissue concentrations of inactive and active forms of tenofovir (TFV) and emtricitabine (FTC) in TGW on GAHT and cisgender men (CGM) not on GAHT. Participants were dosed for 7 days with oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) and underwent blood and colon sampling for pharmacokinetics. We found that GAHT modestly reduced TFV and FTC plasma concentrations.

Combined together, the two studies presented in this thesis contribute to the development of HIV PrEP that values the desires, preferences and needs of MSM and TGW. Further studies are needed to further understand the pharmacokinetics of rectal microbicide gel applied as a sexual lubricant and provide greater insight for TGW on the interaction between PrEP and GAHT.

Dissertation Readers

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Finally, I would like to give a special thanks to my family who instilled in me perseverance, work ethic and a love for learning - values that this thesis demanded. To them, I dedicate this thesis.

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LIST OF ABBREVIATIONS

AK2 – adenylate kinase 2

ANOVA – analysis of variance

ARV – antiretroviral

$AUC_{0\text{-}24}$ – area under the concentration versus time curve from zero to 24 hours

BRAC – Brigham Research Assay Core

C_0 – pre-dose concentration

C_{24} – concentration 24-hours post-dose

CAPRISA – Center for the AIDS Program of Research in South Africa

CGM – cisgender men

CHARM – Combination HIV Antiretroviral Rectal Microbicide

CKM – creatine kinase muscle

CL_{ss}/F – steady-state total clearance

C_{max} – maximum concentration

CPAL – Clinical Pharmacology Analytical Laboratory (CPAL)

CPQA – Clinical Pharmacology Quality Assurance

CPT – cell preparation tube

dATP – deoxyadenosine triphosphate

D_{ave} – mean resident distance. Each symbol represents one participant.

DC_{max} – distance associated with maximum concentration

D_{max} – maximal colorectal distance of the radiosignal

D_{min} – minimal colorectal distance of radiosignal

DTPA – diethylenetriamine pentaacetate

ELISA – enzyme-linked immunosorbent assay

FACTS – Follow-on African Consortium for Tenofovir Studies

FAME – Film Antiretroviral Microbicide Evaluation

fmol – femtomole

FTC – emtricitabine

FTC-TP – emtricitabine-triphosphate

GAHT – Gender affirming hormone therapy

HBsAg – hepatitis B surface antigen

HEC – hydroxyethyl cellulose

HIV – Human Immunodeficiency Virus

HIVNAT – HIV Netherlands Australia Thailand Research Collaboration

HPTN – HIV Prevention Trials Network

iFACT – Interaction between the use of FHT and Antiretroviral agents Concomitantly among TGW

iPERGAY – Intervention Préventive de l'Exposition aux Risques avec et pour les Gays (translation: Action to Prevent Risk Exposure By and For Gay Men)

IPM – International Partnership for Microbicides

iPrEx – Iniciativa Profilaxis Pre-Exposicion (translation: pre-exposure prophylaxis initiative)

IQR – interquartile range

IRAT – Image Response Assessment Team

LC-MS/MS – liquid chromatography-tandem mass spectrometry

LLOQ – lower limit of quantification

Men who have sex with men (MSM)

mg – milligram

mL – milliliter

MTN – Microbicides Trials Network

ng – nanogram

PBMC – peripheral blood mononuclear cells

PBS – phosphate-buffered saline

pg – picogram

PK – pharmacokinetic

PKLR – pyruvate kinase liver and red blood cell

PKM – pyruvate kinase muscle

PMSF – phenylmethylsulfonyl fluoride

PrEP – pre-exposure prophylaxis

PROUD – Pre-exposure Option for Reducing HIV in the UK

RAI – receptive anal intercourse

ROI – region of interest

SPECT/CT – single-photon emission computed tomography with transmission computed tomography

suRAI – simulated unprotected receptive anal intercourse

Tc - technetium

TDF – tenofovir disoproxil fumarate

TDF-FTC – combination of tenofovir disoproxil fumarate and emtricitabine

TFV – tenofovir

TFV-DP – tenofovir diphosphate

TGW – transgender women

UPLC LC-MS/MS – ultra performance liquid chromatography tandem mass spectrometry

VOICE – Vaginal and Oral Interventions to Control the Epidemic

Vz/F – volume of distribution based on the terminal phase

CHAPTER 1: Introduction

The HIV epidemic continues to have a devastating impact globally. Vulnerable populations - men who have sex with men (MSM) and transgender women (TGW) - persons who are designated male at birth and identify as women – are at particularly high risk of HIV. MSM have a 19 times greater odds of HIV infection compared to the general population [1]. TGW are at even higher risk, with 49-fold greater odds of HIV infection than all adults of reproductive age [2]. Both MSM and women engage in unprotected RAI, which has the highest risk per act of HIV acquisition among sexual behaviors, with 10 to 20 times greater risk than unprotected vaginal sex [3, 4]. The persistently high incidence of HIV in spite of condoms and behavioral strategies presents an urgent need for new prevention strategies, such as pre-exposure prophylaxis (PrEP).

1.1 Oral Pre-Exposure Prophylaxis (PrEP) – promises and pitfalls

Daily oral PrEP with tenofovir (TFV) and emtricitabine (FTC) has provided an exciting new approach to HIV prevention. Several studies demonstrate the efficacy of oral tenofovir-based regimens in preventing HIV in diverse HIV risk groups [5-10]. Efficacy improves substantially – as high as 100% - in research cohorts who sustain a high degree of adherence throughout randomized controlled trials [5-10].

Despite the promise of oral PrEP, its efficacy can be crippled by poor adherence in some populations, limiting its potential as a singular HIV prevention method [11]. In some trials, adherence has been as poor as 25% [12]. Maintaining adherence longitudinally also

is problematic, with all randomized controlled trials (RCTs) of oral PrEP, including those demonstrating efficacy, depicting waning adherence over time [13-15].

Even in RCTs showing oral PrEP efficacy, low adherence was observed in MSM (iPrEx) with only 30% consistently having detectable drug levels [15]. In TGW in iPrEx, adherence was even lower at 18% [16]. With study participants incentivized by established prior proof of efficacy, subsequent effectiveness studies in MSM and TGW (iPERGAY and PROUD) had high adherence [17, 18]. The variable levels of medication adherence in these trials are an indirect indication that alternative PrEP dosing formulations, among other potential enhancements, are needed to improve adherence to PrEP and, therefore, improve PrEP impact.

1.2 HIV microbicides

Looking beyond oral PrEP to other formulations, key lessons can be gleaned from the development of HIV vaginal microbicides, which are drugs applied topically to the vagina to prevent HIV transmission in women. TFV vaginal gel was found to have 39% efficacy (improved to 54% with high adherence) in women in CAPRISA 004 [19]. Subsequent studies (VOICE and FACT 001) failed to replicate CAPRISA 004's efficacy, hampered by poor adherence [12, 20]. When asked about reasons for not taking the drug, participants cited having difficulty taking a daily regimen, not knowing if the drug works, not trusting the product personally and being swayed by concerns from their partners and community [21]. While the results were ultimately disappointing, studies of tenofovir vaginal gel at least brought a critical variable affecting efficacy – adherence – to the

forefront of HIV prevention drug development.

1.3 Developing HIV prevention products for MSM and TGW

Learning from the failure of TFV vaginal gel in women, a user-centric approach that takes into account the social and personal context of product use is critical to maximize product use, uptake, adherence and, thereby, efficacy in MSM and TGW. Diverse and complex sexual practices drive HIV risk in MSM and TGW, such that multiple prevention options are needed to accommodate the behavioral contexts of product use [19]. TGW have placed value in having a greater variety of PrEP products [22]. Furthermore, formulation preference varies among individuals [19], such that having both oral and topical PrEP options is likely to more broadly cover the different needs of individuals. By analogy, when options for contraception have increased over time, the population level of effective contraception rises [23-25].

Rectal microbicides, or rectal products containing drugs for preventing rectal HIV transmission through receptive anal intercourse (RAI), are being developed to provide another formulation option for HIV PrEP. Some rectal microbicides are designed to be behaviorally congruent, meaning that utilization requires little behavioral change from typical RAI parasexual practices of using lubricants or douches. Lubricants are frequently used among MSM with 45-77% reporting use with RAI [97-99] with the exception of one study citing a low frequency of 17.4% [100]. Furthermore, MSM have expressed desire for a medicated lube for HIV prevention. Similarly, rectal douches are used frequently among MSM with 88% having ever douched [26] and 43-64% having recently douched

[26-31]. In one survey of MSM, 98% were highly likely to use a rectal microbicide douche [32]. Substituting non-medicated with medicated lubricants or douches does not require changing behavior, only product selection. As rectal microbicides integrate microbicide delivery into typical sexual practices, they may be more acceptable and promote better adherence than daily oral PrEP.

In addition to broadening the menu of formulation options for HIV prevention, understanding the factors that limit or promote uptake and use of HIV PrEP in different populations, such as TGW, is important for enhancing adherence. HIV PrEP development in TGW should recognize the unique social and personal circumstances that make adherence to PrEP challenging. Most TGW use gender-affirming hormone treatment (GAHT), including estrogen, to induce feminization which they often prioritize over PrEP [33]. Among TGW, 86.6% are unwilling to use PrEP due to worries that their hormone regimens may be affected by PrEP [34]. Focus groups with TGW have indicated additional barriers to PrEP uptake and use including pill burden, concerns about side effects, and a dearth of PrEP research focused on TGW. Facilitators to PrEP use include having more research on PrEP in TGW, who have been underrepresented in PrEP trials. Bridging the barriers limiting uptake and use of PrEP in TGW will be a necessary part of successful PrEP development in TGW.

1.4 Chapters 2 and 3

The overarching goal of this thesis is to develop HIV prevention products that are compatible with the lifestyle and needs of their end users, specifically MSM and TGW.

HIV PrEP development that takes into account the social and personal context of product use will be critical to maximize product use, uptake and efficacy in these high-risk groups.

Chapter 2 addresses the desire by MSM for a rectal microbicide that can be used as a sexual lubricant, without the use of an applicator. Specifically, the study asks the question of whether using medicated lubricant, in the typical way a sexual lubricant used, will provide adequate colorectal distribution in sufficient amount to provide rectal HIV prevention.

Chapter 3 aims to address the barriers limiting PrEP uptake and use in TGW by providing TGW-focused research and pharmacokinetic support that daily oral PrEP is likely efficacious in TGW. Chapter 3 is a pharmacokinetic (PK) study of oral Tenofovir-Emtricitabine in TGW on feminizing hormone therapy.

