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Research article

Utility of colposcopy in a phase 2 portion of a microbicide clinical trial of BufferGel and 0.5% PRO 2000 Gel

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Abstract

Background: The majority of new HIV infections are acquired through heterosexual transmission. There is urgent need for prevention methods to compliment behavior change and condom use. Topical microbicide represent a potential strategy for reduction of HIV transmission in women.

Methods: Monthly Colposcopy evaluations were performed during pelvic examinations among 299 women enrolled in the Phase 2 portion of HPTN 035 study at four sites (1 in USA, 3 in Southern Africa). This was a phase 2/2b, multisite, randomized, and controlled clinical trial with four arms: BufferGel, 0.5% PRO2000 Gel, placebo gel and no gel. At two of the sites, pelvic examinations were conducted by the use of naked eye without colposcopy.

Results: A colposcopy finding of any kind was detected in 48% of participants at baseline compared to 40% at 3 months ($p=0.04$). The lower rates were also observed in vaginal discharge (22% at baseline, 16% at 3 months, $p=0.06$), erythema (15% at baseline, 8% at 3 months, $p=0.004$). The trend towards significance at $p=0.05$ disappear when utilizing stringent statistical significance levels. A pelvic finding of any kind was detected in 71% of colposcopy participants compared to 41% of participants who had naked eye examination only conducted at two sites that performed both colposcopy and naked eye without colposcopy. Use of colposcopy yielded significantly higher rates of participants with deep epithelial disruption, erythema and ecchymosis. We observed no cases of incident Chlamydia, Gonorrhea, or Syphilis during the three month follow up. There were 2 cases of incident HIV during 3-month study period neither of which was associated with any abnormal colposcopy evaluation findings.

Conclusion: No safety signals were observed in the 4 study arms, allowing seamless transition from phase 2 to 2b. Colposcopy utility in microbicide clinical trials has minimal value given high rates of background noise findings of no relevant clinical significance.

Keywords: colposcopic evaluations; topical microbicides; genital epithelium; phase 2; HIV; investigational product.

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Background

The AIDS epidemic continues to spread relentlessly, with an estimate of 2.7 million (2.4 to 2.9 million) new HIV infections in 2010 [1]. The majority of these infections are acquired through heterosexual transmission.

Condoms when used correctly and consistently can provide high level of protection against HIV and other sexually transmitted infections (STIs). However, negotiating male condom use is not always feasible for many women, and use of female condom requires the cooperation of the male partner [2,3]. Advances in developing an effective HIV vaccine have been slow with both setbacks [4] and interesting modest results [5]. While there is an ongoing need to scale up known effective prevention measures, there remains a clear need for new technologies to prevent sexual transmission of HIV, particularly for acceptable, affordable, female-controlled prevention methods.

Topical microbicides are chemicals applied to the vagina or rectal mucosa to prevent HIV and STI transmission. The development of vaginal microbicides experienced setbacks when earlier trials with nonoxynol-9 (N-9) formulations showed paradoxically increased risk of HIV acquisition. This increased risk of HIV transmission has been ascribed to the observation that N-9 causes mucosal erosions and ulceration, which can create potential entry portals for HIV [6,7]. In the light of N-9 results, evaluation of integrity of genital epithelium has become an essential step in the development of topical microbicides.

Most drug regulatory authorities require a stringent pathway for microbicide development including preclinical testing that traditionally evaluates local safety in a rabbit vaginal irritation model [8], but this has not always correlated well with human genital tract epithelium toxicity events as seen in the N-9 and cellulose sulphate clinical results [7,9]. The utility

of colposcopy which allows low magnification (4 to 10X) of genital tract was introduced by researchers to assist in identifying potential microbicide-induced epithelium injuries allowing stoppage of testing potential microbicide candidate in phases 1 or 2.

BufferGel contains carbopol 974 P, a gelling agent that provides buffering action and enhances body's natural defences by maintaining a low vaginal pH, and 0.5% PRO 2000 Gel is an HIV entry inhibitor. Both BufferGel and 0.5% PRO 2000 Gel underwent phase 1 clinical trials in several countries showing no safety concerns and good acceptability [10,11].

