

# Genital Tenofovir Concentrations Correlate With Protection Against HIV Infection in the CAPRISA 004 Trial: Importance of Adherence for Microbicide Effectiveness

Angela D. M. Kashuba, BScPhm, PharmD, DABCP,\*† Tanuja N. Gengiah, MClInPharm, PhD, MS(Epi),‡ Lise Werner, MSc,‡ Kuo-Hsiung Yang, PharmD,\*† Nicole R. White, BS,\*† Quarraisha A. Karim, PhD,‡§ and Salim S. Abdool Karim, MBChB, PhD‡§

**Objective:** The CAPRISA 004 trial showed that coitally dosed tenofovir 1% gel reduced HIV acquisition by 39% overall and 54% when used consistently. The objective of this analysis was to ascertain its pharmacokinetic–pharmacodynamic relationship to protect against HIV acquisition.

**Design:** Genital and systemic tenofovir concentrations in 34 women who acquired HIV (cases) were compared with 302 randomly selected women who remained HIV uninfected (controls) during the CAPRISA 004 trial. In total, 336 cervicovaginal fluid (CVF), 55

plasma, and 23 paired cervical and vaginal tissue samples were assayed by validated methods for tenofovir and tenofovir diphosphate (tenofovir-DP) detection.

**Results:** Tenofovir was detected in the genital tract in 8 (23.5%) cases and 119 (39.4%) controls ( $P = 0.076$ ). Among those with detectable genital tract tenofovir, the median CVF concentrations were 97% lower in cases compared with controls, 476 versus 13,821 ng/mL ( $P = 0.107$ ). A total of 14.7% (5/34) of cases and 32.8% (99/302) of controls were found to have tenofovir CVF concentrations above 100 ng/mL [odds ratio (OR): 0.35,  $P = 0.037$ ]. At a higher threshold, 8.8% (3/34) of cases and 26.2% (79/302) of controls were found to have tenofovir CVF concentrations above 1000 ng/mL (OR: 0.27,  $P = 0.036$ ). Plasma tenofovir concentrations were <1 ng/mL in all women and were detected only in controls (16.7%) and not in cases (0%), ( $P = 0.031$ ). Returned used tenofovir gel applicators and CVF concentrations were correlated (Spearman  $r = 0.22$ ,  $P = 0.001$ ).

**Conclusions:** A tenofovir concentration of  $\geq 100$  ng/mL in CVF was associated with 65% (95% CI: 6% to 87%) protection against HIV, whereas a  $\geq 1000$  ng/mL concentration correlated with 76% (95% CI: 8% to 92%) protection against HIV infection.

**Key Words:** tenofovir, genital tract, HIV, protection, adherence

(*J Acquir Immune Defic Syndr* 2015;69:264–269)

## INTRODUCTION

The global HIV epidemic is driven by sexual transmission with an estimated 2.1 million individuals acquiring HIV in 2013.<sup>1</sup> Research efforts to prevent new infections focusing on drug targets, particularly antiretrovirals, are underway.<sup>2</sup> Recent successful trials testing antiretrovirals for prevention have demonstrated the efficacy of oral tenofovir-containing products in preventing HIV infection.<sup>3–5</sup> In 2010, the Centre for the AIDS Program of Research in South Africa (CAPRISA) 004 trial assessed the safety and effectiveness of the 1% vaginal gel formulation of tenofovir. This study found a 39% reduction in risk for acquisition of HIV<sup>6</sup> and a 51% reduction in HSV-2 infection.<sup>7</sup> In 336 subjects in this study who were considered to be at least 80% adherent to tenofovir gel, the reduction in risk for HIV acquisition was 54%.

Received for publication December 19, 2014; accepted March 16, 2015.

From the \*Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC; †University of North Carolina Center for AIDS Research, Clinical Pharmacology and Analytical Chemistry Core, Eshelman School of Pharmacy, Chapel Hill, NC; ‡Centre for the AIDS Program of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa; and §Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY.

The CAPRISA 004 trial was supported by the Centre for the AIDS Programme of Research in South Africa (CAPRISA), the United States Agency for International Development (USAID), FHI (cooperative agreement No. GPO-A-00-05-00022-00, contract No. 132119), and LIFELab, now TIA (Technology Innovation Agency), a biotechnology center of the South African Department of Science and Technology. Support was also received from CONRAD for the product manufacturing and packaging and Gilead Sciences provided the Tenofovir used in the production of gel. The Columbia University-Southern African Fogarty AIDS International Training and Research Programme (CU-SA AITRP), funded by the Fogarty International Center, National Institutes of Health (Grant No. D43TW00231), was used to assist in training of clinical trial staff. The UNC Center for AIDS Research, an NIH-funded program (P30 AI50410) and Gilead Sciences Grant CO-US-106-0104 was used for drug assays costs.

