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BRIEF REPORT

# Effect of On-Demand Oral Pre-exposure Prophylaxis With Tenofovir/Emtricitabine on Herpes Simplex Virus-1/2 Incidence Among Men Who Have Sex With Men: A Substudy of the ANRS IPERGAY Trial

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We evaluated the impact of on-demand oral tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) for pre-exposure prophylaxis (PrEP) on herpes simplex virus (HSV)-1/2 incidence among men who have sex with men (MSM) enrolled in the ANRS IPERGAY trial. Serum samples were tested at baseline and at the last visit for HSV-1/2 antibodies. Overall HSV-1 incidence was 11.7 per 100 person-years; 16.2 and 7.8 per 100 person-years in the TDF/FTC and placebo arm, respectively ($P$ = .19). Overall HSV-2 incidence was 7.6 per 100 person-years; 8.1 and 7.0 per 100 person-years in the TDF/FTC and placebo arm, respectively ($P$ = .75). On-demand oral PrEP with TDF/FTC failed to reduce HSV-1/2 incidence in this population.

**Keywords.** HSV1/2 incidence; HSV1/2 prevalence; MSM; PrEP; TDF/FTC.

Herpes simplex virus (HSV) is a common cause of both genital and oral diseases. Herpes simplex virus type 2 (HSV-2) is mostly sexually transmitted, whereas HSV type 1 (HSV-1) is frequently acquired during early childhood, mainly via oral secretions. However, the epidemiology of HSV is changing, with increased frequency of HSV-1 sexual transmission [1]. Genital herpes is also an important cofactor for human immunodeciency virus (HIV) acquisition [2].

In the Caprisa 004 PrEP study among HIV risk women in South Africa, pericoital application of a topical vaginal-gel formulation of tenofovir was shown to reduce HIV acquisition [3]. In addition to its potent activity as a nucleotide inhibitor of HIV-1 reverse transcriptase, tenofovir has anti-HSV-1 and anti-HSV-2 activity in vitro [4]. Indeed, the use of topical tenofovir gel has been shown to reduce the incidence of HSV-2-infection by 51% in women [5]. Oral tenofovir-based PrEP also reduced HSV-2 acquisition by 30% among heterosexual men and women in the Patrners PrEP study [6]. However, no reduction of HSV-2 incidence was reported in the iPrEx study among men who have sex with men (MSM) with daily tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) [7].

Therefore, this study wished to assess the impact of on-demand oral TDF/FTC for PrEP on HSV-1 and HSV-2 incidence in the ANRS IPERGAY PrEP trial among MSM. We also examined the effect of TDF/FTC on the shedding of HSV-2 in seropositive individuals.

## MATERIALS AND METHODS

### Study Population

From February 2012 through October 2014, 400 MSM were enrolled in the ANRS IPERGAY randomized trial of PrEP for HIV-1 prevention in France and Canada. Details of the IPERGAY trial have been previously published [8]. In brief, participants were randomized 1:1 to receive on-demand oral TDF/FTC or placebo. Tenofovir disoproxil fumarate/FTC was given as a fixed-dose combination of 300 mg of TDF and 200 mg of FTC per pill. Participants were instructed to take a loading dose of 2 pills of TDF/FTC or placebo with food 2 to 24 hours before sex, followed by a third pill 24 hours after the first drug intake and a fourth pill 24 hours later. Study visits were scheduled 4 and 8 weeks after enrollment and every 8 weeks thereafter, and serum and plasma were stored at –80°C.

Before each visit, participants were asked to complete a computer-assisted self-administered questionnaire at home to collect information about sociodemographic characteristics and sexual behavior. At enrollment, participants were screened for syphilis (on serologic analysis) and chlamydia and gonorrhea

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(by means of a specific polymerase chain-reaction [PCR] assay performed on anal and throat swabs and urine samples), which were also stored at –80°C.

#### Analysis of Adherence

Adherence was measured by pill count. Participants were asked to return their study drug bottles at each visit, and pill count of unused medication was performed. The number of pills used per month was then calculated as the total number of pills given, minus total number of pills returned, divided by the number of months.

#### Laboratory Procedures

Stored serum samples from all of the participants enrolled in the ANRS IPERGAY trial were tested at baseline and at their last visit of the randomized phase for HSV-1 and HSV-2 antibodies by using serological tests according to manufacturer's instructions (BioPlex 2200 HSV-1 and HSV-2 immunoglobulin [Ig]G; Bio-Rad). The BioPlex 2200 HSV-1 and HSV-2 IgG kit detects and differentiates IgG antibodies to HSV-1 and HSV-2. As recommended by the manufacturer (Bio-Rad), the results are reported according to their antibody index, with values <0.9 considered negative, values 0.9 to 1.0 considered equivocal, and values >1.0 considered positive [9]. BioPlex has a good specificity and sensitivity compared with HerpeSelect (Focus Technologies), which was used in the iPrEx study. The concordance between the 2 assays was high (agreement = 96.2%; k = 0.80, $P < .0001$). Participants with equivocal results were excluded from the analysis.

