



Published in final edited form as:

*Nat Med.* 2018 May ; 24(4): 491–496. doi:10.1038/nm.4506.

## Genital inflammation undermines the effectiveness of tenofovir gel in preventing HIV acquisition in women

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### Abstract

Several clinical trials have demonstrated that antiretroviral (ARV) drugs, taken as pre-exposure prophylaxis (PrEP), can prevent HIV infection<sup>1</sup>, with the magnitude of protection ranging from -49 to 86%<sup>2–11</sup>. While these divergent outcomes are thought to be due primarily to product

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**Declaration of interests:** None of the authors have any financial or personal relationships to disclose. The study sponsor(s) did not play any role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. The corresponding authors had full access to all of the data in the study and were responsible for the decision to submit for publication.

adherence<sup>12</sup>, biological factors likely contribute<sup>13</sup>. Despite selective recruitment of higher risk participants for prevention trials, HIV risk is heterogeneous, even within higher risk groups<sup>14–16</sup>. To determine whether this heterogeneity could influence PrEP outcomes, we undertook a post-hoc prospective analysis of the CAPRISA 004 tenofovir 1% gel trial (n=774), one of the first trials to demonstrate protection against HIV infection. Concentrations of nine pro-inflammatory cytokines were measured in cervicovaginal lavages at >2,000 visits, and a graduated cytokine score was used to define genital inflammation. In women without genital inflammation, tenofovir was 57% protective against HIV (95% CI: 7 to 80%), compared to 3% (95% CI: –104 to 54%) if genital inflammation was present. Among high gel adherers, tenofovir protection was 75% (95% CI: 25 to 92%) in women without inflammation compared to –10% (95% CI: –184 to 57%) in women with inflammation. Host immune predictors of HIV risk may modify the effectiveness of HIV prevention tools; reducing genital inflammation in women may augment HIV prevention efforts.

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HIV acquisition risk varies widely within a population and is dependent on behavioral and biological factors. Younger women (<25 years), for example, experience higher HIV incidence, likely due to a combination of types and frequencies of partnerships and biological factors such as genital inflammation<sup>17,18</sup>. PrEP effectiveness was lowest in women <25 years of age in the VOICE and Dapivirine ring trials; this sub-group was least adherent to PrEP and did not experience significant protection<sup>2,9</sup>. The route of HIV exposure may also be important, considering better oral PrEP protection was observed in MSM under conditions of high adherence<sup>10,11</sup>, despite higher per-coital acquisition during anal sex<sup>19</sup>. Further, in conditions of lower adherence, protection was still evident in MSM (iPrEx) but not in women (VOICE)<sup>2,4</sup>. Mucosal tissue penetrance and pharmacokinetics may explain some of these differences; for example, active tenofovir levels in colorectal tissue reach 10-times higher concentrations than in the female genital tract (FGT)<sup>20,21</sup>.

Where products are partially effective, protection may not be equal across HIV risk groups. Protection in the RV144 vaccine trial was higher in individuals at low and medium risk but negligible in those at highest risk<sup>22</sup>. In the iPrEX Open Label Extension study in MSM, the “number needed to treat” with PrEP to prevent one infection differed significantly among risk-defined sub-groups<sup>23</sup>. Conversely, in the Partners PrEP trial, participants who consistently used PrEP were protected regardless of risk profile, suggesting that high adherence and/or effectiveness may overcome differences in susceptibility to infection<sup>24</sup>.

Case-control analyses of the three trials that have tested topical tenofovir in women (CAPRISA 004, VOICE, and FACTS001) showed that protection against HIV ranged from 50-60%, if product adherence was high<sup>2,3,8</sup>. These data infer that adherence alone might not fully explain the incomplete efficacy of this product. Here, we evaluate how biological susceptibility, defined by inflammation in the FGT<sup>18</sup>, altered the protective efficacy of tenofovir gel.

