Published in final edited form as:
Science. 2010 September 3; 329(5996): 1168–1174. doi:10.1126/science.1193748.

Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women

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The study was conceptualized by SSAK and QAK and designed by QAK, SSAK, JAF, ACG, LEM, CB, ABMK, SS, SM, NA, and DT. Sections of the data was gathered, analyzed and interpreted by JAF, LEM, CB, ABMK, KPM, ZO, TG, SM, NA, and MM. The overall data was analysed and interpreted by QAK, SSAK, ACG and DT. QAK, SSAK and ACG had full access to all of the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis. LM conducted the resistance testing. SSAK, QAK and CB took responsibility for writing the paper and all co-authors contributed to critical revisions of the paper. Quarraisha Abdool Karim is the Co-Principal investigator of the HPTN Prevention Leadership Group (NIH/NIAID U01 AI068619). Salim S Abdool Karim was the Protocol Chair of the HPTN 035 trial which was supported by the National Institutes of Health (grant # U01AI46749 and U01AI068633). The other authors have no financial conflicts of interest. By arrangement with Gilead Sciences and CONRAD, LIFE*lab* acquired a voluntary non-exclusive royalty-free license for tenofovir gel for low-cost distribution in Africa.

Abstract

The CAPRISA 004 trial assessed effectiveness and safety of a 1% vaginal gel formulation of tenofovir, a nucleotide reverse transcriptase inhibitor, for the prevention of HIV acquisition in women. A double-blind, randomized controlled trial was conducted comparing tenofovir gel (n=445) with placebo gel (n=444) in sexually active, HIV-uninfected 18-40 year-old women in urban and rural KwaZulu-Natal, South Africa HIV serostatus, safety, sexual behavior and gel and condom use were assessed at monthly follow-up visits for 30 months. HIV incidence in the tenofovir gel arm was 5.6 per 100 women-years (wy), i.e. person time of study observation, (38/680.6wy) compared to 9.1 per 100 wy (60/660.7wy) in the placebo gel arm (Incidence Rate Ratio (IRR)=0.61; p=0.017).In high adherers (gel adherence >80%), HIV incidence was 54% lower (p=0.025) in the tenofovir gel arm. In intermediate adherers (gel adherence 50-80%) and low adherers (gel adherence < 50%) the HIV incidence reduction was 38% and 28% respectively. Tenofovir gel reduced HIV acquisition by an estimated 39% overall, and by 54% in women with high gel adherence. No increase in the overall adverse event rates was observed. There were no changes in viral load and no tenofovir resistance in HIV seroconvertors. Tenofovir gel could potentially fill an important HIV prevention gap, especially for women unable to successfully negotiate mutual monogamy or condom use.

Women are disproportionately affected by the Acquired Immunodeficiency Syndrome (AIDS) epidemic in Africa, the region which accounts for 70% of global burden of Human Immunodeficiency Virus (HIV) infection (1). Current HIV prevention behavioral messages on abstinence, faithfulness and condom promotion have had limited impact on HIV incidence rates in women, especially in sub-Saharan Africa, where young women bear the greatest HIV burden (2). The search for new technologies to prevent sexually transmitted HIV infection over the past three decades has had limited success. Only five of 37 randomized controlled trials, which tested 39 HIV prevention strategies, have demonstrated protection against sexual transmission of HIV infection (3). The successful trials tested medical male circumcision in South Africa (4), Kenya (5) and Uganda (6) (combined effectiveness in reducing HIV acquisition = 57%), sexually transmitted infection (STI) treatment in Tanzania (effectiveness in reducing HIV acquisition = 42%) (7) and a HIV vaccine combination in Thailand (effectiveness in reducing HIV acquisition = 31%) (8). Hence, HIV prevention technologies that women can use and control remain a pressing priority (9).

Microbicides are products that can be applied to the vagina or rectum with the intention of reducing the acquisition of STIs including HIV. An effective microbicide has the potential to alter the trajectory of the global HIV pandemic (10). Over the last 20 years of microbicide research, none of the 11 effectiveness trials of six candidate products have demonstrated meaningful protection against HIV infection (11).

Tenofovir, an adenosine nucleotide analog with potent activity against retroviruses (12), was initially developed and tested as a prophylactic in monkeys and was subsequently formulated for oral use as tenofovir disoproxil fumarate (Viread®), which is now widely used for HIV treatment. Tenofovir's efficacy in suppressing viral replication, favorable safety profile and long half-life (13), made it an ideal choice as the first antiretroviral drug to be formulated as a microbicide gel. *In vitro* and *in vivo* assessments of the 1% concentration of tenofovir in a gel formulation have demonstrated its potential as a microbicide (13). Tenofovir has shown efficacy against viral challenge in animal models when administered as pre- or post-exposure prophylaxis (14-15). In monkey challenge studies, tenofovir gel has shown protection with intermittent dosing and with a single pre-exposure dose (16).In early stage clinical trials, tenofovir gel was well tolerated in both HIV negative and HIV positive

women (17), with both daily and coitally-related use of the gel being found to be acceptable and safe (18).

