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Safety and Effectiveness of BufferGel and 0.5% PRO2000 Gel for the Prevention of HIV Infection in Women

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Disclaimer: The study was conceptualized and designed by SSAK, BR, GR, IH, MC, TT, LM, AC, AP, TM, BM, SH, L S-T. Data was gathered by, IH, MC, TT, MK, LM, BM. Data was analysed and interpreted by SSAK, IH, MC, TT, MK, LM, AC, E P-M, BM. SSAK had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. SSAK took responsibility for writing paper and all co authors contributed to critical revision of the paper.
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Abstract

Objective—To determine the safety and effectiveness of BufferGel and 0.5% PRO2000 microbicide gels for the prevention of male to female HIV transmission

Design—Phase II/IIb, randomized, placebo-controlled trial with three double-blinded gel arms and an open label no gel arm.

Methods—Study participants from Malawi, South Africa, Zambia, Zimbabwe and USA were instructed to apply study gel ≤1 hour before each sex act and safety, sexual behavior, pregnancy, gel adherence, acceptability, and HIV serostatus were assessed during follow-up.

Results—The 3101 enrolled women were followed for an average of 20.4 months with 93.6% retention and 81.1% self-reported gel adherence. Adverse event rates were similar in all study arms. HIV incidence rates in the 0.5% PRO2000 Gel, BufferGel, Placebo Gel and No Gel arms were 2.70, 4.14, 3.91 and 4.02 per 100 women-years, respectively. HIV incidence in the 0.5% PRO2000 Gel arm was lower than the Placebo Gel arm (Hazard Ratio (HR)=0.7; p=0.10) and the No Gel arm (HR=0.67; p=0.06). HIV incidence rates were similar in the BufferGel and both Placebo Gel (HR=1.10; p=0.63) and No Gel control arms (HR=1.05; p=0.78). HIV incidence was similar in the Placebo Gel and No Gel arms (HR=0.97; p=0.89).

Conclusions—0.5% PRO2000 Gel demonstrated a modest 30% reduction in HIV acquisition in women. However, these results were not statistically significant and subsequent findings from the MDP 301 trial have confirmed that 0.5% PRO2000 has little or no protective effect. BufferGel did not alter the risk of HIV infection. Both products were safe.

Keywords

Microbicide; PRO 2000 Gel; BufferGel; HIV Prevention; Women

INTRODUCTION

Globally, most new HIV infections are acquired through heterosexual contact [1]. While correct and consistent condom use has been shown to prevent HIV transmission [2], this method may not be applicable for women who are trying to become pregnant or who are unable to negotiate condom use with their male partners [3–5]. Microbicides are products that can be applied to the vagina or rectum with the intention of reducing the acquisition of sexually transmitted infections including HIV. Microbicides could fill an important HIV prevention gap, especially for those women who are unable to successfully negotiate mutual monogamy or condom use.

Before effectiveness trials on PRO2000 Gel and BufferGel were initiated, six candidate microbicides had been assessed for their effectiveness in preventing HIV infection. These were nonoxynol-9 (N9) sponge [6], N9 film [7], N9 gel [8–10], Savvy [11], cellulose suphate [12], and Carraguard [13]. Clinical trials of N9 film, Carraguard and one study of cellulose sulfate showed no impact on HIV acquisition. The Savvy trial was halted early due to futility. Two N-9 studies [6, 10] and a second cellulose sulphate trial [12] showed some increase, though not always statistically significant, in HIV among women randomized to the active arms.

BufferGel and PRO2000 Gel are two novel vaginal products that showed good acceptability and short-term safety in phase I and phase II trials conducted in the USA [14–15], Europe [16], India [17] and several countries in Africa [14, 16] [18–19]. BufferGel was designed to protect against HIV infection by maintaining the normally acidic vaginal pH in the presence

of ejaculate [20]. PRO2000 Gel contains an anionic polymer and was designed to protect against HIV infection by inhibiting viral attachment and entry into susceptible cells [14–15].

