Published in final edited form as:

Future Med Chem. 2011 December ; 3(16): 2101–2116. doi:10.4155/fmc.11.153.

Advances in HIV Microbicide Development

Joanna S. Olsen¹, David Easterhoff¹, and Stephen Dewhurst^{1,*}

¹Department of Microbiology and Immunology, University of Rochester, Rochester, NY 14627

Abstract

There is an urgent need for a way to control the spread of the global HIV pandemic. A microbicide, or topical drug applied to the mucosal environment to block transmission, is a promising HIV prevention strategy. The development of a safe and efficacious microbicide requires a thorough understanding of the mucosal environment and its role in HIV transmission. Knowledge of the key events in viral infection identifies points at which the virus might be most effectively targeted by a microbicide. The cervicovaginal and rectal mucosa play an important role in the innate defense against HIV, and microbicides must not interfere with these functions. In this review we discuss the current research on HIV microbicide development.

Introduction

HIV continues to be one of the most pressing global health threats, with an estimated 33 million people infected worldwide and 2.6 million new infections in 2009 [1]. While great advances have been made in antiretroviral therapy, a top priority is the development of an effective means of preventing virus transmission. A microbicide, in which a drug to block infection would be applied locally to the mucosa, is one possible means to achieve this. Given the nature of the HIV epidemic, an ideal microbicide would be able to inhibit transmission in both the genital and gastrointestinal (rectal) mucosa.

The difficulty in microbicide development is highlighted by the many candidates that have shown efficacy *in vitro*, but failed to protect, and sometimes even enhanced, infection *in vivo* (Table 1). Reasons for these failures have often related to effects on the mucosal environment, including disruption to the epithelial barrier and induction of an inflammatory response [2, 3]. The gap between *in vitro* efficacy and *in vivo* futility highlights the importance of understanding the early events during mucosal transmission of HIV and the role that the mucosal environment plays. This will allow for better development and preclinical testing of microbicide candidates.

In this review we will discuss HIV microbicide development with a focus on the current understanding of mucosal HIV infection, and the ways in which this can influence microbicide design.

The role of the mucosal environment in infection

Considerable recent progress has been made in understanding the role of the mucosal environment in the transmission of HIV. Both the cervicovaginal mucosa and semen contain myriad factors that affect viral transmission.

* To whom correspondence should be addressed, at: Department of Microbiology and Immunology, University of Rochester Medical Center. 601 Elmwood Avenue, Box 672. Rochester, NY 14642 Tel.: (585) 275 3216; Fax: (585) 473 9573
Stephen_dewhurst@urmc.rochester.edu.

In the cervicovaginal mucosa, the epithelial barrier, including both the mucous layer and the epithelial cell layer, is the first line of physical defense against the virus. In the lower reproductive tract the multilayered stratified squamous epithelia of the vagina and ectocervix provides a significant mechanical barrier. The superficial layers of the epithelia are continuously sloughed off, further impeding colonization by pathogens [4]. In the upper reproductive tract, the tight junctions between the columnar epithelial cells of the endocervix and endometrium prevent virus from crossing the single layered epithelia [5]. The initial focus of HIV infection is frequently found in the endocervix or the transformation zone, which is the junction of the endo- and ectocervix, suggesting that this area, with its single layered epithelium and high rate of cell turnover, is most easily crossed [6, 7]. However, the initial focus of infection is certainly not restricted to this region. The area of the vaginal wall and ectocervix is more than 15 times that of the endocervix, providing considerably greater surface area for infection to occur, and HIV infection has been shown to occur both in women who are born without a uterus, as well as in macaques that have had their uteri surgically removed [8, 9].

Physical barriers to virus transmission also include factors present on the surface of mucosal epithelial cells, and mucosal fluids. The epithelial cells of the cervicovaginal mucosa are surrounded by a surface layer consisting of the glycocalyx, a hydrophilic layer of glycoproteins and glycolipids, and a thick, hydrophobic outer layer of mucus [4]. Antimicrobial proteins produced by epithelial cells can be found in this layer [10, 11] (see below), and the cervicovaginal mucus acts as an important defense in its own right. Studies have also shown that cervicovaginal mucus from normal donors traps HIV virions via a charge-dependent mechanism, slowing their diffusion by up to 1000-fold [12]. This may contribute to protection against virus transmission.