We carried out a prospective cohort analysis of all available pre-HIV infection mucosal specimens from CAPRISA 004 (n = 774 women sampled at 2,139 visits). 281 women had genital inflammation when defined as 3 of 9 pro-inflammatory cytokines elevated (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IL-8, IP-10, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ ); 204 had inflammation if defined by 4 elevated cytokines, 140 if 5, 90 if 6, and 45 women if 7 cytokines were

elevated. Those not meeting the criteria for inflammation were automatically placed in the “no inflammation” comparator group, making the denominator  $n = 774$  for all analyses. We carried out Cox regression analysis to determine whether the link between inflammation and HIV risk depends on the definition of inflammation that was used. Each definition of genital inflammation was associated with increased HIV risk adjusted for study arms (tenofovir and placebo); specifically aHR = 1.86 for 3 cytokines elevated (95% CI: 1.11-3.10), aHR = 1.90 for 4 cytokines elevated (95% CI: 1.12-3.22), aHR = 2.38 for 5 cytokines elevated (95% CI: 1.37-4.15), aHR = 2.99 for 6 cytokines elevated (95% CI: 1.64-5.45), and aHR = 3.42 for 7 cytokines elevated (95% CI: 1.62-7.23), all  $p < 0.05$ . While the HIV effect estimates for 3 and 4 elevated cytokines were similar, a step-wise increase in HIV risk of approximately 50% was observed for each elevated cytokine after 4/9. These inflammation-defined strata were used for the subsequent tenofovir efficacy comparisons.

We next determined whether tenofovir gel was protective against HIV infection based on the presence or absence of genital inflammation (Table 1). The HIV incidence in the study was 3.9 per 100 person-years (95% CI: 2.5–5.8), slightly lower than the main trial. In all inflammation-defined strata, the lowest HIV incidence rates were observed in women without inflammation who were randomized to tenofovir. In women with 3 cytokines elevated, HIV incidence was 6.8 per 100 person-years (95% CI: 3.8-11.1) in the tenofovir arm compared to 7.0 (95% CI: 3.7-11.9) in the placebo arm. In contrast, in women with  $< 3$  elevated cytokines, HIV incidence in the tenofovir and placebo arms was 2.3 (95% CI: 1.0-4.4) and 5.4 (95% CI: 3.4-8.2), respectively. Similar results were obtained when additional numbers of cytokines were elevated; in the strata defined by 5, 6, and 7 elevated cytokines, HIV incidence was higher in women with inflammation randomized to tenofovir compared to women with inflammation randomized to placebo (Table 1).

The overall efficacy of tenofovir gel in this study was 34% (95% CI -11-61%). Stratifying by genital inflammation clearly segregated efficacy estimates: women with 3 cytokines elevated had a tenofovir efficacy of 3% (95% CI -104-54%,  $p = 0.936$ ), compared to those with  $< 3$  elevated cytokines, where a tenofovir efficacy of 57% (95% CI 7-80%,  $p = 0.033$ ) was observed (Figure 1a). Tenofovir efficacy was 11% for 4 cytokines elevated, -8% for 5 cytokines, -147% for 6 cytokines, and -37% for 7 cytokines (all  $p > 0.1$ ). In contrast, tenofovir efficacy ranged from 34-56% in the corresponding no-inflammation groups, and these comparisons were statistically significant ( $p < 0.05$ ) for 3, 5, and 6/9 cytokines. Similar results were obtained in multivariate Cox proportional hazards regression analyses, adjusting for age, study site, herpes simplex virus (HSV)-2 serostatus, history of sexually transmitted infections (STIs), number of sex acts and sexual partners, condom and injectable contraception use (Table 2). Interestingly, HSV-2 positivity mattered more for HIV acquisition if inflammation was not present, in line with findings that HSV-2 seroprevalence is not associated with inflammatory cytokines<sup>25</sup>. Conversely, increasing numbers of sex acts was associated with HIV only in those with inflammation. Given that gel dosing was per-coital, it is difficult to disaggregate effects of sex and exposure to tenofovir gel in these analyses. Nevertheless, these data confirm that FGT inflammation predicted the efficacy of tenofovir gel in women.

We further tested for interaction between genital inflammation and study arm in a Cox regression analysis with time-varying covariates, taking into account repeated measures of genital inflammation. In a model that included genital inflammation (3/9 elevated cytokines), study arm, and an interaction term between inflammation and study arm, a significant interaction between genital inflammation and study arm was observed ( $p=0.028$ ). Similar findings were obtained for 4/9 and 5/9, although these were not statistically significant ( $p=0.127$  and  $0.11$ , respectively). Similar results were obtained in models adjusting for potential confounders. These findings support the conclusion that genital inflammation attenuated the efficacy of tenofovir gel.