Soon after the initiation of the HPTN 035 trial, the Microbicide Development Programme (MDP) initiated a large phase III trial of the 0.5% and 2% concentrations of PRO2000 gel; which has, subsequent to the presentation of the HPTN 035 trial results, shown that both concentrations of PRO2000 have little or no protective effect on HIV [21].

The objective of HPTN 035 trial was to determine the safety and effectiveness of BufferGel (ReProtect Inc, Baltimore, Maryland, USA) and 0.5% PRO2000 Gel (Endo Pharmaceuticals Solutions Inc. (formally Indevus Pharmaceuticals Inc.), Lexington, Massachusetts, USA) in preventing HIV infection in women. Here we describe the trial design, key characteristics of the trial participants, and the primary outcome of the effect of BufferGel, PRO 2000 Gel and the hydroxyethylcellulose (HEC) Placebo Gel on HIV acquisition.

METHODS

Study design and population

HPTN 035 was a phase II/IIb, four-arm, multi-site, randomized controlled trial conducted between February 2005 and January 2009, at multiple sites in Blantyre and Lilongwe, Malawi; Durban and Hlabisa, South Africa; Harare and Chitungwiza, Zimbabwe; Lusaka, Zambia; and Philadelphia, USA.

HIV negative non-pregnant women, at least 18 years of age, who were sexually active, defined as having had vaginal intercourse at least once in the past three months were eligible for the study. The exclusion criteria included a history of adverse reactions to latex, use of non-therapeutic injection drugs in the past 12 months, and a history of vaginal intercourse more than an average of two times per day in the past two weeks.

Study procedures

All participants demonstrated adequate understanding of the trial and provided written informed consent. Women were randomly assigned in equal proportions to one of four study arms; BufferGel, 0.5% PRO2000 Gel, and two comparator arms comprising hydroxyethylcellulose (HEC) Placebo Gel or No Gel. All three study gels were similar in appearance and were packaged in identical vaginal applicators. Randomization was stratified by site in blocks of size 12 or 24, distributed randomly. Within each block of size 12 (24), three (6) assignments to each of the four treatment arms were allocated in random order. For the three gel arms, each of the three assignments within a block was associated with a unique 3-digit code which was labeled on the product packaging. In blocks of size 24, each unique 3-digit code was used twice. Each random sequence was determined via generation of uniform random variates in a computer program (SAS) and envelope materials were created and sealed at the Statistical and Data Management Center (SDMC). The three gel groups were double-blinded while the No Gel group was open label. Upon enrollment of a participant at each site, clinic staff opened an envelope revealing assignment to any gel group or to No Gel. For those assigned to any gel group, a corresponding envelope was opened only by the pharmacist to reveal the 3-digit code of the gel product to be prescribed. All persons associated with the study were masked to the product identity of the 3-digit codes throughout the course of the trial, except for the product manufacturers and one independent (not associated with the trial) statistician at the SDMC. Women assigned to a gel arm were requested to insert one applicator of gel intravaginally ≤1 hour before each episode of vaginal intercourse.

The first 799 women enrolled comprised a “lead in” phase II safety study which included intensive safety assessments such as hematological, coagulation, hepatic, renal function tests and monthly pelvic examinations for three months. In addition, 299 of these women underwent monthly colposcopy by trained colposcopists at the Philadelphia, Harare, Chitungwiza, Durban, and Lilongwe sites.

All women were provided comprehensive HIV prevention services, including HIV pre-test, risk reduction, and post-test counseling, condoms and STI testing and treatment as per local standards.

The Data and Safety Monitoring Board (DSMB) reviewed the phase II safety data and recommended proceeding with the phase IIb effectiveness trial. All observations prior to the DSMB review were included as part of the phase IIb analysis.

In phase IIb, study participants were followed monthly for 12 to 30 months based on date of enrollment. At each monthly visit they had a urine pregnancy test before study product was dispensed. Women testing positive for pregnancy were required to temporarily discontinue gel use while continuing follow-up in the trial. Product use was re-initiated when the urine pregnancy test was negative. Self-reported data on gel and condom use during the last coital act and during all coital acts in the last seven days were collected at quarterly visits. We calculated gel adherence as the proportion of women who reported applying gel during their last sex act from the data collected at the quarterly study visits. Condom use was calculated as the proportion of women who reported using a condom during their last sex act from the data collected at the quarterly study visits. Study participants also had quarterly HIV tests and medical and speculum-aided pelvic examinations.