S.S.A.K. and Q.A.K. are coinventors of 2 pending patents (61/354.050 and 61/357.892) of tenofovir gel against HSV-1 and HSV-2. The remaining authors have no conflicts of interest to disclose.

A.D.M.K., L.W., T.N.G., S.S.A.K., and Q.A.K. participated in the design of the study, the collection, analysis, and interpretation of data, and drafting of the paper. A.D.M.K. and N.R.W. participated in the assay of pharmacokinetic samples. A.D.M.K., S.S.A.K., L.W., T.N.G., and K.-H.Y. participated in the statistical analysis and interpretation of data. All authors reviewed and commented on the final manuscript.

Correspondence to: Angela D. Kashuba, BScPhm, PharmD, DABCP, Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, Director, UNC Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Core, University of North Carolina at Chapel Hill, 3318 Kerr Hall, CB# 7569, Chapel Hill, NC 27599-7569 (e-mail: akashuba@unc.edu).

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

The concentration of tenofovir achieved in the vaginal tract in the CAPRISA 004 trial, 24 hours after coitally dependent dosing, was estimated to be  $\sim 10^5$  ng/mL.<sup>8</sup> Two previous animal studies of tenofovir 1% gel in pig-tailed macaques and humanized bone marrow liver thymic (BLT) mice have demonstrated 88%–100% efficacy against viral acquisition.<sup>9,10</sup> However, the limited data on drug exposure in these animals does not allow direct human exposure comparisons. Therefore, it is unclear whether the more modest clinical trial results were due to intermittent adherence, dissimilar drug exposure, or both. Differentiating between these 2 etiologies are difficult using the traditional adherence measures of self-report or, as in the case of CAPRISA 004, returned used gel applicator counts, as none of these directly capture intervention-taking behavior.

Using a pharmacologic measure may allow better interpretation of adherence from samples stored during a clinical trial and could eventually provide real-time information to guide behavioral interventions. Also, because standard phase II drug development dose-ranging pharmacokinetic–pharmacodynamic studies used to generate clinical Emax models (nonlinear model using dose–response analysis) of efficacy are not straight-forward for HIV prevention, pharmacologic data from placebo-controlled clinical trials may provide some insight into the exposure of tenofovir required in the female genital tract to protect against infection.

The purpose of this study was to assess the relationship between local/systemic tenofovir concentrations and HIV acquisition to identify a pharmacokinetic marker of adherence as a correlate of protection in women.

## PATIENTS AND METHODS

### Patients and Study Design

The CAPRISA 004 trial was conducted between May 2007 and March 2010 in KwaZulu-Natal, South Africa, at the CAPRISA Vulindlela Clinical research site in Vulindlela, a rural community located 150 km west of Durban (rural) and at the CAPRISA eThekweni Clinical research site in the Durban city center (urban). The source population for this cohort has been described elsewhere.<sup>11</sup>

Participant screening, enrollment, and randomization procedures have been described in detail previously.<sup>6</sup> Briefly, volunteers were provided with study information in English and/or isiZulu and those agreeing to continue with study participation were eligible if they were 18–40 years of age, willing to provide written informed consent for screening, agreed to provide adequate locator information for study retention purposes, agreed to adhere to study visit schedule, were sexually active (defined as having had vaginal intercourse at least twice within the last 30 days before screening), HIV negative, not pregnant, agreeable to be on a nonbarrier form of contraception, creatinine clearance of  $>50$  mL/min using the Cockcroft and Gault method,<sup>12</sup> and no evidence of deep epithelial disruption on pelvic examination. Volunteers were excluded if they had an untreated sexually transmitted infection. Within 30 days of the first screening visit, returning eligible volunteers were randomly assigned to receive either

1% tenofovir gel or the hydroxyethylcellulose placebo in a 1:1 ratio. Participants received an assigned study gel in quantities guided by the frequency of coital activity.

