



Published in final edited form as:

Lancet Infect Dis. 2008 November ; 8(11): 685–697. doi:10.1016/S1473-3099(08)70254-8.

Vaginal microbicides and the prevention of HIV transmission

Blayne Cutler and Jessica Justman

Department of Medicine (B Cutler MD, J Justman MD), and Department of Epidemiology (J Justman), Mailman School of Public Health, Columbia University, New York, NY, USA

Abstract

Worldwide, nearly half of all individuals living with HIV are now women, who acquire the virus largely by heterosexual exposure. With an HIV vaccine likely to be years away, topical microbicide formulations applied vaginally or rectally are being investigated as another strategy for HIV prevention. A review of preclinical and clinical research on the development of microbicides formulated to prevent vaginal HIV transmission yielded 118 studies: 73 preclinical and 45 clinical. Preclinical research included in-vitro assays and cervical explant models, as well as animal models. Clinical research included phase I and II/IIb safety studies, and phase III efficacy studies. Whereas most phase I and phase II clinical trials have found microbicide compounds to be safe and well tolerated, phase III trials completed to date have not demonstrated efficacy in preventing HIV transmission. Topical microbicides are grouped into five classes of agents, based on where they disrupt the pathway of sexual transmission of HIV. These classes include surfactants/membrane disruptors, vaginal milieu protectors, viral entry inhibitors, reverse transcriptase inhibitors, and a fifth group whose mechanism is unknown. The trajectory of microbicide development has been toward agents that block more specific virus–host cell interactions. Microbicide clinical trials face scientifically and ethically complex issues, such as the choice of placebo gel, the potential for viral resistance, and the inclusion of HIV-infected participants. Assessment of combination agents will most likely advance this field of research.

Introduction

According to recent UNAIDS estimates, in 2007 more than 33 million people were living with HIV and approximately 2.5 million people were newly infected.¹ Worldwide, nearly half of all individuals living with HIV are now women, who acquire the virus largely by heterosexual exposure.^{1–3} Many women, because of limited economic options and gender inequality, cannot reliably negotiate sexual encounters, leaving them vulnerable to unwanted pregnancy and sexually transmitted infections (STIs), including HIV. With clinical deployment of a safe and effective HIV vaccine still likely to be years away, topical microbicide formulations that are applied vaginally or rectally are receiving increasing attention as another strategy for HIV prevention.

Correspondence to: Dr Jessica Justman, Center for Infectious Disease Epidemiologic Research, Mailman School of Public Health, Columbia University, 722 West 168th Street, Room 714, New York, NY 10032, USA jj2158@columbia.edu.

Conflicts of interest JJ has received funding to conduct the following National Institutes of Health-sponsored microbicide clinical trials: HPTN 049 (phase I safety and acceptability study of the vaginal microbicide 6% cellulose sulfate gel among HIV-infected women), HPTN 050 (phase I safety and acceptability study of the vaginal microbicide agent PMPA gel), HPTN/MTN 059 (phase II expanded safety and acceptability study of the vaginal microbicide 1% tenofovir gel) and will soon begin MTN 001 (phase II adherence and pharmacokinetics study of oral and vaginal preparations of tenofovir). She has served as Investigator of Record for the Bronx-Lebanon Hospital Center Clinical Research Site in New York City for all of these trials and was protocol co-chair for HPTN 059. BC assisted with HPTN 059 as a clinical fellow. Gilead was a co-sponsor of HPTN 050 and HPTN/MTN059, and CONRAD a co-sponsor of HPTN 049. Both Gilead and CONRAD are co-sponsors of MTN 001. CONRAD is a non-profit reproductive health organisation and currently holds the Investigational New Drug (IND) for tenofovir gel. Neither JJ or BC have any financial relationships with any companies involved in HIV products, including Gilead.

Until recently, an incomplete understanding of key steps in the sexual transmission of HIV hindered the science of microbicides. Most of the agents that were first developed, including surfactants and acidifying agents, act non-specifically, either by disrupting viral and cellular membranes, or creating a more hostile environment in the genital tract for viral transmission. Progress in understanding how HIV gains entry into the host and establishes lasting infection has permitted the development of compounds that target specific viral–host cell interactions and has allowed for a more tailored approach to microbicide development. At least ten reverse transcriptase inhibitors and 16 entry inhibitor agents have been investigated or are currently being investigated in preclinical or clinical microbicide trials.⁴ The development of these agents will be reviewed here, with a brief overview of current research delineating the sexual transmission of HIV.

The sexual transmission of HIV and microbicide strategies

The sexual transmission of HIV is not uniformly efficient. The type of sexual activity and the phase of disease affect the risk of transmission. Initial estimates of transmission rates per coital act have ranged from 0.0003 to 0.008,^{5–9} with insertive vaginal intercourse associated with lower estimates and receptive anal intercourse associated with estimates as high as 0.01 or 1%.¹⁰ The role of anal intercourse in heterosexual transmission is less well described and the frequency might be greater than previously thought.^{11,12}

Recent investigation has also shown that the rate of sexual transmission depends on cofactors such as circumcision status, genital ulcer disease, and phase of disease.¹³ High serum HIV-1 concentrations during the acute infection period increases the probability of male-to-female heterosexual transmission by up to eight to tenfold.¹⁴ A study of Ugandan serodiscordant couples found the rate of HIV-1 sexual transmission per coital act within 2.5 months after seroconversion of the index partner to be 0.0082, or almost 1%.¹⁵ Although these per-act estimates for HIV-1 transmission risk are not particularly high, the cumulative risk of sexual activity over an extended period of time—with prolonged viral shedding, frequent sexual contact, inflammation or ulcerative lesions of the genital tract, or having sex during a particularly high-risk period, such as acute infection—makes the sexual transmission of HIV-1 increasingly efficient.

Male circumcision status also affects the efficiency of transmission. Three recent randomised clinical trials in Africa have shown that circumcision decreases the risk of female-to-male HIV transmission by 50–76%.^{16–18} Uncircumcised men might acquire HIV at higher rates than circumcised men because of the presence of key target cells in human foreskin: macrophages expressing CD4 receptors and dendritic cells expressing dendritic-cell C-specific intercellular adhesion molecular-3-grabbing non-integrin (DC-SIGN), a mediator of HIV entry into CD4 cells.^{16,19}

By contrast with human foreskin, the intact vaginal epithelium and endocervix each present a different challenge to the entry pathway of the HIV virion. Although vaginal epithelial cells have limited permeability to particles greater than 30 nm (HIV virion is 80–100 nm),²⁰ HIV seems to enter the superficial layers of the squamous epithelium by diffusing across a concentration gradient,²¹ and sequesters itself on the surface of epithelial cells until it can infect other cell types, particularly CD4+ helper cells and Langerhans cells, both of which are found in mucosal epithelium.^{22,23} One recent study using an ex-vivo human organ culture system found that HIV simultaneously enters both intraepithelial CD4+ T cells and Langerhans cells within 2–3 h of challenge and survives within Langerhans cells for 3 days.²⁴ Thus, finding microbicide agents that can disrupt the virus envelope before the initial attachment of the virus to epithelial target cells has been one of the earliest strategies for microbicide development.

The acidity of the vaginal canal is protective against a variety of bacteria and viruses, including *Chlamydia trachomatis*, *Haemophilus ducreyi*, and herpes simplex virus (HSV) type 2.^{25–27} The presence of normal commensal vaginal flora, particularly lactobacilli, and an acidic vaginal pH has been correlated with a decrease in HIV proliferation²⁸ as well as a decrease in HIV acquisition.^{29–31} The development of compounds that protect the acidic vaginal milieu, either by buffering the neutralising effect of semen or maintaining sufficient lactobacilli production in the vaginal canal has been a second strategy in microbicide development.

The subepithelial layer of the genital mucosa is a very favourable environment for HIV replication. Dendritic cells, macrophages, and T cells all densely populate the subepithelial stromal tissues of the male and female genital tract and the rectum. Each of these cell types expresses CD4, CCR5, and in lesser quantities, CXCR4 receptors, making them all vulnerable to HIV-1 binding and entry. Recent research has shown that CCR5-tropic HIV is sexually transmitted more frequently than CXCR4-tropic virus.^{32–34} Epithelial disruption caused by, for example, genital ulcerative disease such as HSV,³⁵ dry sex,³⁶ or trauma^{37,38} therefore increases susceptibility to HIV-1.³⁹ The formulation of microbicide candidates as a protective gel is common to the development of many agents and has the theoretical benefit of minimising mucosal breaks. Additionally, finding specific agents that block viral binding, entry, or viral replication have been other strategies.

The single layer of columnar epithelium lining the endocervix is vulnerable to disruption,⁴⁰ and the cervical transformation zone between the squamous and columnar cells contains many HIV target cells near the surface.⁴¹ The intact endocervix has the capacity to block infection of cell-associated and cell-free HIV and resists internalisation of viral particles, most likely because of a physical barrier created by cervical mucus. Additionally, antiviral proteins contained in the cervical mucus, such as secretory leucocyte protease inhibitor, and high levels of natural ligands to CXCR4 and CCR5 might block HIV-1 binding to local CD4+ cells.^{42,43} Mimicking or augmenting these natural ligands is a fourth modality under investigation.

Microbicide development strategies have also had to account for the ways in which differences in the vaginal and rectal lumen might affect their success. The rectal mucosa seems to be less protective against HIV-1 than the vaginal mucosa. It consists of one layer of columnar epithelium, and the subepithelial lamina propria contains many cell types to which HIV-1 typically binds.⁴⁴ Furthermore, rectal lymphoid follicles contain specialised M cells (microfold cells), which have been shown to bind and present HIV-1 to underlying lymphoid tissue.^{45,46} Finally, unlike the lumen of the vagina, which is ultimately circumscribed at one end, the rectal space, as part of the colon, is open-ended so that vulnerability above the rectal vault might require additional coverage.⁴ Because of the differences in the vaginal and rectal lumens, and the high rates of rectal transmission of HIV with unprotected anal intercourse, certain microbicide compounds are currently being assessed for rectal use in addition to vaginal use.

The evolving conception of mucosal, submucosal, and luminal vulnerability to HIV infection is informing a more targeted approach to microbicide development. Preclinical or non-clinical testing of microbicides before US Food and Drug Administration licensing now includes a battery of at least nine study types, which include: in-vitro assays; animal vaginal irritation tests; pharmacokinetic studies; genetic, general, and reproductive toxicity studies; safety pharmacology studies; carcinogenic studies; hypersensitivity/photosensitivity studies; and condom integrity studies.⁴ Clinical testing includes phase I and phase II dosing, safety and acceptability studies, penile tolerance studies, and phase III trials for efficacy. Each preclinical and clinical phase of testing has its own set of limitations and it remains unclear which set of tests will best predict safety and effectiveness. In this context, we describe the five broad classes of microbicides and some of their most important representative agents.