Local mucosal toxicity was assessed by the incidence of deep epithelial disruption, observed on pelvic exam (speculum and/or colposcopic) as lesions penetrating into and exposing the sub-epithelial tissue and possibly blood vessels [22]. Additional safety outcomes included adverse genital signs and symptoms, as well as haematological, hepatic and renal abnormalities of grade 3 or higher severity based on the Division of AIDS Table for Grading Adult and Paediatric Adverse Events, 2004.

Laboratory tests

HIV infection status was determined using a standardized algorithm which was validated at each site. At the US site, the OraQuickR ADVANCE HIV-1/2 antibody test (Orasure Technologies, Bethlehem, PA, USA) was used. In the African sites, two rapid tests were used; the Determine HIV 1/2 (Abbott Diagnostic Division, Hoofddorp, The Netherlands) test was used with either the OraQuick®, or Uni-Gold Recombigen® HIV test (Trinity Biotech, Wicklow, Ireland). The Zambia site used only the OraQuick assay during follow – up.

Western blot (Genetics systems HIV-1 Western Blot kit, BioRad Laboratories Hercules CA, USA) was performed on samples with any positive HIV result. If the Western blot result was indeterminate or positive, a second blood sample was collected (approximately two weeks later) for further Western blot testing. If the second Western blot result was positive, HIV infection was considered confirmed. For women who tested HIV-positive in their first follow-up visit, plasma stored at study entry was tested by a RNA polymerase chain reaction (PCR) to identify women who may have been in the window period of acute HIV infection at enrolment. Women found to be in the window period at enrolment were deemed ineligible and were excluded from the primary analysis.

Statistical analyses

The Phase II portion of the study was designed to enrol 800 women and follow each of them for 3 months, resulting in approximately 50 person years of follow-up per randomization arm. Assuming a two-sided test with a false positive rate of 0.05, this provides 80% power to detect a three-fold difference between the active and placebo arms in safety measurements with baseline rates of at least 15 per 100 woman years. The Phase IIb portion of the study was designed to enrol 3100 women followed through the study end date or for a maximum of 30 months, whichever occurred first (with minimum follow-up of 12 months). The study end date was set as the date upon which a total of 192 incident HIV infections were observed. The number of incident infections was based on a four point decision guideline for this screening trial: 1) if the estimated effectiveness of a candidate microbicide is less than 15.3%, exclude the candidate microbicide from further testing for HIV prevention; 2) If the estimated effectiveness is greater than 15.3%, but less than or equal to 33%, consider the product plausibly effective and meriting further evaluation; 3) if the estimated effectiveness is between 33% and 43.6%, consider the product effective with strength of evidence equal to that of at least a single Phase III study; and 4) if the estimated effectiveness is greater than 43.6%, consider the product effective with the strength of evidence of at least one-and-a-half Phase III studies. Additional details regarding the statistical rationale for the study design and sample size have been published elsewhere [23].

Due to very low loss to follow-up, complete case analysis was used for all analyses. The primary analysis was intent-to-treat. Discrete time Cox Proportional Hazards models stratified by site were used to assess time to detection of HIV. Cumulative probabilities of infection were estimated using the Kaplan-Meier method. Incidence rates of epithelial disruption were compared using Andersen Gill Proportional Hazards Models stratified by site. Analyses stratified by adherence and condom use were post hoc analyses. All reported p-values are two-sided.

Ethics

The trial (NCT00074425) was approved by 11 institutional review boards that oversee research conducted at the eight study sites as well as regulatory authorities in the USA, South Africa, and Zimbabwe.

RESULTS

Between 2005 and 2007, 5888 women were screened and 3101 were enrolled (Fig. 1). Twelve women were subsequently excluded because they were HIV-infected at the time of enrolment and two women were identified as having enrolled twice. A further 37 women did not attend any follow-up visits. The remaining 3050 women were included in the primary analysis. The baseline characteristics and sexual behaviours were similar across the four study arms (Table 1).