From May 2007 to January 2009, 2160 women were screened and 1085 were enrolled, of whom 889 were included in the primary analysis. Women followed a dosing strategy referred to as “BAT24”: insert 1 dose of gel within 12 hours before sex and a second dose of gel as soon as possible within 12 hours after sex and no more than 2 doses of gel in a 24-hour period. Participants had monthly follow-up visits for 30 months. Participants were requested to return their used (from October 2007 onward) and unused applicators at every visit. Each month, the applicators returned by women as used and unused were counted, reconciled against the number dispensed, and thereafter discarded. At months 3, 12, 24 and at exit, blood plasma and cervicovaginal fluid (CVF) aspirates were collected for pharmacokinetic analysis. Upon detection of HIV seroconversion, vaginal and cervical tissue biopsies were also obtained.

### Sample Processing and Analyses

Tenofovir was quantified in blood plasma and CVF. Tenofovir and tenofovir diphosphate was quantified in vaginal and cervical tissue. For each blood plasma sample, 4 mL of blood was collected in a tube containing K-EDTA. Within 30 minutes of collection, whole blood was centrifuged at 800g for 10 minutes at 4°C. Plasma was pipetted into cryovials and stored at  $-80^{\circ}\text{C}$  until analysis. Specialty collection syringes (UNC CFAR Vaginal Specimen Aspirators) were used to obtain directly aspirated, undiluted CVF samples. After collection, samples were expelled into a cryovial and stored at  $-70^{\circ}\text{C}$  until analysis. To prepare the mucosal tissue for biopsy, the areas of the planned biopsies were gently wiped with a small cotton swab moistened with warm saline followed by a small cotton swab with Betadine. Topical gel containing lidocaine 25 mg/prilocaine 25 mg/g was applied to the biopsy area for anesthesia. A Medium-Tischler biopsy forceps was used to obtain one  $5 \times 3$ -mm biopsy from the vaginal wall and one  $5 \times 3$ -mm biopsy from the ectocervix. Each biopsy was placed in a preweighed individual vial, weighed, snap frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until transport. At the end of the study, samples were shipped on dry ice to the UNC Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Laboratory at the University of North Carolina at Chapel Hill.

### Sample Analysis

Analysis of all samples was performed using an LC–MS/MS method that quantified tenofovir and tenofovir diphosphate simultaneously, as described previously.<sup>13</sup> For blood plasma, the calibration curve for tenofovir ranged from 0.25 to 200 ng/mL. Across 3 quality control concentrations, intraday accuracy and precision was  $>99\%$  and  $<12\%$ , respectively, and interday accuracy and precision was  $>90\%$  and  $<13\%$ , respectively. For CVF, the calibration curve for tenofovir ranged from 2 to 1000 ng/mL. Intraday accuracy and precision was  $>93\%$  and  $<8\%$ , and interday

accuracy and precision was >97% and <5%. For vaginal and cervical tissue, a calibration curve with a range of 2–2000 ng/mL for tenofovir and 10–10,000 ng/mL for tenofovir-DP was prepared. Intraday accuracy and precision for tenofovir was >90% and <10%, respectively; for tenofovir-DP, it was >94% and <9%, respectively. Interday accuracy and precision for tenofovir was >93% and <5%, respectively; for tenofovir-DP, it was >98% and <6%, respectively.

## Statistical Analyses

The demographic characteristics at baseline were summarized using basic descriptive statistics. Fisher exact test was used for the analysis of categorical data, and unpaired *t*-tests or the Wilcoxon rank-sum tests for the analysis of continuous data.

A nested case-control study, post-trial, was conducted to determine a correlate of protection related to drug concentration. For the cases, postinfection samples of the HIV seroconverters on the active arm were selected. Controls were selected randomly from those who did not acquire HIV. Only visits with tenofovir concentrations measured were considered. Detectable concentrations below the limit of quantification were imputed to be one-half of the lower limit of quantification (the lowest point on the standard curve) and values below the limit of detection (BLD) were set to 0 ng/mL. Tenofovir threshold concentration cut points were selected as follows: (1) tenofovir = 0 ng/mL: indicates that tenofovir concentration was BLD, (2) tenofovir  $\geq 1$  ng/mL: indicates that any tenofovir was present, (3) tenofovir  $\geq 100$  ng/mL: tenofovir concentration expected in plasma and CVF after oral dosing, and (4) tenofovir  $\geq 1000$  ng/mL: tenofovir at concentrations at or above the surrogate level of protection identified previously.<sup>8</sup> Logistic regression was performed comparing the different tenofovir thresholds in cases and controls. An adjusted model was fitted controlling for possible confounders: age, site, marital status, education, condom at last sex act, and time on study at sample collection.