The purpose of this study was to assess the effectiveness and safety of tenofovir gel for the prevention of HIV infection in women.

Study design and population

CAPRISA 004, a two-arm, double-blind, randomized, placebo-controlled trial, was conducted from May 2007 to March 2010. Women were enrolled at an urban and a rural clinic in KwaZulu-Natal, South Africa, but the study was not designed to assess the effectiveness of tenofovir in each clinic separately. Urban women were enrolled at the CAPRISA eThekwini Research Clinic, adjacent to an STI clinic located in the Durban city centre. Rural women were enrolled at the CAPRISA Vulindlela Research Clinic, adjacent to a comprehensive primary health care clinic in Vulindlela, a rural community of approximately 90,000 people, about 150km north-west of Durban. Prior to the CAPRISA 004 trial, feasibility studies were conducted to assess HIV incidence and sexual behavior at both sites. Extrapolated HIV incidence rates from prevalence studies in the urban (19) and rural (20) sites were 15.6% and 11.2% respectively. Reported anal sex rates were substantially lower at these two sites than we had observed in previous microbicide trials (21) in female sex workers in this region. Data from these feasibility studies were used as the basis for selecting these sites for the trial as well as for the design and sample size calculations for the CAPRISA 004 trial.

HIV negative women, from 18 to 40 years, who were sexually active (defined as having engaged in vaginal sex at least twice in the 30 days prior to screening), not pregnant, and using a non-barrier form of contraceptive were eligible for enrolment. Participants who had a history of adverse reactions to latex, planned to either travel away from the study site for more than 30 consecutive days, relocate away from the study site, become pregnant, or enroll in any other behavioral or investigational product study were excluded. Participants who had a creatinine clearance <50ml/min, (22), had evidence of genital deep epithelial disruption, had in the past year participated in any research related to any vaginally applied product/s, or had an untreated STI or reproductive tract infection were also excluded. Women who met eligibility criteria and demonstrated adequate understanding of the trial (through a comprehension checklist) were enrolled after providing written informed consent. From May 2007 to January 2009, 2160 women were screened, 1085 were enrolled, of whom 889 were included in the analysis (Figure 1). Further information on the enrollment process and exclusions can be found in SOM

Enrolled women were randomly assigned in equal proportions to one of two study arms; tenofovir gel or placebo gel. Tenofovir gel comprised 40mg of 9- [(R)-2-phosphonomethoxy)propyl]adenine monohydrate (PMPA) in a solution of purified water with edetate disodium, citric acid, glycerin, methylparaben, propylparaben, and hydroxyethycellulose (HEC). The placebo gel was the “universal” HEC placebo gel which has been shown to have minimal anti-HIV activity (23). Tenofovir and placebo gels appeared identical and were dispensed in the same pre-filled vaginal applicators with identical packaging.

A coitally-related dosing strategy was selected to achieve high adherence, based on in-depth consultations with the communities involved. Sexual behavior data showed that women in the key study population had infrequent high-risk sex with migrant partners. Monkey challenge data and perinatal transmission studies informed the timing of doses in relation to sex. The “before and after” sex doses were modeled on the timing of nevirapine in its proven strategy for preventing mother-to-child HIV transmission (24). Women were requested to

insert one dose of gel within 12 hours **b**efore sex and a second dose of gel as soon as possible within 12 hours **a**fter sex and no more than **t**wo doses of gel in a **24**-hour period. Hence the dosing strategy is referred to as “BAT24”. The latter restriction was imposed due to the lack of human safety data on more than two doses of gel per day.

Gel adherence was defined as the estimated proportion of reported sex acts covered by two gel doses and calculated for each woman by dividing half the number of returned used applicators each month by the number of reported sex acts that month. Applicators that were not returned were regarded as unused for the purposes of calculating adherence. When we conducted a sensitivity analysis treating unreturned applicators as used, the results did not change materially. The median of each woman's monthly adherence estimates was assigned as her overall gel adherence. This approach assumed that every reported sex act utilized two doses of gel. While this assumption was not always applicable, adjusting for multiple sex acts within 24 hours made no material difference.

At enrolment and monthly follow-up visits, participants were provided with comprehensive HIV prevention services (HIV pre- and post-test counseling, HIV risk reduction counseling, condoms, and STI treatment), reproductive health services, and assigned study gel.

Participants were requested to return their used (from October 2007 onwards) and unused applicators at every visit. Each month the applicators returned by women as used and unused were counted, reconciled against number dispensed, and thereafter discarded, in accordance with standard requirements for medical waste.