Previous analyses of the CAPRISA 004 trial demonstrated a dose-dependent relationship between gel adherence, measured by the percentage of sex acts covered by two gel doses, and protection<sup>26</sup>. We hypothesized that the combination of having no inflammation and high adherence would provide the best protection from HIV infection, and that high levels of inflammation might supersede the protective effects conferred by adherence. Indeed, tenofovir gel-mediated protection was highest in women without genital inflammation who used the gel at 50% of sex acts (Supplemental Table 1), with efficacy of 75% (95% CI 25-92%,  $p=0.014$ ; <3 cytokines in the upper quartile). In comparison, tenofovir efficacy was -10% (95% CI -184-57%,  $p=0.844$ ) in highly adherent women with genital inflammation (3 elevated cytokines). Tenofovir efficacy was attenuated in women who used gel infrequently (< 50% adherence) irrespective of their inflammation status (25 and 15% efficacy,  $p=0.781$  and  $p=0.656$ , respectively). Similar results were obtained in adjusted models containing the same covariates as described in Table 2. We also obtained similar results in survival analyses. In the strata defined by low adherence, genital inflammation status was the major predictor of HIV acquisition risk (Figure 1b, solid lines) and there was little evidence of tenofovir-mediated protection. However, in those with high adherence to tenofovir (Figure 1c), protection afforded by the gel was restricted to the no inflammation group. Similar results were obtained for all cytokine scores (4, 5, 6, and 7 elevated cytokines; data not shown). These data provide compelling evidence that women without genital tract inflammation largely account for the protective effect of tenofovir gel adherence that was observed in the CAPRISA 004 trial<sup>3</sup>.

The FGT mucosa typically provides an effective barrier against HIV infection, as reflected by the low per-coital rates of male-to-female HIV transmission in epidemiological studies<sup>27,28</sup>. Genital inflammation may decrease natural host defenses against HIV, with the corollary being that individuals with inflammation are more difficult to protect by anti-viral agents such as tenofovir. We have previously described reduced levels of key mucosal barrier proteins and increased numbers of cervical CD4+ T cells, the key targets of HIV, in women with cytokine profiles similar to those used in our inflammation scoring<sup>29</sup>, a finding supported by other studies<sup>30,31</sup>. This barrier susceptibility hypothesis is corroborated by recent data from CAPRISA 004 showing that women with genital inflammation and tenofovir have less fit viruses crossing the barrier and establishing HIV infection<sup>32</sup>. Cellular activation may further increase intracellular dNTP pools and compete with the ability of tenofovir-diphosphate to block HIV reverse transcriptase and prevent infection<sup>33</sup>. Understanding these mechanisms will be critical in designing more effective PrEP strategies, particularly in women.

Strengths of this study include its longitudinal design and large sample size, one of the largest studies of mucosal inflammation in the context of an HIV prevention trial. Genital inflammation was evaluated at repeated measures in participants randomized to tenofovir or placebo gel exposure, and inclusion of the entire available cohort allowed us to calculate HIV incidence and tenofovir efficacy in sub-groups of at-risk individuals. The study tested an *a priori* hypothesis, and immunological analyses were blinded with extensive quality control measures put in place to ensure the accuracy of cytokine measurements across multiple sample runs. While inflammation is difficult to capture by any one measurement, pro-inflammatory cytokines are believed to be central to this process. Our sensitivity analyses support the notion that inflammation was consistently able to differentiate women protected by tenofovir, irrespective of how many cytokines were elevated.

Our study had some limitations. Specimens were available only at certain study visits and were not available for a subset of individuals including some cases who acquired HIV prior to the first available genital sample. However, in the remaining cohort we took a median measure of several visits, and are therefore better able to classify individuals than by a single measurement. Our major conclusions were further borne out by a second time-varying analysis, showing a significant interaction between genital inflammation (3 elevated cytokines) and study arm in predicting HIV acquisition. For adherence, we relied on self-reported return of used applicators. Mucosal tenofovir concentration data are available in a subset of participants<sup>26</sup>, although for too few for comparisons of gel efficacy. We based our adherence analyses on 50% product use; while this has clear clinical implications regarding protection, the study is underpowered for further adherence cut-offs, and for adherence-inflammation interaction analyses. Despite our large sample size, a relatively small proportion of the cohort had genital inflammation, limiting statistical power to definitively conclude that those with inflammation were not protected by tenofovir gel. Further validation in additional cohorts could increase this sample size, but this has logistical challenges. Finally, we deliberately selected composite cytokine outputs based on prior studies<sup>18</sup> to overcome the burden of multiple test correction for individual cytokine concentrations, and used multiple elevated cytokine cut-offs to determine the rigour of these definitions of genital inflammation in assessing HIV outcomes.