Microbicide classes and key compounds

Surfactants/membrane disruptors

Surfactants are the earliest compounds to have been clinically evaluated as topical microbicides. These agents disrupt membranes non-specifically, offering contraceptive properties and activity against a wide range of potential STI pathogens (table 1). Nonoxinol 9 (N-9; nonoxynol-9), an inexpensive and effective spermicide widely available in over-the-counter preparations, disrupts the HIV envelope and early in-vitro efficacy against HIV was initially quite promising.⁵⁸ N-9 was the first microbicide to be formally tested for efficacy in preventing HIV transmission.^{38,47} One of the two blinded, randomised controlled efficacy trials of N-9, which was done among 1292 HIV-negative female sex workers in Cameroon, showed no difference in the rate of HIV infection, but a higher incidence of genital ulcers was associated with N-9 use compared with placebo use.⁴⁷ The second efficacy trial, in 892 female sex workers in four countries, showed an association between N-9 and increased HIV seroincidence when N-9 was used more than three times per day.³⁸ Toxicity to vaginal mucosal tissue at the higher doses was suggested as a possible cause for increased transmission among frequent users. These disappointing results ended the development of N-9 as an anti-HIV microbicide. The experience with N-9 also led to a greater scrutiny of safety studies before the commencement of larger clinical trials.

C31G (Savvy, Cellegy Pharmaceuticals, Quakertown, PA, USA), consisting of cetyl betaine and myristamine oxide, has shown in-vitro safety and broad-spectrum activity against bacteria, including *C trachomatis*, HSV, and especially HIV.^{59–62} C31G had been tested clinically in at least three separate safety trials^{63–65} and was to be assessed in two placebo-controlled, double-blind, phase III clinical trials in Africa (Ghana and Nigeria). The Ghanaian trial was halted in November, 2005, for futility: the HIV seroincidence rate in the study population was lower than anticipated, making the ability to observe statistically significant results highly improbable without doubling the sample size—a costly proposition declined by investigators.⁴⁸ The Nigerian trial was stopped for similar reasons in August, 2006. Analysis of the data from the 2153 participants with 12 months' follow-up found a trend toward higher HIV seroincidence in the C31G users compared with the placebo (hydroxyethylcellulose [HEC]) users, but this trend was not significant.⁴⁹

Sodium lauryl sulfate (Invisible Condom, Université Laval, Quebec, Canada) is a third surfactant compound that has been shown to disrupt both non-enveloped and enveloped viruses.⁶⁶ This agent has been formulated to act as an “invisible condom” in that it can cover the vaginal wall as a liquid at room temperature, and then transform into a gel at body temperature. In this form it can block HIV-1 and STI transmission.^{67,68} Safety of sodium lauryl sulfate has been shown in a rabbit model and in at least two phase I clinical trials.^{68–70} Although results of a phase II study of 200 women in Cameroon are pending (table 1), interest in the development of agents with more virus-specific mechanisms of action continues to progress.

Vaginal milieu protectors

The second broad class of microbicides in development, vaginal milieu protectors, works to maintain, restore, or enhance the natural protective mechanisms within the vaginal canal—the acidic pH maintained by lactobacilli (table 1). A pH between 4.0 and 5.8 has been shown to inactivate HIV.^{71–73} However, a variety of situations, including the presence of semen or bacterial vaginosis, neutralise the baseline acidity of the vagina. The microbicide compounds in this class either operate as direct acidifying agents or as enhancers of lactobacilli production.

Carbopol 974P (BufferGel, ReProtect, Baltimore, MD, USA) is a polyacrylic acid that buffers twice its volume of semen to a pH of 5 or less.⁷⁴ BufferGel has been shown to be spermicidal,

⁷⁴ virucidal in vitro to HIV⁷² and HSV,²⁶ and protective in mouse vaginal models against HSV and *C trachomatis*.⁷⁵ The gel also inhibits human papillomavirus (HPV) in animal models.⁷⁶ BufferGel was found to be safe in two phase I trials.^{77,78} One important ancillary finding of phase I testing was a decline in the prevalence of bacterial vaginosis, reported in 27 (30%) of 90 participants at enrolment and five (6%) of 90 participants after the first week of product use.⁷⁷ BufferGel was safe and acceptable among men in a penile tolerance study in HIV-infected and uninfected men.⁷⁹ A phase II/IIb trial, HPTN 035, is assessing the safety and effectiveness of BufferGel compared with a placebo gel and with condoms and has completed enrolment of 3101 participants in five countries (Malawi, South Africa, USA, Zambia, and Zimbabwe). HPTN 035 will also assess the safety and effectiveness of PRO2000, an HIV entry inhibitor.

Acidform (Amphora, Instead Inc, Dallas, TX, USA) is currently approved as a sexual lubricant gel, but its acid-buffering and bioadhesive properties make it appealing for development as a candidate microbicide. Acidform has undergone two phase I safety studies, as well as a male penile tolerance study.^{80–82} The first phase I study assessed Acidform alone and in combination with N-9. Acidform was well tolerated when used alone, but produced vaginal irritation when combined with N-9.⁸⁰ A second study, done in Brazil, assessed the safety of Acidform used 30 min or 8–10 h before intercourse in 20 women. Mild to moderate vulvar irritation was reported in five colposcopies completed 3 h after intercourse.⁸¹ 36 men participated in a penile tolerance study of Acidform versus K-Y Jelly (Personal Product Co, Skillman, NJ, USA) lubricant.⁸² The Acidform group had fewer genital symptoms (including itching, tingling, burning, and dryness) and both groups had similar rates of genital examination findings (including erythema, ulceration, and vesicles; 8% for each group), which were considered mild. An efficacy trial in Madagascar, testing Acidform's ability to prevent *Neisseria gonorrhoeae* and *C trachomatis*, is currently being planned.

A more recent “probiotic” strategy being developed to protect the vaginal milieu is the use of exogenous lactobacilli for colonisation since lactobacillus colonisation has been shown to correlate with decreased HIV proliferation.^{28,29} Colonisation of macaque vaginal canals was safely achieved with *Lactobacillus crispatus* in one study and a pilot investigation of nine women also showed a 60% colonisation rate.^{83,84} Bioengineered lactobacilli (or “live microbicides”) are also being developed to express proteins that bind to HIV and block either viral—host cell fusion or viral entry into host cells. Three proteins expressed through this type of system are CD4,⁸⁵ a derivative of gp41,⁸⁶ and cyanovirin.⁸⁷ These live microbicides are all in preclinical development. Finally, in certain societies, naturally occurring acidic compounds such as lime juice have been applied with limited effect.⁴ Recent clinical trials evaluating lime juice have shown toxicity.⁸⁸

Entry inhibitors

Viral entry inhibitors form a third broad class of microbicide agents and bind sequences that block either the attachment of HIV-1 to host cells, the fusion of virus and host-cell membranes, or the entry of HIV-1 into host cells (table 1).

Anionic polymers—The first group of viral entry inhibitors to be investigated were anionic polymers.⁸⁹ Through their negative charge, anionic polymers interact with HIV's viral envelope proteins and interfere with the attachment of HIV to CD4+ cells.^{90,91} The greater net positive charge on the gp120 protein of CXCR4-tropic viruses makes them particularly vulnerable to these compounds, but this is not always as reliably the case for CCR5-tropic viruses. For example, dextrin sulfate reduced in-vitro cell infectivity of a CXCR4 virus (HIV-1 HSBc2) by 77%, but did not reduce infectivity of an CCR5 virus (HIV-1 JRCSF).⁹²

Naphthalene sulfonate (PRO2000; Indevus Pharmaceuticals, Lexington, MA, USA), is a sulfonated polymer with in-vitro activity against HIV, *C trachomatis*, *N gonorrhoeae*, and HSV.^{93,94} Phase I clinical trials in Europe,⁹⁵ the USA, and South Africa⁹⁶ showed that PRO2000 was generally well tolerated; however, at the highest concentrations tested (4%), it was associated with a slightly higher incidence of intermenstrual bleeding compared with placebo.⁹⁷ Clinical investigation continues with both a phase II/IIb safety and efficacy study of 3101 participants (HPTN 035), and a phase III efficacy trial (MDP-301). The HPTN 035 trial randomised participants to one of four arms: 0.5% PRO2000, BufferGel, a placebo gel, or a condom; results are expected in early 2009. The 2% PRO2000 arm in the MDP-301 trial was closed early in 2008 because interim results indicated futility; the 0.5% arm continues and will be evaluated for efficacy versus placebo. Target recruitment for the MDP-301 study is 9673 women; as of July, 2008, 9395 women had been enrolled and the trial will be completed in late 2009.

Carrageenan (Carraguard/R515, Population Council, New York, NY, USA) is a sulfated polysaccharide derived from a seaweed extract. In addition to blocking HIV-1 transmission by binding the HIV-1 envelope, Carraguard has been found to prevent HIV-infected mononuclear cells from migrating across vaginal epithelia to pelvic lymph nodes in mouse models.⁹⁸ Phase I safety trials of Carraguard and similar carrageenan-based formulations in 1999,⁹⁹ and more recently in 2006,¹⁰⁰ showed safety in HIV-negative men and women. An additional phase I trial in South Africa showed that Carraguard was safe in HIV-positive men and women.¹⁰¹ Two phase II studies involving 565 women in South Africa and Thailand also demonstrated safety.^{102,103} A placebo-controlled phase III study of 6202 HIV-negative, non-pregnant women enrolled at three sites in South Africa completed data collection in March, 2007. Results released in February, 2008, found that although Carraguard gel was safe when used over a 2-year period, incident HIV infections occurred at a similar rate in the Carraguard and placebo groups (134 new infections for an incidence of 3.3 infections per 100 woman-years in the Carraguard group and 151 new infections for an incidence of 3.7 per 100 woman-years in the placebo group).^{51,104} Although there was a trend towards fewer HIV infections in the Carraguard group, an applicator dye test¹⁰⁵ indicated that gel was used in less than 50% of sex acts,⁵¹ raising major questions about whether poor adherence contributed to the lack of efficacy found in the trial.