We also studied the shedding of HSV-2 from stored anal swabs from HSV-2 seropositive participants. After nucleic acid extraction using QIAsymphony (QIAGEN, Courtaboeuf, France), HSV1/HSV2 (HSV1 HSV2 VZV R-gene kit Argene) PCR was performed at baseline, month 6 (M6) and month 12 (M12). The lower limit of detection of this assay is 200 copies/mL.

#### Statistical Analysis

Comparisons were done using the $\chi^2$ test or Fisher's exact test for categorical variables and the Wilcoxon test for continuous variables. Incidences of HSV-1 and HSV-2 infections were compared between groups with mid-*P* exact. We calculated 95% confidence interval (CI) for incidence by use of a Poisson distribution. We did analyses with Stata/SE 12.1 software and SAS software version 9.3. All *P* values and 95% CIs are 2-sided.

## RESULTS

Baseline characteristics of the 400 participants have been described previously [8]. Of the 400 participants, 70% (280 of 396) were HSV-1 seropositive (4 samples with equivocal results were excluded) and 39% (155 of 397) HSV-2 seropositive (3 samples with equivocal results were excluded). Overall, 82% were seropositive for HSV-1 or HSV-2, and 28% were seropositive for both HSV-1 and HSV-2. Only 18% were seronegative for both HSV-1 and HSV-2.

We compared the baseline behavioral characteristics of the study participants according to the HSV-1 or HSV-2 serology at enrollment (Table 1). Factors associated with testing seropositive for HSV-1 or HSV-2 included older age and higher number of partners in the last 2 months. Condom use at last receptive anal sex in the past 4 weeks was significantly associated with seronegativity for HSV-1 (25% vs 10%, $P = .002$) and for HSV-2 (18% vs 9%, $P = .04$). Ethnic origin, use of recreational drugs, circumcision, and bacterial sexually transmitted infection at enrollment were not associated with HSV-1 or HSV-2 seropositivity.

**Table 1. Behavioral Characteristics of the ANRS IPERGAY Trial Participants According to the HSV-1/HSV-2 Serology at Enrollment**

| Variable | Seropositive for HSV-1 N = 280 | Seronegative for HSV-1 N = 116 | *P* Value | Seropositive for HSV-2 N = 155 | Seronegative for HSV-2 N = 242 | *P* Value |
|---|---|---|---|---|---|---|
| Median age (IQR), yr | 35.9 [30.1–43.3] | 33.0 [25.7–40.9] | .006 | 38.8 [31.1–45.8] | 32.9 [27.2–40.4] | <.0001 |
| Age group, no. (%) | | | | | | |
| <30 yr | 67 (24) | 45 (39) | | 32 (21) | 82 (34) | |
| 30–39 yr | 108 (39) | 36 (31) | | 49 (32) | 95 (39) | |
| ≥40 yr | 105 (37) | 35 (30) | | 74 (47) | 65 (27) | |
| Caucasian origin (n, %) | 252 (90) | 110 (95) | .12 | 146 (94) | 217 (90) | .12 |
| Use of recreational drugs (n, %) | 128 (46) | 53 (46) | 1.00 | 71 (46) | 113 (47) | .66 |
| Circumcision (n, %) | 57 (20) | 22 (19) | .75 | 27 (17) | 52 (21) | .32 |
| No. of partners in past 2 months (median, IQR) | 10 [5.0–17.3] | 6.7 [3.3–12] | .001 | 10 [6.0–20.0] | 8 [4.0–15.0] | .0003 |
| Condom use in men with last receptive anal sex in past 4 weeks (n, %) | 21/204 (10) | 21/85 (25) | .002 | 10/111 (9) | 32/180 (18) | .04 |
| STI[a] at enrollment (n, %) | 73/280 (26) | 36/116 (31) | .31 | 47/155 (30) | 62/242 (26) | .31 |

Abbreviations: HSV, herpes simplex virus; IQR, interquartile range; STI, sexually transmitted infection.

[a]Chlamydia, gonorrhoea, or syphilis.