The causes of inflammation remain unclear. Several groups have shown that bacterial vaginosis (BV) and/or changes in the vaginal microbiome are associated with genital inflammation<sup>34,35</sup>, including in CAPRISA 004<sup>36</sup>. We and others have also recently shown that vaginal dysbiosis impairs tenofovir efficacy, perhaps by reducing levels of tenofovir in the mucosa<sup>37</sup>. Interestingly, the same may not apply to oral PrEP, as BV and/or vaginal dysbiosis did not affect PrEP efficacy in the Partners PrEP study<sup>38</sup>. This could be due to pharmacological differences between oral and topical PrEP. Since not all BV/dysbiosis results in inflammation<sup>39</sup>, repeating our inflammation analyses in other PrEP studies will help to understand the generalizability of our findings.

In summary, the combination of gel adherence and genital inflammation differentiated women who were protected by topical tenofovir from those who were not. This was pronounced in participants who did not have genital inflammation but were highly adherent to gel, who experienced protection levels as high as 75%. However, those with genital

inflammation who were adherent to gel had no protection, underscoring the unlikelihood of any protective effect in the “inflammation/adherent” group. Inflammation is a major risk factor for HIV acquisition; reducing genital inflammation by treating its root causes or by anti-inflammatory agents might further optimize PrEP for women. Genital inflammation should be investigated as a potential effect modifier in trials of novel PrEP products.

## Methods

### Study design

We undertook a prospective cohort study to assess the impact of mucosal cytokine levels on HIV acquisition and tenofovir efficacy using specimens collected during the CAPRISA004 trial, a phase 2B randomized, blinded, placebo-controlled trial that measured the safety and efficacy of tenofovir 1% gel<sup>3,40</sup>. The study was approved by the Biomedical Research Ethics Committee (BREC) at the University of KwaZulu-Natal, and all participants provided informed written consent to participate. The *a priori* objective of these experiments was to compare tenofovir efficacy stratified by inflammation and adherence status of participants, with the hypothesis that tenofovir efficacy would be reduced by mucosal inflammation. The target sample size was all those with available pre-HIV infection specimens from the intent-to-treat (ITT) analysis CAPRISA 004 cohort. The final analysis included 774 women sampled at 2,139 study visits, or 87% of the original intent-to-treat cohort. We were not able to carry out analyses in instances where storage consent was not provided, no specimens were available, or when participants acquired HIV before samples could be obtained. The inclusion/exclusion criteria for CAPRISA 004 have been published previously<sup>40</sup>; these included sexually active women aged 18-40 years living near either an urban or rural study site in KwaZulu-Natal, South Africa. All clinical and epidemiological variables used in these analyses are part of the original locked database generated in the parent clinical trial<sup>3</sup>.

### Cytokine assays

The concentrations of 9 cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IL-8, IP-10, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ ) were measured in undiluted cervicovaginal lavage (CVL) specimens by multiplexed ELISA assays (Bio-Plex; Bio-Rad Laboratories, Inc; Hercules, Ca, USA). For consistency, fresh assays were performed on all participants including those previously published in Masson et al<sup>18</sup>, and all laboratory personnel were blinded to all clinical and epidemiological variables. Cytokine concentrations were measured using a Bio-Plex 200 Array Reader (Bio-Rad Laboratories). The sensitivity of these kits ranged between 0.2 and 45.2 pg/ml for each of the cytokines measured. Data were collected using Bio-Plex Manager software version 6, and a 5 PL regression formula was used to calculate sample concentrations from the standard curves. Cytokine levels below the lower limit of detection of the assay were recorded as half the lowest concentration measured for each cytokine. Similarly, cytokine levels above the detectable limit were recorded as twice the highest concentration measured for each cytokine. To minimize the effect of inter-plate variability, all CVL specimens collected from the same participant were assayed on the same plate.