Cellulose sulfate (Ushercell, Polydex Pharmaceuticals, Toronto, ON, Canada and Topical Prevention of Conception and Disease [TOPCAD], Chicago, IL, USA) is a compound that has shown in-vitro activity against *N gonorrhoeae*, *C trachomatis*, HPV, and *Gardnerella vaginalis*.^{106–109} Cellulose sulfate acts by binding the V3 loop of the gp120 HIV-1 envelope, and it can inhibit both CXCR4 and CCR5-tropic virus types.¹¹⁰ Phase I safety studies of cellulose sulfate, which involved at least 518 women and 48 men in the cellulose sulfate arms, found the gel to be safe.^{111–116} Two phase III efficacy trials of cellulose sulfate versus placebo were initiated in Africa and India, but both studies were halted in 2007 after interim analysis in one of the studies showed a higher HIV seroincidence than expected in the cellulose sulfate arm. Of 1425 women enrolled (717 in the cellulose sulfate arm and 708 in the placebo arm), there were 25 seroconversions in the cellulose sulfate arm compared with 16 seroconversions in the placebo arm.⁵² The second phase III study was halted as a precaution because of safety concerns arising from the first trial, despite the fact that an interim analysis had indicated no effect on the risk of HIV transmission.⁵³

An additional anionic polymer under investigation as a microbicide is cellulose acetate phthalate (CAP). This compound, which blocks gp120 binding sites, has shown in-vitro activity against HIV-1 and HSV (types 1 and 2).¹¹⁷ Like cellulose sulfate, CAP has the ability in tissue explant and animal models to block CXCR4 and CCR5-tropic virus types,^{118,119} and its preclinical evaluation to date shows minimal induction of inflammatory change.¹²⁰ CAP is

being developed as both a film and a micronised gel. In addition to blocking gp120 binding sites, the micronised form of CAP provides an acidic environment, which was shown in one study to cause disintegration and loss of infectivity of HIV-1.¹²¹ A recent phase I CAP trial of a 13% gel was halted because of the occurrence of heavy vaginal discharge in all five participants, a side-effect attributed to the hyperosmolarity of the glycerol-based formulation.⁵⁴

Panel 1: Key epidemiological issues in microbicide development

Seroincidence

Phase III efficacy trials need to be held in settings where HIV seroincidence is high (typically at least 2% per year)

- Even with an HIV seroincidence rate of 2% per year, the study group must be large —eg, over 2000 participants per study arm
- The cost of large trials can be prohibitive, with the estimated cost ranging from US\$46 to 70 million for a phase III microbicide trial^{164,165}
- “Hawthorne” effect: participation in a clinical trial itself, regardless of the study product, might affect the outcome.¹⁶⁶ In the context of HIV prevention trials, attention from study staff, HIV prevention counselling, and access to free condoms decreases HIV seroincidence among participants. The study group must therefore be even larger to detect a significant difference between study product and control

Study populations

Most microbicide trials enrol healthy HIV-uninfected women over 18 years of age who stop using study product if they become pregnant

- HIV-infected women, pregnant women, breastfeeding women, and adolescents all need to be included in study populations, since all are likely to use vaginal microbicides, either intentionally or inadvertently
- Trials in such groups should occur after initial trials show efficacy but before widespread product availability, which poses a challenge
- Any microbicide approved for vaginal use will likely be used rectally by both men and women. It remains to be seen what, if any, are the key safety and efficacy differences between vaginal and rectal use of microbicides

Covert use of microbicides

Vaginal microbicides might offer women control over HIV prevention, including through covert use.¹⁶⁷ Such covert use raises ethical and pragmatic challenges

- Covert use could bring increased mistrust and even physical danger
- Covert use would expose male partners to microbicide products without knowledge or consent
- Some challenge the very idea that covert use would be empowering for women, or the likelihood that women would use microbicides covertly at all¹⁶⁸

The newest category of anionic polymers are dendrimers. These macromolecules contain a central core, interior branches, and terminal surface groups adapted to specific targets. Because of their size and multiple terminal surface groups, they possess the ability to bind to multiple locations on multiple cells. The first dendrimer to be formulated as a microbicide gel and tested clinically, SPL7013 (Vivagel, Starpharma Holdings Ltd, Melbourne, Australia), provided

protection from chimeric simian/human immuno deficiency virus (SHIV) in a macaque model and from HSV2 in two different animal models.^{55,56} SPL7013 has been tested for male tolerance in one Australian phase I safety trial,⁵⁷ and a 3% formulation is undergoing further phase I trials in Kenya and the USA.

CCR5 inhibitors—A second set of entry inhibitors under investigation as topical microbicides for the prevention of HIV transmission are CCR5 inhibitors (table 2). CCR5 is the most important co-receptor for macrophage-tropic viral strains, which can predominate in the early stages of viral transmission.¹²⁶ PSC-RANTES, a potent synthetic inhibitor of the CCR5 co-receptor, exhibits in-vitro antiviral activity against all HIV clades and inhibits HIV-1 infection of Langerhans cells—crucial cells for HIV-1 transmission across the vaginal epithelium.^{127–129} Complete protection from SHIV SF162 was seen in macaques who received the highest dose of PSC-RANTES (1 mmol) tested, with no evidence of systemic absorption or toxicity.¹²²

A second CCR5 receptor antagonist, CMPD167, a cyclopentane-based compound formulated as a 5 mmol vaginal gel, provided protection from vaginal SHIV challenge in eight of ten macaques.¹²³ CMPD167 has been assessed in combination with two peptides that block the viral—host cell interaction at different loci, BMS-378806 and C52-L. BMS-378806 binds viral gp120 and prevents attachment to the CD4 and CCR5 receptors,^{130,131} whereas C52-L, a modified version of enfuvirtide, inhibits gp41-mediated viral—cell fusion.^{123,132} Using two of these agents in combination protected 16 out of 20 macaques from SHIV, and using all three inhibitors together protected all animals tested. There was no evidence of genital irritation or inflammation from these three compounds by colposcopy or biopsy.¹²³ Although these animal studies evaluating combinations of compounds with different mechanisms are promising, it is not yet clear whether they will correlate with protection from HIV in human trials. Additionally, study investigators noted that the concentrations of all three compounds necessary for consistent protection (1–10 mmol) were substantially higher than the in-vitro inhibitory concentrations that were required (1–10 nmol). One reason for the need for higher in-vivo concentrations could be the difficulty in shielding all exposed vaginal surfaces from a high titre viral inoculum, which would be particularly important during sexual encounters with partners who have primary infection.¹²³

An important challenge in considering the CCR5 inhibitors for use as topical microbicides is their inability to block the entry of CXCR4-tropic virus. Although this latter pathway is less important in sexual transmission, it might still have a role. Another concern is the pressure that CCR5-inhibiting compounds might place on HIV-1 to shift toward the use of non-CCR5 pathways/ co-receptors to gain entry into cells. A clinically effective microbicide most likely will need to block all modes of receptor-mediated entry.

Fusion inhibitors—In addition to C52-L, which inhibits gp41-mediated viral—cell fusion,^{123,132} another fusion inhibitor that has undergone early clinical testing as a topical microbicide is cyanovirin-N, a lectin compound purified from cyanobacterium. This compound prevents viral—host cell fusion by binding high mannose residues in the HIV envelope.^{133,134} Cyanovirin-N blocks transmission of SHIV 89.6P both vaginally and rectally in a macaque model, and the compound has also demonstrated efficacy in human cervical explants.^{135,136} However, some lectins have shown unwanted side-effects, such as human red blood cell agglutination, mitogenic stimulation of peripheral blood mononuclear cells, inflammatory activity, and cellular toxicity.¹³⁷ Various formulations of cyanovirin-N, including those expressed by lactobacilli, are under development.¹³⁸

Reverse transcriptase inhibitors

With the success of antiretroviral therapy in the treatment of HIV disease, as well as in the prevention of mother-to-child HIV transmission, interest has grown in using these more targeted drugs for prevention of the sexual transmission of HIV. Relying on compounds that interact with specific viral or cellular receptors, such as the CCR5 inhibitors and fusion inhibitors previously described, offers a more tailored approach than earlier microbicide formulations, with the promise of less toxicity and greater efficacy. The use of such targeted topical compounds has also been suggested as an adjunct strategy in preventing mother-to-child transmission of HIV.¹³⁹

Reverse transcriptase inhibitors bind the HIV-1 reverse transcriptase enzyme and block the conversion of viral RNA into DNA—effectively halting viral replication (table 2). The nucleotide reverse transcriptase inhibitor tenofovir was the first antiretroviral drug to safely demonstrate in animal models both pre-exposure and post-exposure prophylaxis as proof-of-concept against the sexual transmission of HIV.¹⁴⁰ Unlike nucleoside analogues, tenofovir is active as a diphosphate, rather than a triphosphate, and does not act via HIV DNA chain termination. Both of these reasons, coupled with the limited phosphorylation ability of macrophages, explain why the drug might be effective in macrophages and other non-dividing cells.^{141,142} Formulated as a diphosphate, tenofovir has a prolonged intracellular half-life of 9–50 h, depending on cell type.¹⁴³ In 1995, Tsai and colleagues¹⁴⁰ gave tenofovir to macaques before or after intravenous simian immunodeficiency virus (SIV) inoculation and continued treatment for 4 weeks after exposure. None of the treated animals became infected, whereas all ten controls did. Additional studies using vaginal inoculation of macaques with HIV-2 showed transient HIV-2 RNA in early cervicovaginal lavage specimens from five of 12 animals who received tenofovir from 12 to 72 h after the last viral inoculation.¹⁴⁴ Breakthrough systemic infection was noted in one of four animals initially treated 72 h after HIV-2 inoculation. Tsai and colleagues'¹⁴⁰ initial finding of protection against infection in animals treated with tenofovir up to 48 h before inoculation with SIV suggested that pre-exposure prophylaxis with tenofovir in human beings might successfully prevent HIV infection.