Mean follow up was 20.4 months and overall study retention, defined as the proportion of those enrolled (except the window period infections) who had a study exit visit with an HIV test, was 93.6%. Of the 5258 person-years of follow-up accumulated on 3050 women (Fig 1), 240 (6.1%) person-years in 620 women comprised follow up during which study product was temporarily withheld, mostly due to pregnancy, in accordance with the study protocol. Women reported using gel in 81.1% of last sex acts. Gel adherence was similar in the three gel arms. Self-reported condom use during the last sex act was similar in the three gel arms but higher in the No Gel arm (71.7% vs 80.7%; p=<0.0001). Overall, women reported gel use in conjunction with condoms during study follow-up in 61.3% of sex acts. Gel was used in 69.1% of last acts in which a condom was not used.

The HIV incidence rate per 100 person years was 4.1 (54/1304) in the BufferGel arm, 2.7 (36/1332) in the 0.5% PRO2000 Gel arm, 3.9 (51/1305) in the Placebo Gel arm and 4.0 (53/1318) in the No Gel arm (Table 2). The hazard ratio (HR) of HIV incidence rates in 0.5% PRO2000 Gel arm compared to the Placebo Gel arm was 0.70 (p=0.10) and to the No Gel arm was 0.67 (p=0.06). Figure 2 shows how the Kaplan-Meier survival curves from HIV infection in the 0.5% PRO2000 Gel arm differs from the remaining three study arms. The HIV incidence rate in the 0.5% PRO2000 Gel arm was lower, though not statistically significant, than the rate in the Placebo Gel arm at 7 of the 8 sites (Table 3). In the per protocol analysis which excludes follow-up beyond 2 months after initiating product hold, the hazard ratio of HIV incidence rates in the 0.5% PRO2000 Gel arm was 0.71 (p=0.13) compared to the Placebo Gel arm and 0.64 (p=0.04) compared to the No Gel arm. There was no difference in HIV incidence between the BufferGel and both control arms (Table 3).

The HR of the HIV incidence rates in the Placebo Gel arm compared to the No Gel arm was 0.97 (p=0.89), reflecting the similarities in the HIV incidence rates in the Placebo Gel arm (3.9 per 100 person years; CI, 2.9 to 5.1) and in the No Gel arm (4.0 per 100 person years (CI, 3.0 to 5.3). There was little change in this HR after adjusting for baseline sexual behavior and participant characteristics.

In order to assess whether self-reported PRO 2000 Gel use was associated with a lower rate of HIV, a sub-group analysis stratified low and high gel users at the median; women with 85% or more gel use in their quarterly reported last sex acts were categorized as high gel users while those with less than 85% gel use were categorized as low gel users. The HIV incidence rate among low gel users was 3.0 per 100 person-years (18/592) in the 0.5% PRO2000 Gel group and 3.3 per 100 person-years (19/568) in the Placebo Gel group (Hazard Ratio = 1.04; CI, 0.55 to 2.00). However, the HIV incidence rate among high gel users was 2.4 per 100 person-years (18/740) in the 0.5% PRO2000 Gel group and 4.3 per 100 person-years (32/738) in the Placebo Gel group (HR = 0.55; CI, 0.31 to 0.98). In the low condom use (less than 85% condom use in quarterly reported last sex acts) subgroup of the high gel users, the HIV incidence rate was 1.0 per 100 person-years (3/299) in the 0.5% PRO2000 Gel group and 4.6 per 100 person-years (15/324) in the Placebo Gel group (HR = 0.21; CI, 0.06 to 0.73).

After adjusting for multiple comparisons, there were no statistically significant differences in systemic and local adverse events between the four study arms in the intent-to-treat and per protocol analyses. Overall the incidence rate of deep epithelial disruption was 1.55 per 100 person-years; with rates being similar across the four study arms (Table 4). The higher incidence of blood in the vagina with no identified source (Table 4) in the 0.5% PRO2000 Gel arm was statistically significant compared to the Placebo Gel arm (3.5 vs 1.8, p<0.01), but not compared to the No Gel arm (3.5 vs 2.3, p=ns). After adjusting for multiple comparisons these differences were no longer statistically significant. A total of 613 pregnancies occurred during follow-up in the study yielding an overall pregnancy rate of 11.3 per 100 person-years. Pregnancy rates were similar across the four study arms. The lowest pregnancy rate was 9.2 per 100 person-years (CI: 7.0 – 11.4) at the combined Harare and Chitungwiza sites in Zimbabwe and the highest was 16.5 per 100 person-years (CI: 13.4 – 19.5) at the Blantyre site (Table 4).