In addition to these physical barriers, the epithelial cells of the cervicovaginal mucosa also produce biological barriers to HIV infection, including secreted antimicrobial proteins. Secretory leukocyte protease inhibitor, alpha and beta defensins, trappin-2/elafin and lactoferrin have all demonstrated significant anti HIV-1 activity in-vitro [10, 13-16]. Additionally, elevated levels of trappin-2/elafin in genital secretions have been associated with protection from HIV-1 acquisition among highly exposed female sex workers [15], and the alpha defensins human neutrophil peptides (HNP) 1-3 showed increased levels in PBMCs obtained from HIV exposed but seronegative women, as compared to healthy controls [17]. Higher levels of these same peptides in breast milk were also associated with a decrease in the risk of postnatal HIV transmission [18], reinforcing the idea that these peptides may have a protective role in vivo. The normal vaginal flora represents another biological barrier. The dominant lactobacilli in the normal flora maintain an acidic pH in the vaginal environment, which contributes to viral inactivation and trapping of virus in cervical mucus [12]. This low pH also acts to reduce T cell activation, thereby reducing target cell availability [19]. The peroxide producing lactobacilli may also be directly virucidal [20].

The cells of the cervicovaginal mucosa also have a role in innate immunologic defense against HIV. Vaginal and cervical epithelial cells express a wide variety of Toll-like receptors [21]. Activation of these TLRs results in production of cytokines and chemokines, including MIP-1 α , MIP-1 β and Rantes, which are natural ligands of CCR5, and may therefore serve to block the interaction of R5 tropic HIV strains with CCR5 [22, 23].

The defenses of the female reproductive tract provide multiple barriers to HIV transmission, but these defenses are frequently attenuated. The use of hormonal contraception may alter susceptibility to infection by altering immunological signaling [24, 25]. The levels of antibodies in vaginal secretions have been shown to vary throughout the menstrual cycle and in response to exogenous hormones. Progesterone acts as an immunosuppressant by

decreasing both production and transepithelial transport of IgG and IgA antibodies and by inhibiting cytolytic T cell activity [26-28]. Additionally, normal hormone fluctuations can cause cyclical changes in the epithelium. Estrogen causes a thickening of the stratified squamous epithelium, which may exert a protective effect [29, 30], whereas progesterone may cause thinning of the epithelium, although this effect appears to be more pronounced in macaques than in humans [31, 32]. Hormonal variation may also affect HIV transmission through effects on colonization by lactobacillus, and by influencing target cell availability [33, 34]. Other sexually transmitted infections are also associated with increased susceptibility to HIV, due both to disruptive effects on the integrity of the epithelial barrier, and inflammatory effects, which recruit activated HIV target cells to the mucosa [35-37]. Thus, a successful microbicide will have to inhibit the virus without disrupting the intrinsic antiviral functions of the female reproductive tract. Additionally, an ideal microbicide would protect against HIV transmission even when aspects of this protective barrier are impaired or altered.

The rectal epithelium has fewer protections against HIV infection. The single layer columnar epithelium is especially vulnerable to trauma during intercourse. Additionally, as part of the gastrointestinal tract, the rectum contains lymphoid follicles, providing a rich concentration of HIV-1 target cells. The epithelium also contains microfold (M) cells, which are specialized epithelial cells that mediate transepithelial vesicular transport [38] and can transfer virions to rectal lymphoid cells that lie under the epithelial surface [39]. In contrast to peripheral CD4+ T cells, gastrointestinal T cells (including rectal T cells) are characterized by elevated expression of CCR5 and expression of activation markers [40]. Consistent with this increased level of activation, rectal T cells also appear to have a high intracellular concentration of nucleotides, which may be capable of reducing the local effects of microbicides such as tenofovir (i.e., nucleotide reverse transcriptase inhibitors; Table 1) [41].

The role of semen

Semen has an important role in HIV transmission, mediated through interactions with the virus itself, as well as the cervicovaginal mucosa. The alkaline pH of semen serves to neutralize the acidic pH of the vaginal milieu, protecting the virus from inactivation [42]. Neutralized cervical mucus is also no longer able to effectively trap virions [12]. Basic amines in seminal fluid, which function to protect spermatozoa, such as spermine and spermidine, also protect virions from acidic pH [43].