Drug development in HIV prevention cannot occur in isolation and must value the end-user's preferences and lifestyle. By taking into account the end user's preferences, lifestyle and concerns, the studies in this thesis utilize a user-centric approach to developing oral and rectal HIV PrEP in MSM and TGW.

CHAPTER 2

Gel Applied as Lubricant Provides Poor Rectal Mucosal HIV Coverage

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2.1 Abstract

2.11 Background

Given the rising HIV incidence in men who have sex with men (MSM) despite repeatedly proven effectiveness of oral HIV pre-exposure prophylaxis, behaviorally congruent periodic dosing strategies, such as dosing microbicides as lubricants, are now in demand. Rectal microbicide gel studies largely administer gels using vaginal applicators, which have not been well received and do not mimic lubricant use.

2.12 Methods

We compared rectal gel manually dosed as lubricant with applicator dosing in five healthy, HIV-negative MSM who received 10 or 3.5 ml of ^{99m}Tc-DTPA-radiolabeled hydroxyethyl cellulose universal placebo gel intrarectally. After washout, participants received 10 ml of radiolabeled Wet® Original® lubricant to apply to the anus with fingers and/or a phallus in a manner typical of sexual lubricant use with a partner, followed by simulated receptive anal intercourse. Single-photon emission computed tomography with transmission computed tomography was performed 4 h after each gel administration.

2.13 Results

Manual dosing was associated with more variable rectosigmoid distribution, 4.4–15.3 cm from the anorectal junction, compared with more uniform distribution, 5.9–7.4 and 5.3–7.6 cm after 10 and 3.5 ml applicator dosing, respectively. A significantly smaller fraction of the initial 10 ml dose was retained within the colon after manual dosing, 3.4%, compared with 94.9% and 88.4% after 10 and 3.5 ml applicator dosing, respectively

(both $p < .001$).

2.14 Conclusion

Manual dosing of a sexual lubricant delivered a small, variable fraction of the dose with variable rectosigmoid distribution compared with applicator dosing. These results raise concern that dosing a rectal microbicide gel as a sexual lubricant may not provide adequate or predictable mucosal coverage for HIV protection.

2.2 Introduction

The rising incidence of HIV infection in men who have sex with men (MSM) in many regions highlights the need for pre-exposure prophylaxis (PrEP). Recognizing that effectiveness of oral PrEP hinges on high levels of adherence and that MSM practicing receptive anal intercourse (RAI) already commonly use sexual lubricants [35], behaviorally-congruent PrEP in the form of anal lubricant is of great interest.

Studies of rectal microbicide gels largely employ vaginal applicators that neither align with RAI practices [36] nor mimic real-world lubricant use [37]. Trial participants voice concerns about applicator comfort, size, transportability, and visual appeal, potentially limiting acceptability and future adherence [36, 38, 39]. Participants and advocates desire a microbicide gel that can be applied as anal lubricant without an applicator.

Rectal microbicide surrogates dosed with an applicator reach colorectal distributions overlapping that of HIV surrogates and achieve excellent retention [40]. To assess whether similar distribution and retention occur when rectal gels are applied as sexual lubricants without applicators, we performed an open-label cross-over study comparing colorectal distribution, percentage dose retained, and volume retained, among manual and applicator dosing methods.

2.3 Methods

2.3.1 Participants

This study was approved by the Johns Hopkins Medicine Institutional Review Board. Participants provided informed consent before screening. Eligible participants were healthy, HIV-seronegative MSM who had participated in a sequence-randomized cross-over study of a 10 and 3.5 mL hydroxyethyl cellulose (HEC) universal placebo gel administered intrarectally by a study investigator utilizing a 4 cm plastic applicator attached to a syringe [41].

We enrolled five participants from the prior study to evaluate manual dosing of gel as an anal lubricant without an applicator. The lubricant gel, Wet® Original®, is sold over-the-counter and is an aqueous gel like HEC. Wet® Original® was selected based on brand popularity in an International Rectal Microbicide Advocates survey (M. LeBlanc, Personal Communication, September 21, 2015).

2.3.2 Study Design

Participants received a 10 mL lubricant pillow, which is a plastic pouch filled with gel, radiolabeled with 1,000 μCi of ^{99m}Tc (technetium)-DTPA (diethylenetriamine pentaacetate). Participants were asked to apply the gel as they would apply anal lubricant with a sexual partner, using a phallic device as a surrogate for a penis. This was followed by simulated unprotected RAI (suRAI), gamma emission measurements of study materials, and radiolabeled gel imaging with single-photon emission computed tomography with transmission computed tomography (SPECT/CT) 4 hours after dosing, as previously described [42, 43] (Figure 2.1).

2.3.3 SPECT/CT Imaging and Analysis

Colon SPECT data was fit using a three-dimensional curve-fitting algorithm. Colorectal distribution parameters, D_{max} and D_{min} (maximal and minimal colorectal distances of radiosignal), DC_{max} (distance associated with maximum concentration), and D_{ave} (mean resident distance) were determined as previously described [43].

Region of interest (ROI) analysis of SPECT/CT images utilized previously described software [42]. For each axial slice on attenuation-corrected SPECT scans, ROIs were drawn around areas of signal intensity to quantify differences between intraluminal and extracorporeal signal. ROIs proximal to the anorectal junction, approximated as the axial CT slice inferior to air visualization within the rectal ampulla, were labeled intraluminal. For manual dosing, percentage dose retained was estimated as the product of three variables: percentage of gel removed from the pillow, percentage of removed gel applied to the body, and percentage of applied gel that was intraluminal. Percentage removed, applied, and intraluminal were determined by weights, dosimetry, and ROIs, respectively.

For applicator dosing, percentage dose retained was estimated as previously described [41]. Volume retained was estimated as the product of percentage dose retained and volume of the original dose unit. Continuous measures were described as medians and ranges. Differences among dosing arms were tested with Friedman repeated measures analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons, with p-values < 0.05 considered statistically significant.

2.4 Results

Five healthy HIV-negative MSM were enrolled. Median age was 48 years (23-57). For manual dosing, four participants used fingers and phallus to administer lubricant, while one used only the phallus. Similar colorectal distribution was observed among dosing arms, with no statistically significant differences in D_{max}, DC_{max} or D_{ave} (Figure 2.2). D_{min} was 2.8 and 3.2 cm greater for manual than 10 and 3.5 mL applicator dosing, respectively (p=0.01, p<0.01) (Figure 2.2). D_{max} was more variable for manual than 10 and 3.5 mL applicator dosing, with ranges of 4.4–15.3, 5.9–7.4, and 5.3–7.6, respectively.

For manual dosing, participants removed a median of 3.2 mL (1.8, 6.6) from the 10 mL pillow (32%). Then 20.8% (17.1, 39.9) of removed lubricant was applied to the body, and 51% (20, 89) of applied lubricant was intraluminal based on imaging (Figure 2.3 and Figure 2.4). Thus, of the initial 10 mL dose contained in the pillow, 3.4% (0.01, 23.4) was delivered intraluminally.

For applicator dosing, 94.9% (94.3, 95.6) and 88.4% (86.4, 89.5) of the 10 and 3.5 mL dose contained in the syringe were ejected, respectively. For both arms, 100% (100, 100) of ejected gel was delivered intraluminally. Thus, of the 10 and 3.5 mL doses contained in the syringe, 94.9% (94.3, 95.6) and 88.4% (86.4, 89.5) of the full original dose, respectively, were delivered intraluminally. Overall, percentage dose retained for manual dosing was 32-fold and 29-fold less than 10 and 3.5 mL applicator dosing, respectively (both p<0.001). The median intracolonic volume delivered was 0.3, 9.5 and 3.1 mL for manual and 10 and 3.5 mL applicator dosing, respectively.

The number of participants was too small to statistically test for participant variables, like age, which might correlate with measured parameters. However, the participant with the greatest percentage of retained gel and the greatest luminal distribution after manual dosing was the only participant who did not use fingers for gel application and only used the phallus.

2.5 Discussion

We describe the first study evaluating distribution and retention of a rectal gel administered as a sexual lubricant. Compared to applicator dosing, manual dosing delivered a small, variable dose with variable rectosigmoid distribution. Although highly variable, similar median colorectal distribution estimates of the manually applied gel, when compared with the applicator applied gel, were unanticipated in light of the far smaller percentage of dose retained with manual dosing. This distribution similarity may be explained by the gel vehicles having different osmolalities (3,679 and 304 mOsm/kg for Wet® Original® and HEC gels, respectively). The lubricant, with 10-fold greater osmolality due largely to the glycerin content (Table 2.1), likely drew additional fluid intraluminally, increasing volume and colonic spread. For manual dosing, the larger D_{min} was likely related to the radiosignal being below the limit of quantitation because of the small dose retained in the rectum. Although high osmolality gel may provide the better option for increased luminal distribution, it is also associated with significant epithelial toxicity that might increase HIV risk.

The highly variable rectosigmoid distribution of lubricant among participants could be attributed to diverse dosing practices, resulting in heterogeneous application methods and dosing volumes. For example, the finger-free dosing method of one participant achieved nearly a 10-fold greater amount of retained lubricant within the colorectal lumen. However, adapting new methods of gel dosing might also introduce a requirement for behavioral change and our intent was to see how well manual gel dosing fared with existing behaviors.

The study had several limitations including a small sample size. suRAI only occurred with manual dosing; however, based on CHARM-02, suRAI is unlikely to alter colorectal distribution or retention [42]. Investigators administered the gel volume using a syringe/applicator, whereas participants performed manual dosing, removing as much gel from the pillow as needed for lubrication based on personal preference; this contributed to the greater efficiency of applicator dosing. In addition, HEC gel and a lubricant were used, rather than a rectal microbicide gel in development, so no information could be gleaned about the distribution of an active pharmaceutical ingredient. Finally, manual dosing was performed with a restricted maximum gel volume without a sexual partner, diverging from real-world lubricant practices.

This study suggests that, without applicators, manual dosing of a rectal microbicide gel as lubricant may not provide adequate or predictable mucosal coverage, with possible negative impact on HIV protection. A critically complementary study, MTN-033/IPM-044, will address the impact of manual dosing of dapivirine rectal gel on tissue

concentration and *ex vivo* antiviral effect in a larger population. Ultimately, manual dosing of rectal microbicide gel as lubricant may require modifying microbicide formulations in development to achieve adequate antiretroviral delivery. Should rectal gels require applicators to reach HIV-protective colorectal distribution, wide acceptance may be challenging.