A comprehensive adherence support program assisted participants with the mechanics of applicator use, timing and dosing, avoidance of gel sharing, and incorporation of gel use into their daily routines. From October 2008, individualized, motivational interviewing (25-26) was introduced to assist participants to overcome obstacles to gel use and set goals for optimal adherence in the upcoming month. This included individualized adherence support and counseling, customized on the previous month's experience of gel use, which was provided throughout the study. The women in this study were specifically and repeatedly counseled to only use the gel vaginally and the lack of safety with rectal use was highlighted.

Each participant had monthly HIV and urine pregnancy testing (QuickVue One-Step hCG Urine Test Quidel Corporation, San Diego, USA) performed before gel was dispensed. Due to a lack of pregnancy safety data, gel use was temporarily discontinued after a positive pregnancy test and resumed when the pregnancy test returned to negative. Self-reported data on gel use and sexual frequency during the last 30 days were collected at monthly visits, together with gel and condom use on the day of the last sex act, by means of a brief interviewer-administered questionnaire. Two months after study exit, participants attended a post-trial visit to assess HIV status and safety after product withdrawal.

Drug safety was assessed at every study visit by evaluating, grading and recording adverse events experienced by participants. Participants underwent quarterly pelvic examinations and, if needed, colposcopy. Serology was performed for hepatitis B virus (Abbott Architect C8200, Abbott Laboratories, Detroit, MI) and herpes simplex type 2 virus (Kalon Enzyme Immunoassay, Kalon Biologicals, Ashgate, UK). Hematological, hepatic and renal abnormalities were assessed at study months 3, 12 and 24, additionally when clinically indicated, and at study exit. Adverse events were graded for severity via the Division of AIDS Table for Grading Adult and Pediatric Adverse Events, 2004. Product use was temporarily discontinued for an adverse event at the discretion of the study clinician. The trial (NCT00441298) was approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee (E111/06), Family Health International's Protection of Human

Subjects Committee (#9946) and the South African Medicines Control Council (#20060835).

HIV, viral load and genotypic resistance assays

Two HIV rapid tests, Determine HIV 1/2 (Abbott Laboratories, Illinois, USA) and Uni-Gold Recombigen® HIV test (Trinity Biotech, Wicklow, Ireland) were performed at each study visit. Participants with concordantly positive, discordant or indeterminate results were assessed for possible seroconversion by two separate RNA polymerase chain reaction (PCR) (Roche Cobas Amplicor HIV-1 Monitor v1.5, Roche Diagnostics, Branchburg, New Jersey, USA) assays, about one week apart.. When HIV seroconversion was established, product use was discontinued and women were referred to local AIDS treatment services, including the CAPRISA AIDS Treatment Program which provides free antiretroviral therapy. Stored plasma, available from prior study visits by each seroconvertor was tested by RNA PCR to identify the window period for HIV infection (RNA PCR positive but rapid HIV test negative) at prior visits. By protocol, only eligibly enrolled women with HIV infection during study follow-up, confirmed by two independent RNA PCR results, were defined as HIV endpoints. Participants in the HIV window period at study exit were included as HIV endpoints if seropositivity was confirmed post-study. Thus, HIV infections were categorized as follows: (i) HIV endpoints; (ii) HIV infections not meeting the protocol definition for an HIV endpoint (i.e. did not have that two independent RNA PCR tests); iii) window period HIV infections at enrolment (infected prior to study entry); iv) post-trial HIV infections (infected after study exit), and v) HIV infections among women who were enrolled and later found to be ineligible (see SOM for more details).

Tenofovir resistance testing and Western Blot (Genetics systems HIV-1 Western Blot kit, BioRad Laboratories Hercules CA, USA), were performed at the post-seroconversion visit. The HIV-1 *pol* gene was population sequenced by means of a certified (27) in-house assay. Viral RNA was extracted and a 1.7 kb fragment spanning the *po*l gene was amplified by nested PCR using the Expand Long Template PCR System (Roche Diagnostics), as described previously (27). PCR products were sequenced (codons 1-99 of protease and codons 1-350 of reverse transcriptase) using a BigDye Terminator v3.1 cycle sequencing kit and an ABI 3130XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). Consensus sequences were aligned and manually edited using the Sequencher version 4.5 program (GeneCodes, Ann Arbor, MI) and submitted to the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu) to identify mutations.

Statistical analyses

In this endpoint driven trial, participants were followed until 92 HIV infections were observed, providing 90% power to detect a 50% effect (two-sided alpha = 0.05). Originally, the study was designed with 80% power. Prior to their first data review, the Data Safety and Monitoring Board (DSMB) ratified a change to 90% power, adjusted for two pre-planned interim reviews with stringent stopping guidelines.

Upon enrolment, a participant was assigned a sequential identification number, which corresponded to a unique envelope (accessible only to each study site pharmacist) that allocated her randomly, using permuted block randomization of sizes 12 and 18, with no stratification, to one of six codes. The three codes assigned randomly to each of tenofovir and placebo gels were held in confidence by the product manufacturer and independent DSMB statistician.