Of the 116 HSV-1-seronegative participants, 110 had available samples after enrollment and the median of follow-up was 10.2 months (interquartile range [IQR], 6.2–20.5). Results were obtained for 108 (2 samples with equivocal results were excluded) and 14 seroconverted for HSV-1. Overall HSV-1 incidence was 11.7 per 100 person-years (95% CI, 6.4–19.6); 16.2 per 100 (95% CI, 7.4–30.8) in the TDF/FTC arm versus 7.8 per 100 (95% CI, 2.5–18.2) in the placebo arm ($P$ = .19) (Table 2). Considering the serological status of HSV-2 at baseline, we found a higher incidence of HSV-1 in participants seronegative for HSV-2 compared with those seropositive for HSV-2, but this difference was not significant ($P$ = .14).

For HSV-2, of the 242 HSV-2 seronegative participants, 222 had available samples after enrollment, and the median of follow up was 10.2 months (IQR, 6.0–23.5). Results were obtained for 218 (4 samples with equivocal results were excluded) and 19 seroconverted for HSV-2. Overall incidence of HSV-2 infection was 7.6 per 100 person-years (95% CI, 4.6–11.8); 8.1 per 100 (95% CI, 4.0–14.5) in the TDF/FTC arm versus 7.0 per 100 (95% CI, 3.0–13.7) in the placebo arm ($P$ = .76) (Table 3). Considering the serological status of HSV-1 at baseline, no difference of HSV-2 incidence was found in participants seronegative for HSV-1 compared with those seropositive for HSV-1 ($P$ = .76).

Among the 71 participants seronegative for HSV-1 and HSV-2, 15 seroconverted (9 for HSV-1, 4 for HSV-2, and 2 for both HSV-1 and HSV-2). The median of follow up was 9.0 months (IQR, 6.0–18.4). Overall incidence of HSV-1 or HSV-2 infection in this subgroup of participants was 21.4 per 100 person-years (95% CI, 12.0–35.4); 23.4 per 100 person-years (95% CI, 10.7–44.5) in the TDF/FTC arm and 19.0 per 100 person-years (95% CI, 7.0–41.4) in the placebo arm ($P$ = .71).

Considering the number of pills taken in the TDF/FTC arm (≤ or >15 pills/month), no difference was found in the incidence of HSV-1 ($P$ = .43) or HSV-2 ($P$ = .77) infections (Tables 2 and 3). No case of HIV acquisition was reported in participants who seroconverted for HSV-1 or HSV-2 during the study.

Herpes simplex virus type 2 shedding was analyzed in 58 participants with available anal samples (28 in the placebo arm and 30 in the TDF/FTC arm) at baseline, M6 and M12. A total of 172 samples were tested. Only 3 participants (5.2%) had HSV-2 positive PCR, 1 in the placebo arm at enrollment (4 900 copies/mL) and 2 in the TDF/FTC arm, 1 at M12 (115 500 copies/mL) and 1 at M6 (2 816 000 copies/mL) and M12 (595 000 copies/mL).

## DISCUSSION

In this analysis of MSM enrolled in the ANRS IPERGAY trial of on-demand oral TDF/FTC PrEP, we found no association between TDF/FTC use and incidence of HSV-1 or HSV-2 infection. Our results are in accordance with those of the iPrEx study among MSM using daily TDF/FTC [7] but contrast with the 51% reduction in HSV-2 incidence among women randomized to use a 1% tenofovir topical gel in CAPRISA 004 [5] and with the 30% reduction among heterosexual men and women taking oral daily tenofovir in the Patrners PrEP study [6].

The difference between the effects of TDF on HSV-2 incidence seen in the different PrEp trials may be due to differences in populations, route of transmission, and sexual practices (MSM in IPERGAY and iPrEx studies, and women and heterosexual individuals in Caprisa and Partners Prep trial). This could be also due to drug level at the site of HSV acquisition. Indeed, although oral dosing of tenofovir achieves drug concentrations that are 20–100 times higher in rectal tissue than in vaginal and cervical tissue [10], topical application of tenofovir achieves a more than 100-fold higher concentration of the drug in the genital tract than oral dosing [11, 12]. In the Caprisa study, women with high level of vaginal tenofovir exceeding 10 000 ng/mL had a 63% higher rate of protection against HSV-2 infection than women with no detectable vaginal tenofovir [5]. We do not have a clear explanation for the discrepancy with the Partners PreP where oral TDF was also used, but the reduction of HSV-2 acquisition in this trial was moderate and just significant (5.6/100 person-years in TDF arm vs 7.7 in placebo, $P$ = .047). Moreover, adherence to TDF, as measured by plasma drug levels, was higher in Partners PreP (71%) than in iPrEx (50%) and could explain these differences. In the ANRS IPERGAY trial with on-demand oral TDF/FTC, the median number of pills used per month was 15, and we did not see any difference in HSV incidence in those using ≥15 or <15 pills/month.