Spearman correlation was used to assess the relationship between tenofovir plasma and CVF concentrations, as well as between tenofovir and tenofovir-DP concentrations within both vaginal and cervical tissue. The Wilcoxon signed-rank test was used to compare both tenofovir and tenofovir-DP concentrations between vaginal and cervical tissue. Spearman correlation was also used to compare CVF tenofovir concentrations with the number of returned used applicators brought back at monthly study visits. All analyses were performed using 2-sided tests. SAS software version 9.3 (SAS Institute Inc., Cary, NC) was used for analysis while graphs were created using GraphPad Prism version 5.

## Regulatory Considerations

The protocol for the primary study, informed consent forms, and study-related materials were reviewed and approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee, Ref: E111/06, the Protection of Human Subject Committee in the Office of International Research Ethics at FHI360 Ref: 9946, and the South African

Medicines Control Council, Ref: 20060835. The trial was registered with ClinicalTrials.gov, number NCT 00441298.

## RESULTS

### Tenofovir Concentrations in Cases and Controls

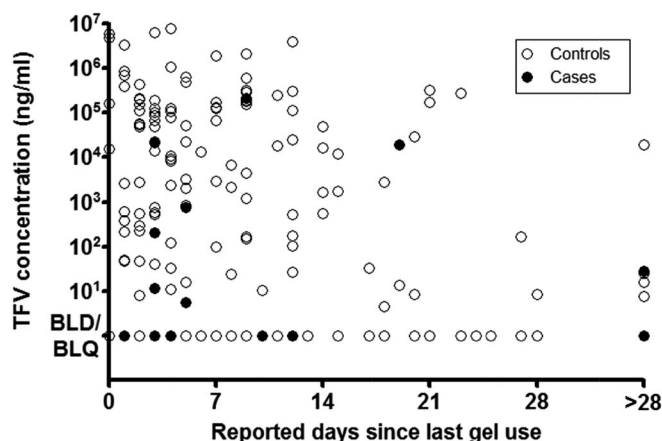
Of the 889 women enrolled in CAPRISA 004, 445 were randomized to the tenofovir gel arm, and of these, 340 had at least 1 tenofovir CVF concentration measured. Of the 340 women with tenofovir concentration data, postinfection samples were available for 34 of the 38 HIV seroconverters (cases). The remaining 4 seroconverters only had preinfection samples available and were therefore excluded. Control samples were selected at a random visit from the remaining 302 women who remained HIV negative. No statistically significant differences in demographic characteristics were seen between HIV cases and controls (Table 1). Median age in both cases and controls were 23 years, with approximately 60% of women representing the rural site. The 336 samples included in this case-control analysis were obtained at a median of 9 days [interquartile range (IQR): 3–27] after the last reported gel use (Fig. 1).

Tenofovir was detected in the genital tract in 23.5% (8/34) cases and 39.4% (119/302) controls ( $P = 0.076$ ). Among those with detectable tenofovir in the genital tract, the median CVF concentrations were 97% lower in cases compared with controls, 476 versus 13,821 ng/mL ( $P = 0.107$ ) (Fig. 2). With 1000 ng/mL as a correlate of protection, we found that 8.8% (3/34) of cases and 26.2% (79/302) of controls had tenofovir CVF concentrations above this threshold [odds ratio (OR): 0.27,  $P = 0.036$ ]. After adjustment for multiple covariates, a concentration of 1000 ng/mL in the CVF was associated with 76% protection against HIV (adjusted  $P = 0.024$ ) (Table 2). At lower tenofovir concentrations, protection against HIV was concomitantly lower, with efficacy of 53% ( $P = 0.076$ ) and 65% ( $P = 0.037$ ) when

**TABLE 1.** Baseline Characteristics of HIV Cases and Controls

Variable	All (N = 336)	Cases (n = 34)	Controls (n = 302)	P
Median age (IQR)	23 (20–27)	23 (20–26)	23 (20–27)	0.478
Rural, % (n)	66.4 (223)	64.7 (22)	66.6 (201)	0.849
Completed high school, % (n)	40.2 (135)	32.4 (11)	41.1 (124)	0.361
Married, % (n)	5.4 (18)	0.0 (0)	6.0 (18)	0.235
Stable partner, % (n)	92.6 (311)	100 (34)	91.7 (277)	0.092
Casual partner, % (n)	8.3 (28)	8.8 (3)	8.3 (25)	1.000
Lives with regular partner, % (n)	13.0 (43)	8.8 (3)	13.5 (40)	0.595
Income per month, % (n)				
None or <R1000	91.4 (307)	88.2 (30)	91.7 (277)	0.597
R1001–R5000	8.0 (27)	11.8 (4)	7.6 (23)	
>R5001	0.6 (2)	0.0 (0)	0.7 (2)	

R, South African Rands.



