Safety and effectiveness of BufferGel and 0.5% PRO2000 gel for the prevention of HIV infection in women

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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 the USA were instructed to apply study gel up to 1 h 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 = 0.7, $P = 0.10$) and the no gel arm (hazard ratio = 0.67, $P = 0.06$). HIV incidence rates were similar in the BufferGel and both placebo gel (hazard ratio = 1.10, $P = 0.63$) and no gel control arms (hazard ratio = 1.05, $P = 0.78$). HIV incidence was similar in the placebo gel and no gel arms (hazard ratio = 0.97, $P = 0.89$).

Conclusion: The 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 Microbicide Development Programme (MDP) 301 trial

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have confirmed that 0.5% PRO2000 gel has little or no protective effect. BufferGel did not alter the risk of HIV infection. Both products were well tolerated.

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Introduction

Globally, most new HIV infections are acquired through heterosexual contact [1]. Although 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 (Endo Pharmaceuticals Solutions Inc. (formerly Indevus Pharmaceuticals Inc.), Lexington, Massachusetts, USA) and BufferGel (ReProtect Inc., Baltimore, Maryland, USA) 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 sulfate [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 owing to futility. Two N-9 studies [6,10] and a second cellulose sulfate trial [12] showed some increase, although 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 HIV Prevention Trials Network (HPTN) 035 trial, the Microbicide Development Programme (MDP) initiated a large phase III trial of 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 and 0.5% PRO2000 gel 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, PRO2000 gel, and hydroxyethylcellulose (HEC) placebo gel on HIV acquisition.

Methods

Study design and population

HPTN 035 was a phase II/IIb, four-arm, multisite, 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 nonpregnant women, at least 18 years of age, who were sexually active, defined as having had vaginal intercourse at least once in the past 3 months were eligible for the study. The exclusion criteria included a history of adverse reactions to latex, use of nontherapeutic 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 2 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 the four study arms: BufferGel, 0.5% PRO2000 gel, and two comparator arms comprising 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 (six) 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 three-digit code that was labeled on the product packaging. In blocks of size 24, each unique three-digit code was used twice. Each random sequence was determined through generation of uniform random variates in a computer program (SAS; Statistical Analysis System Institute Inc., Cary, North Carolina, USA) and envelope materials were created and sealed at the

Statistical and Data Management Center (SDMC). The three gel groups were double-blinded, whereas 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 three-digit code of the gel product to be prescribed. All persons associated with the study were masked to the product identity of the three-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 up to 1 h before each episode of vaginal intercourse.

The first 799 women enrolled comprised a 'lead in' phase II safety study that included intensive safety assessments such as hematological, coagulation, hepatic, and renal function tests and monthly pelvic examinations for 3 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 pretest, risk reduction, and posttest counseling, condoms and sexually transmitted infection 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 12–30 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 7 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 examination (speculum and/or colposcopic) as lesions penetrating into and exposing the subepithelial tissue and possibly blood vessels [22]. Additional safety outcomes included adverse genital signs and symptoms, as well as hematological, 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 OraQuick ADVANCE HIV-1/2 antibody test (Orasure Technologies, Bethlehem, Pennsylvania, 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, California, 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 2 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-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 enroll 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 the 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 enroll 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: if the estimated effectiveness of a candidate microbicide is less than 15.3%, exclude the candidate microbicide from further testing for HIV prevention; 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; 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 if the estimated effectiveness is greater than 43.6%, consider the product

effective with the strength of evidence of at least 1.5 phase III studies. Additional details regarding the statistical rationale for the study design and sample size have been published elsewhere [23].

Owing 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 were 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 behaviors 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 person-years (6.1%) 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 of 1304) in the BufferGel arm, 2.7 (36 of 1332) in the 0.5% PRO2000 gel arm, 3.9 (51 of 1305) in the placebo

