Effect of oral and gel tenofovir on genital HSV-2 shedding in immunocompetent women

Rachel A. Bender Ignacio

A thesis
submitted in partial fulfillment
of the requirements for the degree of
Masters in Public Health

University of Washington
2014

Committee:

Anna Wald
Amalia Magaret
Jeanne Marrazzo

Program authorized to Offer Degree:

Epidemiology

University of Washington

Abstract

Rachel A Bender Ignacio

Effect of oral and gel tenofovir on genital HSV-2 shedding in immunocompetent women

Chair of the Supervisory Committee:
Anna Wald, MD MPH, Professor
Division of Allergy and Infectious Diseases, Department of Medicine
Departments of Epidemiology and Laboratory Medicine

Background: Tenofovir is a potent anti-HIV agent with efficacy in studies of pre-exposure prophylaxis when taken orally or used as intravaginal gel. Studies suggest that tenofovir also reduces the risk of HSV-2 acquisition. Whether tenofovir may be useful in HSV disease management, as a dual agent to decrease synergystic HIV and HSV-2 transmission, is unclear.

Methods: We randomized immunocompetent women with symptomatic HSV-2 infection 2:2:1 to oral tenofovir disoproxil fumarate (TDF)/placebo vaginal gel, oral placebo/tenofovir (TFV) vaginal gel, or double placebo in a one-way cross-over clinical trial. Women collected twice-daily genital swabs for HSV PCR and completed symptom diaries during a 4-week lead-in and 5-week treatment phase. The primary intent-to-treat analysis was within-arm comparison of shedding rates and genital lesions with Poisson generalized linear mixed effects models and shedding quantity with a linear mixed-effects model.

Results: 73 women were enrolled and 64 completed the lead-in phase and were randomized: 24 women to TDF, 27 to TFV gel, and 13 to placebo. Relative to baseline, genital HSV shedding showed a trend toward a small decrease from 22.9% to 19.5% (RR=0.86 p=0.09) in the TDF arm, but did not differ in the TFV gel arm (13.8% versus 12.0%; RR=0.94, p=0.54) or in the placebo arm (21.3% versus 20.4%;

RR=0.90, p=0.45; Table 1). Asymptomatic shedding decreased in the TDF arm only (RR=0.74, p=0.01). There was no change in days with HSV lesions or number of shedding episodes. Shedding quantity decreased by 0.50 $\log_{10}$ copies in the TFV gel arm (p=0.008), but remained consistent in the TDF (p=0.18) and placebo arms (p=0.45). The per-protocol analysis included women who completed ≥33 days of study product with ≥90% adherence. Relative to baseline, the shedding frequency was reduced in the TDF arm (RR=0.74, p=0.006), and quantity was reduced in the TDF (0.41 log) and TFV gel arms (0.6 log); otherwise results were similar to the full cohort. Adherence was 97% by returned product count in all arms.

Conclusions: Oral TDF causes small decreases in shedding and lesion rate, and quantity of virus shed when used consistently. Vaginal TFV gel decreases shedding quantity by 60%. In contrast to evidence that tenofovir reduces HSV acquisition by half, benefit in treatment of established HSV-2 infection are less striking.

Background

An estimated 536 million people worldwide ages 15-49 are infected with herpes simplex virus type 2 (HSV-2) and an additional 23.6 new million new infections occur annually [1]. Classic nucleoside analogs, such as acyclovir and valacyclovir, are effective at preventing and treating clinical genital herpes recurrences, but fail to completely suppress asymptomatic genital shedding and sexual transmission of the virus [2-4]. Apart from the morbidity associated with genital herpes and the morbidity and mortality of disseminated HSV-2 infections in both adults and neonates, up to 40-60% of new HIV-1 acquisitions are attributable to genital herpes co-infection [5]. HSV-2 seropositivity increases the risk of HIV-1 transmission by 2-3 times overall [6, 7].

Despite the epidemiologic synergy between HSV-2 and incident HIV-1 infection [8], randomized trials of acyclovir 400mg twice daily have failed to reduce the risk of HIV-1, despite up to 73% reduction in risk of herpes lesions [9-11]. Surprisingly, randomized controlled trials of the nucleoside reverse transcriptase inhibitor (NRTI) tenofovir disoproxil fumarate (TDF) and tenofovir (TFV) vaginal gel for pre-exposure prophylaxis (PrEP) have shown a similar magnitude of benefit in preventing both HSV-2 and HIV-1 transmission. The CAPRISA 004 study, in which women used tenofovir gel before and after sexual intercourse, demonstrated a 51% reduction in HSV-2 acquisition and 39% reduction in HIV-1 acquisition overall, with 54% reduction in HIV-1 transmission observed in those with high adherence to the product [12, 13]. The VOICE trial, also evaluating TFV 1% vaginal gel, demonstrated a 46% reduction in HSV-2 seroconversion in women with detectable plasma levels of TFV [14]. A subgroup analysis of the Partners in Prevention PrEP study revealed a 31% decrease in risk of HSV-2 acquisition in serodiscordant couples in whom the negative partner took either emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) or TDF alone compared with those taking placebo [15].

With growing evidence that tenofovir products prevent acquisition of new HSV-2 infections, the question remains whether either oral or topical formulation can reduce genital viral shedding and symptomatic lesions in people already infected with HSV-2. Combination oral FTC/TDF reduced the risk of genital

ulcer ulcers by 49% in those who were HSV-2-seropositive and HIV-uninfected at baseline, despite no reduction in HSV-2 acquisition in men who have sex with men (MSM) in the iPREX study [16]. To evaluate the impact of tenofovir on established HSV-2 infection in the absence of HIV-1 infection, we undertook a randomized, double-blind, placebo controlled, cross-over trial of oral and vaginal tenofovir in HIV-uninfected women with symptomatic HSV-2 infection.

Methods

Study Participants

Women aged 18-50 who were HIV-negative and HSV-2 seropositive were recruited at the University of Washington Virology Research Clinic in Seattle, WA. We enrolled women who had symptomatic genital herpes for >1 year, between 4-9 recurrences of genital lesions during the last year (or in the year prior to starting any suppressive antivirals), were using effective contraception, and were willing to perform twice daily genital swabbing. We excluded women with Hepatitis B virus infection or who were at high risk for Hepatitis B or HIV acquisition during the study period, had history of immunosuppressive conditions or medications, major medical problems, contraindication to taking tenofovir products including renal insufficiency, or were unwilling to remain off of suppressive antivirals immediately before and during the study period. Most of these women were recruited from a list of previous study participants who were willing to be contacted for future studies, and therefore were experienced at study sample collection.