The overall incidence rates of infection with *Neisseria gonorrheae, Chlamydia trachomatis, Trichomonas vaginalis, and Treponema pallidum,* as well as bacterial vaginosis were 1.6, 3.9, 15.3, 0.9, and 160.5 per 100 person-years respectively. These infection rates were similar in the four study arms.

DISCUSSION

The HPTN 035 trial showed that 0.5% PRO2000 Gel was safe and, compared to the Placebo Gel, reduced the incidence of HIV infection by a modest 30%, although this finding was not statistically significant (p=0.10). The consistent presence of the 0.5% PRO2000 effect on HIV infection against each of the two comparator arms and in almost all study sites, the greater protection observed in self-reported high PRO2000 Gel users compared to low gel users, and the biological plausibility from animal challenge studies [24–25] suggested a potentially promising signal on PRO2000. However, these data alone were insufficient to conclude that 0.5% PRO2000 Gel protected against HIV infection. Subsequently, our trial's initial encouraging signal was superseded by the finding of no protection against HIV infection in the phase III MDP301 trial [21].

The HEC Placebo Gel [26] was found to be safe and had no demonstrable effect on HIV infection when compared to the No Gel arm, even after adjusting for baseline characteristics, including condom use. This addressed a key concern in microbicide research and makes HEC a suitable "universal" placebo for future microbicide gel trials [26].

The results of microbicide effectiveness trials are impacted by several inherent design and implementation challenges [27], including lengthy periods off-product (mainly due to pregnancy), adherence, other sexually transmitted infections, unprotected and unreported anal sex, use of intravaginal substances which may interfere with the study gel, and difficulties in applying study gel as prescribed.

The 6.1% of follow-up time that was off-product, mainly due to the 11.3% pregnancy rate, had little, if any, effect in the Placebo Gel comparisons. However, it had a small but important impact in the comparisons with the No Gel arm where the effect of PRO2000 changed from 33% (p=0.06) in the intent to treat analysis to 36% (p=0.04) in the per-protocol analysis, highlighting the potential impact of even relatively modest pregnancy rates on microbicide trial outcomes.

Adherence is a major challenge in microbicide trials [28]. In the recent Carraguard trial an applicator dye test revealed a much lower estimate of use compared to self-reported adherence (42.1% vs 96.1%) [13]. Our trial did not have such an objective measure of adherence because the dye test performed poorly on the applicator used in this trial [29]. To increase their chances of success, future microbicides trials will need to enrol a higher proportion of women who will maintain high adherence for both study gel and reliable contraception during the trial.

While interpretation of microbicide trial results can be complicated by the indirect effect of other sexually transmitted infections on HIV infection, this did not apply to this trial as the three study gels did not alter the risk of other sexually transmitted infections. Self-reported unprotected anal sex, intravaginal substance use and concerns about the one hour pre-sex insertion requirement were low and therefore unlikely to have had an impact on the trial result. However, these may have been under-reported, making it difficult to estimate their full potential impact on the study outcome.

BufferGel was found to be safe but did not alter the risk of HIV infection. This could be due to the differential effect of acidity and BufferGel on cell-free [30] compared to cell-associated viruses [31]. Moreover, the duration of action of BufferGel is brief [32]. It was posited that BufferGel would reduce HIV susceptibility by reducing the prevalence of bacterial vaginosis, as observed in a phase I trial [19]; however, no effect on bacterial vaginosis was observed in this trial, possibly due to lower gel use here compared to twice daily use in the phase I trial.