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Table 2.1. Ingredients of gels used for Applicator and Manual Dosing

Dosing Method	Applicator	Manual
Gel	Wet [44] Original	HEC universal placebo
Ingredients	Water Glycerin Carboxymethyl cellulose Pentylene glycol Potassium sorbate	Water Natrosol 250 HX Pharm HEC Sodium chloride Sorbic acid Caramel color Sodium hydroxide

HEC, hydroxyethylcellulose

Figure 2.1. Study Design

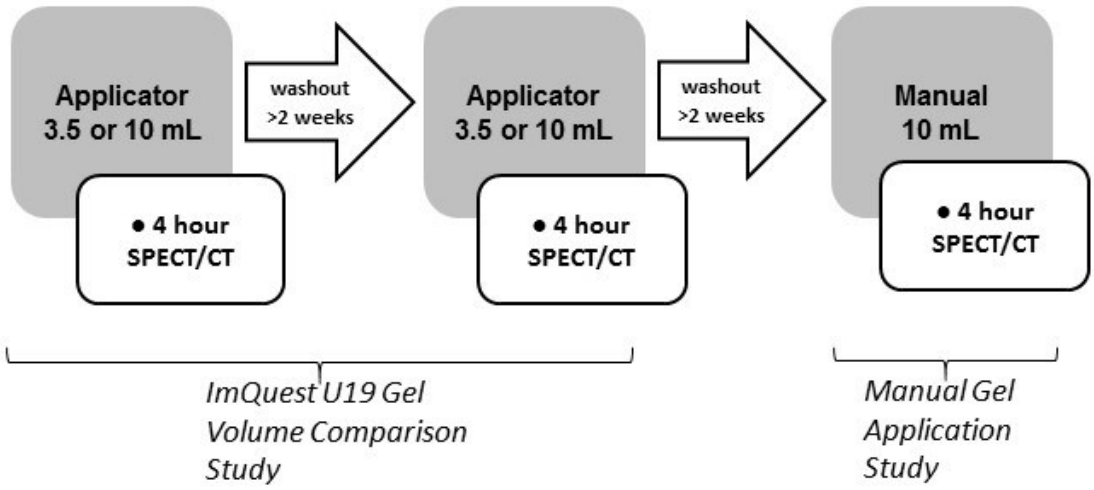
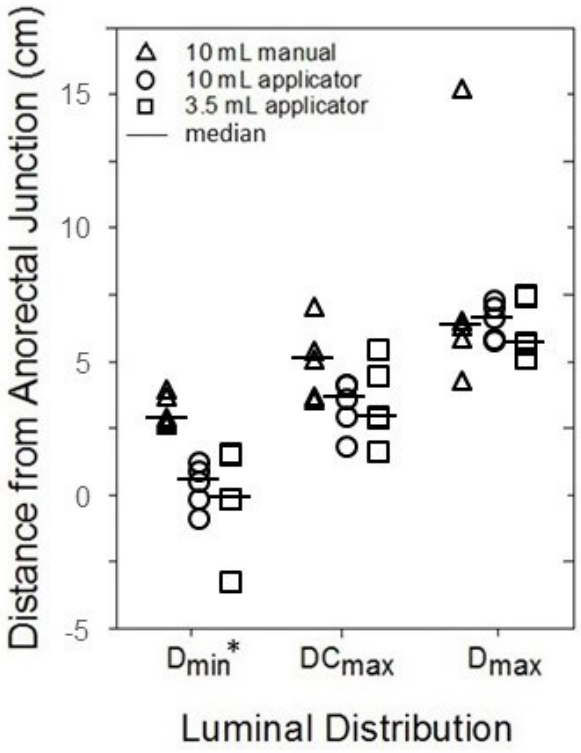
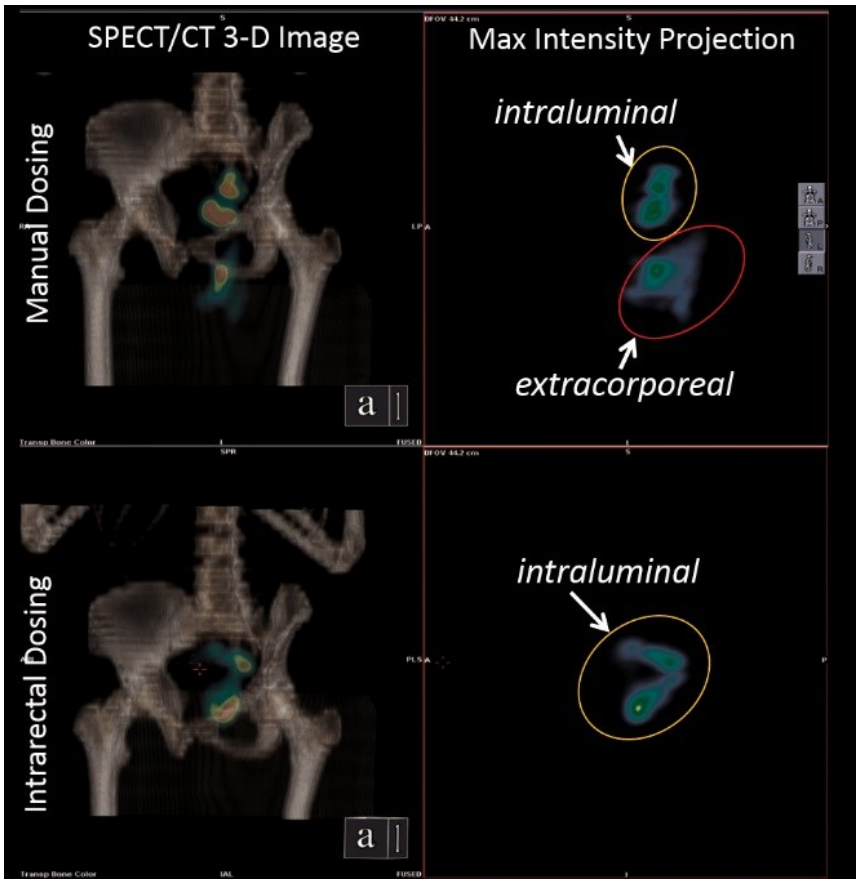


Figure 2.2. Colorectal distance parameters for manual and applicator dosing



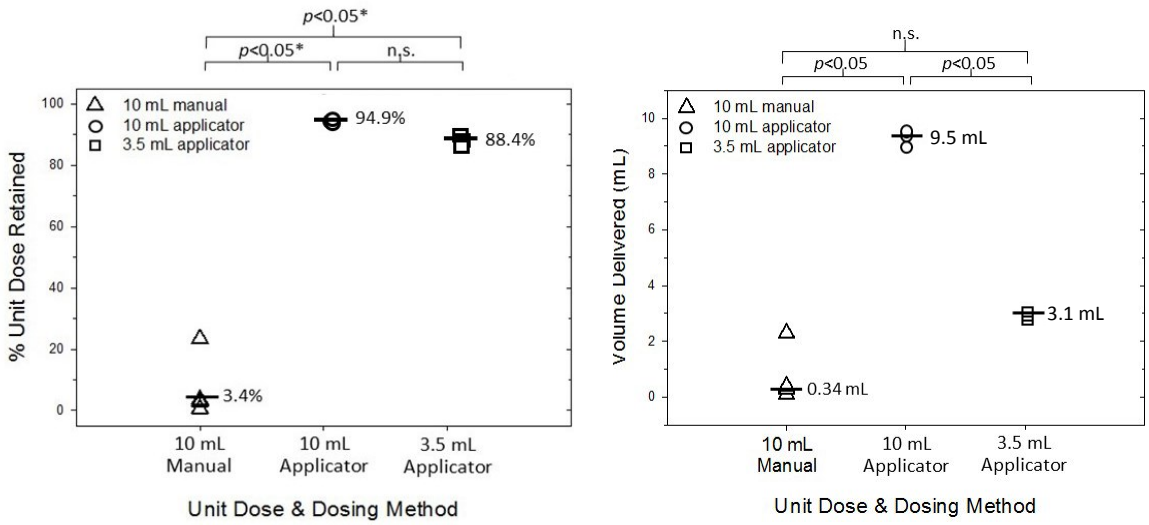
D_{max} is the maximal colorectal distance of the radiosignal. D_{min} is the minimal colorectal distance of radiosignal. DC_{max} is the distance associated with maximum concentration. D_{ave} is the mean resident distance. Each symbol represents one participant.
*Friedman repeated measures ANOVA; Tukey test for multiple comparisons $p<0.05$

Figure 2.3. Distribution of radiolabeled gel within and outside the colon for manual and applicator dosing.



Manual dosing images (top panels) indicate a substantial fraction of the dose falling outside the body (extracorporeal), posteroinferior to the pelvis, mostly in the midline gluteal folds in addition to a fraction within the colonic lumen. The intrarectal dosing images (bottom panels) indicate all visible signals within the colonic lumen within the pelvis. Fused SPECT and CT images (left panels) indicate bone in amber scale with lumbosacral spine, pelvis, and humerus (top to bottom); color in images indicate radiolabel intensity. Right panels are maximum intensity projections of SPECT image (color scale signal intensity) at similar angle of rotation without bony landmarks; labels indicate intraluminal and extracorporeal radiolabel. SPECT, single-photon emission computed tomography; CT, transmission computed tomography.

Figure 2.4. Percentage of dose retained and volume delivered of manual and applicator dosing.



*Friedman repeated measures ANOVA; Tukey test for multiple comparisons $p<0.05$; n.s. not statistically significant

CHAPTER 3

Transgender Women on Oral HIV Pre-exposure Prophylaxis Have Significantly Lower Tenofovir and Emtricitabine Concentrations when also taking Estrogen When Compared to Cisgender Men

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3.1 Abstract

3.11 Introduction.

Oral HIV Pre-Exposure Prophylaxis (PrEP) with tenofovir (TFV) disoproxil fumarate (TDF)/emtricitabine (FTC) is highly effective. Transgender women (TGW) have increased HIV risk, but have been underrepresented in trials. For TGW on estrogens for gender-affirming hormone treatment (GAHT), TDF/FTC-estrogen interactions may negatively affect HIV prevention or gender-affirming goals. Our aim was to evaluate any pharmacokinetic drug-drug interaction between GAHT and TDF/FTC.