**Table 2. HSV-1 Incidence According to Treatment Arm, PrEP Intake, and Results of HSV-2 Serology at Baseline: The ANRS Ipergay Trial**

| Variable | Total | Treatment | | | | Number of Pills/Month[a] (TDF/FTC Arm) | | | | HSV-2 Serology at Baseline | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n = 108 | TDF/FTC n = 53 | Placebo n = 55 | HR (95% CI) | *P* | ≤15 n = 28 | > 15 n = 24 | HR (95% CI) | *P* | Negative n = 69 | Positive n = 39 | HR (95% CI) | *P* |
| HSV-1 incidence, per 100 PY 95% CI | 11.7 [6.4–19.6] | 16.2 [7.4–30.8] | 7.8 [2.5–18.2] | 2.08 (0.63–7.92) | .19 | 20.6 [7.6–44.9] | 11.5 [2.4–33.6] | 1.80 (0.38–11.10) | .43 | 15.6 [7.8–27.9] | 6.1 [1.3–17.9] | 2.55 (0.67–14.23) | .14 |

Abbreviations: CI, confidence interval; FTC, emtricitabine; HR, hazards ratio; HSV, herpes simplex virus; PrEP, pre-exposure prophylaxis; PY, person-years; TDF, tenofovir disoproxil fumarate.
[a]One missing data.

**Table 3. HSV-2 Incidence According to Treatment Arm, PrEP Intake, and Results of HSV-1 Serology at Baseline: The ANRS Ipergay Trial**

| Variable | Total | Treatment | | | | Number of Pills/Month[a] (TDF/FTC Arm) | | | | HSV-1 Serology at Baseline[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n = 218 | TDF/FTC n = 100 | Placebo n = 118 | HR (95% CI) | *P* | ≤15 n = 54 | >15 n = 59 | HR (95% CI) | *P* | Negative n = 69 | Positive n = 149 | HR (95% CI) | *P* |
| HSV-2 incidence, per 100 PY 95% CI | 7.6 [4.6–11.8] | 8.1 [4.0–14.5] | 7.0 [3.0–13.7] | 1.16 (0.43–3.33) | .76 | 7.4 [2.4–17.3] | 8.9 [3.3–19.5] | 1.21 (0.31–5.00) | .77 | 8.4 [3.1–18.2] | 7.3 [3.9–12.4] | 0.87 (0.31–2.78) | .76 |

Abbreviations: CI, confidence interval; FTC, emtricitabine; HR, hazards ratio; HSV, herpes simplex virus; PrEP, pre-exposure prophylaxis; PY, person-years; TDF, tenofovir disoproxil fumarate.

[a]Six missing data.

[b]Two missing data for HSV-1 status at baseline (equivocal results).

In this population of MSM with high risk of sexual behavior, we reported a high baseline seroprevalence of 70% for HSV-1 and 39% for HSV-2, and a high incidence for HSV-1 (11.7 per 100 person-years) and HSV-2 infections during follow up (7.6 per 100 person-years). This incidence increased to 21.4 per 100 person-years when we focused on the 71 participants seronegative for both HSV-1 and HSV-2 at baseline. This is higher than the incidence of 5.9 per 100 person-years of the iPrEx study, which included 1347 participants HSV-2 seronegative at baseline. This is probably explained in our study by the high number of partners in the last 2 month and the lower use of condoms compared with the iPrEx study. We also found that factors associated with HSV-1 and HSV-2 seronegativity at baseline were a lower number of partners and more frequent condom use. Reducing the number of partners and increasing the use of condoms probably represent the best available prevention of HSV acquisition.

We also assessed the role of TDF/FTC on HSV-2 shedding on stored anal samples. The HSV-2 shedding rate among HSV-2 seropositive participants was low (5.2%), and there was no effect of TDF/FTC on HSV-2 shedding as previously described in Tan et al [13]. This is in accordance with the fact that even in HIV-infected patients, most mucosal reactivations are short and subclinical with a median anogenital reactivation duration of 11 hours [14].

Limitations of this study include the limited sample size with a small number of incident cases and a little power to show differences in HSV-1 or HSV-2 incidence between the 2 groups. We also used proxy measures of adherence to the study regimen (number of pills per month) rather than a biologic measure of exposure such as TDF/FTC levels in serum. In addition, we cannot exclude that HSV can spread by skin-to-skin contact instead of through bodily fluids, and people can get infected even in the absence of penetrative sex.

## CONCLUSIONS

In summary, despite the high seroprevalence of HSV-1 or HSV-2 at enrollment, the incidence of both herpes viruses was high in these MSM enrolled in a PrEP trial. On-demand oral PrEP with TDF/FTC failed to reduce HSV-1/2 incidence in this population.


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***Potential conflicts of interest.*** All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.


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