HPTN 035 evaluated the safety and effectiveness of BufferGel and 0.5% PRO 2000 Gel, and this trial showed that 0.5% PRO 2000 reduced the incidence of HIV by a modest 30% ($p = 0.10$) [12], but a subsequent phase 3 MDP 301 trial found PRO 2000 not to be protective against HIV infection [13]. We report on the findings of utility of colposcopic examinations performed during HPTN 035 study, to assess the impact of the two candidate microbicides on the genital epithelium and mucosa.

Methods

HPTN 035 study was as a phase 2/2b, multisite, randomized, controlled clinical trial with four arms: BufferGel, 0.5% PRO 2000 Gel, placebo gel and no gel. A total of 3099 sexually active HIV uninfected women from seven sites in six countries were assigned to the four study treatment groups in a 1:1:1:1 ratio. The three study gel arms were double-blinded; participants assigned to these arms were instructed to apply study gel intravaginally up to one hour before each sex act [12].

The study (NCT 0074425) was approved by each site's Institutional Review Board(s) and drug regulatory authorities. Women were eligible for enrolment if they were sexually active, in good general health, aged 18 or older, not infected with HIV and STIs, not pregnant, and willing and able to provide informed consent for study participation. Women who were diagnosed at screening with an STI received treatment and were required to be symptom free at enrolment. Pap smears were performed at screening and the last quarterly visit with treatment of abnormal pap smears as per local standard of care at selected sites that had the facilities. Sites without facilities to perform and treat women with abnormal pap smears did not participate in the colposcopic evaluations phase 2 portion of HPTN 035.

The phase 2 portion of HPTN 035 was conducted as a ''lead-in'' to the main phase 2b trial. It included the first 793 women enrolled and involved pelvic examinations and intensive laboratory tests performed at screening and during each of the first three months of follow-up. Phase 2 participants completed their three monthly follow-up assessments for safety endpoints and data were analyzed and presented to DSMB in line with predefined stopping rules in the protocol. Accrual from the phase 2 portion of the study into phase 2b continued uninterrupted at study operation sites while also allowing a thorough review of safety data among the smaller target population prior to full-scale exposure of study participants to investigational study products. Monthly colposcopic evaluations were performed during pelvic examinations among a sub-set of 299 women (100 in Philadelphia, USA; 82 in Durban, South Africa; 62 in Lilongwe, Malawi; and 55 in Harare, Zimbabwe) in the Phase 2 portion. The colposcopy evaluations were performed at sites that had a colposcopist with experience in evaluation of female genital tract epithelium disruption. Two of the five sites conducting colposcopy (Durban and Lilongwe) also enrolled participants into the phase 2 study with pelvic exams conducted by the use of naked eye without colposcopy.

Colposcopic evaluations were performed per the CONRAD/WHO [14]. Each participant was examined in a modified lithotomy position. The external genitalia were examined first, followed by insertion of a bi-valve speculum and examination of the cervix and vagina walls. For each anatomical area, naked eye inspection was performed first, followed by colposcopic evaluation (4 to 10 $\times$ magnification). If required for complete visualization, mucus and/or cellular debris were removed from the cervix and vaginal walls with saline (via lavage or using large saline moistened swabs to avoid epithelial trauma). All findings (normal and abnormal) were recorded using terminology based on the CONRAD/WHO manual [14]. For each exam, a determination of whether observed findings involved disruption of blood vessels and/or epithelial tissue was also recorded. Photographs of abnormal epithelium were captured and stored at participating sites.

All phase 2 participants had wet mounts performed for bacterial vaginosis (BV), candidiasis, trichomoniasis as well as complete blood count, hepatic and renal function and coagulation testing as part of safety monitoring. BV diagnosis was by Nugent score. Women with asymptomatic candida and/or asymptomatic BV were allowed entry into the study without treatment.

Urine testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (GC) with Becton Dickson Probe Tec ET strand displacement assay was performed at study entry, and when clinically indicated. Serologic testing for syphilis with rapid plasma reagin screening test followed by confirmatory microhaemagglutinin assay for treponema pallidum or treponema pallidum haemagglutination assay was performed at study entry, annually and at study exit. Plasma stored at study entry and study exit was tested for genital herpes (HSV-2 antibody) with focus enzyme-linked immunosorbent assay.