The importance of considering semen in the development and testing of microbicides has been highlighted in studies of the microbicide candidates PRO 2000 and cellulose sulfate, both of which showed significant promise in vitro but failed to protect in phase III clinical trials. The antiviral activities of these compounds were significantly reduced in vitro if the challenge virus was diluted in pooled human seminal plasma, or if cells were challenged in the presence of 25% semen [44]. Similarly, cervicovaginal lavage (CVL) collected after administration of PRO 2000 gel was able to inhibit HIV-1, however this activity was significantly reduced in CVL collected post-coitally [45].

Semen also affects HIV transmission due to its effects on the cervicovaginal mucosa. Seminal fluid has been shown to stimulate production of chemokines, including CCL 20, from the vaginal epithelium [46, 47]. This results in the recruitment of Langerhans cells and other target cells to the mucosa, facilitating the transfer of virions across the epithelial barrier [46]. Furthermore, semen may inactivate some of the innate antimicrobial peptides produced by the cervicovaginal mucosa. Secretory leukocyte protease inhibitor is an antimicrobial peptide with potent activity against R5 HIV-1 isolates in vitro. However, SLPI

activity is significantly decreased at basic pH [48], and it is proteolytically cleaved by prostate specific antigen, which is found in high concentrations in semen [49].

Studies continue to discover other components of semen that have an effect on HIV, including the semen-derived enhancer of viral infection (SEVI). SEVI consists of cationic amyloid fibrils that are formed from a 39 amino acid cleavage product of the protein prostatic acid phosphatase (PAP[248-286]), which is found at high (>30 µg/ml) concentration in semen. These fibrils are able to enhance HIV infection by more than 40 fold in vitro [43]. The effect of SEVI is independent of the virus and cell type, and is especially pronounced at low multiplicity of infection (MOI) [43].

SEVI is thought to work by binding to both target cells and virions. The highly cationic fibrils form a “charge shield” to reduce electrostatic repulsion between the negatively charged surface of the virion and the negatively charged cell membrane [50]. SEVI has not only been shown to increase the infection of target cells directly, but also to increase infection of T cells in trans by epithelial cells [51]. The ability of semen to enhance HIV infection is donor dependent and has been shown to correspond to the level of SEVI in the sample [51]. This fits well with other data, which have shown that some individuals infected with HIV are better able to transmit the virus than others [52].

Nonetheless, questions remain about SEVI's effects and direct experimental evidence that SEVI and/or semen can enhance HIV-1 transmission in vivo is lacking. Moreover, studies using the simian immunodeficiency virus (SIV)/macaque model have failed to yield definitive data. Two separate studies using low-dose, cell-free vaginal exposure of macaques to SIVmac251 in the presence or absence of human seminal plasma (HSP) were conducted by Miller et al. and Neildez et al. in the 1990s [53, 54]. Both found that a majority of animals exposed to a low dose virus challenge (approx. 10^2 TCID₅₀) in the presence of HSP became persistently infected, while animals exposed to the same dose of virus in the absence of HSP experienced only transient infection [53, 54]. However, the group sizes used were small and the results did not achieve statistical significance. Thus, a larger vaginal challenge study in the presence or absence of SEVI, as proposed by Münch and colleagues, would be informative [51]. It is also possible that the effects of SEVI may partly explain the increased resistance of HIV to microbicides in seminal plasma, as compared to cell culture medium. If so, anti SEVI compounds would be a good addition to microbicide formulations, and might increase the activity of other microbicides in vivo.

Mechanisms of mucosal HIV transmission

An understanding of mucosal HIV transmission is essential to microbicide development, in order to better inform the site and timeframe in which a microbicide must act.

The virus is deposited on the mucosal barrier, and must breach this defense to reach target cells in the submucosa. Microtrauma from sexual intercourse may provide breaks in the epithelium [55] and virus may also cross the epithelial barrier by transcytosis or endocytosis [56-58]. While the epithelial cells of the reproductive tract do not express CD4, or the CCR5 and CXCR4 co-receptors, cell surface glycosphingolipids have been shown to bind gp120 on the virion surface, which could allow transcytosis [59, 60]. Virions may also interact with heparin sulfate moieties on the cell surface, such as syndecans. The immune cells in the cervicovaginal mucosa, most notably Langerhans cells, may also contribute to the ability of HIV virions to cross the epithelial barrier. Gp120 interacts with C-type lectin receptors, such as DC-SIGN and langerin, which can result in “trans” infection of target T cells that interact with virus-bearing Langerhans cells, as well as uptake of virus particles [61]. However, it remains unclear whether Langerhans cells contribute to virus transcytosis across an intact vaginal epithelium [56, 62]. Similar mechanisms allow the virus to cross the rectal mucosa,

where the epithelium is all a single layer columnar epithelium [38] (Figure 1). In the rectal mucosa, interactions between virions and glycosphingolipids on the surface of intraepithelial M cells may also facilitate transport of virus through the epithelia [39].

