Dose-Ranging Phase 1 Study of TMC120, a Promising Vaginal Microbicide, in HIV-Negative and HIV-Positive Female Volunteers

Vicky A. Jespers, MD, Jens M. Van Roey, MD,† Greet I. Beets, MS,† and Anne M. Buvé, MD, PhD**

Objective: To evaluate the short-term safety, tolerability, and systemic exposure of a vaginal microbicide gel containing the nonnucleoside reverse transcriptase inhibitor TMC120.

Design: Randomized, controlled, double-blind, phase 1 trial of a gel containing 3 different concentrations of TMC120 versus placebo.

Methods: Of the 48 HIV-negative and 16 HIV-positive women enrolled, 52 women received active product. Participants applied the gel twice daily for 7 days and were assessed on 6 occasions. Colposcopic evaluation was performed before and after first gel application and on day 8. Laboratory safety assessments were carried out on all visits except day 7. Plasma levels of TMC120 were measured on days 1 and 7.

Results: All TMC120 concentrations were well tolerated, and there were no apparent differences in safety parameters. Four women (6%) had treatment-emergent mild cervical findings (petechiae in 3 women and erythema in 1 woman) of <5 mm. Plasma levels of TMC120 were quantifiable on day 1 in 7 (13%) participants and on day 7 in 39 (75%) participants using TMC120 gel.

Conclusions: The TMC120 vaginal gel was well-tolerated in this short study by HIV-negative and HIV-positive women. The implications of the absorption of TMC120 should be studied further in expanded safety and effectiveness trials.

Key Words: female controlled methods, HIV prevention, microbicides, phase 1 clinical trial, TMC120

(*J Acquir Immune Defic Syndr* 2007;44:154–158)

In populations in which the predominant mode of HIV transmission is heterosexual intercourse, women are more affected than men.[1] The male condom is an efficacious method to prevent the transmission of HIV, but women are often not in a position to negotiate safe sex.[2] Efficacious female-controlled prevention methods are urgently needed to help women protect themselves against HIV infection and other sexually transmitted infections (STIs).[3,4]

Vaginal microbicides are topical formulations designed to block HIV infection when applied vaginally before intercourse.[5] So far, we do not have a microbicide, but modeling suggests that 2.5 million new infections could be averted over 3 years if a 60% efficacious microbicide was used by 20% of all individuals at risk, and this while 50% of their total sexual acts are protected by condoms.[6]

Cellulose sulfate (CS), PRO2000 (0.5% and 2%), and Carraguard are currently being tested in phase 3 clinical trials. These molecules have nonspecific anti-HIV activity in that they are negatively charged polymers that bind to the positively charged areas of X4 and R5 gp120, acting as entry and/or fusion inhibitors.[5,7–9] Newer molecules in development target more specific mechanisms of transmission. Some interact with the viral envelope glycoproteins gp120 and gp41.[10] Other molecules target the virus by inhibiting reverse transcriptase.[11,12]

TMC120, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), was sidelined as a therapeutic compound because of poor absorption and is now in development as a vaginal microbicide. It is active in vitro against wild-type HIV-1 (median effective concentration = 0.9 nM) and drug-resistant HIV.[13,14] An in vivo mouse model showed up to 100% protection against vaginal transmission of cell-associated virus. Cell-free HIV-1 transfer was blocked at 10 nM in an in vitro dendritic model and at 100 nM in a cervical explant model.[15–17] We present here the first clinical trial in women in which a vaginal gel containing TMC120 was evaluated.

METHODS

Study Design

This randomized, phase 1, double-blind, placebo-controlled trial was conducted in 2003 through 2004 at the Institute of Tropical Medicine, Antwerp, Belgium, and was approved by its Ethical Committee.

The trial was designed to assess the safety, tolerability, and systemic exposure of 25 μM, 50 μM, and 150 μM of TMC120 in a vaginal gel compared with the same vaginal gel without TMC120 (placebo). The gel was hydrophilic and consisted of hydroxyethylcellulose (HEC) and glycerol as a base, ethanol as a solvent, and lactic acid and sodium hydroxide for pH adjustment. Methyl-parahydroxybenzoate

Received for publication June 29, 2006; accepted October 13, 2006.
From the *Institute of Tropical Medicine, Antwerp, Belgium; and †Tibotec Pharmaceuticals Ltd., Cork, Ireland.
Tibotec Pharmaceuticals Ltd. provided financial support. J. Van Roey and G. Beets are full-time employees of Tibotec Pharmaceuticals Ltd.
Reprints: Vicky Jespers, MD, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium (e-mail: vjespers@itg.be).

and propyl-parahydroxybenzoate were added as preservatives. All these products are generally recognized as safe.