Study Design and Oversight

The study was designed and implemented by investigators at the University of Washington and was approved by university Institutional Review Board of the Human Subjects Division. The study was sponsored by Gilead Sciences (tenofovir disoporoxil fumarate (TDF; Viread)) and by CONRAD (TFV vaginal gel). Randomizion codes were generated by a biostatistician not otherwise involved in this study. All authors contributed to the work, and take responsibility for the accuracy of data presented. The sponsors reviewed the manuscript but did not participate in the analysis or the decision to submit the manuscript for publication.

Study Drugs

TDF was provided in a single 300 mg tablet daily with matching placebo provided by the manufacturer. The 1% tenofovir (TFV) gel is a clear pH balanced gel provided in a single-use applicator to deliver 4 ml of gel, or 40 mg of tenofovir and applied intravaginally once daily. The matching "universal" placebo gel has been tested in vitro and verified to have no anti-HSV properties; both are the same products that were utilized in the VOICE trial and produced exclusively by Conrad [17, 18].

Study Procedures

All enrolled women were asked to perform twice-daily swabbing of the genital and perianal mucosa and skin and complete daily diary entries of genital symptoms and visualization of lesions [4, 19, 20]. Women who provided ≥90% of the swabs were randomized 2:2:1 to one of three arms: oral TDF tablet and placebo vaginal gel, oral placebo table and TFV vaginal gel, or oral placebo tablet and placebo vaginal gel. Women took both investigational products once daily and continued performing genital swabbing and diary records twice daily for 35 further days. Participants were instructed to present to the study clinic for an exam and lesional swabbing for HSV culture and PCR when experiencing lesions at any point during the study.

Laboratory Studies

HSV-2 infection was confirmed with the University of Washington Western Blot [21]. HSV DNA was extracted from genital swabs containing genital secretions and analyzed with a real-time, quantitative, fluorescent polymerase-chain-reaction (PCR) assay (TaqMan) at the University of Washington Virology Laboratory [22]. Test results were considered to be positive when 150 HSV DNA copies per milliliter or more were observed [23].

Safety Evaluation

Safety evaluation included the nature, rate, duration, and severity of adverse events. Events were graded using the Division of Acquired Immunodeficiency Syndrome Table of adverse events [24]. Participant

report, physical examination, and laboratory investigation contributed to the safety assessment. Laboratory assessments were performed at baseline and after completion of the treatment phase.

Measurement of Study Product Adherence

Adherence to the study regimen was evaluated with two mechanisms. Study staff recorded the fraction of returned pills of those dispensed between study visits and proportion of gel applicators returned unused. Participants also recorded whether and at what time they used the study products and performed genital swabbing as part of the daily diary entry.

Acceptability Questionnaire

Participants completed surveys on acceptability of the vaginal gel investigational product at the mid-way and end points of the study. The survey was administered regardless of randomization arm.

Statistical Methods

Design and Power

We designed this study with a sample size of 22 in each investigational product arm to have 80% power to detect a 50% reduction in primary outcome of HSV-2 shedding with an α of 0.05, assuming a baseline shedding rate of 20% [25]. This study was not powered to detect differences in the secondary outcomes. We included the double placebo arm to demonstrate stability in shedding rates between lead-in and treatment phases when there was no active investigational product.

Primary Analysis

We performed our analyses as an intention-to-treat analysis, wherein all participants with at least 1 swab in the lead-in and 1 swab after day 7 in the treatment phases are included in the primary analysis. In order to allow therapeutic effect of the tenofovir products, we excluded the first 7 days of swabs during the treatment phase from analysis. For each arm of the study in the lead-in and treatment phases, we calculated the median and range for number of swabs and days under observation. Shedding rates are calculated as the proportion of positive swabs out of total swabs collected by all participants within each

phase of each arm. Per-person shedding rate is defined as the number of positive swabs out of all swabs collected for each person during each phase of the study. Asymptomatic shedding rates are defined as number of positive swabs out of all collected swabs coinciding with a diary entry without report of lesions. We similarly define proportion of lesion-days in individual participants and overall for each arm per phase and calculated frequencies. Shedding episodes are defined as discrete periods of positive swabs bounded by 2 negative swabs on each side. Quantitative PCR quantities are given in $\log_{10}$ copies.

We performed Poisson generalized linear mixed effects models (GLME) with an offset for individual exposure duration to evaluate the co-primary outcomes of within-arm differences in shedding rate between the lead-in and treatment phases for each arm. The models were run separately for each arm and no comparisons were performed between arms. We evaluated secondary outcomes including changes in asymptomatic shedding, number of shedding episodes, and frequency of lesions between lead-in and treatment phases, using similar Poisson models each corrected for time under observation. Evaluation of within-arm HSV-2 $\log_{10}$ copy quantity was performed using a linear mixed effects model utilizing only samples with HSV DNA detected.

Per Protocol Analysis

We performed a per-protocol or as-treated analysis given the variability of adherence to the study drugs and small sample sizes. We defined treated "per protocol" post-hoc as participants who had a treatment phase lasting at least 33 days (35 days was target duration) and with ≥90% adherence to the active drug, whether tablet or gel. Participants fulfilling these criteria would therefore have a minimum of 30 days of biologic exposure to the active drug. We repeated each of the previously described analyses using this per protocol criteria.

Results

Study Population

One hundred and four women were screened for this study, and 16 were not enrolled due to abnormalities on pelvic exam (n=6), clinical exam or laboratory tests (2), hepatitis B surface Ag positivity

(1), insufficient frequency of HSV-2 recurrences (3), or lack of HSV-2 infection (5) (Figure 1). Sixteen women declined entry. 73 women began the 4-week lead-in shedding phase and the 64 women who completed ≥90% of genital swabbing were randomized to one of the three treatment arms. The majority of patients in each arm were white; most had a high school diploma and more than one-quarter completed college. The median age was 37.3 years and the median duration of genital herpes was 9.3 years, and the median number of annual clinical recurrences was 4-6 in each arm (Table 1).

24 women were randomized to the oral TDF arm, 22 completed the therapeutic phase, with 20 completing "per protocol" with ≥90% adherence to both the oral and vaginal products.
27 women were randomized to the TFV vaginal gel arm, 22 completed the therapeutic phase and 1 never began either product, and 21 took the oral and 20 took the vaginal products per protocol. 13 women were randomized to the double placebo arm, 2 never began either drug, and 9 completed both products per protocol. Therefore 24, 27, and 13 women are included in the intention-to-treat analysis and 20, 20, and 9 are included in the per-protocol analysis.