Panel 2: Key biological issues in microbicide development

Placebo gels

It is challenging to find a placebo gel identical in appearance, consistency, and odour to the microbicide under investigation

- At least five different gels* have been used in trials, some more as a comparator product than as placebo
- HEC, with no known anti-HIV effect and no buffering properties, has been proposed as a “universal” placebo for vaginal microbicide gel studies to facilitate comparison across studies.^{169,170} The HPTN 035 trial may establish whether HEC is an inert placebo
- Even an inert gel will form a physical barrier over the vaginal mucosa, and the added lubrication makes it less likely that microabrasions will occur during coitus. Both of these properties might reduce HIV transmission

Condom-only arms

Some researchers and regulatory officials endorse a second control arm consisting of no treatment—ie, condom-use only

- Since there is no gel involved in a condom-only arm, it is necessarily unblinded
- Retention of participants in the condom-only arm could be more challenging

- The no-treatment group might be more adherent to condom use than the other study groups

Delivery of microbicides

Microbicides are being formulated in a variety of delivery mechanisms, including gels/creams, rings, tablets, foams, and films.¹⁷¹ Mode of delivery will affect adherence. Considerations in the choice of delivery mode include:

- Leakage, ease of use, potential for covert application, general acceptability, and cost
- Changes in the physiology of the cervix or vagina with age, as well as menstruation
- The physical properties of the microbicide itself

Drug resistance

ART resistance mutations could occur and be passed on by women not aware of their HIV-seropositive status

- The low concentrations of systemic tenofovir seen in the HPTN 050 and HPTN 059 trials suggest that the development of viral resistance is plausible^{124,147}
- It is unclear whether low levels of systemic absorption are necessary for efficacy and whether the systemic levels seen would induce mutations
- More detailed attention to drug resistance and systemic absorption, both of parent drug and metabolites, is needed in future trials⁴

Combinations of prevention strategies

Combinations of microbicide agents with different mechanisms of action may be the best approach to the use of microbicides for HIV prevention

- Combining agents might increase activity across viral subtypes, reduce the development of HIV resistance, and prevent other STIs
- In human beings, safety and efficacy trials of microbicide combinations will require the demonstration of safety and efficacy of each single agent first
- As with HIV therapeutic trials, concurrent trials assessing combinations of agents will be necessary to advance this field of research

ART=antiretroviral therapy. HEC=hydroxyethylcellulose. STIs=sexually transmitted infections. *K-Y Jelly (Personal Product Co, Skillman, NJ, USA), Conceptrol (Personal Products Co, Skillman, NJ, USA), RePlens (LDS Consumer Products, Cedar Rapids, IA, USA), HEC, and methylcellulose.

Based on these animal studies, and with an appreciation for tenofovir's relatively high barrier to resistance compared with other reverse transcriptase inhibitors,¹⁴⁵ the compound became the first antiretroviral drug to be assessed as a vaginal microbicide in a clinical trial. In a phase I study (HPTN 050), 0.3% and 1% vaginal tenofovir gel, formulated as a diphosphate, was used once or twice daily for 14 days by HIV-infected and uninfected women. The gel was found to be safe, well tolerated, and acceptable to participants.¹²⁴ A pharmacokinetic substudy found 14 (56%) of 25 women had low but detectable serum tenofovir levels, with a median C_{max} of 3.4 ng/mL (range 3.0–25.8 ng/mL) and no clear dose-concentration relation. By contrast, the tenofovir concentration associated with the median steady-state 24 h post-dose blood concentration following an oral 300 mg tenofovir dose is approximately 47 ng/mL.¹⁴⁶ Study investigators also assessed the tenofovir gel's capacity to induce resistance mutations. Plasma and cervicovaginal lavage specimens were obtained from 22 of the HIV-positive

women in this study. Genotyping from all specimens with sufficient quantities of HIV RNA showed that none of the samples contained either the K65R or 69SS mutations. Low-level tenofovir resistance mutations (M41L, L210M, and T215I/Y) were detected in the plasma of three women at baseline who were taking nucleoside reverse transcriptase inhibitors at enrolment, and these mutations were unchanged after 2 weeks of tenofovir exposure.¹²⁴ It is not known whether low levels of absorption may be necessary for protection from HIV transmission nor whether longer periods of exposure would result in the induction of resistance mutations.

The preliminary results of a larger, phase II expanded safety trial of tenofovir vaginal gel, done in India and the USA among 200 sexually active HIV-negative women (HPTN 059) found the gel to be safe when applied daily or before each act of sex over a 6-month period.¹⁴⁷ Adherence to gel use between the daily-use and pre-coital groups was found to be similar (83% of 99 women in the daily-use arm reported study gel use in the previous 24 h compared with 80% of 101 women in the pre-coital arm). The two most commonly cited reasons women gave for not using gel were menstruation (41%) and forgetting (23%). Preliminary pharmacokinetic data again indicated low levels of systemic tenofovir absorption in many of the women. An additional pharmacokinetic study by Schwartz and colleagues¹⁴⁸ found tenofovir concentrations in vaginal tissue and vaginal fluid to be higher than blood plasma concentrations after a single 4 g dose, suggesting that tenofovir gel can be administered well in advance of coitus. A phase IIb trial of 1% tenofovir gel in South Africa (CAPRISA 004) with a planned enrolment of 980 women is ongoing, and an efficacy trial currently in development (the Vaginal and Oral Interventions to Control the Epidemic, or VOICE study) will compare oral pre-exposure prophylaxis (tenofovir or a combination of tenofovir plus emtricitabine) with topical pre-exposure prophylaxis (tenofovir gel).

Two non-nucleoside reverse transcriptase inhibitors (NNRTIs), TMC120 and UC781, have proceeded to preclinical or clinical testing as potential topical microbicides and have several features in common: unlike first-generation NNRTIs that only require one mutation before viral resistance occurs, TMC120 and UC781 usually require at least two mutations.^{149–151} Both compounds show minimal systemic absorption, and both had good safety profiles in animal studies.^{152,153} In vitro, TMC120 and UC781 prevent cell-free and cell-associated virus from infecting co-cultures of monocyte-derived dendritic cells and T cells.^{154–156}

TMC120 (4-[[4-[(2,4,6-trimethylphenyl)amino]pyrimidin-2-yl]amino]benzenecarbonitrile), a diarylpyrimidine, was the first NNRTI shown to have in-vivo effectiveness as a topical microbicide. By use of a severe combined immunodeficient mouse model, Di Fabio and colleagues¹⁵³ showed that the most viscous TMC120 gel formulation provided 70–80% protection, while a less viscous formulation protected 100% of mice, whether they were inoculated with CCR5-tropic or CXCR4-tropic viral strains. It was concluded that the thicker gel might have been unevenly distributed across the vaginal mucosa. TMC120 is now being tested in several phase I and II trials. One formulation employs a slow release vaginal ring, which would allow for once monthly, non-coitally dependent dosing.

The thiocarboxanilide UC781 (N-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-furancarbothio-amide), an NNRTI with poor oral bioavailability, showed promise as a vaginal microbicide in rabbit safety studies¹⁵² and in its ability to block cell-free and cell-associated HIV-1 transmission in a human cervical tissue-based organ culture.¹⁵⁷ The latter findings were recently corroborated in a cervical explant model.¹⁵⁴ UC781 has shown decreased activity against NNRTI-resistant HIV-1 virus at some concentrations, although its activity against wildtype and NNRTI-resistant virus at low concentrations (below current microbicide formulations of 25 µmol) has been similar.¹⁵⁸ A recent phase I trial of UC781 demonstrated safety after 6 days of once-daily dosing.¹²⁵ Additional phase I trials are underway.

Search strategy and selection criteria

We undertook a search for English-language preclinical and clinical trials of microbicides, with a focus on recent clinical trials. We used Medline, OVID, PubMed, the Cochrane Library, and systematic reviews to identify trials from 1966 to 2008 and reviewed abstracts from the major meetings in infectious diseases and microbicides during 2004–08. In addition to “microbicides”, we used other terms (eg, “HIV”, “topical”, “vaginal”, “rectal”, as well as specific compound names) to identify sources. 118 studies were identified: 73 preclinical and 45 clinical. Meta-analysis was not done.

Unknown mechanism agents

Several microbicides in development have shown anti-HIV-1 activity, but their mechanism of action remains unknown. The most clinically advanced of these compounds is Praneem (Panacea Biotech Ltd, New Delhi, India)—a combination of extracts originally developed as a spermicide from the Indian neem tree (*Azadirachta indica*), saponins from *Sapindus mukorossi* trees, and menthe citrate oil.¹⁵⁹ Praneem has shown wide-spectrum antimicrobial activity against reproductive tract infections, and has also shown antiretroviral properties.¹⁵⁹ Praneem has undergone phase I and II safety and acceptability studies.^{160–162}

Challenges in microbicide development

This year, the Institute of Medicine issued its first comprehensive report addressing methodological challenges in non-vaccine biomedical HIV prevention trials.¹⁶³ The key epidemiological and biological issues in microbicide development are shown in panel 1 and panel 2. As with HIV therapeutic trials, it is likely that combinations of microbicide agents with different mechanisms of action will be more successful than single agents, and this strategy is gaining momentum. Efficacy trials, however, with HIV seroincidence as an endpoint, need to be large and are expensive to fund. The effect of concomitant sexually transmitted diseases, such as HSV and other ulcerative lesions, on a microbicide’s ability to effectively prevent HIV also remains unknown. Despite these challenges, more than 20 000 HIV-uninfected sexually active women are scheduled to participate in phase II and phase III microbicide studies from 2007 through 2009.

Conclusions

The increasingly specific targets used to develop topical microbicides to prevent HIV infection is a reflection of the advances that have been made in understanding the pathophysiology of HIV sexual transmission. New classes of targeted therapeutic agents, such as integrase inhibitors, are also beginning to move into preclinical investigation.¹⁷² It is clear that the development of a topical microbicide to prevent the sexual transmission of HIV is scientifically, ethically, and culturally complicated. However, the benefit in lives saved may far exceed those risks seen and, as yet, unforeseen.

Acknowledgments

We thank Roberta Black and Wafaa El-Sadr for their helpful comments. This work was funded by US National Institutes of Health grants AI 48016, 5U01 AI048016, and 5T32 AI049821.

References

1. UNAIDS, WHO. AIDS epidemic update 2007. UNAIDS/World Health Organization; Geneva, Switzerland: [accessed Sept 10, 2008].