3.12 Methods.

We performed a pharmacokinetic study, in an urban outpatient setting in 2016-2018, of the effects of GAHT on TFV, FTC and the active forms TFV diphosphate (TFV-DP) and FTC triphosphate (FTC-TP) in eight TGW and eight cisgender men (CGM). At screening, participants were HIV negative. TGW were to maintain their GAHT regimens and have plasma estradiol concentrations >100 pg/mL. Under direct observation, participants took oral TDF/FTC daily for seven days. At the last dose, blood was collected pre-dose, 1, 2, 4, 6, 8 and 24 hours, and colon biopsies were collected at 24 hours to measure drug concentration. TGW vs. CGM concentration comparisons used non-parametric tests. Blood and colon tissue were also obtained to assess kinase expression.

3.13 Results.

Plasma TFV and FTC C_{24} (trough) concentrations in TGW were lower by 32% (p=0.010) and 32% (p=0.038), respectively, when compared to CGM. Plasma FTC 24-hr area under the concentration-time curve in TGW was significantly lower by 24% (p=0.028). Plasma TFV 24-hr area under the concentration-time curve, plasma TFV and FTC concentrations, as well as all other pharmacokinetic measures, were not statistically significant when comparing TGW to CGM. Estradiol concentrations were not different comparing before and after TDF/FTC dosing. Plasma estrogen concentration, renal function (estimated creatinine clearance and glomerular filtration rate), and TFV and FTC plasma concentrations (trough and area under the concentration-time curve) were all correlated.

3.14 Conclusion.

GAHT modestly reduces both TFV and FTC plasma concentrations. In TGW taking GAHT, it is unknown if this reduction will impact the HIV protective efficacy of a daily PrEP regimen. However, the combination of an on demand (2+1+1) PrEP regimen and GAHT may result in concentrations too low for reliable prevention of HIV infection.

3.2 Introduction

Transgender women (TGW) are at high risk for HIV transmission with a worldwide prevalence of 19% [2], making them a critical population for HIV prevention with pre-exposure prophylaxis (PrEP). While PrEP willingness in TGW is high [45, 46], several challenges limit PrEP use and efficacy. Barriers to PrEP uptake in TGW include concerns about side effects and the effect of PrEP drugs on gender-affirming hormone treatment (GAHT), limited transgender-inclusive PrEP promotion, and medical mistrust [47]. The iPrEx trial of oral daily tenofovir disoproxil fumarate (TDF) 300 mg/emtricitabine (FTC) 200 mg (Truvada^{TM} Gilead Sciences, Inc. Foster City, CA) showed efficacy in men who have sex with men (MSM) and TGW, collectively [48]. However, a subgroup analysis of TGW on GAHT showed lower PrEP efficacy and lower adherence (as measured by pharmacologic assessment) when compared to MSM and TGW not on GAHT; also, the pharmacologically-based adherence benchmarks used were established in persons not on GAHT [16]. Most TGW use GAHT, including estrogen, to induce feminization [49]. In one survey, 86.6% of TGW cited unwillingness to use PrEP due to worries that their hormone regimens may be affected [50]. Surgical interventions for both hormonal modification (e.g., orchiectomy) and anatomic modification ranging from silicone injections to gender reassignment surgery are also elected, though with less frequency.

The impact of GAHT on TDF/FTC metabolism is poorly understood. The activation of tenofovir (TFV) and emtricitabine (FTC) requires intracellular phosphorylation by different nucleotide kinases in different tissues. In cell culture, estrogen increases the activity of creatine kinase, which phosphorylates TFV-MP in colon cells [51]. Cisgender

female genital tract CD4+ cells show decreased TFV-diphosphate (TFV-DP) concentrations in the presence of progesterone and estradiol [52]. Ex vivo, estrogen increases cytosolic 5'-nucleotidase activity in only some human cervicovaginal cell types, but there are no data in colorectal cells [10]. In vitro, the uptake of TFV and FTC into cervicovaginal cell lines increases or decreases varying with timing of estrogen addition and cell type [53]. Clinically, pregnancy is associated with high levels of endogenous estrogen compared to the non-pregnant state and changes in renal physiology significantly increase the clearance of many renally cleared drugs. The complex relationship between hormones and TDF/FTC uptake, activation, and clearance make it challenging to predict the pharmacokinetic (PK) interaction between GAHT and TDF/FTC.

The aim of the study was to investigate whether drug in blood plasma, peripheral blood mononuclear cells (PBMCs), and colon tissue, following daily oral TDF/FTC dosing differed in TGW on GAHT when compared to cisgender men (CGM).

3.3 Methods

3.31 Objectives

This was a phase one open-label PK interaction study of oral TDF/FTC and GAHT in TGW and CGM. The study was conducted in an urban outpatient setting in Baltimore, Maryland, from April 2016-April 2018, after approval by the Johns Hopkins Medicine Institutional Review Board. The primary objective was to compare steady-state plasma, intracellular PBMC, colon tissue homogenate, and colon tissue cell TFV, TFV-DP, FTC,

and FTC-TP concentrations in TGW on estrogen and CGM not on estrogen. Other objectives included assessing the effect of exogenous estrogen on both nucleotide kinase expression of enzymes relevant for intracellular phosphorylation of TFV and FTC and colon tissue HIV infectivity assessed by ex vivo HIV challenge of colon tissue explants.

3.32 Study Participants

Eligible participants were healthy HIV-seronegative self-identified TGW and CGM aged 18 to 65 years recruited from Baltimore, Maryland. TGW had to be taking estrogen and have a serum total estradiol concentration >100 pg/mL indicating consistent estradiol use. The estrogen formulation, dose, route, and frequency were not otherwise restricted; other GAHT medications could be taken. Exclusion criteria included enrollment in HIV clinical trials, colorectal symptoms, rectal infection, hepatitis B surface antigen (HBsAg), altered gastrointestinal anatomy, and concomitant medications with potential for toxicity or interactions.

3.33 Study Procedures

After informed consent, participants were screened with history, physical, and laboratory evaluation for hematology, chemistries, HBsAg, HIV, and syphilis; TGW had serum estradiol measurements. Eligible participants underwent daily directly observed dosing of oral TDF/FTC in the research clinic each morning from Day 0 to Day 7 to assure TFV-DP and FTC-TP reached steady state [54]. Due to varied GAHT regimens, the only GAHT dose managed by the study team was on Day 7 when the dose was held until after pre-dose (C_0) blood collection. Plasma was sampled for TFV and FTC on Day 7 pre-

dose, 1, 2, 4, 6, 8 and 24 hours (C_{24}) post-dose. PBMCs were collected pre-dose, 2, 8, and 24 hours post-dose for TFV-DP, FTC-TP, and kinase expression. Serum hormones were collected 24 hrs after the final TDF/FTC dose (Day 7, C_{24}). Flexible sigmoidoscopy with 30 rectosigmoid biopsies was performed approximately 24-26 hours after the final TDF/FTC dose for histology, PK, kinase expression, and ex vivo explant challenge.

3.34 Histology

Histology was pathologist scored for surface denudation (0-3 scale indicating thirds of epithelial surface affected), lamina propria hemorrhage (0-3 scale indicating thirds of lamina propria affected), and number of apoptotic bodies per 20x field as previously described [55].

3.35 Pharmacokinetic Sample Processing

For blood processing in all participants, plasma was prepared by centrifugation of coagulated blood in serum separator tubes at 1,500 × *g* for 10 min at 4°C, aliquoted into cryovials, and stored at −80°C until analysis. PBMCs were isolated via centrifugation of a cell preparation tube (CPT) at 1,800 × *g* for 20 min at 20–25°C, washed once with phosphate-buffered saline (PBS), and centrifuged at 400 × *g* for 15 min at 4°C. Cells were resuspended in 10 ml PBS for cell counting. Cells were centrifuged again at 400 × *g* for 15 min at 4°C. Cell pellets were lysed with 2 mL of 70% ice cold methanol in water and stored at −80°C until analysis.

Colon biopsies were collected via flexible sigmoidoscopy 10–20 cm from the anus using 3.7-mm pinch biopsy forceps (Microvasive no. 1599; Boston Scientific Corp., Natick, MA). Biopsies were placed in RPMI medium with L-glutamine and 10% fetal bovine serum (R10 media) until processing. Biopsies for drug concentration were weighed and stored at -80˚C prior to analysis. To release colon tissue cells for intracellular drug analysis, biopsies were incubated with an enzyme cocktail (collagenase type II, DNase I) in RPMI containing L-glutamine, HEPES, and 10% fetal bovine serum (FBS) using the 37C_h_TDK3 tumor dissociation program on a GentleMACS (Miltenyi) tissue dissociator [56]. Cells were counted via the Guava/Millipore EasyCyte Plus (Millipore, Billerica, MA). Thereafter, cells were processed similarly to the PBMCs.

3.36 Hormone Sample Processing and Analysis

Serum for hormone analysis was per protocol of Brigham Research Assay Core (BRAC; Brigham and Women's hospital, Boston, MA), which performed the assays, including LC-MS for total estradiol and total testosterone, tracer equilibrium dialysis for free testosterone, and immunoassay for LH and FSH.

3.37 Drug Concentration Analysis

TFV, FTC, TFV-DP, and FTC-TP concentrations were determined by previously described liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods validated for biological matrix by the Clinical Pharmacology Analytical Laboratory (CPAL) [56-58]. CPAL participates in the National Institutes of Health-supported Clinical Pharmacology Quality Assurance (CPQA) program of assay method review and

approval and periodic proficiency testing [18]. Assays were validated based on the Food and Drug Administration Guidance for Industry, Bioanalytical Method Validation and met all acceptability criteria. Assay lower limits of quantification (LLOQ) were: plasma TFV and FTC, 0.31 ng/mL; tissue TFV, 0.05 ng/sample (median LLOQ based on biopsy weights, 0.002 ng/mg); tissue FTC, 0.25 ng/sample (median LLOQ based on biopsy weights, 0.010 ng/mg); PBMC and tissue TFV-DP, 5 fmol/sample; PBMC and tissue FTC-TP, 50 fmol/sample. Intracellular metabolite concentrations were normalized to cell counts and tissue weights and reported as fmol/10^6 cells and fmol/mg, respectively. Median LLOQ values for TFV-DP and FTC-TP concentrations in PBMCs, colonic tissue cells, and tissue homogenates were 0.446 fmol/10^6, 0.721 fmol/10^6, and 0.208 fmol/mg and 4.46 fmol/10^6, 7.21 fmol/10^6, and 2.08 fmol/mg, respectively.