In addition to naked eye inspection and colposcopic examination, clinically observed genital ulcers were swabbed and tested with multiple PCR for chancroid, HSV-2 and syphilis at the Network Central Lab (Pittsburgh, USA).

Statistical methods

Descriptive statistics were used to summarize proportions of participants in each arm with baseline characteristics and follow-up pelvic exam findings. Chi-squared or Fisher's exact tests were used to assess differences between sites or between cohorts (colposcopy vs. naked eye). Changes in the prevalence of findings over time were assessed by McNemar's test, and differences in changes over time between two study treatment arms were assessed by Fisher's exact test, according to the methods of Levin and Serlin

[15], and confidence intervals, according to Newcombe [16]. To account for the multiple testing among the pelvic exam findings, *p*-values were adjusted across findings using the false discovery rate method [17]. All analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

The phase 2 portion enrolled 793 participants among whom 299 women underwent colposcopic evaluations, 75 were assigned to BufferGel, 75 to 0.5% PRO 2000 Gel, 76 to placebo gel and 73 to no gel. Colposcopy was performed on all women at screening and was performed at 94% of the 897 expected monthly follow-up visits. Six (2%) participants had no colposcopy performed during the three months of follow-up, yielding a total of 293 women with at least one follow-up colposcopic evaluation. Eighty-eight percent of women underwent colposcopy at all three follow-up visits.

Baseline characteristics and follow-up findings

The 299 participants had similar characteristics at baseline across the four study arms (Table 1a). The mean age was 30.2 years (8.7 SD), with 55% of the women aged between 18 and 29 years (varying from 48% in the No Gel arm to 69% in the BufferGel arm, $p = 0.03$), and mean parity of 2.8 ± 2.1 SD. The majority (74%) reported that they had some secondary education or more, varying from 6% in Lilongwe to between 86 and 99% in Durban, Harare and Philadelphia (Table 1b) and 84% received financial support from a husband or partner. Forty-nine percent were married (varying from 15 to 23% in Philadelphia and Durban to 94 to 98% in Harare and Lilongwe) and 51% had a regular sexual partner. The husband/partner mean age was 4.1 years older than the mean age of participants. At baseline the median vaginal pH was 4.7. Thirty-five percent had BV, syphilis (4%), gonorrhoea (0%), Chlamydia (3%), trichomoniasis (5%) and Candida (8%).

Table 1a. Baseline demographic characteristics, sexually transmitted infections (STI), and sexual behaviour and vaginal practices of study participants in the colposcopy cohort of the HPTN 035 trial (all sites)

	BufferGel (n = 75)	PRO 2000 Gel (n = 75)	Placebo Gel (n = 76)	No Gel (n = 73)
Baseline characteristics				
Mean age, years (SD)	28.6 (8.7)	30.5 (8.8)	31.6 (9.4)	29.9 (7.6)
% Aged 18 to 29 years[a]	69%	53%	49%	48%
% Some secondary education or more	75%	71%	75%	77%
% Own Income	53%	48%	57%	45%
% Financial support from husband/partner	84%	88%	80%	85%
% Married	48%	52%	43%	52%
% Regular partner	52%	48%	57%	47%
Mean partner age, years (SD)	32.8 (9.3)	35.1 (9.8)	34.6 (9.3)	34.7 (9.3)
Mean parity (SD)[b]	2.4 (2.1)	3.1 (2.1)	2.8 (2.1)	2.8 (2.1)
Sexually transmitted infections				
% Bacterial vaginosis (BV) by microscopy[c]	47%	37%	26%	30%
% BV by Amsel's criteria	21%	23%	14%	19%
Median vaginal pH	4.7	4.7	4.7	4.7
% with homogeneous vaginal discharge	25%	19%	25%	15%
% Syphilis	3%	5%	4%	3%
% Gonorrhoea	0%	0%	0%	0%
% Chlamydia	3%	3%	1%	4%
% Trichomonas vaginalis (TV)	4%	7%	5%	3%
% Candida[d]	1%	11%	12%	7%
Sexual behaviour and vaginal practices				
Mean (SD) # of vaginal sex acts in past seven days	2.5 (2.6)	2.5 (1.8)	2.4 (2.0)	2.5 (2.3)
% Condom use in the last sex act	53%	63%	66%	60%
% Inserted anything in vagina, past month	55%	56%	61%	55%
% Inserted water, past month	17%	12%	18%	19%
% Inserted water with vinegar (douching), past month	12%	7%	11%	8%
% Inserted water with soap, past month	7%	9%	7%	3%
% Inserted paper, cloth, cotton, etc., past month[c]	5%	12%	13%	3%
% Inserted tampons, past month	24%	17%	20%	15%
% Inserted fingers, past month	15%	16%	21%	21%