## Statistical analyses

HIV was the primary endpoint for all analyses. Inflammation, study arm, and adherence were the main explanatory variables, with some models adjusted for additional variables. Inflammation was defined by the number of cytokines in the upper quartile, using our published scoring criteria<sup>18</sup>. For stratified analyses, we calculated median cytokine values across multiple HIV- study visits (intra-individual), and determined whether the median cytokine concentration was in the upper quartile for each cytokine based on data from the entire cohort (Supplemental Figure 1). To diagnose inflammation, a score was calculated based on the number of pro-inflammatory cytokines in the upper quartile. Inflammation groups that included 3, 4, 5, 6, and 7 or more elevated cytokines were used to stratify individuals, and to examine the effect of different cytokine cut-offs on HIV risk and tenofovir protection. All those not meeting each “inflammation” cut-off were considered to be in the “no inflammation” group; all analyses therefore included all 774 participants. For time-varying analyses, we calculated whether genital inflammation was present at each visit, and assigned that value as the absence or presence of inflammation (0/1) to all person-time preceding that visit. All person-time that occurred following the final visit was determined to be the same as the final inflammation measurement.

Stratification by adherence was carried out using a cut-off of 50% of sex acts covered by returned used applicators, as increases in the proportion meeting this threshold were shown to correlate with protection against HIV in the original analysis<sup>3</sup>. Analyses of tenofovir efficacy were carried out using the subset of the intent-to-treat population, stratified by either inflammation status and/or gel adherence. Follow-up time was calculated from randomization to the estimated date of HIV infection or termination date, whichever occurred first.

We used Poisson distributions to calculate confidence intervals for incidence rates and incidence rate ratios (IRR). Efficacy was reported as 1-IRR. A z-test was used to compare IRRs between the two study arms. Univariate and multivariate Cox proportional hazard regression was used to calculate adjusted hazard ratios for a range of covariates as indicated in the relevant tables, including inflammation modeled as time-varying and interaction analyses between inflammation and study arm. We inspected the plausibility of the proportional hazards assumption by visual inspection of log [-log(survival)] curves. All p-values are reported as two-sided and without adjustment for multiple testing.

## Data availability

All data will be made available upon publication via an online repository.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

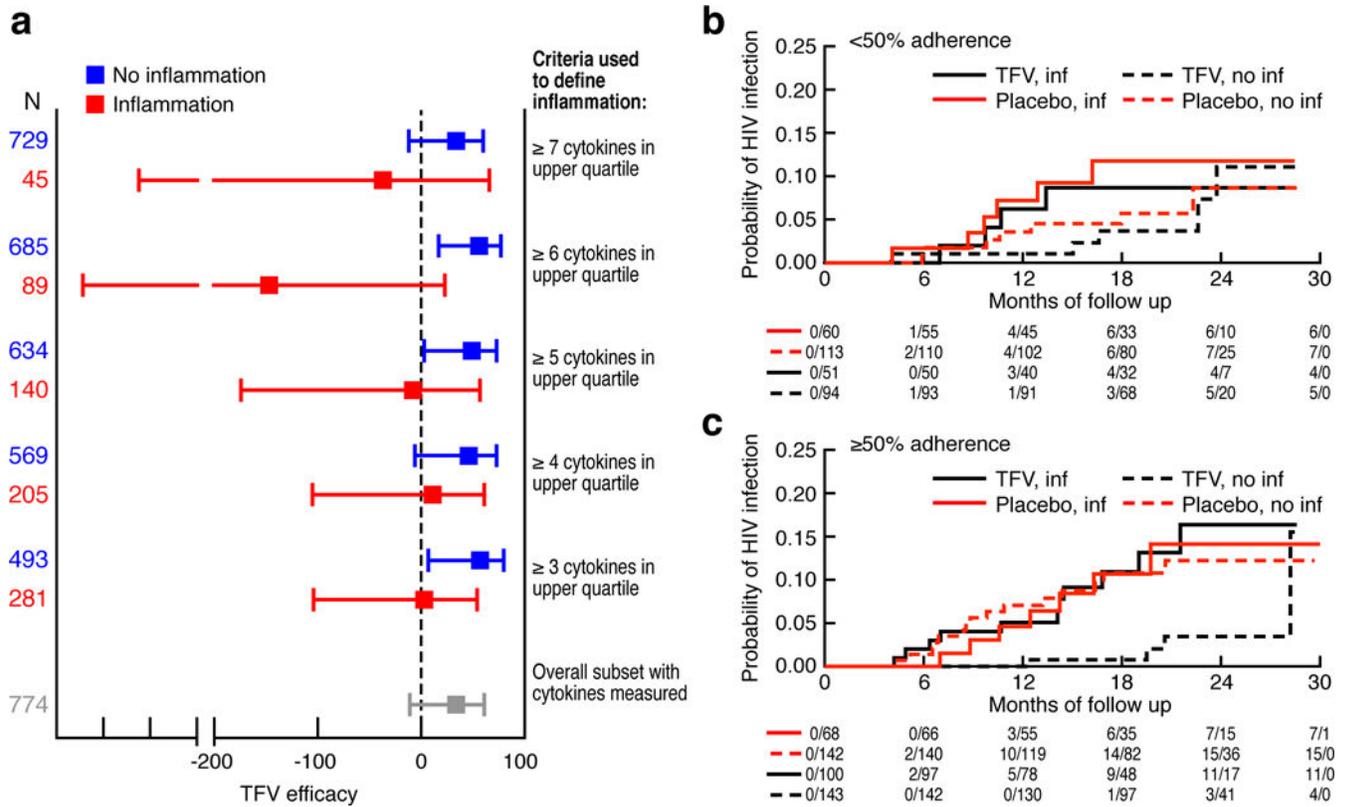
We would like to thank Walid Heneine and Douglas Kwon for important discussions regarding these data. The study was funded by the NIH (5R01AI111936 to JSP) and the DST-NRF Centre of Excellence in HIV Prevention at CAPRISA. The original CAPRISA 004 1% tenofovir gel trial was funded principally by the United States Agency