2. UNAIDS, WHO. AIDS epidemic update 2004. UNAIDS/World Health Organization; Geneva, Switzerland: [accessed Sept 10, 2008].
3. Walker PR, Worobey M, Rambaut A, Holmes EC, Pybus OG. Epidemiology: sexual transmission of HIV in Africa. *Nature* 2003;422:679. [PubMed: 12700750]
4. McGowan I. Microbicides: a new frontier in HIV prevention. *Biologicals* 2006;34:241–55. [PubMed: 17097303]
5. DeGruttola V, Seage GR, Mayer KH, Horsburgh CR. Infectiousness of HIV between homosexual partners. *J Clin Epidemiol* 1989;42:849–56. [PubMed: 2789269]
6. Peterman TA, Curran JW. Sexual transmission of human immunodeficiency virus. *JAMA* 1986;256:2222–26. [PubMed: 3531561]
7. Padian NS, Shiboski SC, Jewell NP. Female-to-male transmission of human immunodeficiency virus. *JAMA* 1991;266:1664–67. [PubMed: 1886189]
8. Downs AM, De Vincenzi I. Probability of heterosexual transmission of HIV: relationship to the number of unprotected sexual contacts. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;11:388–95. [PubMed: 8601226]
9. Chakraborty H, Sen PK, Helms RW, et al. Viral burden in genital secretions determines male-to-female sexual transmission of HIV-1: a probabilistic empiric model. *AIDS* 2001;15:621–27. [PubMed: 11317000]
10. Vittinghoff E, Douglas J, Judson F, McKirnan D, MacQueen K, Buchbinder SP. Per-contact risk of human immunodeficiency virus transmission between male sexual partners. *Am J Epidemiol* 1999;150:306–11. [PubMed: 10430236]
11. Wilson TE, Massad LS, Riestler KA, et al. Sexual, contraceptive, and drug use behaviors of women with HIV and those at high risk for infection: results from the Women's Interagency HIV Study. *AIDS* 1999;13:591–598. [PubMed: 10203384]
12. Mosher WD, Chandra A, Jones J. Sexual behavior and selected health measures: men and women 15–44 years of age, United States. Report No 362. 2002National Center for Health StatisticsHyattsville, MD
13. Powers KA, Poole C, Pettifor AE, Cohen MS. Rethinking the heterosexual infectivity of HIV-1: a systematic review and meta-analysis. *Lancet Infect Dis* 2008;8:553–63. [PubMed: 18684670]
14. Pilcher CD, Tien HC, Eron JJ, et al. Brief but efficient: acute HIV infection and the sexual transmission of HIV. *J Infect Dis* 2004;189:1785–92. [PubMed: 15122514]
15. Wawer MJ, Gray RH, Sewankambo NK, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. *J Infect Dis* 2005;191:1403–09. [PubMed: 15809897]
16. Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, Puren A. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. *PLoS Med* 2005;2:e298. [PubMed: 16231970]
17. Bailey RC, Moses S, Parker CB, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *Lancet* 2007;369:643–56. [PubMed: 17321310]
18. Gray RH, Kigozi G, Serwadda D, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. *Lancet* 2007;369:657–66. [PubMed: 17321311]
19. Soilleux EJ, Coleman N. Expression of DC-SIGN in human foreskin may facilitate sexual transmission of HIV. *J Clin Pathol* 2004;57:77–78. [PubMed: 14693841]
20. Shattock RJ, Griffin GE, Gorodeski GI. In vitro models of mucosal HIV transmission. *Nat Med* 2000;6:607–08. [PubMed: 10835650]
21. Hope, TJ. Latest understanding of HIV sexual transmission. *Microbicides 2008 Conference*; New Delhi, India. Feb 24–27, 2008;
22. Dezzutti CS, James VN, Ramos A, et al. In vitro comparison of topical microbicides for prevention of human immunodeficiency virus type 1 transmission. *Antimicrob Agents Chemother* 2004;48:3834–44. [PubMed: 15388443]
23. Miller CJ, Shattock RJ. Target cells in vaginal HIV transmission. *Microbes Infect* 2003;5:59–67. [PubMed: 12593974]
24. Hladik F, Sakchalathorn P, Ballweber L, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity* 2007;26:257–70. [PubMed: 17306567]

25. Sturm AW, Zanen HC. Characteristics of *Haemophilus ducreyi* in culture. *J Clin Microbiol* 1984;19:672–74. [PubMed: 6610690]
26. Croughan WS, Behbehani AM. Comparative study of inactivation of herpes simplex virus types 1 and 2 by commonly used antiseptic agents. *J Clin Microbiol* 1988;26:213–15. [PubMed: 2830306]
27. Yasin B, Pang M, Wagar EA, Lehrer RI. Examination of *Chlamydia trachomatis* infection in environments mimicking normal and abnormal vaginal pH. *Sex Transm Dis* 2002;29:514–19. [PubMed: 12218842]
28. Klebanoff SJ, Coombs RW. Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. *J Exp Med* 1991;174:289–92. [PubMed: 1647436]
29. Martin HL, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis* 1999;180:1863–68. [PubMed: 10558942]
30. Sewankambo N, Gray RH, Wawer MJ, et al. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. *Lancet* 1997;350:546–50. [PubMed: 9284776]
31. Taha TE, Hoover DR, Dallabetta GA, et al. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS* 1998;12:1699–706. [PubMed: 9764791]
32. Kish-Catalone TM, Lu W, Gallo RC, DeVico AL. Preclinical evaluation of synthetic -2 RANTES as a candidate vaginal microbicide to target CCR5. *Antimicrob Agents Chemother* 2006;50:1497–509. [PubMed: 16569870]
33. Muciaccia B, Padula F, Gandini L, Lenzi A, Stefanini M. HIV-1 chemokine co-receptor CCR5 is expressed on the surface of human spermatozoa. *AIDS* 2005;19:1424–26. [PubMed: 16103775]
34. Berlier W, Bourlet T, Lawrence P, et al. Selective sequestration of X4 isolates by human genital epithelial cells: implication for virus tropism selection process during sexual transmission of HIV. *J Med Virol* 2005;77:465–74. [PubMed: 16254974]
35. Strathdee SA, Hogg RS, O’Shaughnessy MV, Montaner JS, Schechter MT. A decade of research on the natural history of HIV infection: part 2. Cofactors. *Clin Invest Med* 1996;19:121–30. [PubMed: 8697671]
36. Baleta A. Concern voiced over “dry sex” practices in South Africa. *Lancet* 1998;352:1292. [PubMed: 9788473]
37. Norvell MK, Benrubi GI, Thompson RJ. Investigation of microtrauma after sexual intercourse. *J Reprod Med* 1984;29:269–71. [PubMed: 6716372]
38. Van Damme L, Ramjee G, Alary M, et al. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomized controlled trial. *Lancet* 2002;360:971–77. [PubMed: 12383665]
39. Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1 infection. *Nat Rev Microbiol* 2003;1:25–34. [PubMed: 15040177]
40. Greenhead P, Hayes P, Watts PS, Laing KG, Griffin GE, Shattock RJ. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. *J Virol* 2000;74:5577–86. [PubMed: 10823865]
41. Pudney J, Quayle AJ, Anderson DJ. Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. *Biol Reprod* 2005;73:1253–63. [PubMed: 16093359]
42. Moriyama A, Shimoya K, Ogata I, et al. Secretory leukocyte protease inhibitor (SLPI) concentrations in cervical mucus of women with normal menstrual cycle. *Mol Hum Reprod* 1999;5:656–61. [PubMed: 10381821]
43. Agace WW, Amara A, Roberts AI, et al. Constitutive expression of stromal derived factor-1 by mucosal epithelia and its role in HIV transmission and propagation. *Curr Biol* 2000;10:325–28. [PubMed: 10744978]
44. Poles MA, Elliot J, Taing P, Anton PA, Chen IS. A preponderance of CCR5(+) CXCR4(+) mononuclear cells enhances gastrointestinal mucosal susceptibility to human immunodeficiency virus type 1 infection. *J Virol* 2001;75:8390–99. [PubMed: 11507184]

45. Amerongen HM, Weltzin R, Farnet CM, Michetti P, Haseltine WA, Neutra MR. Transepithelial transport of HIV-1 by intestinal M cells: a mechanism for transmission of AIDS. *J Acquir Immune Defic Syndr* 1991;4:760–65. [PubMed: 1856788]
46. Neutra MR. Interactions of viruses and microparticles with apical plasma membranes of M cells: implications for human immunodeficiency virus transmission. *J Infect Dis* 1999;179(suppl 3):S441–43. [PubMed: 10099115]
47. Roddy RE, Zekeng L, Ryan KA, Tamoufe U, Weir SS, Wong EL. A controlled trial of nonoxynol-9 film to reduce male-to-female transmission of sexually transmitted diseases. *N Engl J Med* 1998;339:504–10. [PubMed: 9709043]
48. Lorenzo A. Cellegy stops Ghana HIV trial; broader plans moving ahead. *BioWorld Today*. 2005; (Nov 9)
49. Feldblum PJ, Adeiga A, Bakare R, et al. Savvy vaginal gel (C31G) for prevention of HIV infection: a randomized controlled trial in Nigeria. *PLoS One* 2008;3:e1474. [PubMed: 18213382]
50. Alliance for Microbicide Development. Microbicide candidates and ancillary devices in planned and funded clinical trials summary as of September 2008. <http://www.microbicide.org/galleries/clinical-trials/Microbicides.Planned.Funded.Clinical.Trials2Sept2008.pdf> [accessed Sept 28, 2008]. <http://www.microbicide.org/galleries/clinical-trials/Microbicides.Planned.Funded.Clinical.Trials2Sept2008.pdf>
51. Johansson, E. Results of phase III Carraguard trial. Microbicides 2008 Conference; New Delhi, India. Feb 24–27, 2008;
52. Van Damme L, Govinden R, Mirembe FM, et al. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. *N Engl J Med* 2008;359:463–72. [PubMed: 18669425]
53. Halpern V, Wang L, Obunge O, et al. Effectiveness of cellulose sulfate gel for prevention of HIV: results of the phase III trial in Nigeria. 4th IAS Conference Sydney, Australia July 22–25, 2007 Abstract LB WESS302.
54. Lacey C. Unacceptable side effects of a hyperosmolar vaginal microbicide in a Phase I trial. Microbicides 2008 Conference New Delhi, India Feb 24–27, 2008 Abstract B08-527.
55. Jiang YH, Emau P, Cairns JS, et al. SPL7013 gel as a topical microbicide for prevention of vaginal transmission of SHIV89.6P in macaques. *AIDS Res Hum Retroviruses* 2005;21:207–13. [PubMed: 15795526]
56. Bernstein DI, Stanberry LR, Sacks S, et al. Evaluations of unformulated and formulated dendrimer-based microbicide candidates in mouse and guinea pig models of genital herpes. *Antimicrob Agents Chemother* 2003;47:3784–88. [PubMed: 14638483]
57. Paull J, Chen M, Millwood I, et al. SPL7013 gel (VivaGel(TM)), a topical microbicide in development for prevention of HIV and genital herpes, shown to be well tolerated and comparable with placebo after seven days administration in healthy males. 4th IAS Conference Sydney, Australia July 22–25, 2007 Abstract TUAC1LB.
58. Bourinbaiar AS, Lee-Huang S. The efficacy of nonoxynol-9 from an in vitro point of view. *AIDS* 1996;10:558–59. [PubMed: 8724057]
59. Calis S, Yulug N, Sumnu M, Ayhan A, Hincal AA. A non-antibiotic antimicrobial mixture (C31G): evaluation of the antimicrobial efficiency of C31G on vaginal cultures. *Boll Chim Farm* 1992;131:335–38. [PubMed: 1492969]
60. Krebs FC, Miller SR, Malamud D, Howett MK, Wigdahl B. Inactivation of human immunodeficiency virus type 1 by nonoxynol-9, C31G, or an alkyl sulfate, sodium dodecyl sulfate. *Antiviral Res* 1999;43:157–73. [PubMed: 10551374]
61. Wyrick PB, Knight ST, Gerbig DG, et al. The microbicidal agent C31G inhibits *Chlamydia trachomatis* infectivity in vitro. *Antimicrob Agents Chemother* 1997;41:1335–44. [PubMed: 9174195]
62. Thompson KA, Malamud D, Storey BT. Assessment of the antimicrobial agent C31G as a spermicide: comparison with nonoxynol-9. *Contraception* 1996;53:313–18. [PubMed: 8724622]
63. Bax R, Douville K, McCormick D, Rosenberg M, Higgins J, Bowden M. Microbicides—evaluating multiple formulations of C31G. *Contraception* 2002;66:365–68. [PubMed: 12443968]
64. Ballagh SA, Baker JM, Henry DM, Archer DF. Safety of single daily use for one week of C31G HEC gel in women. *Contraception* 2002;66:369–75. [PubMed: 12443969]