3.38 Kinase Expression

Tissues samples and PBMCs were lysed using 1mM phenylmethylsulfonyl fluoride (PMSF) and protease/phosphatase inhibitor cocktail (Cell Signaling Technology). Lysate was centrifuged at 6000 × *g* for 10 min at 4°C and supernatant was collected for protein determination using a BCA protein assay kit (ThermoFisher). A 50 µg protein sample was resolved by SDS gel electrophoresis and transferred to a nitrocellulose membrane. Membranes were probed using primary antibodies for adenylate kinase 2 (AK2), creatine kinase muscle (CKM), pyruvate kinase liver and red blood cell (PKLR), pyruvate kinase muscle (PKM) (all kinase antibodies from Fisher Scientific), GAPDH (Cell Signaling), or β-actin (Cell Signaling). Visualization was performed using SuperSignal West Dura or Femto ECL Substrate (ThermoFisher) and a BioRad Gel Doc XR (BioRad).

3.39 Ex vivo Colon Tissue Explant HIV Challenge

Four colon biopsies were incubated for 2hr each with 10^3 $TCID_{50}$ per mL of HIV-1, strain Ba-L from Advanced Biotechnologies, Inc. (Columbia, MD) in a 24-well plate after which they were placed in four individual wells on Surgifoam rafts (Ethicon Endo-Surgery, Inc., Somerville, NJ) in culture media for 15 days. On days 4, 7, 10, and 14, all culture media were harvested and replaced (except final day 14) with fresh media. Harvested media were assayed for HIV-1 p24 antigen using the Alliance HIV-1 ELISA kit (PerkinElmer, Waltham, MA) according to manufacturer. Cumulative p24 antigen produced in supernatant over the incubation period was divided by biopsy weight for weight-adjusted cumulative p24 antigen. Median (of four) biopsy weight-adjusted cumulative p24 produced over 14 days was the unit of analysis [19].

3.40 Pharmacokinetic and Statistical Analysis

PK parameters in plasma and PBMC (area under the concentration versus time curve from zero to 24 hours [AUC_{0-24}], peak concentration [C_{max}], trough concentration [C_{24}], steady-state total clearance [CL_{ss}/F], and volume of distribution based on the terminal phase [Vz/F]) and colon tissue concentration 24-hours post-dose (C_{24}) were estimated using non-compartmental analysis (Pharsight WinNonlin v. 8.1, Certara, Inc., Cary, NC) and summarized using median and interquartile range (IQR). Differences between gender groups were compared using non-parametric Wilcoxon rank sum test; correlations between variables were assessed using Spearman rank correlation test (IBM SPSS, v. 25.0. Armonk, NY). We referred to p values <0.05 as statistically significantcant. The

sample size enabled detection of a 1.4 effect size with 80% power and 5% two-sided alpha error.

3.4 Results

3.41 Demographics and Clinical characteristics

Eight TGW and eight CGM were enrolled. Three-quarters of participants in both categories were of African ancestry. TGW had 35% higher BMI than the CGM (p=0.061; Table 3.1), but the effect was not statistically significant. TGW self-reported GAHT regimens varied widely, and GAHT use ranged in duration from 1 to 27 years (Table 3.2). Six (75%) TGW were taking spironolactone in combination with various estrogen regimens. At screening, median (IQR) plasma estradiol concentrations were 756 (187 – 1,370) pg/dL. No participants had previously elected gender-affirming surgical interventions.

3.42 PrEP PK

Concentration-time profiles of plasma TFV and FTC (Figure 3.1) indicate biphasic decline after peak concentration with TGW median concentrations for both drugs falling below the concentrations for CGM at all times and progressively so after peak concentrations are achieved. PBMC TFV-DP concentrations are nearly flat, and FTC-TP concentrations show more gently rounded peak concentrations compared to plasma FTC and demonstrated far greater variability compared to plasma concentrations.

Plasma TFV trough concentrations (C_{24}) in TGW were lower by 32% (p=0.010) when compared to CGM. AUC_{0-24} was 27% lower (p=0.065) and clearance (CL_{ss}/F) was 38% higher (p=0.065) in TGW compared to CGM (Table 3.3). Plasma TFV C_{max} and V/F were not different between gender cohorts. In TGW, plasma FTC trough (C_{24}) and AUC_{0-24} were lower by 32% (p=0.038) and 24% (p=0.028), respectively, while CLss/F was higher by 31% (p=0.028) when compared to CGM; V/F was higher in TGW by 26% (p=0.065) when compared to CGM (Table 3.4), but the effect was not statistically significant. FTC C_{max} was not different between gender cohorts. There were no statistically significant PK differences between TGW and CGM in PBMC or colon tissue for any analytes.

3.43 Kinase Expression

Expression of adenylate kinase 2 (AK2), pyruvate kinase muscle (PKM) and pyruvate kinase liver and red blood cell (PKLR) in PBMC did not differ over time at 0, 2, 8 and 24 hours between CGM and TGW (Figure 3.2). Expression of creatine kinase muscle (CKM) and AK2 in colon tissue did not differ between CGM and TGW (Figure 3.2).

3.44 Pharmacodynamics

There were no significant differences in median (IQR) cumulative p24 produced by colorectal tissue explants between TGW and CGM (9.3 [8.1-13.6] vs 6.8 [5.5-13.3] pg/mg tissue, p=0.366).

3.45 Hormone Concentrations

After seven days of TDF/FTC dosing (Day 7), concentrations for all measured hormones were unchanged compared to baseline (all p values > 0.15) (Table 3.5). However, the heterogeneity in GAHT regimens and the absence of directly observed GAHT dosing meant hormone sampling before and after the TDF/FTC dosing period may not have been at steady state. After seven days of TDF/FTC dosing, estradiol concentrations were 25-fold higher in TGW compared to CGM. FSH, LH, total and free testosterone in CGM ranged from 2% to 8% of the concentrations measured in TGW after seven days of TDF/FTC dosing (all p values < 0.05).

3.46 Correlation between PrEP PK parameters and Hormones

Estradiol dose and plasma FTC AUC_{0-24} (r=-0.68, p=0.007) were strongly negatively correlated. Plasma TFV trough concentrations were negatively correlated with both estradiol dose (r=-0.53, p=0.04) and Premarin dose (r = -0.54, p=0.03). Correlation between GAHT doses and all other PK parameters were not statistically significant. Serum estradiol concentrations were moderately correlated with plasma C_{24} concentrations of TFV (r = -0.54, p=0.03) and FTC (r = -0.52, p=0.04). FSH measurements were moderately correlated with plasma C_{24} FTC concentrations (r=0.61, p=0.02) and PBMC TFV-DP C_{max} (r = 0.58, p=0.02). Other hormone and TDF/FTC PK parameters were not statistically significantly correlated.

3.47 Renal function

Renal function in TGW was significantly higher when compared to CGM using all three measures - creatinine clearance (CrCl) estimated with the Cockcroft-Gault equation and

glomerular filtration rate (GFR) estimated using both MDRD and CKD-EPI equations (all $p<0.01$) (Table 3.6). When we substituted the female term for the male term in the two GFR estimating equations for TGW on GAHT, the differences between TGW and CGM largely disappeared and were no longer statistically different. Estimated CrCl remains significantly higher for TGW compared to CGM when substituting female for male for TGW. Plasma TFV and FTC trough concentrations (C_{24}) and exposure (AUC_{0-24}) were all strongly negatively correlated with all three measures of renal function (r range -0.65 to -0.82, p values ≤ 0.02 in all comparisons). When the formulas for females were substituted for TGW, the correlation dropped slightly.

3.48 Histology

Surface denudation, lamina propria hemorrhage, and apoptotic bodies scores did not differ between TGW and CGM.

3.5 Discussion

We found a 24-32% reduction in plasma TFV and FTC AUC_{0-24} and C_{24} in TGW on a variety of estrogen regimens when compared to CGM. The iFACT study also showed a reduction in plasma TFV – 18% C_{24} and 12% AUC_{0-24} – in 20 TGW on a standardized estrogen/cyproterone regimen [20]. The different magnitude of TFV reductions may have been due to study differences, e.g., higher estrogen doses in some of our participants, different anti-androgens, and use of parallel group design in our study. Another PrEP-GAHT interaction study found a 7-fold reduction in rectal tissue homogenate TFV-DP:dATP ratio in TGW on GAHT, also raising concern regarding PrEP efficacy [21]. In

this regard, our active phosphorylated TFV and FTC analytes were not statistically significant. However, our assessment of these intracellular readouts are limited by greater assay variability, small sample size, and sparse sampling. In addition, detailed examination of the plasma ARV and hormone concentrations indicate GAHT non-adherence in some participants possibly related to a drug-drug interaction not at steady-state, which would result in a delayed impact on the relatively longer half-life of the phosphorylated drug analytes when compared to parent drug in plasma.

The magnitude of reduced plasma TFV and FTC exposure associated with GAHT regimens in these studies is consistent with taking only five doses (Hopkins) or six doses (iFACT) per week when compared to taking 7 daily TDF/FTC doses each week, based on HPTN 066 directly observed TDF/FTC dosing [54]. In HPTN 066, which reported combined results for CGM and cisgender women not on GAHT, the median (IQR) steady-state TFV and FTC serum concentrations were 52 ng/mL (49-56) and 71 ng/mL (68-82), respectively [12]. The iPrEx study demonstrated highly effective oral TDF/FTC as PrEP with only four doses per week, motivating the Ipergay and Prevenir on demand 2+1+1 TDF/FTC regimens, which demonstrated very high 86% and 100% HIV protection, respectively, in MSM and TGW [59, 60]. However, there were too few TGW in those studies to draw conclusions regarding PrEP effectiveness on GAHT. Theoretically, one would expect the combination of reduced concentrations from both the 2-1-1 regimen and GAHT to result in a dose frequency equivalent of two to three doses per week. At this dose frequency, PrEP efficacy in the iPrEx models would predict reductions in PrEP efficacy [61]. Accordingly, it seems prudent to recommend against the

2-1-1 regimen in TGW on GAHT, at least until more rigorous PK studies address optimal PrEP dosing in TGW.