The primary intent-to-treat analysis was based on a log-rank test, stratified by site. Duration of time on study was calculated from randomization to estimated date of HIV infection or

date of withdrawal, whichever occurred first. A Poisson distribution was assumed for confidence intervals (CI) of incidence rates and incidence rate ratios (IRR). Fisher's exact test and the unpaired t-test/Wilcoxon two-sample test were performed where appropriate. Proportional hazards regression models were used to calculate hazard ratios while adjusting for potentially important covariates. All reported p-values are two-sided and all CIs are 95%. The statistical analysis was performed using SAS® (SAS Institute Inc., Cary) Version 9.1.3.

Results

A total of 611 rural and 278 urban women met eligibility criteria, were enrolled and followed-up for a total of 1341 women-years (mean=18 months), with an overall study retention rate of 94.8%. Rural women were younger and poorer with fewer lifetime sexual partners, and had lower sexual frequency, and lower condom use (Table 1). At enrolment, there were no significant differences in the demographic characteristics and sexual behavior of women in the tenofovir (n=445) and placebo (n=444) gel arms (Table 1).

HIV incidence and effectiveness

The tenofovir and placebo gel arms had 38 and 60 HIV endpoints respectively. The HIV incidence rate in the tenofovir gel arm was 5.6 per 100 women-years (wy) (CI: 4.0, 7.7) compared to 9.1 per 100 wy (CI: 6.9, 11.7) in the placebo gel arm (IRR=0.61; CI: 0.40, 0.94; p=0.017).

HIV infection trends (Figure 2) show that the tenofovir gel effect was evident soon after initiation of gel use. The steadily declining HIV incidence rates in placebo gel arm were 11.2, 10.5, 10.2, 9.4, and 9.1 per 100 wy after follow-up for 6, 12, 18, 24, and 30 months respectively (Figure 2). In contrast, the HIV incidence rate in the tenofovir gel arm remained in a narrow range between 5.2 and 6.0 per 100 wy during the study. The HIV incidence rate in the tenofovir gel arm, when compared to the placebo gel arm, was 50% (p=0.007) lower after 12 months of follow-up and 40% (p=0.013) lower after 24 months of follow-up (Figure 2).

After adjusting for baseline covariates including, age, site, anal sex history, contraceptive method, HSV-2 antibody status, and condom use, the hazard ratio was 0.63 (CI: 0.42, 0.94; p=0.025). All 98 HIV endpoints were Western Blot positive. One additional HIV infection did not meet the protocol endpoint requirement of two independent RNA PCR results. There were five HIV infections among ineligibly enrolled women, 10 window period HIV infections (two among ineligibly enrolled women) and five post-trial HIV infections. Sensitivity analyses (Table 2), which include these additional HIV infections do not differ appreciably from the overall 39% level of effectiveness.

Gel adherence and sexual behavior

Over the entire duration of the study, 181 340 applicators were dispensed and 95.2% of these were returned. Each month, study participants returned an average of 6.0 used applicators, and reported a mean of 5.0 sex acts. Coital frequency, gel adherence and condom use during the trial were similar in the tenofovir and placebo gel arms. Gel acceptability was high; 97.4% of the study participants found the gel to be acceptable and 97.9% indicated that they would use it if it prevented HIV.

Five women reported having no sex during follow-up in the study. Adherence estimates based on applicator returns for the remaining 884 women indicate that, on average, 72.2% (median = 60.2%) of self-reported sex acts in the last 30 days were covered by two doses of gel. In the 336 high gel adherers, HIV incidence was 54% lower (IRR 0.46, CI: 0.20, 0.94, p=0.025) in the tenofovir gel arm (Table 2). In intermediate gel adherers and low gel

adherers, the HIV incidence reduction was 38% (p=0.343) and 28% (p=0.303) respectively. The mean number of sex acts in the high, intermediate and low gel adherers was 3.2, 5.0 and 6.7 per month respectively.

Over the 30 months of follow-up, reported coital frequency declined steadily (Figure 3) from 7.2 sex acts per month in the first 6 months to 3.1 sex acts per month in months 18-24 (p<0.001). In women who did not acquire HIV, overall median gel adherence was 61.3%, increasing from 55.0% in the first 6 months to 75.0% in months 18-24 (p<0.001). In HIV seroconvertors, overall median adherence (until product discontinuation following HIV infection) was 59.2%, ranging from 56.9% in the first 6 months to 61.3% in months 18-24 (p=0.735). Overall, condoms were reportedly used in 80.3% of sex acts; increasing from 78.5% in the first 6 months to 84.3% in months 18-24 (p<0.001) (Figure 3).