Oral Tenofovir Arm:

Overall, 2,587 swabs were collected in the oral TDF arm. The shedding rate during the lead-in phase was 22.9%; HSV DNA was detected in 66.7% of women at least once prior to treatment. The median $\log_{10}$ copy number of positive swabs was 4.02 (IQR 3.21, 5.29) in the lead-in phase. Women reported genital lesions on 11.8% of days and 42.7% of women had lesions on at least one day during the lead in phase (Tables 2a-b). Change in shedding rates between lead-in and treatment phases in each arm is demonstrated in Figure 2 a-c. The variability in shedding rates observed in the study is exemplified by separate bar plots for each arm in Figures 3a-c.

The relative risk (RR) for shedding during treatment compared with the lead-in phase was 0.86 (95%CI 0.72, 1.01; p=0.086) (Table 4). The risk for asymptomatic shedding was 0.74 (0.59, 0.92; p=0.010). There was no difference in days with lesions RR= 0.98 (0.70, 1.37; p= 0.90). Oral TDF did not change number of episodes of HSV-2 shedding or quantity of HSV-2 shed on days where shedding was detected. In the per-protocol analysis, the risk for HSV-2 shedding, lesions, asymptomatic shedding, and quantity of shedding were significantly reduced (Table 5). The RR for shedding on treatment was 0.74 (0.60, 0.91;

p=0.006), risk for asymptomatic shedding was 0.69 (0.54, 0.89; p=0.006), and risk for lesions was 0.75 (0.57, 0.97; p=0.032). The quantity on days where shedding was detected was decreased by 0.41 log_{10} copies (p=0.004).

Tenofovir Gel Arm

2,841 total swabs were collected in the TFV gel arm. During the lead-in phase, shedding was present on 13.8% of days and 63.0% of women had HSV DNA detected at some point. Median log_{10} copy number (and IQR) on positive swabs was 4.47 (2.92, 6.24). Women had lesions on 8.7% of days, and 37.0% of women had lesions on at least one day during the lead-in phase (Table 2a-b).

We found no decrease in risk for HSV-2 shedding (RR 0.94, p=0.54), asymptomatic shedding (RR 1.30, p=0.90), or frequency of lesions in those using TFV gel (RR 0.80, p=0.25) (Table 4). TFV gel was associated with a 0.50 log_{10} copy decrease on days with shedding (-0.86, -0.13; p=0.008).

The per-protocol analysis similarly showed no decrease in risk for HSV-2 shedding, asymptomatic shedding, lesions, or number of shedding episodes. The magnitude of copy number decrease on positive swabs was somewhat greater at -0.60 log_{10} copies (-.0.98, -0.21; p=0.003) (Table 5).

Double Placebo Arm

1,223 total swabs were collected in the double-placebo arm. During the lead-in phase, the shedding rate was 21.3%; 84.6% of women shed HSV on at least one day. Median log_{10} copy number on positive swabs was 3.71 (IQR 2.78, 5.54). In the lead-in phase, lesions were present on 13.6% of days and 69.2% of participants had lesions on at least one day (Table 2a-b).

There were no significant changes between the lead-in and treatment phases in the arm that received both placebo gel and placebo tablets (Tables 4 & 5).

Study Drug Adherence

The median number of days of treatment was 35.5 (range 13, 53) in the oral TDF arm, 36 (13, 43) in the TFV gel arm, and 35 (2, 37) in the double placebo arm (Table 3). Adherence to the active tablet was

97.1% (IQR 91.0, 100.0%) in the oral TDF arm, to the gel 97.3% (IQR 94.3, 100.0%) in the TFV gel arm, and 97.1% (IQR 94.4, 100.0%) to both placebo products in that arm.

Adverse Events

Eleven people in the oral TDF, 7 in the TFV gel and 3 in the placebo arm reported 64 adverse events considered possibly or definitely related to an investigational product; 60 events were Grade 1 or 2. Four Grade 3 events were reported, and no Grade 4 or SAEs were observed. Two Grade 3 events occurred in the same participant, who experienced severe abdominal pain and diarrhea, beginning as moderate pain on the 2nd day of oral TDF and escalating over 2 weeks. The second participant experienced severe diarrhea beginning on the 3rd day of oral TDF. Both participants reported spontaneous resolution of symptoms after 15 and 22 days and did not discontinue the study products. A different participant in the oral TDF arm terminated the study drugs on day 13 due to Grade 1-2 diarrhea and abdominal cramping that began on the first day of therapy. The last Grade 3 event was an increase in frequency of chronic migraines in a participant randomized to TFV gel that had stopped using the gel 8 days earlier because of a urinary tract infection, and was only taking oral placebo at time of migraine onset. Only these last two participants terminated the study early due to AEs. The most commonly reported symptoms in the oral TDF arm were diarrhea (16.7%), nausea, and abdominal pain (both 12.5%) (Table 6). In the TFV gel arm, 11.1% experienced vaginal burning, itching, or discomfort and 11.1% experienced headache. In the placebo arm 15.4% experienced vaginal burning, itching, or discomfort and 7.7% developed vulvovaginitis or candidiasis. Rates of abnormalities in post-treatment chemistries (serum sodium, potassium, bicarbonate, phosphate, glucose, or amylase), liver function tests (SGOT, SGPT, alkaline phosphatase, total bilirubin) and complete blood count appeared similar in all three arms (Table 7). There were no abnormalities in serum creatinine before or after the study in any arm.

Acceptability of Vaginal Gel Product

26.3% of women “liked a lot” or “somewhat liked” the gel product, 30.2% were neutral, and the remainder somewhat (23.8%) or very much (21.4%) disliked the product. Most women felt the gel was very easy to use, but somewhat less so during menses. Most women reported they would use the gel if it were proven

to reduce either their own signs and symptoms (69.7%) or risk of transmitting genital herpes (67.4%); however, fewer women (19.3%) would prefer to use the gel if an oral option was equally effective. Women noted that the gel was noticeable both to herself and the partner during intercourse, and thus its use could not be easily concealed. Most felt that the gel did not change the experience of sexual intercourse, though several women found the lubrication provided by the gel beneficial. Women frequently noted that mess from the gel was problematic, soiled undergarments and bedclothes, and often leaked out from the vagina after application.

Discussion

We tested the efficacy of both oral and vaginal tenofovir products to prevent genital HSV shedding and clinical lesions in a randomized controlled trial of immunocompetent women with symptomatic genital herpes. In the intention to treat analysis, neither tenofovir preparation was protective against HSV shedding or genital lesions. However, among women with very high adherence by self-report, oral TDF reduced the risk of HSV shedding, asymptomatic shedding, and lesions by 25-30%. Vaginal TFV gel did not reduce these risks. Both tenofovir products approximately halved the quantity of HSV DNA shed in the genital tract on days in which shedding occurred.