The mechanism behind lower plasma TFV and FTC in the setting of exogenous estrogen (and many also with spironolactone) is not clear. The TFV plasma clearance (CLss/F) was higher in TGW, though the effect size was not statistically significant, without parallel difference in volume of distribution (Vz/F). Because clearance and volume are influenced by oral bioavailability when dosing is oral, the difference in clearance and volume changes suggests bioavailability was not a major factor. For FTC, both clearance and volume changed similarly (with small differences in magnitude and statistical significance). Therefore, bioavailability or volume may differ between cohorts. However, we have no explanation for this and find more consistent evidence for a difference in clearance. TGW had markedly higher creatinine clearance and eGFR when compared to CGM participants, which might account for the major mechanism for reduced concentrations of TFV and FTC, both of which are primarily eliminated through renal clearance. We also observed a correlation of serum estrogen concentration with both TFV and FTC reduction and renal glomerular function increase. We found that estradiol does not affect the kinases that phosphorylate TFV in plasma and colon tissue, but this might have been the result of off-setting decreases in plasma parent drug PK and increases in kinase effects (predicted by in vitro data) [9].

Whether this difference in renal function is due to estradiol or simply an association is unclear. Prior studies have shown both increasing renal function with acute use of

estrogens and decreasing renal function with chronic estrogen dosing [62, 63]. A more mechanistic explanation may involve the estrogen effect in upregulation of angiotensin-2 receptor (AT_2R) expression resulting in vasodilation and increased clearances shown in rodents, though supraphysiologic estrogen concentrations result in vasoconstriction [64-68]. In addition, testosterone downregulates AT_2R expression, resulting in decreased vasodilation [69]. Since testosterone concentrations are reduced by a variety of drugs used in GAHT regimens, resulting in greater than 20-fold lower free testosterone in our TGW compared to CGM participants, this may be an additional mechanism of enhanced renal clearance of TFV and FTC. We did not assess angiotensin receptor expression to explore this potential explanation.

While we did not identify an impact of PrEP drug on estrogen concentrations, the heterogeneity of estrogen formulations and doses among the GAHT prevented us from sampling plasma to assess estrogen concentrations reliably at times coinciding with pseudo steady-state conditions. In addition, we did not use directly observed dosing of all GAHT drugs (unlike with TDF/FTC dosing). As a result, we are not highly confident about the finding of no TDF/FTC impact on estrogen concentrations. In contrast, the iFACT study team was able to employ a standardized GAHT regimen of estradiol and cyproterone acetate and demonstrated that TDF/FTC dosing did not affect estrogen concentration. Further, since GAHT regimens are often adjusted to achieve desired subjective and objective effects in clinical practice, even modest TDF/FTC impacts on estrogen concentration would be corrected by usual clinical practice.

Our study also suggests caution when using pharmacologically-based adherence benchmarks established in one population when applied to another. In iPrEx, some of the concentration difference noted in TGW on GAHT may be attributable to GAHT-PrEP interactions in addition to adherence. A clearer example of adherence mischaracterization would be in pregnant women in whom plasma TFV concentrations are 58% lower than in non-pregnant women when controlling for adherence [33].

3.6 Conclusions

We demonstrated that GAHT regimens taken by TGW in our study resulted in greater than 20% reduction of both TFV and FTC taken as oral daily PrEP, possibly because of increases in renal clearance. The iFACT study made similar findings. We caution that the combination of a GAHT regimen and the on demand 2-1-1 PrEP regimen may result in TFV and FTC concentrations that fall below those associated with high levels of HIV protection. Conversely, neither our study nor the iFACT study demonstrated any effect of daily TDF/FTC on estrogen concentrations providing needed reassurance for TGW concerned that PrEP may negatively affect their GAHT regimen. More rigorous study designs are essential to explore the mechanism of the estrogen effect, better define the magnitude of related active TFV and FTC concentration changes in colorectal tissue, and confirm our dosing cautions with regard to GAHT and on demand PrEP regimens.

Conflict of Interest Statement:

Craig Hendrix, Edward Fuchs, Jennifer Breakey, Mark Marzinke, and Rahul Bakshi receive research support from Gilead Sciences through a contract with and managed by Johns Hopkins University School of Medicine. The other authors note no conflicts of interest.

Authorship:

Author Contributions:

ES, MAM, EJF, AH, RB, WA, JB, TP, TB, NNB, and CWH performed the research. ES, MAM, EJF, AH, JB, NNB, and CWH designed the research study. ES and CWH analyzed the data and drafted the manuscript. All authors read successive versions of the manuscript and approved the final manuscript.

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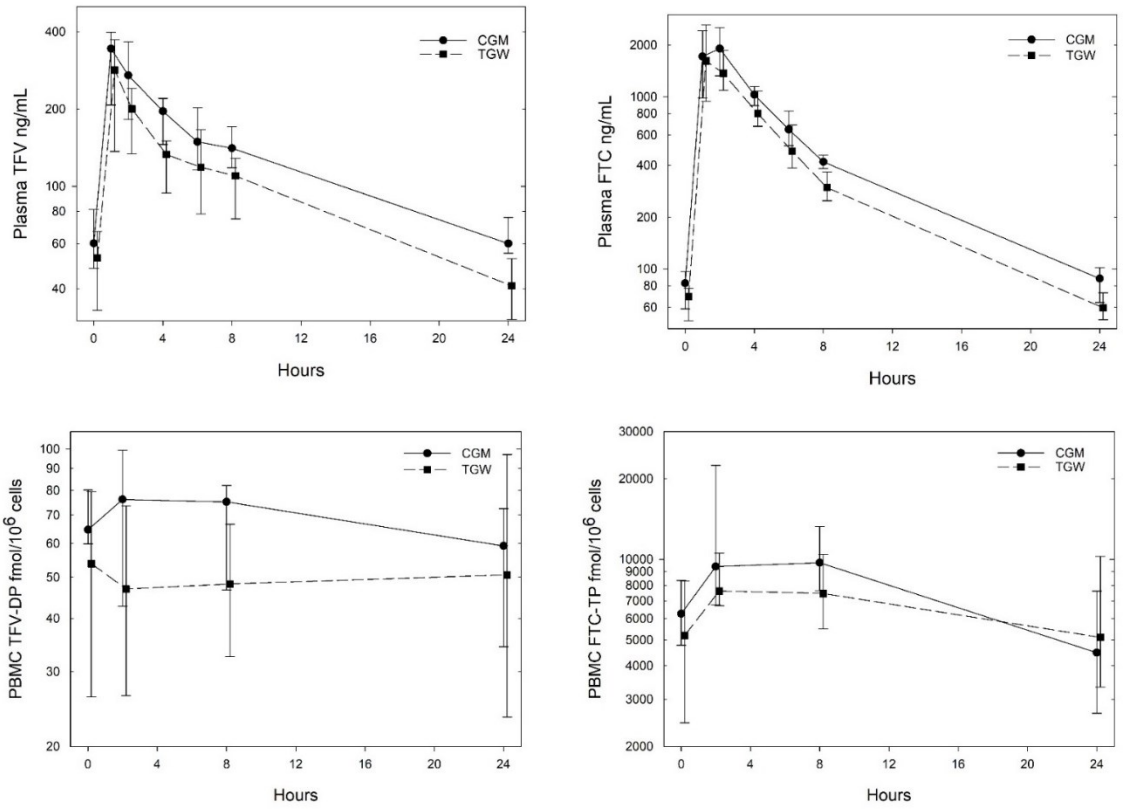
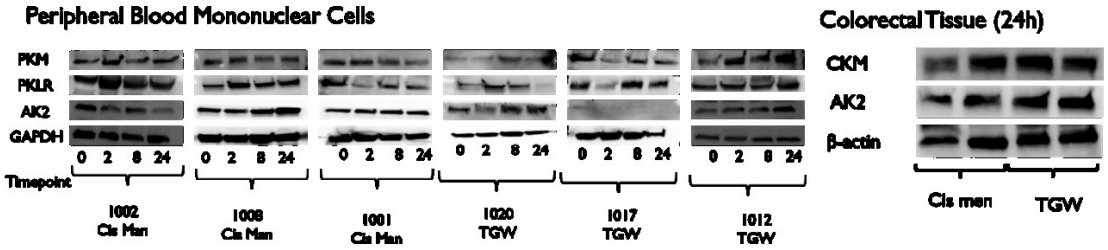
Figure 3.1. Concentration vs. time plots for TFV, FTC, TFV-DP, and FTC-TP on blood and colon tissue.



Concentration vs. time plots for plasma tenofovir (TFV, Panel A) and emtricitabine (FTC, Panel B), and peripheral blood mononuclear cell (PBMC) TFV diphosphate (TFV-DP, Panel C) and FTC triphosphate (FTC-TP, Panel D) comparing transgender women (TGW; dashed lines, squares) and cisgender men (CGM; solid lines, circles). Data are medians with error bars indicating lower and upper quartiles. Time values are slightly offset to avoid overlap of data.

Figure 3.2. Kinase expression analyzed via immunoblotting for TFV-activating kinases in PBMC and colon tissue.



Kinase expression analyzed via immunoblotting for tenofovir-activating kinases in peripheral blood mononuclear cells (PBMC) and colon tissue. PBMC and colon tissue were lysed and immunoblotting was performed using 50 µg of total protein lysate as described under Methods. Panel A. Immunoblotting of PBMC lysate for pyruvate kinase muscle (PKM), pyruvate kinase liver and red blood cell (PKLR), adenylate kinase 2 (AK2), and glyceraldehyde 3-phosphatase dehydrogenase (GAPDH). Panel B. Immunoblotting of colon tissue lysate for creatine kinase muscle (CKM), adenylate kinase 2 (AK2), and β-actin. Participant identification numbers are indicated (1001, 1002, 1008, 1012, 1017, and 1020). CGM, cisgender male; TGW, transgender woman.