Safety and pregnancy outcomes

There were 4692 adverse events reported during the study, with 94.3% (838/889) of the study participants reporting at least one adverse event. Adverse event rates were 3.55 per wy in the tenofovir and 3.44 per wy in the placebo gel arms (p=0.265). Women in the tenofovir gel arm reported more instances of diarrhea (Table 3) than those using placebo gel (16.9% vs 11.0%, p=0.015). There were 39 serious adverse events, including one death. In the 37 hepatitis B virus carriers, (20 randomized to tenofovir gel and 17 to placebo gel) there were two cases of “hepatic flares” (ie. alanine aminotransferase > 5 times the upper limit of normal) in each arm. Further information on grading of the hepatic, renal, and bone adverse events can be found in SOM.

Five participants discontinued gel use for a total of 1.04 wy because of adverse events; four were due to genital findings and one due to congestive cardiac failure.

The overall pregnancy rate was 4.0 per 100 wy; 3.2 per 100 wy in the tenofovir arm and 4.7 per 100 wy in the placebo arm (p=0.183) (Table 3). At the time of analysis, there were 6 ongoing pregnancies while 58.3% of the remaining 48 pregnancies had resulted in a full-term live birth. There were no significant differences in pregnancy outcomes by study arm and there were no congenital anomalies. A total of 20.9 wy of follow-up occurred while women were not using gel due to pregnancy.

Viral load and tenofovir resistance

The mean log HIV viral load at the time when HIV seroconversion was identified was 4.65 (Inter quartile range (IQR): 4.04 - 5.39) and 4.30 (IQR: 3.56 – 5.17) log copies/ml in the tenofovir gel arm (n=38) and placebo gel arm (n=60) respectively (p=0.147).

It is estimated that the HIV seroconvertors were exposed to gel episodically for about 3-4 weeks post-infection and the resistance assays (n=35) were performed on average 20 weeks after the estimated date of infection. There were no tenofovir-related resistance mutations (K65R, K70E) detected and none of the women had thymidine analogue mutations (M41L, L210W, T215Y/F, D67N, K70R, and K219Q/E) or mutations that confer multi-NRTI resistance.

Discussion

Tenofovir gel reduced HIV infection by an estimated 39%. The protective effect of coitally-related tenofovir gel use was evident soon after initiation and peaked at 50% after 12 months of gel use. This protective effect is evident irrespective of sexual behavior, condom use, herpes simplex type 2 virus infection or urban/rural differences. A trend of higher

effectiveness was observed as gel adherence improved; high adherers had a 54% lower HIV incidence rate in the tenofovir gel arm.

The observed level of effectiveness is dependent on both the efficacy of the product and the participants' willingness and ability to use it as prescribed. Inadequate adherence is the most serious challenge to obtaining an accurate estimate of product efficacy (28). To address this, we implemented an intensive adherence support program with motivational strategies which depended on reliable measurement of adherence. Monitoring of this key behavior in the trial included an objective count of used and unused applicators returned each month and did not rely solely on self-reported use. Despite this adherence program and high gel acceptability, about 40% of the women in this study had below 50% gel adherence. Future trials will need to place greater emphasis on enhancing and objectively measuring adherence, in light of its substantial influence on the trial outcome.

In this study population, women with the highest gel adherence tended to have the lowest reported coital frequency. Despite their lower coital frequency, these women had HIV incidence rates comparable (in the placebo gel arm) to those in women with much higher coital frequencies, highlighting the importance of infrequent but very high-risk sex with migrant men. The impact of coitally-related tenofovir gel was substantial in this group, indicating its potential to alter the course of the HIV epidemic in southern Africa, where young women engaging in sex with migrant men is the key driver in the spread of HIV infection (29). On a cautionary note, the effectiveness of coitally-related tenofovir gel appeared to decline after 18 months, reasons for this are unclear and factors, including the possibility of declining number of gel applications and/or adherence over time, need further investigation.

HIV incidence rates observed in this study population were high, as KwaZulu-Natal province is at the epicenter of South Africa's “explosive” HIV epidemic (30). While the women in the tenofovir gel arm had a substantially lower HIV incidence rate than the placebo arm, they still had an unacceptably high HIV incidence rate, consistently above 5 per 100wy. This highlights the need to seek higher levels of adherence and effectiveness with tenofovir gel and/or to develop other effective prevention strategies for use in combination with tenofovir gel. Encouragingly, there was no evidence of risk compensation (31), the phenomenon whereby individuals increase their HIV risk by reducing their use of proven prevention modalities, such as condoms, in favor of less effective or unproven prevention strategies. Instead, we observed declining HIV incidence rates in the placebo gel arm. This may have been due to their declining coital frequency and increasing condom use. However, the consistently high levels of self-reported condom use in the last sex act need to be interpreted cautiously as these may be affected by inaccurate recall and may be indicative of condom use in only the last sex act and not all sex acts.

We found no empiric evidence for the theoretical concern that tenofovir gel may mask HIV infection and that withdrawal of tenofovir gel use after study exit may lead to the unmasking of these infections.