for International Development (USAID) through FHI360 and CONRAD, with additional support provided by the South African Department of Science and Technology (DST). We would like to thank all study participants and CAPRISA staff for making the CAPRISA 004 trial possible. LRM is supported by a Canadian Institutes of Health Research (CIHR) New Investigator Award. L.J.L, DA, and LM are funded by South African National Research Foundation (NRF) RCAF awards.

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**Figure 1.** Tenofovir efficacy stratified by inflammation status, defined by the number of elevated cytokines that were detected in female genital tract secretions (n = 774) **a**). Those meeting the cytokine cut-off for inflammation are indicated in red boxes (efficacy estimate) and whiskers (95% confidence intervals), with those falling below this cut-off indicated in blue boxes and whiskers. Tenofovir efficacy is shown on the x-axis, with a dotted black line indicating 0% efficacy. Overall tenofovir efficacy of the participants included in this analysis is indicated by the grey box and whiskers. Efficacy was calculated as 1-incident rate ratio (IRR) multiplied by 100, as shown in Table 1. Kaplan-Meier survival plots indicating the probability of seroconversion stratified by gel adherence (< and ≥ 50%, panels **b** and **c**, respectively). Separate lines are indicated for participants in the tenofovir arm of the study, with (solid black) and without genital inflammation (dotted black), and the placebo arm of the study, with (solid red) and without genital inflammation (dotted red). Genital inflammation in this analysis was defined by ≥ 3 of 9 cytokines in the upper quartile. The number of HIV infections/the number at risk in each strata and time point are shown below each graph. All statistical tests were two-sided and unadjusted for multiple comparisons.

**Table 1**

Incidence rates of HIV infection among women assigned to tenofovir or placebo gel, stratified by the number of elevated cytokines in genital secretions