Table 3.1. Demographic characteristics

	TGW median (IQR)[†]	CGM median (IQR)	*TGW/CGM* %	*p* value
Age, years	29 (26, 41)	46 (28, 52)	63	0.195[‡]
Weight, kg	98 (83, 123)	83 (71, 91)	118	0.130
BMI	31 (24, 36)	23 (21, 27)	133	0.061
Race, n (%)				
Asian ancestry	1 (12)	0 (0)		1.000[§]
African ancestry	6 (75)	6 (75)		
European ancestry	1 (12)	2 (25)		

[†]IQR, interquartile range

[‡]Exact 2-sided *p* value, Wilcoxon rank sum test, comparing TGW and CGM

[§]Fisher's exact test

Table 3.2. **Hormone Regimens in TGW**

PID	Estradiol (oral)	Estradiol Valerate (IM)	Premarin	Spironolactone	Medroxyprogesterone
1010			1.25 mg qd		
1011	6 mg q day			200 mg q day	
1012			1.25 mg qd	50 mg q day	
1017		0.5 mg q 2 weeks		50 mg q day	
1018	6 mg q day	20 mg q 2 weeks		200 mg q day	
1019	2 mg q day	40 mg q 2 weeks	6.25 mg qd		
1020		20 mg q 2 weeks		100 mg q day	5 mg q day
1021		1.5 mg q 1 week		200 mg q day	

Table 3.3. Tenofovir (TFV) pharmacokinetic parameters

Analyte	Matrix	Units	Parameter	TGW median (IQR†)	CGM median (IQR)	*TGW/CGM* %	*p* value‡
TFV	Plasma	ng/mL	C_{max}	301 (210, 392)	349 (269, 398)	86	0.721
TFV	Plasma	ng/mL	C_{24}	41 (30, 52)	60 (55, 76)	68	0.010
TFV	Plasma	ng-hr/mL	AUC_{0-24}	2,500 (1,712, 3,001)	3,431 (2,720, 3,649)	73	0.065
TFV	Plasma	L/hr	CL_{ss}/F	120 (100, 177)	87 (82, 110)	138	0.065
TFV	Plasma	L	Vz/F	2,110 (1,645, 3,391)	2,009 (1,442, 2,504)	105	0.574
TFV-DP	PBMC	fmol/10^6 cells	C_{max}	69 (51, 101)	83 (67, 118)	83	0.328
TFV-DP	PBMC	fmol/10^6 cells	C_{24}	51 (23, 97)	56 (34, 82)	90	0.982
TFV-DP	PBMC	fmol-hr/10^6 cells	AUC_{0-24}	1,239 (755, 1,560)	1,637 (1,404, 1,995)	76	0.121
TFV	Colon homogenate	ng/mg	C_{24}	0.48 (0.17, 0.54)	0.31 (0.16, 0.83)	153	0.977
TFV-DP	Colon homogenate	fmol/mg	C_{24}	701 (368, 2,694)	1,329 (290, 4,231)	53	0.798
TFV-DP	Colon cells	fmol/10^6 cells	C_{24}	1,238 (286, 4,811)	3,398 (881, 7,894)	36	0.442

Abbreviations: TGW, transgender women; CGM, cisgender men; TFV-DP, TFV diphosphate; PBMC, peripheral blood mononuclear cells; C_{max}, peak concentration; C24, trough concentration; AUC_{0-24}, area under the concentration versus time curve from zero to 24 hours; CL_{ss}/F, steady-state total clearance divided by bioavailability; V_z/F, volume of distribution based on the terminal phase

†IQR is interquartile range

‡Exact 2-sided p value, Wilcoxon rank sum test, comparing TGW and CGM

Table 3.4. Emtricitabine (FTC) pharmacokinetic parameters

Analyte	Matrix	Units	Parameter	TGW median (IQR†)	CGM median (IQR)	*TGW/CGM* %	*p* value‡
FTC	Plasma	ng/mL	C_{max}	2,047 (1,442, 2,615)	2,284 (1,415, 2,889)	90	0.645
FTC	Plasma	ng/mL	C_{24}	60 (51, 73)	88 (64, 102)	68	0.038
FTC	Plasma	ng-hr/mL	AUC_{0-24}	9,682 (8,512, 10,926)	12,698 (11,114, 13,683)	76	0.028
FTC	Plasma	L/hr	CL/F	20.6 (18.3, 23.6)	15.8 (14.6, 18.0)	131	0.028
FTC	Plasma	L	Vz/F	187 (173, 230)	148 (140, 162)	126	0.065
FTC-TP	PBMC	fmol/10^6 cells	C_{max}	10,617 (7,918, 12,880)	10,943 (8,702, 22,409)	97	0.382
FTC-TP	PBMC	fmol/10^6 cells	C_{24}	5,110 (3,332, 10,255)	4,479 (2,659, 6,820)	114	0.645
FTC-TP	PBMC	fmol-hr/10^6 cells	AUC_{0-24}	180,869 (122,399, 203,741)	204,467 (159,963, 272,918)	88	0.281
FTC	Colon homogenate	ng/mg	C_{24}	§BLQ (BLQ, 0.26)	BLQ (BLQ, 0.25)	-	0.706
FTC-TP	Colon homogenate	fmol/mg	C_{24}	123 (46, 183)	203 (84, 237)	60	0.279
FTC-TP	Colon cells	fmol/10^6 cells	C_{24}	79 (BLQ¶, 297)	180 (121, 251)	44	0.393

Abbreviations: TGW, transgender women; CGM, cisgender men; FTC-TP, FTC triphosphate; PBMC, peripheral blood mononuclear cells; C_{max}, peak concentration; C24, trough concentration; AUC_{0-24}, area under the concentration versus time curve from zero to 24 hours; CL_{ss}/F, steady-state total clearance divided by bioavailability; V_z/F, volume of distribution based on the terminal phase

†IQR is interquartile range

‡Exact 2-sided p value, Wilcoxon rank sum test, comparing transgender women (TGW) and cisgender men (CGM)

§Values below the lower limit of assay quantitation (BLQ) are included in the median (IQR) estimates; 5 CGM and 4 TGW are BLQ for FTC colon homogenates.

¶Among colon tissue cell FTC-TP, 1 CGM and 3 TGW are BLQ.

Table 3.5. Hormone Concentrations in transgender women (TGW) and cisgender men (CGM). Data other than *p* values are median (interquartile range).

Hormone	TGW Day 0	TGW Day 7	CGM Day 7	Pre vs. Post TDF/FTC *p* value†	TGW vs. CGM *p* value†
Estradiol (pg/mL)	221 (60, 615)	380 (208, 437)	15 (12, 23)	0.669	<0.001
FSH (mIU/mL)	0.17 (0.10, 3.23)	0.10 (0.10, 3.87)	4.02 (2.23, 5.83)	0.806	0.047
LH (mIU/mL)	0.88 (0.13, 4.16)	0.46 (0.16, 5.63)	5.45 (2.98, 7.62)	0.626	0.048
Total Testosterone (ng/dL)	15 (10, 90)	17 (10, 297)	422 (346, 605)	0.151	0.028
Free Testosterone (ng/dL)	0.34 (0.19, 2.03)	0.35 (0.30, 3.48)	12.65 (8.10, 17.70)	0.375	0.011

†Exact 2-sided *p* value, Wilcoxon rank sum test, comparing pre-TDF/FTC dosing (Day 0) to post-TDF/FTC dosing (Day 7) or comparing TGW to CGM

‡All TGW had estradiol concentrations greater than 100 pg/ML per protocol at screening. One subject fell below this value on both Day 0 and Day 8 (day of biopsy), while another fell below only on Day 0.

Table 3.6. Estimated creatinine clearance (CrCl) and estimated glomerular filtration rate (eGFR) on study day 7.

Physiologic Variables	TGW median (IQR[†¶])	CGM median (IQR)	TGW/CGM %	*p* value[‡]
Serum Creatinine (mg/dL)	0.80 (0.80, 0.88)	1.05 (1.00, 1.18)	76	0.002
CrCl Cockcroft-Gault (mL/min)	204 (120, 218)	108 (83, 125)	188	0.008
eGFR MDRD (mL/min/1.73m^2)	130 (117, 142)	89 (79, 98)	146	0.002
eGFR CKD-EPI (mL/min/1.73m^2)	133 (124, 142)	114 (109, 118)	116	0.003
[§]CrCl Cockcroft-Gault [TGW=F] (mL/min)	174 (102, 185)	109 (83, 125)	160	0.035
[§]eGFR MDRD [TGW=F] (mL/min/1.73m^2)	97 (86, 105)	89 (79, 98)	109	0.442
[§]eGFR CKD-EPI [TGW=F] (mL/min/1.73m^2)	107 (100, 118)	98 (81, 106)	109	0.315

Abbreviations: TGW, transgender women; CGM, cisgender men; MDRD, Modification of Diet in Renal Disease Study equation;

CKDEPI, Chronic Kidney Disease Epidemiology Collaboration equation

[†]IQR – interquartile range (lower quartile, upper quartile)

[‡]Exact 2-sided *p* value, Wilcoxon rank sum test, comparing TGW and CGM

[§]CrCL and eGFR estimated using the female term replacing the male term in the equation for TGW

CHAPTER 4: Conclusion

The vulnerability of oral and vaginal PrEP efficacy to adherence points to the need to improve current PrEP options and develop alternate formulations. Regardless of formulation, a successful PrEP strategy has to take into account the lifestyle, concerns and personal preferences of its users. The failure of vaginal PrEP showing no efficacy in women [12, 20] has made it clear that HIV PrEP cannot succeed without user buy-in and without taking lifestyle and preferences into account. Furthermore, the poor adherence of oral daily PrEP in TGW leading to no efficacy in subgroup analyses suggest that PrEP will not succeed if merely adapted from MSM to TGW [16]. TGW are a unique population with different social, biological, psychological and lifestyle needs that impact their views, uptake and use of HIV PrEP.

With the varying needs of individuals in mind, this thesis embraces a user-centric approach to the clinical pharmacology of HIV prevention in MSM and TGW with a broader view that this strategy will improve uptake and adherence. Chapter 2 addresses the feasibility of developing a medicated lubricant for HIV prevention, which is desired by many MSM, while Chapter 3 addresses a specific high level concern of TGW that HIV PrEP will adversely impact their gender-affirming hormonal treatment.

Chapter 2 asks the question of whether using a medicated lube in the same manner as a sexual lubricant can achieve adequate colorectal distribution for rectal HIV prevention. The study found that dosing a rectal microbicide gel in the typical way a sexual lubricant is applied, or manual dosing, delivers a small, variable fraction of the dose with variable

rectosigmoid distribution compared to applicator dosing. Designed for immediate use prior to potential rectal HIV-1 exposure, rectal microbicide products should rapidly achieve and sustain a targeted surface area in the colon. This study suggests that manual dosing with rectal microbicide formulations currently in development may not reliably provide sufficient drug delivery to the area of likely HIV infection needed for HIV prevention.