The trial consisted of 2 parts, with a review of data after the first part (HIV-uninfected women) (Table 1). The different concentrations between groups in part B were chosen to generate the maximum of safety and pharmacokinetic information. The gel was packaged in 7 aluminum tubes, and each tube was sufficient for 2 daily applications of 2.4 mL of gel. The gel was applied with an applicator for 7 consecutive days in the morning and in the evening. The last evening dose in part B was omitted to assess pharmacokinetics on day 8.

Enrollment of Study Participants

All participants provided written informed consent before starting the study procedures. They were between 18 and 50 years of age and had a regular menstrual cycle or amenorrhea as a result of long-acting progesterone use. The confirmed HIV-negative women in the study were at low risk of acquiring STIs. The HIV-positive women had a CD4 count of $\geq$200 cells/mm^3 and an undetectable viral load ($<$50 RNA copies/mL). Because low plasma levels of TMC120 were detected in part A, the selection criteria for HIV-positive women were reviewed: they were on stable antiretroviral (ARV) therapy, including an NNRTI and/or had documented genotypic evidence of NNRTI resistance. The HIV-negative women in part B had a regular male partner with whom they had intercourse at least twice a week protected by a condom. They used hormonal contraception or had an intrauterine device. Exclusion criteria were pregnancy or breast-feeding. Women who had a noniatrogenic deep disruption of the vaginal epithelium at enrollment were excluded.

Participants were randomized on day 1 by entering their name on the next line of the respective trial register. A prelabeled box was then distributed accordingly.

Assessment of Study Participants

Participants were assessed on 6 occasions: at screening, before enrollment (day 1), 4 hours after the first gel dose (day 1), on day 7, on day 8, and by telephone 1 week after finishing the gel. Pelvic examinations, including colposcopy, were performed according to the World Health Organization (WHO) criteria[18] at screening, on day 1 (at baseline and 4 hours after application), and on day 8. All colposcopy examinations were done by the same physician and were documented by digital imaging. Symptoms of genital irritation were elicited by interview at each visit and by evaluation of each participant's diary. Systemic toxicity was determined by liver enzyme tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], bilirubin, and alkaline phosphatase), urea, creatinine, electrolytes, and blood cell counts. From phase 1 therapeutic studies (doses ranging from 50–1000 mg), it is known that TMC120 can cause reversible mild changes in liver test results. Plasma concentrations of TMC120 were determined by a validated liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS) method with a detection limit of 0.05 ng/mL. Plasma levels of TMC120 were measured on days 1 and 7 at 1, 2, 4, and 8 hours after gel application. Additionally, a 24-hour postdose sample was taken on day 8 in part B. Tolerability and acceptability were assessed by questionnaire on day 8.

All women were screened for STIs with the following tests: a wet mount for *Trichomonas vaginalis* (TV) and yeast cells, polymerase chain reaction (PCR) on an endocervical swab for *Neisseria gonorrhoeae* (NG) *and Chlamydia trachomatis* (CT), and a rapid plasma reagin test (RPR) for syphilis. Vaginal ecology (pH and Nugent score) was assessed at screening and 12 to 24 hours after last gel application.[19] A midstream urine sample was examined for infection by dipstick.

For HIV-1–positive participants, a plasma viral load was measured at screening, day 1, and day 8. If the viral load was greater than 1000 HIV-1 RNA copies/mL on day 1 or day 8, resistance testing (genotype and phenotype) had to be performed.

Because this was a phase 1 study, the sample size was based on the usual number enrolled in phase 1 microbicide safety trials and on the number that was thought feasible to enroll rather than on statistical considerations. The sexually active subjects were included to assess whether sexual activity would cause irritation in HIV-negative women. The HIV-1–positive women were included to assess whether HIV infection affects the toxicity profile. The data were analyzed on an intent-to-treat basis. Groups were compared using Kruskal-Wallis analysis of variance or Fisher exact probability testing. A 2-sided 5% significance level was used. Because this was an exploratory trial, no adjustment for multiple comparisons was done.

TABLE 1. Numbers of Study Participants by Dose of Product Application

	Dose	No. Women	Discontinued
Part A			
HIV-negative/abstinent	Placebo	4	1*
	25 μM	8	
	50 μM	8	
	150 μM	8	1*
Part B			
Group 1: HIV-negative/sexually active	Placebo	4	
	50 μM	8	
	150 μM	8	
Group 2: HIV-positive/abstinent	Placebo	4	
	25 μM	6	
	150 μM	6	

*Discontinued per protocol because of an AE.