65. Mauck CK, Weiner DH, Creinin MD, Barnhart KT, Callahan MM, Bax R. A randomized phase I vaginal safety study of three concentrations of C31G vs extra strength gynol II. *Contraception* 2004;70:233–40. [PubMed: 15325893]
66. Piret J, Desormeaux A, Bergeron MG. Sodium lauryl sulfate, a microbicide effective against enveloped and nonenveloped viruses. *Curr Drug Targets* 2002;3:17–30. [PubMed: 11899262]
67. Haineault C, Gourde P, Perron S, et al. Thermoreversible gel formulation containing sodium lauryl sulfate as a potential contraceptive device. *Biol Reprod* 2003;69:687–94. [PubMed: 12724280]
68. Roy S, Gourde P, Piret J, et al. Thermoreversible gel formulations containing sodium lauryl sulfate or n-lauroylsarcosine as potential topical microbicides against sexually transmitted diseases. *Antimicrob Agents Chemother* 2001;45:1671–81. [PubMed: 11353610]
69. Trottier S, Omar RF, Desormeaux A, et al. Phase I clinical trial to evaluate the safety, tolerance and acceptability of the invisible condom when applied intravaginally to healthy female subjects. XIV International AIDS Conference Barcelona, Spain July 7–12, 2002 Abstract LbPp2212.
70. Omar RF. Phase I/II randomized, double-blinded, placebo-controlled trials on the safety, tolerance and acceptability of the invisible condom, a physical and chemical barrier vaginal gel against HIV, in healthy volunteers in Cameroon. Microbicides 2008 Conference New Dehli, India Feb 24–27, 2008 Abstract B01-86.
71. Martin LS, McDougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. *J Infect Dis* 1985;152
72. Ongradi J, Ceccherini-Nelli L, Pistello M, Specter S, Bendinelli M. Acid sensitivity of cell-free and cell-associated HIV-1: clinical implications. *AIDS Res Hum Retroviruses* 1990;6:1433–36. [PubMed: 2078421]
73. O'Connor TJ, Kinchington D, Kangro HO, Jeffries DJ. The activity of candidate virucidal agents, low pH and genital secretions against HIV-1 in vitro. *Int J STD AIDS* 1995;6:267–72. [PubMed: 7548290]
74. Olmsted SS, Dubin NH, Cone RA, Moench TR. The rate at which human sperm are immobilized and killed by mild acidity. *Fertil Steril* 2000;73:687–93. [PubMed: 10731526]
75. Achilles SL, Shete PB, Whaley KJ, Moench TR, Cone RA. Microbicide efficacy and toxicity tests in a mouse model for vaginal transmission of Chlamydia trachomatis. *Sex Transm Dis* 2002;29:655–64. [PubMed: 12438901]
76. Zeitlin L, Hoen TE, Achilles SL, et al. Tests of BufferGel for contraception and prevention of sexually transmitted diseases in animal models. *Sex Transm Dis* 2001;28:417–23. [PubMed: 11460027]
77. Van de Wijgert JH, Fullem A, Kelly C, et al. Phase I trial of the topical microbicide BufferGel: safety results from four international sites. *J Acquir Immune Defic Syndr* 2001;26:21–27. [PubMed: 11176265]
78. Mayer KH, Peipert J, Fleming T, et al. Safety and tolerability of BufferGel, a novel vaginal microbicide, in women in the United States. *Clin Infect Dis* 2001;32:476–82. [PubMed: 11170957]
79. Tabet SR, Callahan MM, Mauck CK, et al. Safety and acceptability of penile application of 2 candidate topical microbicides: BufferGel and PRO 2000 gel: 3 randomized trials in healthy low-risk men and HIV-positive men. *J Acquir Immune Defic Syndr* 2003;33:476–83. [PubMed: 12869836]
80. Amaral E, Faundes A, Zaneveld L, Waller D, Garg S. Study of the vaginal tolerance to Acidform, an acid-buffering, bioadhesive gel. *Contraception* 1999;60:361–66. [PubMed: 10715372]
81. Amaral E, Perdigao A, Souza MH, et al. Vaginal safety after use of a bioadhesive, acid-buffering, microbicidal contraceptive gel (ACIDFORM) and a 2% nonoxynol-9 product. *Contraception* 2006;73:542–47. [PubMed: 16627043]
82. Schwartz JL, Poindexter A, Schmitz SW, Mauck C, Callahan MM. Male tolerance of ACIDFORM gel. *Contraception* 2005;71:443–46. [PubMed: 15914134]
83. Patton DL, Sweeney YT, Cosgrove, Antonio MA, Rabe LK, Hillier SL. Lactobacillus crispatus capsules: single-use safety study in the Macaca nemestrina model. *Sex Transm Dis* 2003;30:568–70. [PubMed: 12838085]
84. Antonio MA, Hillier SL. DNA fingerprinting of Lactobacillus crispatus strain CTV-05 by repetitive element sequence-based PCR analysis in a pilot study of vaginal colonization. *J Clin Microbiol* 2003;41:1881–87. [PubMed: 12734221]

85. Chang TL, Chang CH, Simpson DA, et al. Inhibition of HIV infectivity by a natural human isolate of *Lactobacillus jensenii* engineered to express functional two-domain CD4. *Proc Natl Acad Sci USA* 2003;100:11672–77. [PubMed: 12972635]
86. Rao S, Hu S, McHugh L, et al. Toward a live microbial microbicide for HIV: commensal bacteria secreting an HIV fusion inhibitor peptide. *Proc Natl Acad Sci USA* 2005;102:11993–98. [PubMed: 16040799]
87. Lagenaur LA, Berger EA. An anti-HIV microbicide comes alive. *Proc Natl Acad Sci USA* 2005;102:12294–95. [PubMed: 16118279]
88. Hemmerling A, Potts M, Walsh J, et al. Lime juice as a candidate microbicide? An open-label safety trial of 10% and 20% lime juice used vaginally. *J Womens Health* 2007;16:1041–51.
89. Balzarini J, Van Damme L. Microbicide drug candidates to prevent HIV infection. *Lancet* 2007;369:787–97. [PubMed: 17336656]
90. Schols D, Pauwels R, Desmyter J, De CE. Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T-cells persistently infected with HIV-1. *Virology* 1990;175:556–61. [PubMed: 1691563]
91. Mitsuya H, Looney DJ, Kuno S, Ueno R, Wong-Staal F, Broder S. Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. *Science* 1988;240:646–49. [PubMed: 2452480]
92. Moulard M, Lortat-Jacob H, Mondor I, et al. Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120. *J Virol* 2000;74:1948–60. [PubMed: 10644368]
93. Indevus Pharmaceuticals. PRO2000: product description. Indevus Pharmaceuticals; Lexington, NA, USA: 2006.
94. Keller MJ, Zerhouni-Layachi B, Cheshenko N, et al. PRO 2000 gel inhibits HIV and herpes simplex virus infection following vaginal application: a double-blind placebo-controlled trial. *J Infect Dis* 2006;193:27–35. [PubMed: 16323128]
95. 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;76:126–30. [PubMed: 10858715]
96. Mayer KH, Karim SA, Kelly C, et al. Safety and tolerability of vaginal PRO 2000 gel in sexually active HIV-uninfected and abstinent HIV-infected women. *AIDS* 2003;17:321–29. [PubMed: 12556685]
97. Joshi S, Dutta S, Bell B, et al. Documenting intermenstrual bleeding in a vaginal microbicide study: case reports and lessons learned. *AIDS Res Hum Retroviruses* 2006;22:294–96. [PubMed: 16545017]
98. Perotti ME, Pirovano A, Phillips DM. Carrageenan formulation prevents macrophage trafficking from vagina: implications for microbicide development. *Biol Reprod* 2003;69:933–39. [PubMed: 12773428]
99. 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;76:480–83. [PubMed: 11221133]
100. Bollen L, Blanchard K, Kilmarx PH, et al. No increase in cervicovaginal proinflammatory cytokines after Carraguard use in a placebo-controlled randomized clinical trial. *J Acquir Immune Defic Syndr* 2008;47:253–57. [PubMed: 18025996]
101. Van de Wijgert JH, Braunstein SL, Morar NS, et al. Carraguard vaginal gel safety in HIV-positive women and men in South Africa. *J Acquir Immune Defic Syndr* 2007;46:538–46. [PubMed: 18193495]
102. Kilmarx PH, van de Wijgert J, Chaikummao S, et al. Safety and acceptability of the candidate microbicide Carraguard in Thai women: findings from a phase II clinical trial. *J Acquir Immune Defic Syndr* 2006;43:327–34. [PubMed: 16980907]
103. Pistorius AG, van de Wijgert J, Sebola M, et al. Microbicide trials for preventing HIV/AIDS in South Africa: phase II trial participants' experiences and psychological needs. *SAHARA J* 2004;1:78–86. [PubMed: 17601013]
104. Population Council. Trial shows anti-HIV microbicide is safe, but does not prove it effective. 2008 http://www.popcouncil.org/mediacenter/newsreleases/Carraguard_Findings.html [accessed Sept 28, 2008]. http://www.popcouncil.org/mediacenter/newsreleases/Carraguard_Findings.html