Chapter 3 is a pharmacokinetic (PK) study examining the two-way drug-drug interaction of oral TFV-FTC and GAHT in TGW. Chapter 3 provides pharmacokinetic evidence that daily oral PrEP with TDF/FTC has no impact on GAHT in TGW, consistent with another study conducted in parallel. Surprisingly, the study suggests that GAHT modestly reduces TFV and FTC plasma concentrations though not to a degree that likely impacts the efficacy of daily oral PrEP in HIV prevention. While the concentration decrease in TFV and FTC in TGW on GAHT was modest, it suggests that adherence to daily oral PrEP is even more critical, as missing doses or using PrEP on demand while on GAHT may not provide drug concentrations sufficient for HIV prevention. Because of the modest concentration decrease, increasingly popular on demand 2+1+1 regimens, such as those studied in Ipergay and Prevenir, may not provide high levels of HIV protection.

4.1 Future Directions

MSM desire a rectal microbicide gel that fits seamlessly with their lifestyle. Chapter 2 raises concerns that applying lubricant gel in this manner, or manual dosing, may need further alterations to achieve the colonic distribution and amount needed for HIV

prevention. The findings in Chapter 2, however, are based solely on imaging studies. A larger study MTN-033/IPM-044 is currently in progress to compare the effect of manual and applicator dosing of dapivirine rectal gel on tissue concentration and ex vivo HIV efficacy. In combination with the results of Chapter 2, the results of MTN-033/IPM-044 will inform the feasibility of manual dosing of a current rectal microbicide formulation in development. Should this study show that manual dosing does not achieve HIV-protective tissue concentrations, future studies should look into increasing the concentration of antiviral drug in the rectal formulation to achieve HIV-protective tissue concentrations and adequate colorectal distribution with manual dosing (as lubricant). Without manual dosing, product acceptance and adherence of rectal microbicide gel for use in RAI may be challenging. Several anal douche formulations – incorporating either tenofovir or griffithsin - are also in development as behaviorally-congruent, on demand rectal microbicide strategies.

Whereas MSM-directed HIV PrEP research is bountiful in MSM, there has been a dearth of TGW-focused HIV PrEP research, a gap that TGW cite as a barrier to PrEP uptake and use. Chapter 3 not only provides much-needed TGW-focused PrEP research, but also reassuring pharmacokinetic evidence for TGW on GAHT that daily oral PrEP does not adversely affect their GAHT. In addition, daily oral TDF/FTC forPrEP likely provides concentrations sufficient to protect against HIV. The decreased TFV and FTC concentrations seen in TGW also on GAHT seen in this study, as well as the Thai Red Cross/HIVNAT iFACT study [70], while modest, should be assessed further to determine whether they translate into decreased efficacy. Given that that less intensive on-demand,

2+1+1 oral PrEP regimens may not provide sufficient HIV-concentrations in TGW on GAHT and as the burden of a daily dosing regimen may not be acceptable to all individuals, alternate formulations should be explored in TGW on GAHT.

4.2 Conclusion

Drug development in HIV prevention cannot occur in isolation and must value the end-user's preferences and lifestyle. Without taking account of varied preferences and lifestyles of individuals at risk of HIV infection, HIV PrEP development has shown that HIV prevention product development may not succeed. Altogether, this thesis takes a step toward developing a medicated lubricant for MSM and provides TGW-focused research that takes into account GAHT use. By considering the end user's preferences, concerns and lifestyle, the studies in this thesis utilize a user-centric approach to developing oral and rectal HIV PrEP in MSM and TGW.

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RESEARCH ACTIVITIES

Peer-Reviewed Publications

1. Castoreno AB, Wang Y, Stockinger W, Jarzylo LA, Du H, Pagnon JC, **Shieh EC**, Nohturfft A. Transcriptional regulation of phagocytosis-induced membrane biogenesis by sterol regulatory element binding proteins. *Proc Natl Acad Sci U S A* 2005; 102(37):13129-34.
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6. Yang J, Chen YI, Friedland S, Holmes I, Paiji C, Law R, Hosmer A, Stevens T, Matheus F, Pawa R, Mathur N, Sejpal D, Inamdar S, Berzin TM, DiMaio CJ, Gupta S, Yachimski PS, Anderloni A, Repici A, James T, Jamil LH, Ona M, Lo SK, Gaddam S, Dollhopf M, Alammar N, **Shieh E**, Bukhari M, Kumbhari V, Singh V, Brewer O, Sanaei O, Fayad L, Ngamruengphong S, Shin EJ, Baron TH, Khashab MA. Lumen-apposing stents versus plastic stents in the management of pancreatic pseudocysts: a large, comparative, international, multicenter study. *Endoscopy*; 2019; 51(11):1035-1043
7. Nguyen E, Trinh S, Trinh H, Nguyen H, Nguyen K, Do A, Levitt B, Do S, Nguyen M, Purohit T, **Shieh E**, Nguyen MH. Sustained virologic response rates in patients with chronic hepatitis C genotype 6 treated with ledipasvir+sofosbuvir or sofosbuvir+velpatasvir. *Aliment Pharmacol Ther;* 2019; 49(1):99-106.
8. **Shieh E,** Marzinke MA, Fuchs EJ, Hamlin A, Bakshi R, Aung W, Breakey J, Poteat T, Brown T, Bumpus NN, Hendrix CW. Transgender Women on Oral HIV Pre-exposure Prophylaxis Have Significantly Lower Tenofovir and Emtricitabine Concentrations when also taking Estrogen When Compared to Cisgender Men. J Int AIDS Soc. 2019 Nov; 22(11): e25405. PMID: 31692269.

Case Reports

1. Chen A, **Shieh E**, Brinkley S, Blankson JN. Candida esophagitis in a human immunodeficiency virus-1-positive elite controller with hepatitis C virus cirrhosis. *Open Forum Infect Dis.* 2014 Dec 23;1(3).
2. **Shieh EC**, Mullin GE (in press). Tapeworms Diagnosed on Capsule Endoscopy: An Unusual Cause of Abdominal Pain in a Child. *American Journal of Gastroenterology.* 2017 Apr:112(4)

Chapters

1. **Shieh EC**, Lee L. (2014). Constipation. In S. McKean, J. Ross, D. Dressler, D. Brotman, J. Ginsberg (Eds.), *The Principles and Practice of Hospital Medicine*. American College of Physicians (pp. 541-547). China: McGraw-Hill.

CLINICAL ACTIVITIES

Board Certification

2013	Internal Medicine
2017	Gastroenterology

EDUCATIONAL ACTIVITIES

Classroom Instruction

2006-2007	Medical Chinese Course for Beginners, Johns Hopkins School of Medicine
2016	Small Group Preceptor, Gastrointestinal Pathophysiology (2nd year Medical School)

ORGANIZATIONAL ACTIVITIES

Editorial Activities

2004-2006	Communications Editor, The Next Generation, an Online Journal on Medicine, a collaboration with Editors of the New England Journal of Medicine
2016, 2018	Reviewer: American Journal of Gastroenterology

Committees

2009-2010	Student Committee on Admissions, Johns Hopkins School of Medicine
2016	Program Evaluation Committee, Johns Hopkins Division of Gastroenterology & Hepatology

Professional Societies

2004-2005	President, Harvard Pre-Medical Society
2006	Co-National Education Officer, Asian Pacific American Medical Student Association
2013-present	American Gastroenterological Association
2016-present	American College of Gastroenterology

RECOGNITION

Honors

2003	Detur Book Prize, awarded to the top 10% of the Harvard freshman class
2007	Medical Student Training in Aging Research Summer Scholar

OTHER PROFESSIONAL ACCOMPLISHMENTS

Oral Presentation

1. **Shieh EC**, Weld E, Fuchs EJ, Hiruy H, Buckheit K, Buckheit Jr. RW, Breakey J, Hendrix CW. Gel Applied as Anal Lube Without Applicator Provides Poor Rectal Mucosal HIV Coverage. Conference on Retroviruses and Opportunistic Infections 2016.

Abstracts

1. **Shieh EC**, Ying HS, Grebe RR, Mahmoud M, Kim SY, Goldberg MF, Handa, JT, de Juan E . Genistein reduces retinal pigment epithelial and Bruch's membrane degeneration induced by prolonged blue light exposure in senescence-accelerated mice: light microscopy results. American Geriatrics Society Annual Meeting, Washington D.C., Poster Presentation, 2008
2. Kim K, Alawad AS, Hutfless S, Singh VK, Lennon AM, Sharaiha RZ, Shin EJ, Juneja R, **Shieh EC**, Canto MI, Okolo PI, Kalloo AN, Khashab M. A Comparative Evaluation of Uncovered Partially and Fully-Covered Self-Expandable Metal Stents in the Palliation of Malignant Biliary Strictures. Digestive Disease Week 2012.
3. Sharaiha RZ, Shin EJ, Kim K, **Shieh E**, Halazun HJ, Singh VK, Kalloo AN, Hruban RH, Canto MI, Khashab M. EUS accurately predicts endoscopic local resectability regardless of tumor size. Digestive Disease Week 2012.
4. Kazzi ES, Gresham G, **Shieh E**, Harer K. Water Intubation Method vs. Air Intubation for Colonoscopy Insertion: A Systematic Review and Meta-Analysis. Digestive Disease Week 2016.
5. **Shieh E**, Stein E, Clarke J, Nandwani M, Dhalla S. Role of Achalasia Subtype in Radiographic Bird-Beak Narrowing of the Gastroesophageal Junction. Digestive Disease Week 2016.
6. Chen Y, Yang J, Friedland S, Holmes I, Law R, Hosmer A, Stevens T, Franco M, Jang S, Pawa R, Mathur, Sejpal D, Inamdar S, Trindade A, Nieto J, Berzin T, DeSimone M, DiMaio J, Gupta S, Yachimski P, Anderloni A, Baron T, James T, Jamil L, Ona M, Alammar A, **Shieh E**, Bukhari M, Gutierrez O, Sanaei O, Fayad L, Zhou D, Ngamruengphong S, Kumbhari V, Singh V, Repici A, Khashab M. Lumen apposing stents are superior to plastic stents in the management of pancreatic walled-off necrosis: A large international multicenter study. Digestive Disease Week 2017
7. Wei M, Dao A, Le Angelica, Nguyen T, Tran B, Trinh L, Nguyen H, Nguyen K, Levitt B, **Shieh E**, Purohit T, Cheung R, Nguyen MH, Trinh H. What to do when fecal immunochemical test (FIT) is positive following normal colonoscopy? Comparison of adenoma detection rate (ADR) of standard FIT-colonoscopy and relook colonoscopy. American College of Gastroenterology 2019 (accepted)