Our findings are consistent with what is known about the pathophysiology of established HSV-2 infection. Following primary infection, HSV-2 becomes latent in the sacral ganglia. Periodic reactivation of the latent infection then reaches the skin and mucosa via sensory nerve roots, where the infection propagates between adjacent epithelial cells leading to both formation of lesions and asymptomatic shedding [26, 27]. It is likely that high levels of tenofovir, or any antiviral, must be delivered to central and peripheral nervous system to achieve sufficient concentrations to prevent reactivation. The 50% inhibitory concentration (IC50) of tenofovir for HSV-2 is 50 to 100 times higher than that for HIV-1 [28-30]. Therefore, standard HIV-targeted dosing of tenofovir will likely not reach sufficient inhibitory concentrations in this privileged anatomic site, and providing higher systemic doses is likely unfeasible given consequential increases in toxicity. TFV gel applied vaginally results in much higher local concentration of the drug within the tissue than that achieved with systemic administration of standard doses, and therefore may be better able to

inhibit propagation of the infection between epithelial cells. Conversely, the topical gel is unlikely to inhibit initiation of the reactivation event in the ganglia, as topical vaginal administration results in 56-fold lower serum concentrations than standard oral dosing [31]. This is consistent with our finding that TFV gel produced minimally larger reductions in quantity of virus shed, but failed to reduce shedding rate, frequency of shedding episodes, or lesions. Genital mucosal samples and cervical lavage examined in prior studies of oral TDF demonstrate that the concentration available in tissue easily achieves inhibitory concentrations for HIV-1 but not HSV-2, and exemplifies why even with excellent adherence, oral TDF may have limited utility in preventing shedding or lesion formation in established HSV-2 infection [28-30].

Prior to our study, the only study directly evaluating the effect of tenofovir on shedding in established HSV-2 infection was a single observational study performed in HIV-infected women taking tenofovir as part of their backbone antiretroviral regimen. This study demonstrated no decrease in genital shedding of HSV-2 in women with infrequent symptomatic genital herpes recurrences [32]. However, this study was limited by the fact that only patients with infrequent genital symptoms were enrolled, and enrollment occurred when patients were already receiving TDF or non-TDF containing ART. Therefore it is not clear whether the same patient may have had more lesional events, and consequently higher frequency of HSV shedding, if receiving non-TDF containing ART. Conversely, a sub-group analysis of HSV-2-infected/HIV-uninfected MSM in the iPREX trial demonstrated a 49% decrease in genital ulcers among those taking TDF for PrEP; genital HSV shedding was not assessed in this study [16]. Adding our study results to these previous findings demonstrates that there is some effect of tenofovir on established HSV-2 infection, but not clinically significant enough to replace the antivirals currently in use.

In contrast to the minimal effects of tenofovir on established HSV-2 infection, risk of HSV-2 acquisition in participants receiving any of the tenofovir preparations in Partners PrEP, CAPRISA 004, and VOICE was decreased by 30-50% [12, 14, 15]. It has been hypothesized that HSV-2 acquisition occurs through the skin and mucosa of the genitalia and perineum at the same sites where reactivation later occurs. Interesting insights can be drawn from the results of this study taken together with those from the HIV-1 PrEP trials. The first is that if topical TFV only effects propagation of HSV-2 within the epithelium at

locations at which it has been applied, these results may suggest a different route of HSV-2 acquisition than traditionally recognized in women. That is to say, if TFV gel only provokes local effects and is only applied intravaginally, the cervix may be a common initial site of HSV-2 acquisition, rather than the external genitalia where the lesions and subsequent shedding ultimately occur. It is plausible that infection occurs through the cervix, ascends retrograde to the sacral ganglia and only returns to the genital and perineal skin or mucosal area by virtue of peripheral nerve supply from the same ganglion that was infected. Primary infection occurring at the cervix may in part explain why intra-vaginal application of TFV can prevent HSV-2 acquisition but does not produce more profound decreases in shedding quantity and lesions in external genital and perineal areas. Inferring a new pathway for acquisition of primary HSV-2 infection however is not clear cut in that, as reported by the women in this study, the TFV gel often did not stay intravaginally, and therefore likely coated the external genitalia and perineal area as well. Also complicating the hypothesis that HSV-2 is acquired cervically, is that the use of a diaphragm did not prevent HSV-2 acquisition in seronegative participants in the MIRA trial [33]. Additionally, the drug routes and dosages of tenofovir (and perhaps other antivirals) sufficient to prevent HSV-2 acquisition may not provide adequate concentration or distribution to prevent reactivation of established infection.

Limitations of our study include small study size with person-time to detect differences in the less-frequent genital lesion recurrences. Additionally, we used pill counts and diaries to determine adherence to the study regimen, rather than a biologic measure of exposure, such as serum or vaginal fluid tenofovir levels. However, we feel that it is unlikely that our study had significant issues with adherence because swab compliance was excellent and we believe it to be highly unlikely that a participant would selectively adhere to twice daily swabbing while not adhering daily administration of study product. A high proportion of women returned swabs with HSV DNA present and there was good correlation between swab numbers and pill and gel applicator counts. An analysis of self-sampled swabs in similar participants at our research clinic demonstrated that 93-99% of returned swabs contained β-globin, a control marker for exposure to human host cells [34]. Strengths of this study include rigorous and well-documented methods for measurement of genital HSV shedding. Additionally, our cross-over study design utilized participants as their own controls and provided the additional control of a double placebo arm, which

performed the cross-over to inactive investigational products to confirm the stability of shedding rates across study phases.

Conclusions

This study is the first to evaluate the effect of tenofovir on symptomatic and asymptomatic genital recurrences in persons infected with HSV-2. While tenofovir and acyclovir or valacyclovir have not been compared head-to-head, nor tested in combination, tenofovir appears inferior to these nucleoside analogs in both outcomes of interest. Our study supports several relevant points. First, vaginally applied tenofovir, despite its potential to achieve high local tissue concentrations, is unlikely to be able to inhibit initiation of the HSV-2 reactivation events at their source. Oral TDF, or vaginal tenofovir products alone or co-formulated with acyclovir, remain promising in the prevention of HSV-2 and HIV-1 in persons who are seronegative for both infections [35, 36]. Second, for HIV-negative HSV-2 seropositive persons, either systemic or topical routes are unlikely to prevent clinical disease or transmission of HSV-2. Future studies should evaluate whether combination acyclovir and tenofovir products can both decrease HIV-1 acquisition further than tenofovir alone, and also further reduce clinical HSV-2 disease and shedding beyond the effects of acyclovir alone. Because oral TDF demonstrated some effect on both shedding and clinical disease in persons without HIV-1, larger studies of TDF as a component of ART in HSV-2/HIV-infected persons may be considered. Future trials of vaginal tenofovir products may also consider use of TDF rather than TFV to improve anti-HSV-2 activity given improved cellular uptake and efficacy of TDF compared with TFV in animal models of HSV-2 infection [30]. In summary, while our study showed minimal effect of either oral TDF or vaginal TFV in established HSV-2 infection in HIV-1 women, our results contribute to the knowledge of how tenofovir may be clinically applicable in interrupting the joint epidemics of HSV-2 and HIV-1.