Coitally-related tenofovir gel use was safe. There were no increases in renal, hepatic, pregnancy-related or genital adverse events. The increased risk of diarrhea in women using tenofovir gel may possibly have been due to a local tenofovir effect; further investigation is needed to establish the mechanism for this observed adverse effect. The reported cases of diarrhea were mild and self-limiting, rarely requiring medication.

There was no renal toxicity, the most important tenofovir-related safety concern (32), though it should be noted that the study excluded women with compromised creatinine clearance at enrollment. Increases in hepatic flares, which have been reported upon cessation

of oral tenofovir use in hepatitis B-infected individuals (33), was not observed in this study, possibly due to the low systemic absorption of tenofovir from the gel formulation (17). No safety concerns were identified in the 22 women exposed to tenofovir gel in early pregnancy, providing further evidence to support the analysis of the Antiretroviral Pregnancy Register (34) which showed no increases in congenital anomalies. No tenofovir-related resistance was found in the 35 women exposed to tenofovir gel early in acute HIV infection. Further studies to identify tenofovir resistance at earlier timepoints post-infection, in both the genital and systemic compartments, are needed. Coitally-related tenofovir gel use showed no impact on viral load in HIV seroconvertors.

This test-of-concept study had several limitations; the relatively small sample size and the small number of study sites restrict the broad generalizability of the results. The study's adherence program needed to attain higher and sustained levels of adherence. The co-enrollment challenge was a set back at the urban site. It did not, however, impact on the estimated effectiveness of tenofovir gel when infections in co-enrolled women were included in the analysis. It is not possible to derive from this study any conclusions on the safety and effectiveness of tenofovir gel for anal sex. Similarly, it is not possible to make any conclusions on the effectiveness of tenofovir gel in relation to the timing of gel applications because when gel was applied, BAT 24 was usually followed.

Currently there are five large-scale trials assessing oral pre-exposure prophylaxis with tenofovir or tenofovir-emtricitabine (35) in men who have sex with men, intravenous drug users and heterosexual men and women. One of these, the MTN 003 trial (36), is assessing the effectiveness of daily tenofovir gel for HIV prevention. This critically important study will provide urgently needed evidence on whether more frequent dosing can improve adherence and effectiveness of tenofovir gel without compromising safety. Additional studies are needed to corroborate the findings of the CAPRISA 004 trial and to assess the safety, effectiveness, adherence and/or cost advantages and disadvantages of coitally-related tenofovir gel compared to daily tenofovir in either the gel or oral formulation, for HIV prevention in women.

Conclusion

Coitally-related tenofovir gel appears safe and effective in preventing HIV infection. Once these promising findings have been corroborated, this antiretroviral microbicide could potentially fill an important HIV prevention gap, especially for women unable to successfully negotiate mutual monogamy or condom use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We pay tribute to the women who participated in this trial; their dedication and commitment made this study possible. We thank the Health Department of the City of Durban, the traditional leadership of Vulindlela, specifically, Nkosi Sondelani Zondi and Nkosi Nsikayezwe Zondi; and members of the Community Advisory Boards at the Vulindlela and eThekwini Research Clinics.

The CAPRISA 004 Tenofovir gel trial was supported by the Centre for the AIDS Program of Research in South Africa (CAPRISA), the United States Agency for International Development (USAID), FHI (co operative agreement # GPO-A-00-05-00022-00, contract # 132119), and LIFElab, a biotechnology centre of the South African Department of Science and Technology. Support from CONRAD for the product manufacturing and packaging as well as support from Gilead Sciences for the tenofovir used in the production of gel is gratefully acknowledged. We thank the US National Institutes for Health's Comprehensive International Program of Research on AIDS (CIPRA grant # AI51794) and the Columbia University-Southern African Fogarty AIDS International

Training and Research Programme (AITRP grant # D43TW00231) for the research infrastructure and training that made this trial possible.

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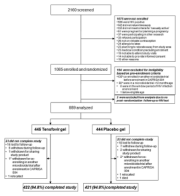

Figure 1.
Screening, enrolment, randomization and follow-up of the study participants in the CAPRISA 004 tenofovir gel trial.
* *Note*: co-enrolment occurred only in the urban clinic

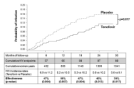

Figure 2.
Kaplan-Meier estimates of cumulative probability of HIV infection in the tenofovir and placebo gel arms. The table provides the cumulative number of HIV endpoints, corresponding HIV incidence rates and effectiveness of tenofovir gel for each additional 6-months of follow-up.

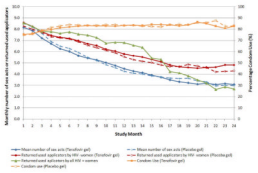

Figure 3.
Trends in coital frequency, condom use and gel use (gel use by HIV status) in relation to duration of follow-up in the CAPRISA 004 tenofovir gel trial

Science. Author manuscript; available in PMC 2011 September 3.