RESULTS

Baseline Data

Baseline characteristics and data are summarized in Table 2. No STI was detected at screening. A vaginal finding was seen at baseline in 9 (14%) study participants. The different treatment groups were well balanced for all demographic and baseline characteristics.

Compliance and Withdrawals

Compliance was good. Only 1 dose was missed, and 2 extra doses were used erroneously. No protocol deviations

TABLE 2. Baseline Characteristics of Study Participants

No. Randomized	Placebo (n = 12)	25 μM (n = 14)	50 μM (n = 16)	150 μM (n = 22)	All Subjects (n = 64)
Mean (median) age in years (range)	30 (30) (20–47)	35 (28) (23–47)	26 (25) (21–36)	32 (33) (19–47)	31 (27) (19–47)
Mean (median) BMI (range)	23 (18) (18–30)	24 (22) (17–34)	20 (20) (17–25)	22 (21) (19–35)	22 (21) (17–35)
Ethnic group (%)					
African/Caribbean	4 (33.3)	4 (28.6)	0 (0)	6 (27.3)	14 (21.9)
White	8 (66.7)	10 (71.4)	16 (100)	15 (68.2)	49 (76.6)
Asian	0 (0)	0 (0)	0 (0)	1 (4.5)	1 (1.6)
Mean pH (range)	4.7 (4.1–5.4)	4.5 (3.8–5.1)	4.3 (3.0–5.1)	4.5 (3.8–5.1)	4.5 (3.0–5.4)
Nugent score ≥7 (%)	0 (0)	1 (7.1)	1 (6.2)	1 (4.5)	3 (4.7)
Yeast cells (%)	0 (0)	0 (0)	1 (6.2)	0 (0)	1 (1.6)

BMI indicates body mass index.

were noted concerning sexual activity. A participant in part A (placebo) had to stop gel use because of treatment for a urinary tract infection. Another participant had an early menstrual period and could not use the last dose.

Treatment-Emergent Adverse Events

Thirty-nine (61%) participants (75% of the placebo users and 58% of the TMC120 users) presented with at least 1 adverse event (AE). Four women (6%) had a vaginal finding (petechiae in 3 women and erythema in 1 woman) of <5 mm. One was in the placebo group, and 3 were in the sexually active group. Table 3 lists the numbers of the other most significant AEs. The most common AEs emerging during treatment were related to the genitourinary system. Six participants (9%) complained about headache during treatment, and this was the most commonly reported individual AE.

One serious AE, considered to be unrelated to gel use, occurred in a diabetic patient needing hospital admission for a diabetic foot. Two of the HIV-positive women (12%) had a detectable viral load (63 and 192 copies/mL) on day 8. Laboratory grade I AEs classified as not clinically significant were seen in 3 women (5%).

Systemic Exposure of TMC120

Plasma concentrations were detectable in 13% of women on day 1 and in 75% of women on day 7 (Table 4). The highest detected level was 0.16 ng/mL. The linear mean plasma concentration-time curves were flat without a clear absorption or distribution and/or elimination phase. Plasma concentrations declined for most participants in the first hour after the morning application, and maximum concentrations were reached at 4 or 8 hours. Plasma concentrations were still quantifiable 24 hours after the last application for 11 women (79%) using the 150 μM gel and for 2 women (25%) using the 50 μM gel.

Acceptability

Gel insertion was described as easy by all participants. Forty-two percent of women using the placebo could feel the gel after insertion versus 25% of women using TMC120 gel ($P = 0.3$). Gel leakage was reported by 96% of women and was rated as moderate or severe by 75% of women in the placebo group and by 78% of women using TMC120 gel ($P = 1$). Seventy-five percent of participants in the placebo group and 71% of women using TMC120 gel stated that leakage would not prevent them from using the gel if it were available. No relevant differences in acceptability were noted among the different treatment groups or trial parts.