Acknowledgments:

This study benefited from significant contributions by Dr. Tara Perti, Claire Stevens, Sharanya Rajagopal, Dr. Meei-Li Huang, Dr. Christine Johnston, and Stacy Selke.

References

1. Looker, K.J., G.P. Garnett, and G.P. Schmid, *An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection.* Bull World Health Organ, 2008. **86**(10): p. 805-12, A.
2. Hobson, A., et al., *Evaluation of a quantitative competitive PCR assay for measuring of HSV DNA in genital tract secretions.* J Clin Microbiol, 1997. **35**: p. 548-552.
3. Gupta, R., et al., *Valacyclovir and Acyclovir for Suppression of Shedding of Herpes Simplex Virus in the Genital Tract.* J Infect Dis, 2004. **190**(8): p. 1374-1381.
4. Corey, L., et al., *Once-daily valacyclovir to reduce the risk of transmission of genital herpes.* N Engl J Med, 2004. **350**(1): p. 11-20.
5. Wald, A. and K. Link, *Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis.* J Infect Dis, 2002. **185**(1): p. 45-52.
6. Serwadda, D., et al., *Human immunodeficiency virus acquisition associated with genital ulcer disease and herpes simplex virus type 2 infection: a nested case-control study in Rakai, Uganda.* J Infect Dis, 2003. **188**(10): p. 1492-7.
7. Freeman, E.E., et al., *Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies.* Aids, 2006. **20**(1): p. 73-83.
8. Rollenhagen, C., et al., *Herpes simplex virus type-2 stimulates HIV-1 replication in cervical tissues: implications for HIV-1 transmission and efficacy of anti-HIV-1 microbicides.* Mucosal immunology, 2014.
9. Mujugira, A., et al., *Daily acyclovir to decrease herpes simplex virus type 2 (HSV-2) transmission from HSV-2/HIV-1 coinfected persons: a randomized controlled trial.* J Infect Dis, 2013. **208**(9): p. 1366-74.
10. Celum, C., et al., *Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2.* N Engl J Med, 2010. **362**(5): p. 427-39.
11. Watson-Jones, D., et al., *Effect of herpes simplex suppression on incidence of HIV among women in Tanzania.* N Engl J Med, 2008. **358**(15): p. 1560-71.
12. Abdool Karim, Q., et al., *Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women.* Science, 2010. **329**(5996): p. 1168-74.
13. Karim, S.S.A. *Results of the CAPRISA 004 trial of tenofovir gel*. in *XVIII International AIDS Conference 18–23 July*. 2010.
14. Marrazzo, J., et al., *Association of Tenofovir (TFV) Detection with Reduced Risk of Herpes Simplex virus Type-2 (HSV-2) Acquisition in the VOICE (MTN 003) Study.* AIDS Res Hum Retroviruses, 2014. **30 Suppl 1**: p. A31.
15. Celum, C., et al., *Daily oral tenofovir and emtricitabine-tenofovir preexposure prophylaxis reduces herpes simplex virus type 2 acquisition among heterosexual*

HIV-1-uninfected men and women: a subgroup analysis of a randomized trial. Ann Intern Med, 2014. **161**(1): p. 11-9.
16. Marcus, J.L., et al., *Daily oral emtricitabine/tenofovir preexposure prophylaxis and herpes simplex virus type 2 among men who have sex with men.* PLoS One, 2014. **9**(3): p. e91513.
17. Marrazzo, J., et al. *Pre-exposure prophylaxis for HIV in women: daily oral tenofovir, oral tenofovir/emtricitabine, or vaginal tenofovir gel in the VOICE study (MTN 003)*. in *20th Conference on Retroviruses and Opportunistic infections*. 2013.
18. Schwartz, J.L., et al., *A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel.* PLoS One, 2011. **6**(10): p. e25974.
19. Johnston, C., et al., *Standard-dose and high-dose daily antiviral therapy for short episodes of genital HSV-2 reactivation: three randomised, open-label, cross-over trials.* Lancet, 2012. **379**(9816): p. 641-7.
20. Wald, A., et al., *Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir.* Ann Intern Med, 1996. **124**(1 Pt 1): p. 8-15.
21. Ashley, R.L., et al., *Comparison of Western blot (Immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera.* J Clin Microbiol, 1988. **26**: p. 662-7.
22. Wald, A., et al., *Polymerase Chain Reaction for Detection of Herpes Simplex Virus (HSV) DNA on Mucosal Surfaces: Comparison with HSV Isolation in Cell Culture.* J Infect Dis, 2003. **188**(9): p. 1345-51.
23. Magaret, A.S., et al., *Optimizing PCR positivity criterion for detection of herpes simplex virus DNA on skin and mucosa.* J Clin Microbiol, 2007. **45**(5): p. 1618-20.
24. National Institutes of Health. *Divison of AIDS table for grading the severity of adult and pediatric adverse events.*
25. Tronstein, E., et al., *Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection.* JAMA, 2011. **305**(14): p. 1441-9.
26. Schiffer, J.T., et al., *Frequent release of low amounts of herpes simplex virus from neurons: results of a mathematical model.* Sci Transl Med, 2009. **1**(7): p. 7ra16.
27. Schiffer, J.T., et al., *Rapid localized spread and immunologic containment define Herpes simplex virus-2 reactivation in the human genital tract.* Elife, 2013. **2**: p. e00288.
28. Andrei, G., et al., *Topical tenofovir, a microbicide effective against HIV, inhibits herpes simplex virus-2 replication.* Cell Host & Microbe, 2011. **10**(4): p. 379-89.
29. Herold, B.C., et al., *Antiviral activity of genital tract secretions after oral or topical tenofovir pre-exposure prophylaxis for HIV-1.* J Acquir Immune Defic Syndr, 2014. **66**(1): p. 65-73.