[a]Significant difference among arms at the $p = 0.05$ level; [b]Based on n = 199 (Data on baseline parity not collected in Philadelphia); [c]$p = 0.06$; [d]$p = 0.07$.

Table 1b. Baseline demographic characteristics, sexually transmitted infections (STI), and sexual behaviour and vaginal practices of study participants in the colposcopy cohort of the HPTN 035 trial (per site)

	Durban (n = 83)	Lilongwe (n = 65)	Philadelphia (n = 100)	Zimbabwe (n = 51)
Baseline characteristics				
Mean age, years (SD)	24.4 (4.5)	33.5 (9.3)	33.5 (9.6)	28.5 (5.4)
% Aged 18 to 29 years	82%	37%	43%	57%
% Some secondary education or more	86%	6%	99%	94%
% Own income	45%	48%	70%	27%
% Financial support from husband/partner	83%	95%	71%	100%
% Married	23%	98%	15%	94%
% Regular partner	77%	2%	84%	6%
Mean partner age, years (SD)	29.1 (4.8)	37.1 (10.6)	38.0 (11.2)	33.7 (6.1)
Mean parity (SD)[a]	1.5 (1.1)	4.7 (2.3)	–	2.3 (1.1)
Sexually transmitted infections				
% Bacterial vaginosis (BV) by microscopy	22%	38%	45%	35%
% BV by Amsel's criteria	19%	12%	17%	33%
Median vaginal pH	4.8	4.8	4.6	4.8
% with homogeneous vaginal discharge	37%	11%	16%	18%
% Syphilis	4%	3%	5%	2%
% Gonorrhoea	0%	0%	0%	0%
% Chlamydia	5%	0%	2%	4%
% *Trichomonas vaginalis* (TV)	2%	12%	1%	6%
% Candida	12%	5%	4%	12%
Sexual behaviour and vaginal practices				
Mean (SD) # of vaginal sex acts in past seven days	1.8 (1.4)	2.5 (1.7)	2.3 (2.5)	3.8 (2.6)
% Condom use in the last sex act	65%	51%	59%	69%
% Inserted anything in vagina, past month	19%	69%	83%	49%
% Inserted water, past month	11%	15%	15%	31%
% Inserted water with vinegar (douching), past month	0%	0%	28%	0%
% Inserted water with soap, past month	4%	14%	7%	0%
% Inserted paper, cloth, cotton, etc., past month	2%	12%	6%	18%
% Inserted tampons, past month	4%	0%	52%	4%
% Inserted fingers, past month	11%	12%	26%	22%

[a]Based on n = 199 (data on baseline parity not collected in Philadelphia).

There were no statistically significant differences in genital tract infections across study arms at baseline. However, there were differences in BV prevalence at baseline, which varied from 26% in the Placebo Gel arm to 47% in the BufferGel arm ($p = 0.06$), and candida, which varied from 1% in the BufferGel arm to 12% in the Placebo Gel arm. No statistically significant differences were observed in BV, candidiasis and trichomoniasis between the four study arms throughout the three-month study period.