**FIGURE 1.** CVF concentrations of tenofovir versus reported days since last gel use in cases and controls. BLD, below the limit of detection; TFV, tenofovir.

tenofovir CVF concentrations were  $\geq 1$  and  $\geq 100$  ng/mL, respectively. For the latter tenofovir cutoff concentrations, the efficacy against HIV was 53% (adjusted  $P = 0.089$ ) and 67% (adjusted  $P = 0.034$ ), after adjusting for age, site, marital status, education, condom at last sex act, and time on study at sample collection.

There was a correlation, though not very strong, between CVF tenofovir concentrations and the number of returned used applicators (Spearman  $r = 0.22$ ,  $P < 0.001$ ) in women (331/336) with applicator data available.

### Blood Plasma Samples

Plasma tenofovir concentrations were measured in 55 women randomized to tenofovir gel. Of these, 93% (51/55) were BLD or below the limit of quantification. In all subjects, plasma concentrations were less than 1 ng/mL but nonetheless

were detected less frequently in cases than in controls. Tenofovir was detected in plasma in none (0/31) of the HIV-positive women and in 16.7% (4/24) of the HIV-negative women. Of the 55 plasma tenofovir concentrations, 54 had a tenofovir CVF concentration on the same collection date. Plasma and CVF tenofovir concentrations were moderately correlated with a Spearman correlation coefficient of 0.46 ( $P \leq 0.001$ ). In the 4 women with detectable tenofovir in plasma, their median tenofovir concentration in CVF was 312 482 ng/mL (IQR: 121 298–510 512). The 4 women with detectable concentrations reported gel use at a median of 5 days before sampling (IQR: 3–18), whereas the remaining undetectable plasma concentrations were in visits where women reportedly used the gel at a median of 10 days ago (IQR: 4–83) ( $P = 0.313$ ).

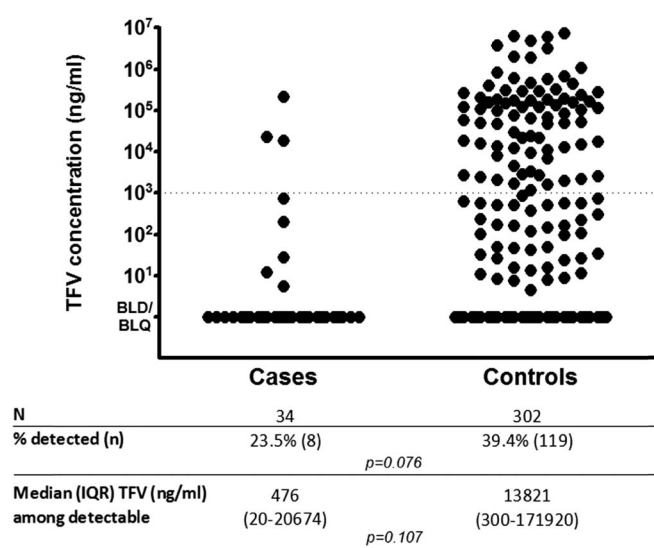
### Cervical and Vaginal Biopsies in Seroconverters

Cervical and vaginal biopsies were collected in 23 of 38 HIV seroconverters at a median of 37 days after infection (IQR: 29–67) and a median of 43 (IQR: 20–93) days after the last reported gel insertion. In total, 6 women had detectable tenofovir and 6 women had detectable tenofovir-DP from the 23 cervical and 23 vaginal biopsies collected. Tenofovir-DP concentrations correlated with tenofovir concentrations in both vaginal and cervical tissue ( $\rho = 0.61$ ,  $P = 0.002$ ; and  $\rho = 0.53$ ,  $P = 0.009$ , respectively). The median tenofovir concentration was 10 ng/g (IQR: 7.1–16.7) and 10 ng/g (IQR: 8.3–25.0) in cervical and vaginal tissues, respectively. Among those with detectable tenofovir-DP, median (IQR) tenofovir-DP concentrations in vaginal and cervical biopsies were 79 fmol/mg (IQR: 0–186) and 0 fmol/mg (IQR: 0–93), respectively.