30. Nixon, B., et al., *Vaginally delivered tenofovir disoproxil fumarate provides greater protection than tenofovir against genital herpes in a murine model of efficacy and safety.* Antimicrob Agents Chemother, 2014. **58**(2): p. 1153-60.
31. Hendrix, C.W., et al., *MTN-001: Randomized Pharmacokinetic Cross-Over Study Comparing Tenofovir Vaginal Gel and Oral Tablets in Vaginal Tissue and Other Compartments.* PLoS One, 2013. **8**(1).
32. Tan, D.H., et al., *No impact of oral tenofovir disoproxil fumarate on herpes simplex virus shedding in HIV-infected adults.* Aids, 2011. **25**(2): p. 207-10.
33. de Bruyn, G., et al., *The effect of the vaginal diaphragm and lubricant gel on acquisition of HSV-2.* Sex Transm Infect, 2011. **87**(4): p. 301-5.
34. Mujugira A, H.M., Selke S, Drolette L, Magaret AS, Wald A, Corey L, *"Validation of self-sampling for HSV diagnosis using beta globin PCR*.
35. Mesquita, P.M., et al., *Intravaginal ring delivery of tenofovir disoproxil fumarate for prevention of HIV and herpes simplex virus infection.* J Antimicrob Chemother, 2012. **67**(7): p. 1730-8.
36. Shankar, G.N. and C. Alt, *Prophylactic treatment with a novel bioadhesive gel formulation containing aciclovir and tenofovir protects from HSV-2 infection.* J Antimicrob Chemother, 2014. **69**(12): p. 3282-93.

Table 1: Baseline characteristics of participants

Characteristic	**Oral TDF Placebo Gel N=24**	**Oral Placebo TFV Gel N=27**	**Double Placebo N=13**
Age			
Median	39.1	36.7	41.0
Range	20.8-50.8	21.1-50.7	19.2-48.7
Race N (%)			
White	14 (58.3)	14 (51.9)	9 (69.2)
Black	5 (20.8)	10 (37.0)	2 (15.4)
Asian/PI	1 (4.2)	0	1 (7.7)
Other/multiple	4 (16.7)	3 (11.1)	1 (7.7)
Education N (%)			
Less than HS	1 (4.2)	0	1 (7.7)
HS Graduate	16 (66.7)	20 (74.1)	6 (46.2)
College grad +	7 (29.2)	7 (25.9)	6 (46.2)
Recurrences in 1 year*			
Median	5	4	6
Range	1-24	0-6	0-12
Missing -- N (%)	1	2	0
Years since HSV Dx			
Median	15.3	10.0	7.1
Range	1.4-29.7	1.1-31.8	1.0-32.5
Missing – N (%)	1 (4.2)	1(3.7)	--

*Recurrences listed are from the first year of enrollment at VRC, which may not correspond to the immediate 12 months prior to study. Therefore some persons had no recurrences reported in the above statistic but were study-eligible by more recent recurrence frequency and/or reported frequency in the absence of antiviral therapy.

Table 2a: Shedding and lesions, overall by arm

Variable	Oral TDF Placebo gel	Oral Placebo TFV Gel	Double Placebo
Total # of swabs	2587	2841	1223
Shedding Rate: + swabs/tot. (%)			
Lead-in phase	295/1291 (22.9)	204/1477 (16.0)	151/708 (21.3)
Treatment phase	253/1296 (19.5)	164/1364 (12.0)	105/515 (20.4)
Asymptomatic Shedding Rate: + swabs/tot (%)			
Lead-in phase	200/1140 (17.5)	102/1345 (7.6)	88/611 (14.4)
Treatment phase	157/1145 (13.7)	104/1266 (8.2)	53/438 (12.1)
Number of shedding episodes			
Lead-in phase	34	36	27
Treatment phase	32	36	16
Log-quantity shed on positive swabs: median (IQR)			
Lead-in phase	4.02 (3.21, 5.29)	4.47 (2.92, 6.24)	3.71 (2.78, 5.54)
Treatment phase	4.11 (3.23, 5.39)	4.40 (3.37, 5.13)	4.22 (3.35, 5.45)
Lesion rate: + days/tot. (%)			
Lead-in phase	79/667 (11.8)	68/784 (8.7)	50/368 (13.6)
Treatment phase	78/670 (11.6)	50/709 (7.1)	39/265 (14.7)

Table 2b: Shedding and lesions, by individual within arm

Variable	TDF Oral Placebo gel	Oral Placebo TFV gel	Double Placebo
Median # of swabs per person collected (range)			
Lead-in phase	54 (47, 59)	54 (42, 71)	54 (50, 61)
Treatment phase	57 (11,85)	55.5 (11,69)	56 (10,59)
Median observation days (range)			
Lead-in phase	28.0 (25,31)	27.5 (24,41)	28.0 (25,34)
Treatment phase	35.5 (13,53)	36 (14,43)	35 (2, 37)
Persons with any shedding: n (%)			
Lead-in phase	16 (66.7)	17 (63.0)	11 (84.6)
Treatment phase	19 (79.2)	14 (53.8)	8 (80.0)
Shedding rate: median (range)			
Lead-in phase	12.4 (0, 42.0)	5.7 (0, 26.7)	14.8 (5.3, 39.8)
Treatment phase	14.0 (1.7, 39.2)	2.7 (0, 23.2)	23.9 (8.2, 33.9)
Asymptomatic shedding rate: median (range)			
Lead-in phase	10.1 (0, 97.6)	1.9 (0, 49.1)	8.6 (0, 71.7)
Treatment phase	4.9 (0, 100.0)	0 (0, 40.0)	10.9 (0, 32.7)
Mean log-copy number on positive days (range)			
Lead-in phase	3.9 (2.9, 5.7)	4.0 (2.5, 6.9)	4.0 (3.0, 5.1)
Treatment phase	4.3 (2.3, 7.9)	3.8 (3.0, 4.4)	3.9 (2.6, 4.4)
Proportion of persons with HSV lesions: n (%)			
Lead-in phase	10 (42.7)	10 (37.0)	9 (69.2)
Treatment phase	15 (54.2)	9 (34.6)	9 (60.0)
Lesion Rate: Median (range)			
Lead-in phase	0 (0, 39.2)	0 (0, 46.4)	11.1 (0, 48.1)
Treatment phase	11.4 (0, 66.6)	0 (0.46.4)	8.4 (0, 71.4)