Colposcopic findings

Colposcopy examinations were conducted every month for the three-month study period on 279 of the 299 colposcopy participants. The prevalence of colposcopy pelvic exam findings at the baseline and month 3 time points is presented for these 279 participants in Table 2 by study treatment arm. In general, the proportion of findings at the month 3 visit was lower than at baseline. Overall, a colposcopic finding of any kind was detected in 48% of participants at baseline compared to 40% at the month 3 visit ($p = 0.04$). The rate of erythema reduced significantly from baseline (15%) to month 3 (8%, $p = 0.004$). There were also trends towards lower rates of abnormal vaginal discharge (16%, from 22% at baseline, $p = 0.06$) and petechia (8% from 12% at baseline, $p = 0.09$). On the other hand, increased rates were observed at month 3 with respect to blood from the cervical os (3%, from 1% at baseline, $p = 0.03$) and blood-tinged discharge (2%, from 0% at baseline, $p = 0.06$). In all cases, statistical significance at the $p = 0.05$ level and the trend towards significance disappear when utilizing the more stringent statistical significance levels provided by the adjustment made for comparing multiple pelvic exam findings.

Within each study treatment arm, we also observed changes in the rates over time for many of the individual

Table 2. Prevalence of main pelvic exam findings at baseline and at month 3 of follow-up among participants in the colposcopy cohort of the HPTN 035 trial

	BufferGel		PRO 2000 Gel		Placebo Gel		No Gel		Overall	
	Baseline	Month 3	Baseline	Month 3	Baseline	Month 3	Baseline	Month 3	Baseline	Month 3
# **Participants with colposcopy**	69	69	73	73	69	69	68	68	279	279
% Participants with any pelvic exam finding	55%	54%	44%	40%	48%	30%	46%	37%	48%	40%
% Participants with >1 pelvic exam finding	23%	12%	12%	12%	17%	10%	13%	9%	16%	11%
% Participants with:										
Disrupted epithelium										
Blood from cervical os	3%	3%	0%	3%	0%	1%	0%	4%	1%	3%
Abrasion	1%	1%	1%	3%	0%	0%	4%	0%	2%	1%
Deep epithelial disruption	3%	0%	0%	0%	0%	1%	3%	1%	1%	1%
Bleeding from site of epithelial disruption	1%	0%	0%	0%	0%	1%	3%	0%	1%	0%
Intact epithelium										
Abnormal vaginal discharge	28%	22%	19%	16%	28%	14%	15%	13%	22%	16%
Erythema	13%	7%	12%	10%	17%	6%	16%	7%	15%	8%
Petechia	13%	9%	12%	8%	10%	4%	12%	10%	12%	8%
Blood-tinged discharge	0%	7%	0%	1%	1%	0%	0%	0%	1%	2%
Abnormal cervical discharge	9%	1%	1%	3%	1%	3%	4%	0%	4%	2%
Ecchymosis	3%	1%	1%	0%	3%	1%	3%	4%	3%	2%
Blood in vagina-no identified source	0%	1%	0%	3%	0%	0%	0%	0%	0%	1%

Note: The following pelvic exam findings were also observed through three months of follow-up at an overall rate of 1% or less: ulceration, enlarged/tender inguinal lymph nodes, warts, cervical motion tenderness, abnormal cysts, laceration, peeling, and vesicles.
For each finding, treatment group differences in the rate of change of each finding from baseline to the month 3 visit were calculated and tested for the four pairwise comparisons of each active product (BufferGel, PRO 2000 Gel) with each control arm (Placebo Gel, No Gel). No significant differences were detected in any of the pairwise comparisons of the rates of change. For example, among the proportions of participants with any pelvic exam finding, the rate of change from baseline to month 3 for BufferGel, −1.4%, and for Placebo Gel, −17.4%, produce a difference of 15.9%, which has a *p*-value of 0.16 for the Fisher's Exact test of the cells indicating change over time from 2x2 table for each treatment arm. See methods of Levin and Serlin.

pelvic exam findings reported in Table 2. Our analysis addressed whether these changes over time occurred at different rates among pairwise comparisons of active gel arms (BufferGel, PRO 2000) compared to the control arms (Placebo Gel, No Gel). For each finding, treatment group differences in the rate of change of each finding from baseline to the month 3 visit were calculated and tested for the four pairwise comparisons of each active product with each control arm. No significant differences were detected in any of the pairwise comparisons of the rate of change. For example, among the proportions of participants with any pelvic exam finding, the rate of change from baseline to month 3 for BufferGel, −1.4%, and for Placebo Gel, −17.4%, produce a difference of 15.9% [95% C.I. = (3.9%, 36.4%), $p = 0.16$].