The pregnancy rates in all arms were similar. While HPTN 035 was not specifically designed to assess contraceptive efficacy due to the high background rates of effective contraception use in the study population, 0.5% PRO2000 Gel and BufferGel did not demonstrate a contraceptive effect in this study.

Viewed jointly, the HPTN 035 and MDP 301 trials suggest that 0.5% PRO2000 gel may have little or no effect on reducing a woman's risk of HIV infection. Hope is now being placed in the topical use of antiretroviral agents, such as tenofovir gel[33], as the next class of candidate microbicides and on new formulations to improve adherence, such as vaginal rings. Most recently, results from the CAPRISA 004 trial, showed that tenofovir gel had a protective effect of 39% against HIV [33]. The protective efficacy of tenofovir gel has demonstrated that microbicides can prevent HIV infection and could potentially alter the course of the HIV epidemic[33]. While efforts to bring tenofovir gel into widespread public health use are underway, there are limitations to using prescription-only medications, including their potential for drug resistance, and potential adverse effects on concomitant viral infections such as hepatitis B. For these reasons, the microbicide field should not abandon the search for a safe, single-use with sex, over-the-counter microbicide, as had been hoped for when we undertook this study of PRO2000 Gel and BufferGel.

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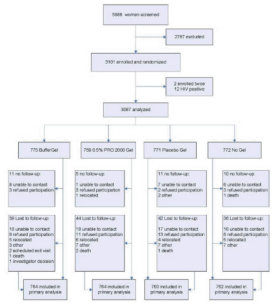

Figure 1.
Screening, randomization, and follow up of the study participants in the HPTN 035 trial.

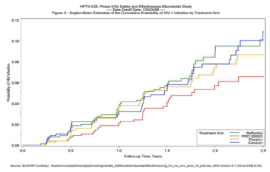

Figure 2.
Kaplan-Meier graph of HIV-1-free survival in each of the four study arms in the HPTN 035 trial

Table 1

Baseline demographic characteristics, sexual history and contraceptive use by enrolled study participants in the HPTN 035 trial

	BufferGel (n=775)	0.5% PRO2000 Gel (n=769)	Placebo Gel (n=771)	No Gel (n=772)
BASELINE CHARACTERISTICS				
Mean Age (years)	26.2	26.3	26.5	26.3
Age Range (years)	18–55	18–52	18–53	17–56
% that were between 17 and 24 years	45%	46%	44%	46%
Married	62%	62%	63%	63%
Own Income	43%	39%	42%	40%
At least some secondary school	63%	64%	62%	63%
SEXUAL BEHAVIOUR				
Mean # of vaginal sex acts in past 7 days	2.8	2.9	2.8	3.0
Condom use in the last sex act	67%	68%	69%	67%
Ever had anal sex	4%	4%	5%	5%
Anal sex in past 7 days	1%	<1%	1%	1%
For last sex act - douched before sex	24%	27%	24%	26%
For last sex act - douched after sex	30%	29%	26%	27%
CONTRACEPTION				
Hormonal contraception (Oral)	20%	20%	19%	20%
Hormonal contraception (Injectable)	49%	46%	49%	47%

Table 2

Retention, gel and condom use, and HIV incidence rates for each treatment arm in the HPTN 035 trial.

	0.5% PRO2000 Gel	BufferGel	Placebo Gel	No Gel	Overall
N	769	775	771	772	3087
Retention rate (%)	93.6	93.5	93.1	94.0	93.6
% condom use in last sex act	71.8	71.8	71.3	80.7	71.7
% gel use in last sex act*	80.5	81.5	81.3	--	81.1
% gel use in sex acts with no condoms*	68.2	69.3	69.9	--	69.1
Person-years of follow-up	1332.0	1303.8	1305.0	1317.5	5258.3
Number of HIV seroconversions	36	54	51	53	194
HIV incidence rates	2.7	4.1	3.9	4.0	3.7

*Calculated for the three gel arms only.

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Table 3

Retention, gel and condom use, and HIV incidence rates for each site in the HPTN 035 trial.