Table 3: Days on Study Drug and Drug Adherence

	Oral TDF Placebo gel	Oral Placebo TFV Gel	Double Placebo
Days on study drug median (range)	35 (13,53)	35.5 (13, 43)	36 (2, 37)
Tablet adherence* % median (range)	97.1 (78.6, 100.0)	97.3 (83.3, 100.0)	97.1 (45.7, 100)
Gel adherence product* % median (range)	97.3 (57.1, 100.0)	97.1 (42.9, 100.0)	97.1 (57.1, 100)

*Adherence measured by proportion of returned tablets or unused gel applicators of those dispensed between visits. For the

Table 4: Intra-participant risk of HSV shedding and genital lesions on study drug (ITT)

Outcomes	Oral TDF Placebo Gel		Oral Placebo TFV Gel		Double Placebo	
	RR (95% CI)	P value	RR (95% CI)	P value	RR (95%CI)	P value
Shedding	0.86 (0.72, 1.02)	0.086	0.94 (0.75, 1.16)	0.54	0.90 (0.67, 1.22)	0.47
Asymptomatic shedding	0.74 (0.59, 0.92)	0.010	1.30 (0.96, 1.76)	0.090	0.75 (0.49, 1.14)	0.15
Shedding episodes	0.94 (0.56, 1.57)	0.81	1.17 (0.71, 1.92)	0.52	0.84 (0.41, 1.71)	0.58
Lesions	0.98 (0.70, 1.37)	0.90	0.80 (0.54, 1.18)	0.25	0.95 (0.57, 1.57)	0.82
	Change in log quantity (95% CI)		Change in log quantity (95% CI)		Change in log quantity (95% CI)	
Quantity	-0.16(-0.40, 0.07)	0.18	-0.50 (-0.86, -0.13)	0.008	0.16 (-0.27, 0.60)	0.45

Table 5: Intra-participant risk of HSV shedding and genital lesions on study drug: Per protocol analysis (≥33 drug days with ≥90% adherence to active drug, or placebos in double placebo arm*)

Outcomes	Oral TDF Placebo Gel		Oral Placebo TFV Gel		Double Placebo	
	RR (95%CI)	P value	RR (95%CI)	P value	RR (95%CI)	P value
Shedding	0.74 (060, 0.91)	0.006	0.95 (0.74, 1.22)	0.69	0.78 (0.57, 1.07)	0.11
Asymptomatic shedding	0.69 (0.54, 0.89)	0.006	1.38 (0.95, 1.99)	0.085	0.73 (0.48, 1.11)	0.12
Shedding episodes	0.83 (0.47, 1.47)	0.50	1.25 (0.71, 2.20)	0.41	0.73 (0.33, 1.58)	0.37
Lesions	0.75 (0.57, 0.97)	0.032	0.81 (0.61, 1.09)	0.15	0.75 (0.51, 1.08)	0.11
	Change in log quantity (95% CI)		Change in log quantity (95% CI)		Change in log quantity (95% CI)	
Quantity	-0.41 (-0.70, -0.13)	0.004	-0.60 (-0.98, -0.21)	0.003	0.18 (-0.27, 0.64)	0.42

*Applying these rules to usage of either product in the placebo arm resulted in inclusion of the same number of participants. We did not therefore need to employ a tie-breaking rule.

Table 6: Possibly and definitely related adverse events, excluding events unrelated to study product

Individual events in the upper portion of the table are numbers of persons with each event. The lower half of the table gives events. Participants experiencing an adverse event could contribute to more than one category of event (for example, several persons with nausea also had abdominal pain and/or diarrhea). Grades listed are the most severe grade of that event experienced by an individual participant.

Event Category	Oral TDF Placebo Gel N=24	Oral Placebo TFV Gel N=27	Double Placebo N=13	Total N=64
		Persons with Events N (%)		
Genital Burning, Itching, Irritation	1 (4.2)	3 (11.1)	2 (15.4)	9 (14.1)
Headache	1 (4.2)	3 (11.1)	0	3 (4.7)
Diarrhea	4 (16.7)	0	0	4 (6.3)
Vulvovaginitis/Candidiasis	1 (4.2)	2 (7.4)	1 (7.7)	3 (4.7)
Abdominal Pain	3 (12.5)	0	0	3 (4.7)
Vaginal spotting	1 (4.2)	0	0	1 (1.6)
Perianal Fissure	1 (4.2)	0	0	1 (1.6)
Nausea	3 (12.5)	0	0	3 (4.7)
Fatigue	1 (4.2)	0	0	1 (1.6)
Lightheadedness	1 (4.2)	0	0	1 (1.6)
Difficulty concentrating	1 (4.2)	0	0	1 (1.6)
Vomiting	1 (4.2)	0	0	1 (1.6)
Urinary tract infection (UTI)	0	1 (3.7)	0	1 (1.6)
Skin rash	1 (4.2)	0	0	1 (1.6)
Reflux	1 (4.2)	0	0	1 (1.6)
Uterine cramping after gel application	0	1 (3.7)	0	1 (1.6)
		Events N		
Any Adverse Event	21	10	3	34
Grade 1 (Mild) Events	9	8	2	19
Grade 2 (Moderate) Events	9	1	1	11
Grade 3 (Severe) Events	3	1	0	4
Serious Adverse Events (SAE)	0	0	0	0
Events prompting early termination[†]	1	1	0	2

[†]In the Oral TDF arm, 1 person discontinued the study products after 13 days of Grade I/II diarrhea and abdominal pain. In the TFV gel arm on participant discontinued the gel following a urinary tract infection. She continued in the study but discontinued the oral tablet 8 days later when she developed worsening of her chronic migraine headaches (Grade III); these headaches were noted in screening history, but severity not clear.

Table 7: Laboratory abnormalities following treatment phase

Lab Category	Oral TDF Placebo Gel N=24	Oral Placebo TFV Gel N=24	Double Placebo N=10
	N (%)	N (%)	N (%)
Amylase, total	1 (4.2)*	2 (8.3)	1 (10.0)
Glucose†	2 (8.3)	2 (8.3)	0
Potassium	1 (4.2)	0	0
Phosphorus	0	0	1 (10.0)
Sodium	7 (29.2)	5 (20.8)	1 (10.0)
Total bilirubin	0	0	1 (10.0)
Hemoglobin	1 (4.2)	0	0

This table includes any abnormality detected on post-treatment blood work, regardless of whether the abnormality was present on screening laboratories. No abnormalities in creatinine were detected either at screening or post-treatment in any participant. All abnormalities are Grade 1 unless noted otherwise.