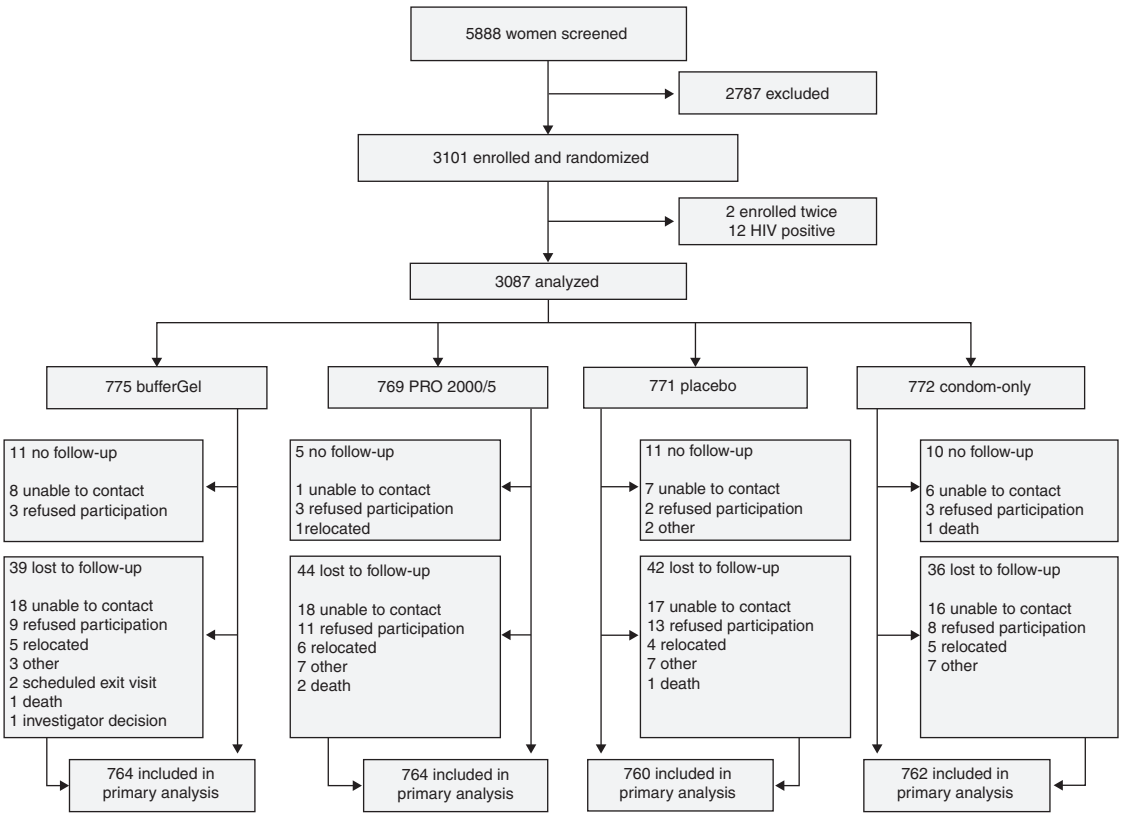

Fig. 1. Screening, randomization, and follow-up of the study participants in the HIV Prevention Trials Network (HPTN) 035 trial.

Table 1. Baseline demographic characteristics, sexual history, and contraceptive use by enrolled study participants in the HIV Prevention Trials Network (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
Percentage 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 behavior				
Mean number 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

gel arm, and 4.0 (53 of 1318) in the no gel arm (Table 2). The hazard ratio of HIV incidence rates in 0.5% PRO2000 gel arm compared with the placebo gel arm was 0.70 ($P=0.10$) and with 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 seven of the eight sites (Table 3). In the per-protocol analysis that 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 with the placebo gel arm and 0.64 ($P=0.04$) compared with the no gel arm. There was no difference in HIV incidence between the BufferGel and both control arms (Table 3).

The hazard ratio of the HIV incidence rates in the placebo gel arm compared with 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, 95% confidence interval (CI) 2.9–5.1] and in the no gel arm (4.0 per 100 person-years, 95% CI 3.0–5.3). There was little change in this hazard ratio after adjusting for baseline sexual behavior and participant characteristics.

In order to assess whether self-reported PRO2000 gel use was associated with a lower rate of HIV, a subgroup 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, whereas 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 of 592) in the 0.5% PRO2000 gel group and 3.3 per 100 person-years (19 of 568) in the placebo gel group (hazard ratio = 1.04, 95% CI 0.55–2.00). However, the HIV incidence rate among high gel users was 2.4 per 100 person-years (18 of 740) in the 0.5% PRO2000 gel group and 4.3 per 100 person-years (32 of 738) in the placebo gel group (hazard ratio = 0.55, 95% CI 0.31–0.98). 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 (three of 299) in the 0.5% PRO2000 gel group and 4.6 per 100 person-years (15 of 324) in the placebo gel group (hazard ratio = 0.21, 95% CI 0.06–0.73).

After adjusting for multiple comparisons, there were no statistically significant differences in systemic and local adverse events among the four study arms in the intent-to-treat and per-protocol analyses. Overall, the incidence

Table 2. Retention, gel and condom use, and HIV incidence rates for each treatment arm in the HIV Prevention Trials Network (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
Percentage condom use in last sex act	71.8	71.8	71.3	80.7	71.7
Percentage gel use in last sex act[a]	80.5	81.5	81.3	–	81.1
Percentage gel use in sex acts with no condoms[a]	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

[a]Calculated for the three gel arms only.