TABLE 3. Incidence of Genitourinary and Abdominal AEs and Headache During the Study Period

	No. AEs (%)				
	25 μM (n = 14)	50 μM (n = 16)	150 μM (n = 22)	Placebo (n = 12)	Active Product* (n = 52)
Genitourinary system					
Genital itch	1	2	0	0	3
Smelly vaginal discharge	0	0	0	1	0
Vaginal burning sensation	0	0	2	1	2
Vaginal pain	0	0	1	0	1
Brown vaginal discharge	0	1	1	1	2
Dysuria	1	1	1	0	3
Vaginal erythema	0	1	1	0	2
Cervical petechiae	0	0	1	1	1
Nugent score ≥7	2	0	0	0	2
Trichomonas vaginalis infection	0	0	0	1	0
Total	4 (29)	5 (36)	7 (32)	5 (42)	16 (31)
Abdominal system					
Loose stools	1	2	2	0	5
Abdominal pain	3	0	0	2	3
Bloated abdomen	0	1	0	0	1
Total	4 (29)	3 (19)	2 (9)	2 (17)	9 (17)
Headache	2 (14.3)	2 (12.5)	1 (4.5)	1 (8.3)	5 (9.6)

*AEs for all 3 TMC120 gel concentrations (25 μM, 50 μM, and 150 μM) combined.

TABLE 4. Number of Participants With at Least 1 Quantifiable TMC120 Plasma Level on Day 1 and Day 7

	25 μM		50 μM		150 μM		
No. Randomized	Part A HIV$^-$ (n = 8)	Part B HIV$^+$ (n = 6)	Part A HIV$^-$ (n = 8)	Part B HIV$^-$ (n = 8)	Part A HIV$^-$ (n = 8)	Part B HIV$^+$ (n = 6)	Part B HIV$^-$ (n = 8)
Day 1							
No. women	0	0	0	2	2	1	2
%	0	0	0	25	25	16.7	25
Day 7							
No. women	1	2	8	6	8	6	8
%	12.5	33.3	100	75	100	100	100

DISCUSSION

This short phase 1 clinical trial found that all 3 different TMC120 concentrations used twice a day for 1 week were safe and well tolerated and that there were no apparent differences in safety parameters between the 3 concentrations of TMC120 and placebo gel.

A remarkably low number of vaginal findings (6%) occurred during treatment. One was in the placebo group, and 3 were possibly caused by sexual activity.[20] This number of findings compares favorably with results from other phase 1 trials.[21–25] For instance, in a control group of sexually active women, an incidence of 7.3% has been reported.[24] The reported AEs were all mild and short and did not affect compliance. Although local safety assessed by colposcopy in this study did not show product-related toxicity, further studies to detect subtle changes in the mucosal barrier evaluating inflammatory cells and cytokines need to be considered.[26,27]

Product acceptability was high despite the common occurrence of leakage present in all treatment groups independent of sexual activity. Several studies showed that increased lubrication improved intercourse and acceptability of the gel.[28,29] It should be noted that in some cultures, however, dry sex is favored.[30]

Serum TMC120 levels were detectable in 75% of women on day 7. The highest detected level (0.16 ng/mL) is 1000 times lower than the lowest trough plasma level (C_{min}) after 7-day monotherapy with 50 mg of oral TMC120 administered twice daily. This dose regimen was shown to decrease the viral load, with 1.44 log (mean, n = 13) in treatment-naive individuals.[31] In the tenofovir vaginal gel safety study, the active product was also detected in plasma.[22] Local vaginal mucosal measurements of TMC120 were not performed in this study; however, on the basis of the plasma levels, we can assume that TMC120 was present locally. TMC120 is a highly lipophilic (log P = 5.27) NNRTI, and it may thus prevent HIV infection by embedding and crossing the cell membranes and virus envelope.[32,33] A prolonged presence of TMC120 in the mucosa may be an advantage in preventing HIV infection. In an in vitro model, Fletcher et al[34] demonstrated a lasting effect up to 6 days of prevention of infection by a tight-binding NNRTI.

Researchers now agree that a combination product acting at different steps in the transmission process (prebinding, binding, entry, fusion, uncoating, and reverse transcription) would have a better chance of blocking infection than a single product acting at a single step.[3,11,35–38] TMC120 could be a potential component of a combination microbicide because it works 1 step after viral entry blockers. It might also prevent HIV-1 infection of migratory cells, including dendritic cells, which are thought to be responsible for the dissemination of HIV to the regional lymph nodes.[39,40]

Although the lipophilic characteristics of TMC120 seem to have several positive aspects, the absorption of TMC120 requires further investigation. Theoretically, a low serum concentration of TMC120 might lead to the selection of HIV strains that are resistant not only to TMC120 but to other NNRTIs in women who are HIV-1 infected and use the gel.[38] At present, we have no in vivo knowledge of the concentrations leading to selection of resistant strains. Nevertheless, the finding that TMC120 is absorbed after vaginal application needs to be taken into account when testing TMC120 in large-scale trials.[36]

In conclusion, TMC120 vaginal gel was found to be safe in this short study , and further evaluation is appropriate. The systemic exposure of TMC120 should be studied further in ongoing and planned expanded safety and effectiveness studies.