*Grade 1 elevation at screening increased to Grade 2 post-treatment
†Non-fasting glucose levels. 1 of 2 events in the Oral TDF arm was Grade 2. Both events in the TFV Gel arm were Grade 3; one of these was in a known Type II diabetic with elevated glucose (Grade 2) at screening that was increased mildly on the single random value obtained. The second Grade 3 event was hypoglycemia with a random glucose of 54 mg/dl without accompanying symptoms recorded.

Figure 1: Study Flow Diagram

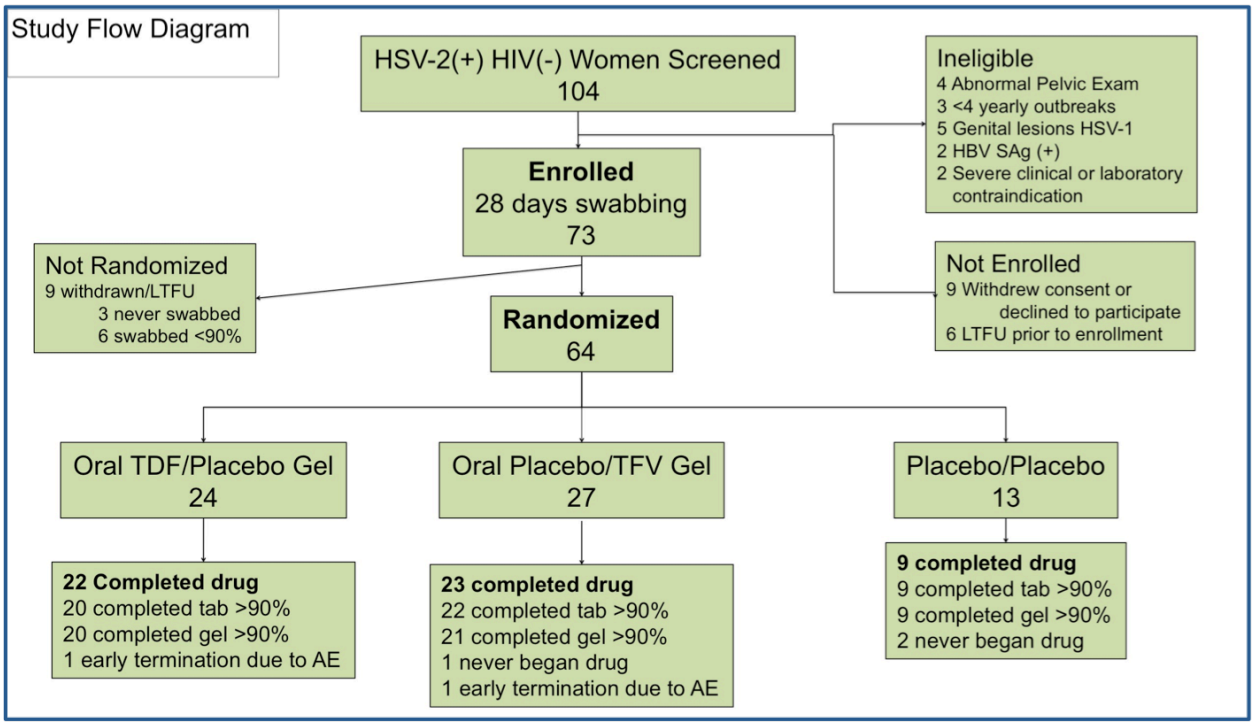
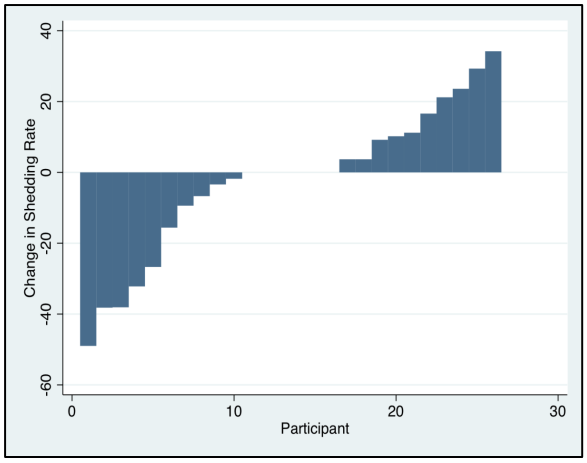


Figure 2: Change in within-person shedding rates from lead-in to treatment phase in each arm

2a: Oral TDF Arm



2b: TFV gel arm

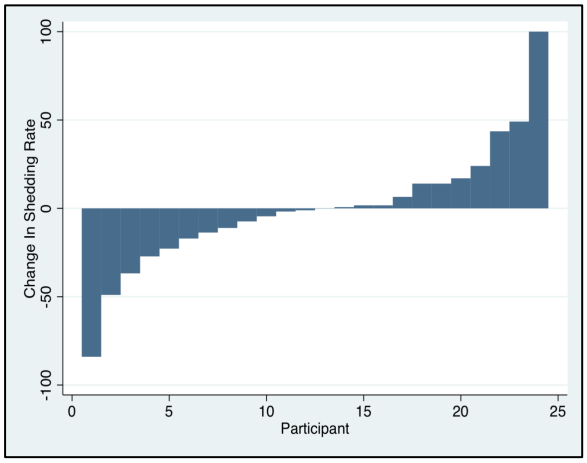
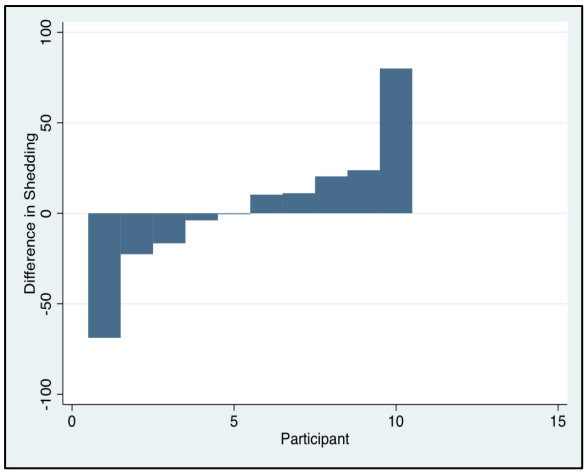


Figure 2c: Double placebo arm



Shedding rates represented as 0-100%. More bars below the axis (negative change in shedding rate) favors treatment benefit; bars in the positive region demonstrate an increase in shedding rate during treatment. All three figures demonstrate large variability in treatment effect. A modest visual trend toward negative shedding differences can be seen in Figure 2a.

Figure 3: Matched bar plots demonstrate wide variability in shedding rates more so than a distinct trend towards treatment effect in each arm. Figure 3a: Oral TDF arm 3b: TFV vaginal gel arm 3c: Double placebo arm