105. Wallace AR, Teitelbaum A, Wan L, et al. Determining the feasibility of utilizing the microbicide applicator compliance assay for use in clinical trials. *Contraception* 2007;76:53–56. [PubMed: 17586138]
106. Anderson RA, Feathergill KA, Diao XH, et al. Preclinical evaluation of sodium cellulose sulfate (Ushercell) as a contraceptive antimicrobial agent. *J Androl* 2002;23:426–38. [PubMed: 12002445]
107. Su H, Caldwell HD. Sulfated polysaccharides and a synthetic sulfated polymer are potent inhibitors of *Chlamydia trachomatis* infectivity in vitro but lack protective efficacy in an in vivo murine model of chlamydial genital tract infection. *Infect Immun* 1998;66:1258–60. [PubMed: 9488423]
108. Christensen ND, Reed CA, Culp TD, et al. Papillomavirus microbicidal activities of high-molecular-weight cellulose sulfate, dextran sulfate, and polystyrene sulfonate. *Antimicrob Agents Chemother* 2001;45:3427–32. [PubMed: 11709319]
109. Simoes JA, Citron DM, Aroutcheva A, et al. Two novel vaginal microbicides (polystyrene sulfonate and cellulose sulfate) inhibit *Gardnerella vaginalis* and anaerobes commonly associated with bacterial vaginosis. *Antimicrob Agents Chemother* 2002;46:2692–95. [PubMed: 12121959]
110. Scordi-Bello IA, 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;49:3607–15. [PubMed: 16127029]
111. Mauck C, Weiner DH, Ballagh S, et al. Single and multiple exposure tolerance study of cellulose sulfate gel: a phase I safety and colposcopy study. *Contraception* 2001;64:383–91. [PubMed: 11834238]
112. El-Sadr WM, Mayer KH, Maslankowski L, et al. Safety and acceptability of cellulose sulfate as a vaginal microbicide in HIV-infected women. *AIDS* 2006;20:1109–16. [PubMed: 16691061]
113. Schwartz JL, Mauck C, Lai JJ, et al. Fourteen-day safety and acceptability study of 6% cellulose sulfate gel: a randomized double-blind phase I safety study. *Contraception* 2006;74:133–40. [PubMed: 16860051]
114. Jaspers V, Buve A, van Damme L. Safety trial of the vaginal microbicide cellulose sulfate gel in HIV-positive men. *Sex Transm Dis* 2007;34:519–22. [PubMed: 17297382]
115. Mauck C, Frezieres R, Walsh T, Robergeau K, Callahan M. Cellulose sulfate: tolerance and acceptability of penile application. *Contraception* 2001;64:377–81. [PubMed: 11834237]
116. Malonza IM, Mirembe F, Nakabiito C, et al. Expanded phase I safety and acceptability study of 6% cellulose sulfate vaginal gel. *AIDS* 2005;19:2157–63. [PubMed: 16284466]
117. Neurath AR, Strick N, Li YY. Water dispersible microbicidal cellulose acetate phthalate film. *BMC Infect Dis* 2003;3:27. [PubMed: 14617380]
118. Lu H, Zhao Q, Wallace G, et al. Cellulose acetate 1,2-benzenedicarboxylate inhibits infection by cell-free and cell-associated primary HIV-1 isolates. *AIDS Res Hum Retroviruses* 2006;22:411–18. [PubMed: 16706617]
119. Kawamura T, Cohen SS, Borris DL, et al. Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants. *J Exp Med* 2000;192:1491–500. [PubMed: 11085750]
120. Fichorova RN, Zhou F, Ratnam V, et al. Anti-human immunodeficiency virus type 1 microbicide cellulose acetate 1,2-benzenedicarboxylate in a human in vitro model of vaginal inflammation. *Antimicrob Agents Chemother* 2005;49:323–35. [PubMed: 15616312]
121. Neurath AR, Strick N, Li YY, Lin K, Jiang S. Design of a “microbicide” for prevention of sexually transmitted diseases using “inactive” pharmaceutical excipients. *Biologicals* 1999;27:11–21. [PubMed: 10441398]
122. Lederman MM, Veazey RS, Offord R, et al. Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5. *Science* 2004;306:485–87. [PubMed: 15486300]
123. Veazey RS, Klasse PJ, Schader SM, et al. Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. *Nature* 2005;438:99–102. [PubMed: 16258536]
124. Mayer KH, Maslankowski LA, Gai F, et al. Safety and tolerability of tenofovir vaginal gel in abstinent and sexually active HIV-infected and uninfected women. *AIDS* 2006;20:543–51. [PubMed: 16470118]

125. Schwartz JL, Kovalevsky G, Lai JJ, et al. A randomized six day safety study of an antiretroviral microbicide candidate UC-781, a nonnucleoside reverse transcriptase inhibitor. *Sex Transm Dis* 2008;35:414–19. [PubMed: 18362865]
126. Maeda K, Nakata H, Ogata H, Koh Y, Miyakawa T, Mitsuya H. The current status of, and challenges in, the development of CCR5 inhibitors as therapeutics for HIV-1 infection. *Curr Opin Pharmacol* 2004;4:447–52. [PubMed: 15351348]
127. Torre VS, Marozsan AJ, Albright JL, et al. Variable sensitivity of CCR5-tropic human immunodeficiency virus type 1 isolates to inhibition by RANTES analogs. *J Virol* 2000;74:4868–76. [PubMed: 10775626]
128. Kawamura T, Bruse SE, Abraha A, et al. PSC-RANTES blocks R5 human immunodeficiency virus infection of Langerhans cells isolated from individuals with a variety of CCR5 diplotypes. *J Virol* 2004;78:7602–09. [PubMed: 15220435]
129. Kawamura T, Gulden FO, Sugaya M, et al. R5 HIV productively infects Langerhans cells, and infection levels are regulated by compound CCR5 polymorphisms. *Proc Natl Acad Sci USA* 2003;100:8401–06. [PubMed: 12815099]
130. Lin PF, Blair W, Wang T, et al. A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding. *Proc Natl Acad Sci USA* 2003;100:11013–18. [PubMed: 12930892]
131. Guo Q, Ho HT, Dicker I, et al. Biochemical and genetic characterizations of a novel human immunodeficiency virus type 1 inhibitor that blocks gp120-CD4 interactions. *J Virol* 2003;77:10528–36. [PubMed: 12970437]
132. Lu, M. Stabilizing peptides and their use in the preparation of stabilized HIV inhibitors. World Intellectual Property Organization Patent WO-04/106364A1. 2004.
133. Bewley CA, Otero-Quintero S. The potent anti-HIV protein cyanovirin-N contains two novel carbohydrate binding sites that selectively bind to Man(8) D1D3 and Man(9) with nanomolar affinity: implications for binding to the HIV envelope protein gp120. *J Am Chem Soc* 2001;123:3892–902. [PubMed: 11457139]
134. Bewley CA. Rapid validation of the overall structure of an internal domain-swapped mutant of the anti-HIV protein cyanovirin-N using residual dipolar couplings. *J Am Chem Soc* 2001;123:1014–15. [PubMed: 11456652]
135. Tsai CC, Emau P, Jiang Y, et al. Cyanovirin-N gel as a topical microbicide prevents rectal transmission of SHIV89.6P in macaques. *AIDS Res Hum Retroviruses* 2003;19:535–41. [PubMed: 12921090]
136. Tsai CC, Emau P, Jiang Y, et al. Cyanovirin-N inhibits AIDS virus infections in vaginal transmission models. *AIDS Res Hum Retroviruses* 2004;20:8–11.
137. Balzarini J, Van Laethem K, Peumans WJ, et al. Mutational pathways, resistance profile, and side effects of cyanovirin relative to human immunodeficiency virus type 1 strains with n-glycan deletions in their gp120 envelopes. *J Virol* 2006;80:8411–21. [PubMed: 16912292]
138. Xu Q. Development of a live topical microbicide for women. *Microbicides 2008 Conference New Delhi, India Feb 24–27, 2008 Abstract 221*.
139. Wiysonge CS, Shey MS, Shang J, Kongnyuy EJ, Brocklehurst P. Vaginal microbicides for preventing mother-to-child transmission of HIV infection—no evidence of an effect or evidence of no effect? *S Afr Med J* 2007;97:530–33. [PubMed: 17805456]
140. Tsai CC, Follis KE, Sabo A, et al. Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl)adenine. *Science* 1995;270:1197–99. [PubMed: 7502044]
141. Balzarini J, Zhang H, Herdewijn P, Johns D, De Clercq E. Intracellular metabolism and mechanism of antiretrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. *Proc Natl Acad Sci USA* 1991;88:1499–503. [PubMed: 1705039]
142. Aquaro S, Caliò R, Balzarini J, Bellocchi MC, Garaci E, Perno CF. Macrophages and HIV infection: therapeutic approaches toward this strategic virus reservoir. *Antiviral Res* 2002;55:209–25. [PubMed: 12103427]
143. Robbins BL, Srinivas RV, Kim C, Bischofberger N, Fridland A. Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate

- 9-R-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(isopropylloxymethylcarbonyl)PMPA. *Antimicrob Agents Chemother* 1998;42:612–17. [PubMed: 9517941]
144. Otten RA, Smith DK, Adams DR, et al. Efficacy of post exposure prophylaxis after intravaginal exposure of pig-tailed macaques to a human-derived retrovirus (human immunodeficiency virus type 2). *J Virol* 2000;74:9771–75. [PubMed: 1100253]
 145. Wainberg M. The prospect for RT inhibitors as topical microbicides. *Microbicides 2004 Conference London, UK March 28–31, 2004 Abstract MMM-03.*
 146. Barditch-Crovo P, Deeks SG, Collier A, et al. Phase I/II trial of the pharmacokinetics, safety, and antiretroviral activity of tenofovir disoproxil fumarate in human immunodeficiency virus-infected adults. *Antimicrob Agents Chemother* 2001;45:2733–39. [PubMed: 11557462]
 147. Hillier S. Safety and acceptability of daily and coitally dependent use of 1% tenofovir over six months of use. *Microbicides 2008 Conference New Delhi, India Feb 24–27, 2008 Abstract 655.*
 148. Schwartz JL, Kashuba A, Rezk N, et al. Preliminary results from a pharmacokinetic study of the candidate vaginal microbicide candidate 1% tenofovir gel. *Microbicides 2008 Conference New Delhi India; Feb 24–27, 2008 Abstract B011-210.*
 149. Balzarini J, Pelemans H, Aquaro S, et al. Highly favorable antiviral activity and resistance profile of the novel thiocarboxanilide pentenyloxy ether derivatives UC-781 and UC-82 as inhibitors of human immunodeficiency virus type 1 replication. *Mol Pharmacol* 1996;50:394–401. [PubMed: 8700148]
 150. Borkow G, Barnard J, Nguyen TM, Belmonte A, Wainberg MA, Parniak MA. 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;71:3023–30. [PubMed: 9060662]
 151. Buckheit RW, Snow MJ, Fliakas-Boltz V, et al. Highly potent oxathiin carboxanilide derivatives with efficacy against nonnucleoside reverse transcriptase inhibitor-resistant human immunodeficiency virus isolates. *Antimicrob Agents Chemother* 1997;41:831–37. [PubMed: 9087499]
 152. Balzarini J, Naesens L, Verbeken E, et al. Preclinical studies on thiocarboxanilide UC-781 as a virucidal agent. *AIDS* 1998;12:1129–38. [PubMed: 9677161]
 153. Di Fabio S, Van RJ, 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;17:1597–604. [PubMed: 12853741]
 154. 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;79:11179–86. [PubMed: 16103169]
 155. Van HY, Michiels J, Van RJ, et al. In vitro evaluation of nonnucleoside reverse transcriptase inhibitors UC-781 and TMC120-R147681 as human immunodeficiency virus microbicides. *Antimicrob Agents Chemother* 2004;48:337–39. [PubMed: 14693562]
 156. Van HY, 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;48:3684–89. [PubMed: 15388420]
 157. Zussman A, Lara L, Lara HH, Bentwich Z, Borkow G. Blocking of cell-free and cell-associated HIV-1 transmission through human cervix organ culture with UC781. *AIDS* 2003;17:653–61. [PubMed: 12646787]
 158. Hossain MM, Parniak MA. In vitro microbicidal activity of the nonnucleoside reverse transcriptase inhibitor (NNRTI) UC781 against NNRTI-resistant human immunodeficiency virus type 1. *J Virol* 2006;80:4440–46. [PubMed: 16611904]
 159. Talwar GP, Raghuvanshi P, Mishra R, et al. Polyherbal formulations with wide spectrum antimicrobial activity against reproductive tract infections and sexually transmitted pathogens. *Am J Reprod Immunol* 2000;43:144–51. [PubMed: 10735590]
 160. Joshi SN, Katti U, Godbole S, et al. Phase I safety study of Praneem polyherbal vaginal tablet use among HIV-uninfected women in Pune, India. *Trans R Soc Trop Med Hyg* 2005;99:769–74. [PubMed: 16084547]