	Malawi	Malawi	South Africa	South Africa	United States	Zambia	Zimbabwe	Zimbabwe	All sites
	Blantyre	Lilongwe	Durban	Hlabisa	Philadelphia	Kamwala	Chitungwiza	Harare	
N	441	596	702	346	200	319	260	223	3087
Retention rate (%)	95.0	92.4	93.3	96.8	94.5	94.4	89.6	92.4	93.6
% condom use in last sex act*	71.8	62.9	74.8	74.6	72.8	67.1	76.7	79.6	71.7
% gel use in last sex act*	82.6	75.4	79.0	79.2	76.7	82.5	93.5	91.0	81.1
% gel use in sex acts with no condoms*	64.8	68.7	63.5	61.7	56.0	81.6	89.9	78.1	69.1
Person-years of follow-up	708.75	1128.25	1239.25	637.50	416.25	439.25	367.75	321.25	5258.25
Number of HIV seroconversions	26	16	57	58	2	18	9	8	194
HIV incidence rates [95% Confidence Interval]									
Overall	3.67 [2.4 – 5.4]	1.42 [0.8 – 2.3]	4.60 [3.5 – 6.0]	9.10 [6.9 – 11.8]	0.48 [0.1 – 1.7]	4.10 [2.4 – 6.5]	2.45 [1.1, 4.6]	2.49 [1.1 – 4.9]	3.69 [3.2 – 4.2]
0.5% PRO2000 Gel	1.09 [0.1 – 3.9]	0.70 [0.1 – 2.5]	5.43 [3.2 – 8.7]	6.18 [3.0 – 11.4]	0.00 [0.0 – 3.5]	3.73 [1.0 – 9.5]	0.00 [0.0 – 4.0]	1.20 [0.0 – 6.7]	2.70 [1.9 – 3.7]
BufferGel	3.45 [1.3 – 7.5]	2.10 [0.8 – 4.6]	4.23 [2.3 – 7.2]	11.24 [6.5 – 18.0]	0.00 [0.0 – 3.7]	4.60 [1.5 – 10.7]	3.17 [0.7, 9.3]	4.89 [1.3 – 12.5]	4.14 [3.1 – 5.4]
Placebo Gel	5.31 [2.4 –10.1]	1.09 [0.2 – 3.2]	4.92 [2.8 – 8.1]	7.74 [4.1 – 13.2]	1.92 [0.2 – 6.9]	4.49 [1.5 – 10.5]	3.33 [0.7, 9.7]	1.21 [0.0 – 6.8]	3.91 [2.9 – 5.1]
No Gel	4.97 [2.3 – 9.4]	1.78 [0.6 – 4.2]	3.82 [2.0 – 6.7]	11.50 [6.8 – 18.2]	0.00 [0.0 – 3.4]	3.57 [1.0 – 9.1]	3.29 [0.7, 9.6]	2.71 [0.3 – 9.8]	4.02 [3.0 – 5.3]
Hazard Ratios [95% Confidence Interval]									
PRO2000 vs No Gel	0.22 [0.05 – 1.00]	0.39 [0.08 –2.02]	1.43 [0.68 – 3.00]	0.53 [0.25 – 1.17]	- -	1.06 [0.26 – 4.27]	0.00 [0.0 – ∞]	0.45 [0.04 – 4.93]	0.67 [0.44 – 1.02]
PRO2000 vs Placebo	0.21 [0.04 – 0.96]	0.63 [0.11 –3.77]	1.11 [0.55 – 2.22]	0.81 [0.35 – 1.86]	0.00 [0.0 - ∞]	0.83 [0.22 – 3.11]	0.00 [0.0 – ∞]	0.96 [0.06 – 15.62]	0.70 [0.46 – 1.08]
BufferGel vs No Gel	0.69 [0.25 – 1.96]	1.18 [0.36 –3.88]	1.10 [0.50 – 2.43]	1.04 [0.53 – 2.05]	- -	1.30 [0.35 – 4.88]	0.95 [0.19, 4.75]	1.82 [0.33 – 10.02]	1.05 [0.72 – 1.55]
BufferGel vs Placebo	0.65 [0.23 – 1.84]	1.91 [0.48 –7.66]	0.86 [0.41 – 1.81]	1.57 [0.74 – 3.31]	0.00 [0.0 - ∞]	1.02 [0.29 – 3.55]	0.95 [0.19, 4.75]	4.00 [0.45 – 35.91]	1.10 [0.75 – 1.62]