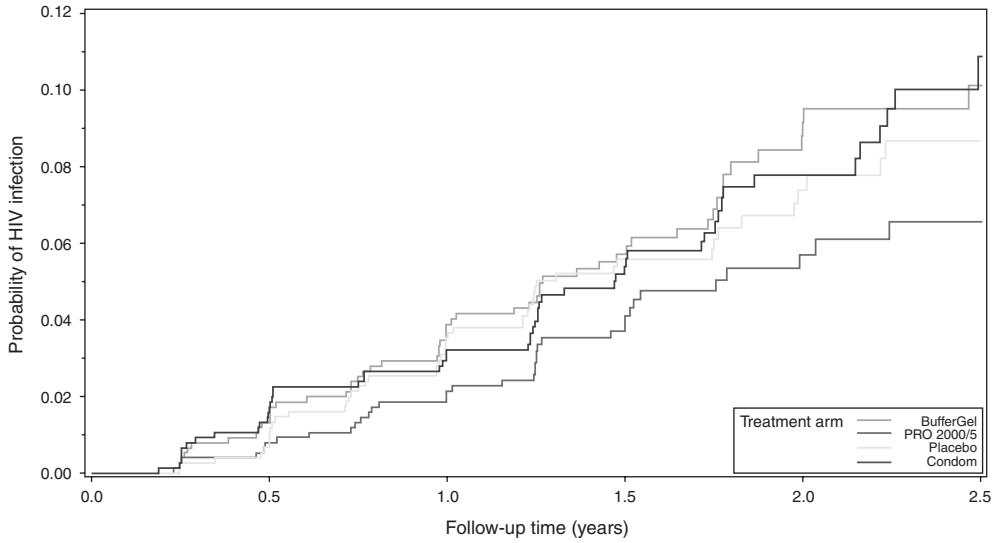

Fig. 2. Kaplan–Meier graph of HIV-1-free survival in each of the four study arms in the HIV Prevention Trials Network (HPTN) 035 trial.

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 with the placebo gel arm (3.5 vs. 1.8, $P < 0.01$), but not compared with 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 (95% CI 7.0–11.4) at the combined Harare and Chitungwiza sites in Zimbabwe and the highest was 16.5 per 100 person-years (95% 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 well tolerated and, compared with 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 with 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 well tolerated and had no demonstrable effect on HIV infection when compared with 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 that 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 in which 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

Table 3. Retention, gel and condom use, and HIV incidence rates for each site in the HIV Prevention Trials Network (HPTN) 035 trial.

	Malawi Blantyre	Malawi Lilongwe	South Africa Durban	South Africa Hlabisa	USA Philadelphia	Zambia Kamwala	Zimbabwe Chitungwiza	Zimbabwe Harare	All sites
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
Percentage condom use in last sex act[a]	71.8	62.9	74.8	74.6	72.8	67.1	76.7	79.6	71.7
Percentage gel use in last sex act[a]	82.6	75.4	79.0	79.2	76.7	82.5	93.5	91.0	81.1
Percentage gel use in sex acts with no condoms[a]	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 gel	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)

[a]Calculated for the three gel arms only.

Table 4. Main safety outcomes by study arm in the HIV Prevention Trials Network (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%)*	36 (5%)	51 (7%)	195 (6%)
Cervix hemorrhage 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%)**	63 (8%)	53 (7%)	70 (9%)	264 (9%)
Intermenstrual bleeding events	56 (7%)	63 (8%)***	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 (or 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 examination 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†	7.9	7.4	10.9	8.2
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‡	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%

*$P=0.04$ vs. placebo.
**$P=0.02$ vs. placebo.
***$P=0.01$ vs. placebo.
†$P=0.02$ vs. no gel.
‡$P<0.01$ vs. placebo.

Carraguard trial, an applicator dye test revealed a much lower estimate of use compared with 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 microbicide trials will need to enroll a higher proportion of women who will maintain high adherence for both study gel and reliable contraception during the trial.

Although 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 1-h presex 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 well tolerated, 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 with 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 with twice-daily use in the phase I trial.

The pregnancy rates in all arms were similar. Although 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, like tenofovir gel [33], as the next class of candidate microbicides and on new formulations to improve adherence, like vaginal rings. Most recently, results from the Centre for the AIDS Programme of Research in South Africa (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 [34]. Although 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 well tolerated, 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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The study was conceptualized and designed by S.S.A.K., B.A.R., G.R., I.F.H., Z.M.C., T.T., L.M., A.C., A.P., T.R.M., B.M., S.L.H., L.S.-T. Data was gathered by I.F.H., Z.M.C., T.T., M.K., L.M., B.M. Data was analyzed and interpreted by S.S.A.K., I.F.H., Z.M.C., T.T., M.K., L.M., A.C., E.P.-M., B.M. S.S.A.K. 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. S.S.A.K. took responsibility for writing the article and all co-authors contributed to critical revision of the article.

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