# of cytokines in the upper quartile	Inflammation present	Tenofovir arm				Placebo arm				Arm comparison	
		No of women	No of HIV events	Person-years	Incidence rate (95% CI)	No of women	No of HIV events	Person-years	Incidence rate (95% CI)	Incidence rate ratio (IRR) (95% CI)	p-value
3/9	No	238	9	392.4	2.3 (1.0-4.4)	255	22	408.5	5.4 (3.4-8.2)	<b>0.43 (0.2-0.93)</b>	<b>0.033</b>
	Yes	153	15	221.9	6.8 (3.8-11.1)	128	13	186.8	7.0 (3.7-11.9)	0.97 (0.46-2.04)	0.936
4/9	No	280	13	459.0	2.8 (1.5-4.8)	290	24	457.5	5.2 (3.4-7.8)	0.54 (0.27-1.06)	0.074
	Yes	111	11	155.2	7.1 (3.5-12.7)	93	11	137.8	8.0 (4.0-14.3)	0.89 (0.39-2.05)	0.785
5/9	No	314	14	509.5	2.7 (1.5-4.6)	320	27	505.0	5.3 (3.5-7.8)	<b>0.51 (0.27-0.97)</b>	<b>0.041</b>
	Yes	77	10	104.8	9.5 (4.6-17.5)	63	8	90.3	8.9 (3.8-17.5)	1.08 (0.43-2.74)	0.871
6/9	No	344	14	553.4	2.5 (1.4-4.2)	340	31	535.0	5.8 (3.9-8.2)	<b>0.44 (0.23-0.83)</b>	<b>0.011</b>
	Yes	47	10	60.9	16.4 (7.9-30.2)	43	4	60.3	6.6 (1.8-17.0)	2.47 (0.77-7.88)	0.126
7/9	No	371	20	591.2	3.4 (2.1-5.2)	358	31	563.6	5.5 (3.7-7.8)	0.62 (0.35-1.09)	0.096
	Yes	20	4	23.1	17.3 (4.7-44.3)	25	4	31.7	12.6 (3.4-32.3)	1.37 (0.34-5.48)	0.656
<b>Overall</b>		<b>391</b>	<b>24</b>	<b>614.3</b>	<b>3.9 (2.5-5.8)</b>	<b>383</b>	<b>35</b>	<b>595.3</b>	<b>5.9 (4.1-8.2)</b>	<b>0.66 (0.40-1.12)</b>	<b>0.123</b>

\* Analysis based on n = 774 women sampled at 2,139 visits. Poisson distributions were used to calculate confidence intervals for incidence rates and incidence rate ratios (IRR). A z-test (two-sided) was used to compare IRRs between the two study arms, with all p-values reported without adjustment for multiple testing.

**Table 2**

Adjusted Hazard Ratios of HIV incidence from a multivariate model, stratified for the presence and absence of female genital tract inflammation \*

Inflammation	Parameter	aHR (95% CI)	p-value
No Inflammation (<3 elevated cytokines, n=493)	Tenofovir vs. Placebo	0.45 (0.20-0.98)	<b>0.044</b>
	Age (years)	0.93 (0.85-1.01)	0.087
	Urban vs. rural site	1.27 (0.49-3.30)	0.626
	HSV-2 seropositive	3.90 (1.66-9.12)	<b>0.002</b>
	Sex acts in last 30 days	1.03 (0.93-1.15)	0.547
	Contraceptive use, DMPA vs. oral	4.26 (0.57-31.86)	0.158
	Contraceptive use, NET-EN vs. oral	2.55 (0.28-23.36)	0.409
	Abnormal vaginal discharge	0.82 (0.37-1.84)	0.634
	Condom use, always vs. not always	0.85 (0.37-1.97)	0.709
Inflammation present (≥ 3 elevated cytokines, n=281)	Tenofovir vs. Placebo	0.88 (0.40-1.93)	0.757
	Age (years)	0.95 (0.87-1.04)	0.305
	Urban vs. rural site	1.19 (0.46-3.07)	0.727
	HSV-2 seropositive	1.21 (0.53-2.75)	0.654
	Sex acts in last 30 days	1.13 (1.04-1.23)	<b>0.004</b>
	Contraceptive use, DMPA vs. oral	5.23 (0.69-39.81)	0.110
	Contraceptive use, NET-EN vs. oral	5.95 (0.69-51.28)	0.105
	Abnormal vaginal discharge	2.16 (0.96-4.84)	0.063
	Condom use, always vs. not always	1.66 (0.75-3.65)	0.210

\* This analysis is based on inflammation being defined as ≥ 3 elevated cytokines; the results are similar when definition of inflammation is based on a higher number (up to 7 of 9) of elevated cytokines (data not shown). Multivariate Cox proportional hazard regression was used to calculate adjusted hazard ratios for a range of covariates as indicated in the table (n = 774 women sampled at 2,139 visits). P values are two-sided and unadjusted for multiple testing.

NET-EN = Norethisterone enantate

DMPA = Depot-medroxyprogesterone