### DISCUSSION

After topical application, the tenofovir concentration in the genital compartment provides a potential clinical correlate of protection against HIV infection, with 76% effectiveness demonstrated when genital concentrations are in excess of 1000 ng/mL. In women with no detectable tenofovir in the genital tract, protection against HIV was not observed while the detection of any genital tenofovir ( $>1$  ng/mL) was associated with 53% effectiveness. The increasing levels of effectiveness associated with log increases in tenofovir concentrations highlights the importance of adherence to attain high levels of protection against HIV. This is further supported by the recent outcome of the VOICE trial that failed to demonstrate the protective efficacy of tenofovir gel because of low adherence to the prescribed daily gel application schedule.<sup>14</sup>

Tenofovir was seldom detected systemically in the CAPRISA 004 trial, confirming that intermittent topical application achieves limited and short-lived systemic tenofovir exposure. With intermittent topical application, there was no observed correlation between random systemic tenofovir concentrations and protection against HIV. Because the median serum tenofovir  $C_{max}$  is 3.0 ng/mL at 2.1 hours after gel insertion and was  $<1$  ng/mL at 24 hours after insertion,<sup>15</sup> a coital dosing strategy would not produce consistently



**FIGURE 2.** Cervicovaginal tenofovir concentrations for HIV cases and controls.

**TABLE 2.** Efficacy of Various Tenofovir Concentration Cut Points Against HIV Infection

Tenofovir Concentration, ng/mL	HIV Cases		HIV Controls		Odds Ratio*		
	No. Women	% (n) With Specified Tenofovir Concentration	No. Women	% (n) With Specified Tenofovir Concentration	(95% CI) of HIV Above Versus Below Tenofovir Concentration	Estimated Efficacy (1 – OR) × 100, %	P
0	34	76.6 (26)	302	60.6 (183)	2.11 (0.93 to 4.82)	–111	0.076
≥1	34	23.5 (8)	302	39.4 (119)	0.47 (0.21 to 1.08)	53	0.076
≥100	34	14.7 (5)	302	32.8 (99)	0.35 (0.13 to 0.94)	65	0.037
≥1000	34	8.8 (3)	302	26.2 (79)	0.27 (0.08 to 0.92)	73	0.036

\*After adjustment for age, site, marital status, education, condom use at the last sex act, and time on study, ORs and *P* values remained similar: tenofovir ≥1 ng/mL, OR: 0.47 (95% CI: 0.19 to 1.12, *P* = 0.089); tenofovir ≥100 ng/mL, OR: 0.33 (95% CI: 0.12 to 0.92, *P* = 0.034); tenofovir ≥1000 ng/mL, OR: 0.24 (95% CI: 0.07 to 1.82, *P* = 0.024).

detectable tenofovir systemically. Furthermore, the lack of detectable tenofovir in blood plasma in this analysis supports the findings of limited drug-related systemic adverse effects<sup>16</sup> and may explain why no tenofovir resistance was observed in plasma viruses, even in women who continued applying tenofovir gel, for approximately 3–4 weeks, after HIV infection in the CAPRISA 004 trial.<sup>6</sup>

The assays on the vaginal and cervical tissue from the genital biopsies showed that concentrations of the active form of tenofovir, tenofovir-DP, correlated with concentrations of the tenofovir prodrug, noting that the gel contains only the tenofovir pro-drug. Despite the biopsies being sampled a median of 43 days since last gel insertion, tissue concentrations were detected in 30.4% of women sampled.

The specified concentration of genital tract tenofovir or intracellular tenofovir-DP required to protect women from HIV infection is unknown. Protective concentrations modulated by additional acquisition risk factors such as genital inflammation and presence of sexually transmitted infection(s) are yet to be described. Two animal studies have previously demonstrated >80% efficacy of tenofovir 1% gel. In 2009, 100% protection from SHIV<sub>SF162P3</sub> acquisition was found in 6 pig-tailed macaques given 3 mL of a tenofovir 1% vaginal gel 30 minutes before viral challenge twice weekly for 10 weeks.<sup>9</sup> In 2011, 88% protection from HIV-1<sub>JR-CSF</sub> acquisition was demonstrated in 8 humanized BLT mice given 20 µL of a tenofovir gel 4 hours before and 4 hours after a single viral challenge.<sup>10</sup>

In the macaque model, Dobard et al<sup>17</sup> analyzed tenofovir-DP concentrations in isolated vaginal lymphocytes at necropsy after dosing with 3 mL of tenofovir 1% gel. The authors demonstrated high concentrations (median, 1810; range, 1312–2684 fmol/10<sup>6</sup> cells) 4 hours after dose, where tenofovir 1% gel is 100% efficacious in macaques. At 72 hours after a dose of tenofovir gel, when protective efficacy in macaques is 67%, tenofovir-DP concentrations are 86% lower [252 (196–295) fmol/10<sup>6</sup> cells]. These data cannot be directly compared with those of women using tenofovir gel, as isolated vaginal lymphocytes cannot be obtained in sufficient quantities by standard biopsy techniques. However, they do demonstrate that a tissue concentration–effect relationship exists for tenofovir gel and should be defined in human tissue.