Table 1

Baseline demographic characteristics, sexual history and contraceptive use by study participants in the CAPRISA 004 tenofovir gel trial

	Site			Study arms		
	Rural (n=611)	**Urban (n=278)**	**p-value**	**Tenofovir (n=445)**	**Placebo (n=444)**	**p-value**
Demographic characteristics						
Mean Age (years)	23.3	25.1	<0.001	24.2	23.6	0.131
Monthly income <R1000	86.1%	69.1%	<0.001	81.1%	80.4%	0.799
Married	6.5%	3.6%	0.085	5.8%	5.4%	0.884
Stable partner	77.0%	93.1%	<0.001	87.6%	88.5%	0.756
Sexual behavior						
Mean age sexual debut	17.3	17.7	0.014	17.4	17.4	0.782
Mean number sexual partners (lifetime)	2.1	6.0	<0.001	3.0	3.6	0.780
Mean age of oldest partner (past 30 days)	26.4	29.6	<0.001	27.7	27.1	0.299
Reported sex in the past 7 days	58.9%	68.3%	0.007	63.6%	60.1%	0.301
Always use condom during sex	22.9%	42.8%	<0.001	28.8%	29.5%	0.825
New partner (past 30 days)	0.5%	2.5%	0.014	1.3%	0.9%	0.753
Anal sex (past 30 days)	0.5%	0.4%	1.000	0.4%	0.5%	1.000
HSV-2 prevalence	47.6%	59.6%	0.001	53.5%	49.2%	0.202
Contraception						
Injectable	83.1	79.9	0.606*	80.7%	83.6%	0.288*
Oral	14.6	17.6		16.4%	14.6%	
Tubal Ligation	2.1	2.5		2.9%	1.6%	
Hysterectomy	0.2	0.0		0	0.2%	

* p-value applicable to comparison for all forms of contraception

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Table 2

Impact of adherence and non-endpoint HIV infections on the effectiveness of tenofovir gel in HIV prevention in the CAPRISA 004 tenofovir gel trial.

	# HIV infections / women years		N	HIV incidence		Incidence Rate Ratio	Effectiveness	95% Confidence Interval	p-value
	Tenofovir	Placebo		Tenofovir gel (95% CI)	Placebo gel (95% CI)				
Overall effectiveness of tenofovir gel									
HIV endpoints	38 / 680.6	60 / 660.7	889	5.6 (4.0, 7.7)	9.1 (6.9, 11.7)	0.61	39%	6, 60	0.017
Site-specific effectiveness									
Rural	25 / 484.7	42 / 461.2	611	5.2 (3.3, 7.6)	9.1 (6.6, 12.3)	0.57	43%	5, 67	0.023
Urban	13 / 195.9	18 / 199.5	278	6.6 (3.5, 11.3)	9.0 (5.3, 14.3)	0.74	26%	-59, 67	0.380
HIV endpoints by levels of adherence^									
High adherers (>80% gel adherence)	11 / 259.2	25 / 269.4	336	4.2 (2.1, 7.6)	9.3 (6.0, 13.7)	0.46	54%	4, 80	0.025
Intermediate adherers (50-80% adherence)	10 / 159.8	10 / 99.7	181	6.3 (3.0, 11.5)	10.0 (4.8, 18.4)	0.62	38%	-67, 77	0.343
Low adherers (<50% gel adherence)	16 / 258.5	25 / 290.6	367	6.2 (3.5, 10.1)	8.6 (5.6, 12.7)	0.72	28%	-40, 64	0.303
Effectiveness of tenofovir gel by study exit HSV-2 status•									
HSV-2 positive	29 / 397.7	46 / 410.7	541	7.3 (4.9, 10.5)	11.2 (8.2, 14.9)	0.65	35%	-6, 61	0.070
HSV-2 negative	9 / 272.8	14 / 248.0	339	3.3 (1.5, 6.3)	5.6 (3.1, 9.5)	0.58	42%	-45, 78	0.209
Sensitivity analyses									
HIV endpoints *plus* HIV infection not meeting protocol definition	39 / 680.6	60 / 660.7	889	5.7 (4.1, 7.8)	9.1 (6.9, 11.7)	0.63	37%	4, 59	0.023
HIV endpoints *plus* ineligibly enrolled	40 / 720.1	63 / 698.6	1075	5.6 (4.0, 7.6)	9.0 (6.9, 11.5)	0.62	38%	7, 60	0.015
HIV endpoints *plus* women with post-trial infection	39 / 680.6	64 / 660.7	889	5.7 (4.1, 7.8)	9.7 (7.5, 12.4)	0.59	41%	11, 61	0.015
Per protocol analysis#	32 / 589.2	53 / 575.4	889	5.4 (3.7, 7.7)	9.2 (6.9, 12.0)	0.59	41%	7, 63	0.017
All HIV infections*	43 / 720.1	76 / 698.8	1085	6.0 (4.3, 8.0)	10.9 (8.6, 13.6)	0.55	45%	19, 63	0.003
Adjusted analysis♣	38	60	889		Hazard Ratio=0.63		37%	6, 58	0.025