ACKNOWLEDGMENTS

The authors thank all the participants, without whom the study would not have been possible; Tessa James, our dedicated study nurse; and Guido Vanham for reviewing the article.

REFERENCES

1. Joint United Nations Program on HIV/AIDS and World Health Organization. *AIDS Epidemic Update*. UNAIDS/05 19E. Geneva, Switzerland: Joint United Nations Programme on HIV/AIDS and World Health Organization; 2005.
2. Heise L, Elias C. Transforming AIDS prevention to meet women's needs: a focus on developing countries. *Soc Sci Med*. 1995;40:931–943.
3. Ramjee G. Microbicides and other prevention strategies. Presented at: XVI International AIDS Conference; 2006; Toronto. Available at: http://www.aids2006.org/PAG/PSession.aspx?s=653. Accessed September 5, 2006.
4. Stein Z. HIV prevention: the need for methods women can use. *Am J Public Health*. 1990;80:460–462.
5. Stone A. Microbicides: a new approach to preventing HIV and other sexually transmitted infections. *Nat Rev Drug Discov.* 2002;1:977–985.
6. Watts C, Vickerman P. The impact of microbicides on HIV and STD transmission: model projections. *AIDS*. 2001;15(Suppl):S43–S44.
7. Phase III trial updates. Presented at: Microbicides 2006, Cape Town, South Africa; 2006. Available at: http://www.microbicides2006.org/Feedback.htm. Accessed September 5, 2006.
8. Alliance for Microbicides. Microbicides clinical trials summary table. Available at: http://www.microbicide.org/publications/digest/MicrobicideClinicalTrialsSummaryTable.September.pdf. 2006. Accessed September 5, 2006.
9. Scordi-Bello I, Mosoian A, He C, et al. Candidate sulfonated and sulfated topical microbicides: comparison of anti-human immunodeficiency virus