In the CAPRISA 004 study, as expected, tenofovir CVF exposures were highly variable on 0–3 days after dose. For those women who reported using the gel within 24 hours of

their visit, CVF concentrations were ~100,000 ng/mL. These concentrations are similar to a recent phase I investigation of tenofovir 1% gel by Schwartz et al.<sup>18</sup> However, 1 to 3 days after dose, CVF concentrations in the women in the CAPRISA 004 study were up to 100-fold lower (~1000 ng/mL). This is not unexpected because of the phase I investigation being conducted under rigorous restricted conditions while the CAPRISA 004 study participants were exposed to sexual activity and accompanying practices. Yet this concentration of 1000 ng/mL was useful in predicting efficacy in this case–control analysis. Based on the previously established ~10:1 relationship between CVF and vaginal tissue concentrations, and the 3% molar conversion rate between tenofovir and tenofovir-DP in vaginal tissue,<sup>18</sup> we estimate that the CVF concentration of 1000 ng/mL should yield 500 fmol/mg of tissue of tenofovir-DP. Whether this concentration will be directly predictive of efficacy is yet to be determined.

These case–control dose–response data are consistent with preliminary analysis of the CAPRISA 004 CVF concentrations<sup>8</sup> and recently published data in PrEP trials testing oral tenofovir-containing products. In the Partners PrEP trial, 81% of blood samples had detectable tenofovir and HIV efficacy was 75% in that trial.<sup>3</sup> Similarly, in the TDF2,<sup>19</sup> iPREX,<sup>5</sup> and FEM-PrEP<sup>20</sup> trials, 79%, 51%, and 26% of blood samples had detectable tenofovir with a corresponding HIV protection point estimate of 62%, 44%, and 6%, respectively. Most recently, the MTN003 trial failed to show efficacy of oral TDF, oral Truvada, and topical tenofovir 1% gel with tenofovir detected in only 28%, 29%, and 23% of blood samples, respectively.<sup>14</sup>

Finally, objective measures of adherence are critical to the interpretation of clinical trial data. Measurement of adherence in HIV prevention trials using self-reports or pill counts have been shown to overestimate adherence.<sup>21,22</sup> Using the counts of applicators used, the CAPRISA 004 investigators determined that tenofovir 1% gel could confer 52% protection against HIV acquisition in those who were >80% adherent. However, in this analysis, CVF concentrations taken at a single visit only weakly correlated with the number of gel applicators used in the previous month. Self-reports of gel insertion compared with concentrations and model simulated data indicate that self-report of gel use are prone to bias. Based on a PK model developed for tenofovir gel dosing,<sup>23</sup> 62.5% of participants on active product could be

considered adherent based on their tenofovir CVF concentrations (data not shown).

Limitations of this analysis include the inability to determine a threshold correlate of protection from tissue concentrations as genital tissue biopsies were not permitted in HIV-uninfected women; furthermore, tissue biopsies from seroconverters were performed on average more than 6 weeks after the last gel insertion. It is also acknowledged that the time of last gel insertion was collected from participant self-report and is subject to recall bias. There was poor correlation between time of last dose and drug level in this trial. This was not unexpected because gel use was intermittent (coitally dependent), unscheduled, and not associated with clinic visits when PK samples were collected or seroconversion was detected.

In conclusion, an analysis of tenofovir concentrations in the CAPRISA 004 trial demonstrated a potential correlation between genital tract concentrations of the drug and its effectiveness, with CVF concentrations >1000 ng/mL being associated with 76% protection against HIV infection. Tenofovir 1% vaginal gel is still under clinical trial evaluation for safety and effectiveness in the current Follow-on African Consortium for Tenofovir Studies (FACTS) 001 trial, which is required for its registration for use as a topical PrEP agent. Based on this analysis, the attainment of this concentration during sexual exposure consistently may be necessary to achieve high levels of protection against HIV. Achieving high levels of gel adherence is therefore going to be essential for PrEP trials, without which, an efficacious product may be found spuriously to be ineffective. Importantly, maintaining high adherence in HIV prevention trials is critical in light of the urgent need for the prevention of sexually transmitted HIV in young women in Africa.