#excludes all visits after 3 months interruption of drug supply;

^Adherence could not be calculated for the 5 women who reported no sex during their follow-up in the study

*All HIV infections = *Protocol defined HIV endpoints (n=98), + HIV infection not meeting protocol definition (n=1 who did not have 2 RNA PCR results), + HIV infections among ineligibly enrolled women (n=5), + post-trial HIV infections (n=5), + window period HIV infections in eligible women (n=8), + window period HIV infections in ineligibly enrolled women (n=2).*

♣Adjusted for the following baseline covariates: age, site, parity, number of sexual partners (past 30 days), presence of STI, anal sex, contraceptive method, HSV-2 antibody status, and condom use;

•HSV-2 status is indeterminate in 4 women and missing in 5 women

Table 3

Adverse events and other safety markers in the CAPRISA 004 tenofovir gel trial

	Tenofovir gel	Placebo gel	p-value
	# events/ # participants / (% with ≥ 1 event)	# events / # participants / (% with ≥ 1 event)	
Number of adverse events	2419 / 423 / (95.1%)	2273 / 415 / (93.5%)	0.32
Deaths	0 / 0/ (0%)	1 / 1 / (0.2%)	0.50
Serious adverse events:			
Total serious adverse events	23 / 21 /(4.7%)	16 / 16 / (3.6%)	0.50
Pregnancy related serious adverse events	8 / 8 / (1.8%)	8 / 8 / (1.8%)	1.00
Grade 3* adverse events	19 / 15 / (3.4%)	18 /16 / (3.6%)	0.86
Grade 4* adverse events	4 / 4 / (0.9%)	4 / 3 / (0.7%)	1.00
Common adverse events			
Influenza	365 / 216 / (48.5%)	314 / 220 / (49.6%)	0.85
Vaginal discharge	203 / 156 / (35.1%)	239 /156 / (35.1%)	1.00
Vaginal candidiasis	156 /114 / (25.6%)	187 /130 / (29.3%)	0.28
Headache	126 / 93 / (20.9%)	133 /102 / (23.0%)	0.53
Urinary tract infection	135 / 100 / (22.5%)	120 /93 / (21.0%)	0.63
Diarrhea and gastrointestinal infections	91 / 75 / (16.9%)	65 / 49 / (11.0%)	0.02
Upper respiratory tract infections	162 / 114 / (25.6%)	145 / 100 / (22.5%)	0.31
Genital adverse events			
Disrupted Epithelium e.g.genital ulceration	18 / 18 / (4.0%)	14 / 13 / (2.9%)	0.47
Intact epithelium, e.g. erythema	48 / 41 / (9.2%)	42 / 33 / (7.4%)	0.40
Urogenital symptoms e.g.menorrhagia	312 /210 / (47.2%)	394 / 238 / (53.6%)	0.06
Vaginal candidiasis	156 / 114 / (25.6%)	187 / 130 / (29.3%)	0.23
Other	182 / 131 / (29.4%)	176 / 123 / (27.7%)	0.60
Laboratory parameters - any abnormality post-randomization			
Hepatic			
- aspartate aminotransferase (AST)	29 / 21 / (4.7%)	36 / 29 / (6.5%)	0.25
- alanine transaminase (ALT)	42 / 33 / (7.4%)	50 / 40 / (9.0%)	0.38
Renal			
- raised creatinine	4 / 4 / (0.9%)	1 / 1 / (0.2%)	0.37
- low potassium	119 / 95 /(21.4%)	99 / 83 / (18.7%)	0.36
- raised sodium	54 / 48 / (10.8%)	43 / 41 / (9.2%)	0.50
Hematological			
- anemia	52 / 34 / (7.6%)	46 / 29 / (6.5%)	0.60
- neutropenia	19 / 16 / (3.6%)	13 /11/ (2.5%)	0.44
Bone			
- low phosphate	79 / 62 / (13.9%)	65 / 51 / (11.5%)	0.31
- low calcium	16 / 15 / (3.4%)	14 / 13 / (2.9%)	0.85
Fractures	3 / 2 / (0.5%)	3 / 3 / (0.7%)	0.69
Pregnancy rate per 100 women-years	3.2	4.7	0.18

	Tenofovir gel	Placebo gel	p-value
	# events/ # participants / (% with ≥ 1 event)	# events / # participants / (% with ≥ 1 event)	
Proportion of pregnancies resulting in live births	66.7	51.9	0.38

* Grade 3 and 4 adverse events refer to the grading for severity according to the Division of AIDS Table for Grading Adult and Pediatric Adverse Events, 2004