activities and mechanisms of action. *Antimicrob Agents Chemother*. 2005; 49:3607–3615.
10. Shattock R, Solomon S. Microbicides—aids to safer sex. *Lancet*. 2004; 363:1002–1003.
11. Parniak M. Nonnucleoside reverse transcriptase inhibitors as anti-HIV-1 microbicides. *AIDS*. 2001;15(Suppl):S56.
12. Van Herrewege Y, Vanham G, Michiels J, et al. A series of diaryltriazines and diarylpyrimidines are highly potent nonnucleoside reverse transcriptase inhibitors with possible applications as microbicides. *Antimicrob Agents Chemother*. 2004;48:3684–3689.
13. de Bethune M, Andries K, Ludovici D, et al. TMC120 (R147681), a next generation NNRTI, has potent in vitro activity against NNRTI resistant HIV variants. Presented at: 8th Conference on Retroviruses and Opportunistic Infections (CROI) 2001; Chicago.
14. Jonckheere H, Azijn H, Roobaert F, et al. TMC120 and TMC125: NNRTIs active against resistant HIV as the result of a new and accelerated screening strategy. Presented at: 8th European Conference on Clinical Aspects and Treatment of HIV Infection, 2001; Athens.
15. Di Fabio S, Van Roey J, Giannini G, et al. Inhibition of vaginal transmission of HIV-1 in hu-SCID mice by the non-nucleoside reverse transcriptase inhibitor TMC120 in a gel formulation. *AIDS*. 2003;17:1597–1604.
16. Harman S, Watts P, Shattock R. TMC120 blocks HIV-1 infection in cellular and human cervical tissue models [abstract 02591]. Presented at: Microbicides 2004 Conference; 2004; London.
17. Van Herrewege Y, Michiels J, Van Roey J. In vitro evaluation of nonnucleoside reverse transcriptase inhibitors UC-781 and TMC120-R147681 as human immunodeficiency virus microbicides. *Antimicrob Agents Chemother*. 2004;48:337–339.
18. CONRAD/WHO. *Manual for the Standardization of Colposcopy for the Evaluation of Vaginal Products. Update*. 2004; Geneva, Switzerland.
19. Nugent R, Krohn M, Hillier S. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 1991;29:297–301.
20. Mauck C, Baker J, Birnkrant D, et al. The use of colposcopy in assessing vaginal irritation in research. *AIDS*. 2000;14:2221–2227.
21. Mauck C, Weiner D, Ballagh S, et al. Single and multiple exposure tolerance study of cellulose sulfate gel: a Phase I safety and colposcopy study. *Contraception*. 2001;64:383–391.
22. Mayer K, Maslankowski L, Gai F, et al. Safety and tolerability of tenofovir vaginal gel in abstinent and sexually active HIV-infected and uninfected women. *AIDS*. 2006;20:543–551.
23. Schwartz J, Mauck C, Lai J, et al. Fourteen-day safety and acceptability study of 6% cellulose sulfate gel: a randomized double-blind Phase I safety study. *Contraception*. 2006;74:133–140.
24. Van Damme L, Niruthisard S, Atisook R, et al. Safety evaluation of nonoxynol-9 gel in women at low risk of HIV infection. *AIDS*. 1998;12:433–437.
25. Van Damme L, Wright A, Depraetere K, et al. A phase I study of a novel potential intravaginal microbicide, PRO 2000, in healthy sexually inactive women. *Sex Transm Infect*. 2000;76:126–130.
26. Keller M, Klotman M, Herold B. Rigorous pre-clinical evaluation of topical microbicides to prevent transmission of human immunodeficiency virus. *J Antimicrob Chemother*. 2003;51:1099–1102.
27. WHO/CONRAD. Technical consultation on nonoxynol-9. *Reprod Health Matters*. 2002;10:175–181.
28. Coggins C, Blanchard K, Alvarez F, et al. Preliminary safety and acceptability of a carrageenan gel for possible use as a vaginal microbicide. *Sex Transm Infect*. 2000;76:480–483.
29. Vandebosch A, Goetghebeur E, Ramjee G, et al. Acceptability of COL-1492, a vaginal gel, among sex workers in one Asian and three African cities. *Sex Transm Infect*. 2004;80:241–243.
30. Ramjee G, Abdool Karim S, Morar N, et al. Acceptability of a vaginal microbicide among female sex workers. *S Afr Med J*. 1999;89:673–676.
31. Van't Klooster G, Gruzdev B, Horban A, et al. The novel non-nucleoside reverse transcriptase inhibitor TMC120 has potent antiretroviral activity after short-term monotherapy in treatment naive, HIV-1 infected subjects [abstract 9]. Presented at: 14th International Conference on Antiviral Research; 2001; Seattle. *Antiviral Res*. 2001;50:A40.
32. Borkow G, Barnard J, Nguyen TM, et al. Chemical barriers to human immunodeficiency virus type 1 (HIV-1) infection: retrovirucidal activity of UC781, a thiocarboxanilide nonnucleoside inhibitor of HIV-1 reverse transcriptase. *J Virol*. 1997;71:3023–3030.
33. Motakis D, Parniak M. A tight-binding mode of inhibition is essential for anti-human immunodeficiency virus type 1 virucidal activity of non-nucleoside reverse transcriptase inhibitors. *Antimicrob Agents Chemother*. 2002;46:1851–1856.
34. Fletcher P, Kiselyeva Y, Wallace G, et al. The nonnucleoside reverse transcriptase inhibitor UC-781 inhibits human immunodeficiency virus type 1 infection of human cervical tissue and dissemination by migratory cells. *J Virol*. 2005;79:11179–11186.
35. Liu S, Lu H, Neurath A, et al. Combination of candidate microbicides cellulose acetate 1,2-benzenedicarboxylate and UC781 has synergistic and complementary effects against human immunodeficiency virus type 1 infection. *Antimicrob Agents Chemother*. 2005;49:1830–1836.
36. Martinez J, Coplan P, Wainberg M. Is HIV drug resistance a limiting factor in the development of anti-HIV NNRTI and NRTI-based vaginal microbicide strategies? *Antiviral Res*. 2006;71:343–350.
37. Van Damme L. Clinical microbicide research: an overview. *Trop Med Int Health*. 2004;9:1290–1296.
38. Wainberg M. Issues and implications for ARV-containing products. Presented at: Microbicides 2006, Cape Town, South Africa; 2006. Available at: http://www.microbicides2006.org/PDF/MarkWainberg.pdf.
39. Hu Q, Frank I, Williams V, et al. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J Exp Med*. 2004;199:1065–1075.
40. Masurier C, Salomon B, Guettari N, et al. Dendritic cells route human immunodeficiency virus to lymph nodes after vaginal or intravenous administration to mice. *J Virol*. 1998;72:7822–7829.