## ACKNOWLEDGMENTS

*This study would not have been possible without the support of the women participating in this trial and their contributions and commitment is acknowledged with deep appreciation. Special thanks to the CAPRISA Research Support Group members at the CAPRISA Vulindlela and eThekweni Clinical Research Sites. The authors acknowledge the dedication and commitment of staff at the CAPRISA eThekweni and Vulindlela Clinical Research Sites in implementing this trial; the CAPRISA Research Laboratory staff for undertaking the laboratory testing and archiving of samples; CAPRISA Data Management and Statistics staff for management and quality assurance of case report forms.*

## REFERENCES

- UNAIDS. *The Gap Report*. 2014. Available at: [http://www.who.int/tb/publications/global\\_report/en/](http://www.who.int/tb/publications/global_report/en/). Accessed September 1, 2014.
- Padian NS, Buve A, Balkus J, et al. Biomedical interventions to prevent HIV infection: evidence, challenges, and way forward. *Lancet*. 2008;372:585–599.
- Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N Engl J Med*. 2012;367:399–410.
- Choopanya K, Martin M, Suntharasamai P, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*. 2013;381:2083–2090.
- Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med*. 2010;363:2587–2599.
- Abdool Karim Q, Abdool Karim SS, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science*. 2010;329:1168–1174.
- Abdool Karim SS; on behalf of the CAPRISA 004 Trial Group. CAPRISA 004: effectiveness and safety of vaginal microbicide 1% tenofovir gel for prevention of HIV infection in women: Paper presented at: XVIII International AIDS Conference; July 18–23, 2010; Vienna, Austria.
- Karim SS, Kashuba AD, Werner L, et al. Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women. *Lancet*. 2011;378:279–281.
- Parikh UM, Dobard C, Sharma S, et al. Complete protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine. *J Virol*. 2009;83:10358–10365.
- Denton PW, Othieno F, Martinez-Torres F, et al. One percent tenofovir applied topically to humanized BLT mice and used according to the CAPRISA 004 experimental design demonstrates partial protection from vaginal HIV infection, validating the BLT model for evaluation of new microbicide candidates. *J Virol*. 2011;85:7582–7593.
- Karim QA, Kharsany AB, Frohlich JA, et al. Stabilizing HIV prevalence masks high HIV incidence rates amongst rural and urban women in KwaZulu-Natal, South Africa. *Int J Epidemiol*. 2011;40:922–930.
- Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16:31–41.
- Patterson KB, Prince HA, Kraft E, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. *Sci Transl Med*. 2011;3:112re114.
- Marrazzo J, Ramjee G, Nair G, et al; VOICE Study Team. Pre-exposure prophylaxis for HIV in women: daily oral tenofovir, oral tenofovir/emtricitabine, or vaginal tenofovir gel in the voice study (MTN003). Conference of Retroviral and Opportunistic Infections, Georgia World Congress Centre; March 3–6, 2013; Atlanta, GA. Abstract#26LB.
- Hendrix CW, Chen BA, Guddera V, 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:e55013.
- Sokal DC, Karim QA, Sibeko S, et al. Safety of tenofovir gel, a vaginal microbicide, in South African women: results of the CAPRISA 004 Trial. *Antivir Ther*. 2013;18:301–310.
- Dobard C, Sharma S, Martin A, et al. Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. *J Virol*. 2012;86:718–725.
- Schwartz JL, Rountree W, Kashuba AD, et al. A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel. *PLoS One*. 2011;6:e25974.
- Thigpen MC, Kebaabetswe PM, Paxton LA, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *The New England journal of medicine*. 2012;367(5):423–434.
- Van Damme L, Corneli A, Ahmed K, et al. Preexposure prophylaxis for HIV infection among African women. *N Engl J Med*. 2012;367:411–422.
- Berg KM, Arnsten JH. Practical and conceptual challenges in measuring antiretroviral adherence. *J Acquir Immune Defic Syndr*. 2006;43(suppl 1):S79–S87.
- Amico KR, Liu A, McMahan V, et al. Adherence Indicators and pre-exposure prophylaxis (PrEP) drug levels in the iPrEx study. Paper presented at: Conference on Retroviruses and Opportunistic Infections; 2011; Boston, MA.
- Yang KH, Schwartz JL, Sykes C, Doncel GF, et al. Population pharmacokinetic model of vaginal tenofovir 1% gel in the cervicovaginal fluid. HIV research for prevention; February 28–March 2, 2011; Cape Town, South Africa. Abstract# OA13.04.