161. Joglekar NS, Joshi SN, Navlakha SN, Katti UR, Mehendale SM. Acceptability of Praneem polyherbal vaginal tablet among HIV uninfected women and their male partners in Pune India—phase I study. *Indian J Med Res* 2006;123:547–52. [PubMed: 16783046]
162. Josh SN, Dutta S, Kumar BK, et al. Expanded safety study of Praneem polyherbal vaginal tablet among HIV-uninfected women in Pune, India: a phase II clinical trial report. *Sex Transm Infect* 2008;84:343–37. [PubMed: 18426844]
163. Lagakos, SW.; Gable, AR., editors. Methodological challenges in biomedical HIV prevention trials. National Academy of Sciences—The National Academies Press; Washington, DC: 2008.
164. Pharmaco-Economics Working group of the Microbicide Initiative. The economics of microbicide development: a case for investment. Rockefeller Foundation Microbicide Initiative; New York: 2002. p. 27
165. Global Campaign for Microbicides. Funding needs. <http://www.global-campaign.org/fundingneeds.htm> [accessed Sept 28, 2008]. <http://www.global-campaign.org/fundingneeds.htm>
166. Grufferman S. Complexity and the Hawthorne effect in community trials. *Epidemiology* 1999;10:209–10. [PubMed: 10230825]
167. Bentley M, Morrow KM, Fullem A, et al. Acceptability of a novel vaginal microbicide during a safety trial among low-risk women. *Fam Plann Perspect* 2000;32:184–88. [PubMed: 10942354]
168. Woodsong C. Covert use of topical microbicides: implications for acceptability and use. *Perspect Sex Reprod Health* 2004;36:127–31. [PubMed: 15306271]
169. Moench TR, Doncel GF, Cone RA. The HEC placebo: designed for “no effect”. Microbicides 2004 Conference London, UK March 28–31, 2004 Abstract 02609.
170. Tien D, Schnaare RL, Kang F, et al. In vitro and in vivo characterization of a potential universal placebo designed for use in vaginal microbicide clinical trials. *AIDS Res Hum Retroviruses* 2005;21:845–53. [PubMed: 16225411]
171. Garg S, Kandarapu R, Vermani K, et al. Development pharmaceuticals of microbicide formulations. Part I: preformulation considerations and challenges. *AIDS Patient Care STDS* 2003;17:17–32. [PubMed: 12614517]
172. Terrazas-Aranda K, Van Herrewege Y, Hazuda D, et al. Human immunodeficiency virus type 1 (HIV-1) integration: a potential target for microbicides to prevent cell-free or cell-associated HIV-1 infection. *Antimicrob Agents Chemother* 2008;52:e2544–54.

Table 1

Selected non-specific microbicide agents

	Advantages	Disadvantages	Examples in class	Clinical trial status
Surfactants				
Non-specific disruption of cellular and microbial membranes	Active against wide range of pathogens; often spermicidal	Potentially toxic to host cells	Nonoxinol 9 (nonoxynol-9) C31G (Savvy)	No current clinical trials for HIV prevention. Two phase III efficacy trials completed in 1996 and 2000, one of which showed increased HIV-1 seroincidence with nonoxinol 9 when used more than three times per day ^{38,47} Two phase III trials in Ghana (n=2142) and Nigeria (n=1800) halted in November, 2005, and August, 2006, because of low HIV seroincidence rate in the study population ^{48,49} Phase II safety trial in Cameroon completed. Results pending (clinicaltrials.gov identifier NCT00136643) Phase II/III trial assessing efficacy in high-risk women planned ⁵⁰
Vaginal milieu protectors/acidifying agents				
Restores protective acidic pH of vagina by buffering semen	Spermicidal; activity against HIV, HSV, <i>C trachomatis</i>	None known	Carbopol 974P (BufferGel) Acidform (Amphora)	Phase II/III trial (HPTN 035) ongoing; 3101 women in five countries (Malawi, South Africa, USA, Zambia, and Zimbabwe; clinicaltrials.gov NCT00074425) Phase III trial in Madagascar testing diaphragm with Acidform for prevention of <i>N gonorrhoeae</i> and <i>C trachomatis</i> is planned ⁵⁰
Entry inhibitors: anionic polymers				
Negative charge causes interaction with HIV's viral envelope proteins and interferes with attachment of HIV to CD4+ cells	Many have activity against other STI pathogens (including <i>C trachomatis</i> , <i>Neisseria gonorrhoeae</i> , and HSV)	Not all virus types respond equally well to negative charge properties of these compounds	Naphthalene sulfonate (PRO2000) Carrageenan (Carraguard/PC-515) Cellulose sulfate (Ushercell) Cellulose acetate phthalate (CAP) Dendrimers: SPL7013 (Vivagel)	Phase II/III trial (HPTN 035) ongoing; 3101 women in five countries (Malawi, South Africa, USA, Zambia, and Zimbabwe; clinicaltrials.gov NCT00074425) Phase III (MDP-301, UK Medical Research Council); PRO2000 originally in two concentrations (0.5% vs 2.0%) vs placebo gel. 2.0% arm stopped in February, 2008. Enrollment of 9395 women completed in July, 2008, and trial to be completed in late 2009 (clinicaltrials.gov NCT00262106) Phase III trial completed in South Africa (n=6202). Results released in February, 2008, show gel to be safe with no difference in HIV incidence between study and placebo groups ⁵¹ Two phase III trials in Africa and India halted in January, 2007, for increased HIV seroincidence during interim analysis of one trial ^{52,53} Phase I trial of 13% gel halted because of heavy vaginal discharge in multiple participants ⁵⁴ Showed protection from HIV in a macaque model and from HSV in two animal models. ^{55,56} Completed phase I male tolerance study. ⁵⁷ Phase I safety trial completed in Kenya with results pending (clinicaltrials.gov NCT00331032). Phase I trial ongoing in the USA (clinicaltrials.gov NCT00442910)

HSV=herpes simplex virus. STI=sexually transmitted infection. More information about the ongoing clinical trials can be found on the clinicaltrials.gov website.

Table 2

Selected specific microbicide agents

Advantages	Disadvantages	Examples in class	Clinical trial status
Entry inhibitors: CCR5 blockers			
Block CCR5 co-receptor and interfere with attachment of HIV to host cells	No activity against other STI pathogens	PSC-RANTES CMPD167	Protected macaques from SHIV (SF162) with no evidence of systemic absorption or toxicity ¹²² Full protection of macaques from SHIV (162P4) not achieved alone, but only with addition of BMS-378806 and C52-L, two peptides that block the viral—host cell interaction at different loci (gp120 and gp41, respectively) ¹²³
Reverse transcriptase inhibitors			
Interfere with HIV reverse transcriptase enzyme	No activity against other STI pathogens	Tenofovir (PMPA; nucleotide analogue)	Phase I safety trial testing 0.3% and 1% gel formulations in HIV-positive and HIV-negative sexually active and sexually abstinent women found gel to be safe and well tolerated ¹²⁴ Two phase I pharmacokinetic trials and a third phase I trial evaluating the effect of tenofovir gel on mediators of mucosal immunity are ongoing (clinicaltrials.gov identifiers NCT00561496, NCT00540605, and NCT00594373) Phase II expanded safety trial in India and USA completed in 2007; results pending (clinicaltrials.gov NCT00111943) Phase IIb trial in South Africa ongoing (CAPRISA 004; clinicaltrials.gov NCT00441298) Phase IV/IIb trial (MTN 003) in South Africa comparing two oral antiretroviral drugs (tenofovir and emtricitabine) vs 1% tenofovir gel is planned (clinicaltrials.gov NCT00705679)
Tenofovir: active in multiple cell types. TMC-120 and UC781 (NNRTIs): delayed development of resistance compared with first-generation NNRTIs			
		TMC120 (NNRTI)	Phase III efficacy study (IPM 009) and at least eight phase I/II safety trials planned ⁵⁰
		UC781 (NNRTI)	Phase I study completed, indicating safety after 6 days of daily dosing ¹²⁵ Three phase I trials assessing safety and acceptability of 0.1% or 0.25% formulation applied vaginally are ongoing (clinicaltrials.gov NCT00441909, NCT00132444, and NCT00385554) Phase I trial assessing safety and acceptability with rectal use in HIV-negative adults ongoing (clinicaltrials.gov NCT00408538) Male tolerance study ongoing (A06-104; clinicaltrials.gov NCT00385554)

SHIV=chimeric simian/human immunodeficiency virus. STI=sexually transmitted infection. NNRTI=non-nucleoside reverse transcriptase inhibitor. More information about the ongoing clinical trials can be found on the clinicaltrials.gov website.