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* Calculated for the three gel arms only

Table 4

Main safety outcomes by study arm in the HPTN 035 trial

	BufferGel (n=775)	0.5% PRO2000 Gel (n=769)	Placebo Gel (n=771)	No Gel (n=772)	Overall (n=3087)
Participants with adverse events					
- *Deaths*	2 (0.3%)	2 (0.3%)	1 (0.1%)	2 (0.3%)	7 (0.2%)
- *Hospitalizations*	37 (5.6%)	30 (3.9%)	30 (3.9%)	33 (4.3%)	130 (4.2%)
- *Reproductive system events*	412 (53%)	393 (51%)	387 (50%)	375 (49%)	1567 (51%)
- Vaginal discharge	229 (30%)	221 (29%)	202 (26%)	223 (29%)	875 (28%)
- Vulvovaginal pruritus	115 (15%)	97 (13%)	105 (14%)	90 (12%)	407 (13%)
- Metrorrhagia	53 (7%)	55 (7%)[1]	36 (5%)	51 (7%)	195 (6%)
- Cervix haemorrhage uterine	39 (5%)	37 (5%)	36 (5%)	40 (5%)	152 (5%)
- Menorrhagia	34 (4%)	31 (4%)	29 (4%)	35 (5%)	129 (4%)
- *Adverse event categories*					
- Genital infection events	563 (73%)	577 (75%)	557 (72%)	561 (73%)	2258 (73%)
- Genital irritation events	317 (41%)	308 (40%)	302 (39%)	281 (36%)	1208 (39%)
- Genital bleeding abnormality events	140 (18%)	135 (18%)	116 (15%)	143 (19%)	534 (17%)
- Urinary tract events	126 (16%)	132 (17%)	109 (14%)	106 (14%)	473 (15%)
- Genital pain events	79 (10%)	78 (10%)	73 (9%)	65 (8%)	295 (10%)
- Genital lesion events	78 (10%)[2]	63 (8%)	53 (7%)	70 (9%)	264 (9%)
- Intermenstrual bleeding events	56 (7%)	63 (8%)[3]	39 (5%)	54 (7%)	212 (7%)
- Pregnancy-related events	41 (5%)	39 (5%)	30 (4%)	40 (5%)	150 (5%)
- *Coagulation abnormalities*	2 (0.3%)	4 (0.5%)	2 (0.3%)	2 (0.3%)	10 (0.3%)
Systemic liver, renal and coagulation abnormalities during Phase II (/ participants in Phase II)	1/195 (0.5%)	2/201 (1.0%)	1/201 (0.5%)	1/196 (0.5%)	5/793 (0.6%)
Pelvic Exam Findings (events per 100 person-years)					
- Deep epithelial disruption	1.1	1.7	1.5	1.9	1.5
- Abnormal vaginal discharge	77.4	78.2	73.7	73.0	75.6
- Any blood-related finding	17.4	16.1	15.3	14.9	15.9
- Blood from cervical os	10.7	9.8	10.5	8.5	9.8
- Erythema	6.3 [4]	7.9	7.4	10.9	8.2

	BufferGel (n=775)	0.5% PRO2000 Gel (n=769)	Placebo Gel (n=771)	No Gel (n=772)	Overall (n=3087)
- Petechia	5.0	3.9	4.6	5.2	4.7
- Blood-tinged discharge	4.0	2.4	2.6	3.8	3.2
- Blood in vagina – no identified source	2.5	3.5 [5]	1.8	2.3	2.5
- Ulceration	2.6	1.9	1.9	3.5	2.5
Pregnancy rate (per 100 person-years)	11.2	12.0	9.9	12.2	11.3
Proportion of pregnancies resulting in live births	70%	68%	71%	68%	69%

[1] p=0.04 vs. Placebo;

[2] p=0.02 vs. Placebo;

[3] p=0.01 vs. Placebo;

[4] p=0.02 vs. No Gel;

[5] p<0.01 vs. Placebo