No statistically significant differences were observed in BV, candidiasis and trichomoniasis between the four study arms throughout the three-month study period. We observed no cases of incident Chlamydia, Gonorrhoea or Syphilis during the three-month follow-up. There were 2 cases of incident HIV infections during the three-month study period, neither of which was associated with any abnormal colposcopy evaluation findings.

There were 40 participants with incident Candidiasis, all not associated with any pelvic exam findings. With small numbers, erythema was detected in two of the five (40%) cases of incident Trichomoniasis cases compared to 3% of participants without new Trichomoniasis ($p = 0.01$). Lastly, bleeding from the site of epithelial disruption was observed in 4 of the 78 participants (5%) with incident BV during the three-month study period, compared to 1% without new BV ($p = 0.05$).

Across the two sites (Durban and Lilongwe) that performed colposcopy and naked eye without colposcopy pelvic exams with colposcopy generally detected a significantly higher proportion of participants with observed findings (Table 3). A pelvic exam finding of any kind was detected during the three-month follow-up period in 71% of colposcopy participants compared to 41% of non-colposcopy participants ($p = < 0.0001$). With respect to individual pelvic findings,

Table 3. Main pelvic exam findings of colposcopy participants versus naked eye inspection participants during phase 2 portion of the HPTN 035 trial, Durban and Lilongwe sites only

	Durban		Lilongwe		Overall		*p*-Value for overall comparison
	Colposcopy	Naked eye	Colposcopy	Naked eye	Colposcopy	Naked eye	
# **Participants**	82	109	62	152	144	261	–
% Participants with any pelvic exam finding	73%	66%	68%	23%	71%	41%	<0.0001
% Participants with >1 pelvic exam finding	13%	26%	34%	2%	22%	13%	0.01
% Participants with:							
Disrupted epithelium							
Blood from cervical os	5%	9%	0%	5%	3%	7%	0.10
Abrasion	2%	2%	13%	3%	7%	2%	0.02
Deep epithelial disruption	1%	0%	8%	0%	4%	0%	0.002
Bleeding from site of epithelial disruption	0%	0%	3%	0%	1%	0%	0.06
Intact epithelium							
Abnormal vaginal discharge	60%	53%	16%	7%	41%	26%	0.003
Erythema	0%	2%	32%	2%	14%	2%	<0.0001
Petechia	1%	6%	34%	3%	15%	4%	<0.0001
Blood-tinged discharge	2%	3%	2%	3%	2%	3%	0.71
Abnormal cervical discharge	6%	4%	8%	0%	7%	2%	0.008
Ecchymosis	1%	3%	10%	0%	5%	1%	0.04
Blood in vagina – no identified source	2%	2%	0%	0%	1%	1%	0.62

use of colposcopy yielded significantly higher rates of participants with deep epithelial disruption, bleeding from site of epithelium disruption, erythema, petechia, blood tinged discharge, ecchymosis and a trend towards significance of abrasion.

Discussion

This study was the second microbicide trial designed as an intense safety evaluation of genital epithelium disruption by naked eye pelvic examinations together with colposcopic evaluations and laboratory tests over an extended three-month period unlike the classical two-week phase 1 studies [10,11,18]. The first was COL-1492 trial, a phase 2 study that assessed safety of multiple daily applications of nonoxynol-9 among female sex workers and colposcopy evaluations were discontinued after finding no significant safety differences between placebo and active product 7. However, the phase 3 portion of COL-1492 found that noxynol-9 increased risk of HIV-1 infection compared with placebo, a finding attributable to study gel causing higher incidence of lesions with epithelial disruption [7]. This observation had not been seen during colposcopic evaluations in phase 2 portion of the study [7].

This extended safety portion of HPTN 035 demonstrated that women in the 0.5% PRO 2000 Gel and BufferGel arms had no significant safety signals compared to women in the Placebo Gel and No Gel arms as evidenced by pelvic examinations and colposcopy findings. We also observed no statistical differences in genital tract infections across the four arms.

The observation of at least one pelvic exam finding at colposcopy in 48% of participants across the four arms are consistent with reports from other previous colposcopy studies as these are the most frequently observed outcomes related to previous sexual intercourse [19,20]. The most common finding of vaginal discharge found at pelvic examinations among this cohort may be related to cultural practice of douching (9% douching at baseline) and a high burden of BV prevalence (35% at baseline). The erythematic and petechia findings have also been observed in other cohorts to be normal findings in vagina and cervix before insertion of applicator or gel, and these findings disappear within a few days without treatment [20]. Importantly, the findings of erythema and petechia were observed in women with intact epithelium and, therefore, had no clinical significance for increased risk to HIV/STI acquisitions. The women (1%) observed to have superficial ulceration and laceration at baseline had no evidence of deep epithelium disruption and these findings could have been attributed to be a result of sexual trauma or other intravaginal practices also observed in other previous studies [19]. The cumulative observation of 4% women with deep epithelial disruption at colposcopy in the three months of study period did not differ across arms suggesting an uncommon finding not related to product use. It should be noted, however, that since none of the agents tested in this study have been shown to cause significant toxicity, it is not known what differences in the incidence rate of deep epithelial disruption should be assessed.

We must, however, point out the limitation of these colposcopic findings whose significance is not completely known and seem to have no clinical relevance for increased

HIV acquisitions as seen after application of N-9 [6,7]. Colposcopy picked up significantly more abnormal genital epithelium findings than examination by naked eye (69% compared to 51%, $p = <0.001$). These findings commonly termed ''background noise'' do not seem to contribute much to detecting safety signals, and we therefore do not see much added value in continuing to add colposcopy examinations in future phase 2 topical microbicides trials. The added cost of colposcopy procurement, training and limited utility in predicting clinical outcome [7] does not justify its continued use in microbicide development pathway. Suggestions to add collection of cervico-vaginal lavage specimens to assess inflammatory changes as early safety signals have not demonstrated significant correlation with sub-clinical inflammation, and HIV acquisition thus far has remained in use in preclinical animal models [21].

The intense extended monitoring with pelvic examinations, colposcopy for three months in the first portion of HPTN 035 provided us with evidence that there were no safety signals of concern in the cervico-vaginal compartments of women using BufferGel and PRO 2000 for three months of intense follow-up. The DSMB allowed a seamless transition from phase 2 to phase 2b after review of the safety data. The follow-on efficacy component of the HPTN 035 study went to completion and confirmed that both BufferGel and PRO 2000 Gel were safe and without local genital safety concerns [12].

Conclusions

The current clinical trial design for microbicide development requires phase 1/2 portion of participants that have been evaluated by colposcopy examination to detect potential epithelial disruption from investigational product. This study demonstrates that intense colposcopy evaluations result in identification of non-significant clinical findings. Colposcopy evaluations are costly, uncomfortable to participants and therefore of questionable use in future microbicide phase 1/2 clinical trials.

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Competing interests

The authors declare that they do not have any competing interests.

Authors' contributions

ZMC participated in the development of the study protocol, data collection instruments and procedures, monitored field operations and data, led the analysis, and drafted the manuscript. AC oversaw field implementation across all participating sites, and reviewed and edited the manuscript. BM coordinated and managed the data collection and analysis. CK data analysis, drafting and editing of the manuscript. GR oversaw field implementation at Durban site, reviewed and edited the manuscript. TM oversaw the field implementation at Harare site, reviewed and edited the manuscript. LM provided clinical expertise and field implementation at Philadelphia site, reviewed and edited the manuscript. SAK participated in the development of study protocol, provided clinical technical assistance in the oversight of safety issues, and reviewed and edited the manuscript. TNT oversaw field implementation at Lilongwe site, reviewed and edited the manuscript. LST participated in the development and design of the project and protocol, provided technical expertise in the development and interpretation of safety assessments, and reviewed and edited the manuscript. SLH participated in the development and design of the protocol, reviewed and edited the manuscript.

All co-authors read and approved the final version of the manuscript.

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