Studies in the SIV/maaque model have provided a detailed picture of the early events following mucosal exposure to the virus, with the caveat that these experiments have used a large virus inoculum (to ensure reproducible infection of all animals). In this model, virus can cross the mucosal epithelial barrier within hours, and perhaps as rapidly as 30-60 min [63]. Once across to the submucosa, a small founder population of infected cells is established. In the macaque model, these initial infected cells are CD4⁺ T cells with a “resting” phenotype - meaning that they lack the expected markers of activation, but are not truly resting cells, because they presumably express CCR5 and must possess at least a minimally activated phenotype in order to support productive virus infection [64]. Studies using a human cervical tissue explant system also found that CD4⁺ T cells were the initial infected cells; in the cervical explants, HIV positive CD4⁺ cells could be detected within 6 hours of virus exposure [65].

“Resting” CD4⁺ T cells are considerably more common in the submucosa than macrophages and dendritic cells, which presumably explains why the initial phase of infection selectively targets these cells [66]. Once the small founder population of infected cells is established, the initial spread of infection in the macaque model occurs by accumulation of new infected cells around the first cluster of infection [7]. This expansion occurs by recruitment of new CD4⁺ T cell target cells to the site of infection, which is likely driven by expression of chemokines from the endocervical epithelium and plasmacytoid DCs [7]. Local expansion of infection continues through the first week, but by week two, extensive replication in lymphoid tissues can already be seen, accompanied by establishment of disseminated latent infection [67].

Taken together, the early events in mucosal transmission of HIV provide an ideal target to inhibit virus transmission. Furthermore, the virus may continue to be vulnerable to inhibition after crossing the epithelial barrier, during the local expansion phase. If a microbicide could prevent formation of a self-sustaining local infection, for example, by limiting the recruitment of additional target cells, a productive infection would not be established [7]. This early vulnerability of HIV is underscored by the relatively low frequency of virus transmission per sexual encounter, which is estimated at .0003 to .008 [68-70].

Attributes of a Successful Microbicide

A successful microbicide will have to be efficacious, but must also be acceptable, affordable and safe. A microbicide must have little to no toxicity to the reproductive epithelium, including pro-inflammatory effects, and must maintain the antiviral milieu of the cervicovaginal mucosa, including the normal flora. Reproductive safety will also be important, since a large portion of the target population for a microbicide are women of childbearing age. The MSM community will likely adopt any microbicide approved for vaginal use, so it is key to understand the safety profile in the GI tract as well as the female reproductive tract.

Acceptability is also important, as an effective microbicide will be of little use if people do not choose to use it. Factors such as color, odor, feel and ease of use have a strong impact on whether a microbicide will actually be used [71]. Finally, if a microbicide is to be widely distributed, and afford protection to women in developing countries, it must also be affordable. In order to do so, it should be easy to manufacture, store and transport.

Classes of microbicides

HIV microbicides under development generally fall into one of the following 5 classes: Surfactants, entry inhibitors, vaginal milieu protectors, reverse transcriptase inhibitors and other molecules, whose mechanism of action is either unknown or falls outside these classes. Key clinical trials of microbicide candidates are summarized in Table 1.

Surfactants are able to non-specifically disrupt membranes, meaning they are active against a wide variety of microorganisms (and often also act as effective contraceptives) [68]. Surfactants were the first molecules to enter clinical trials as candidate HIV microbicides, but have also been associated with some of the most disappointing results. Nonoxynol-9 resulted in an increased rate of HIV infection in phase III clinical trials, due to toxicity to the cervicovaginal mucosa [3, 72]. Clinical trials of another surfactant, C3IG (Savvy), were halted when an unexpectedly low rate of HIV infection in the study population made it nearly impossible to achieve statistically significant results [73]. At the time of study termination there was, however, a non-statistically significant trend toward a higher rate of infection among participants using C3IG [74].

Entry inhibitors encompass a wide range of agents that act to prevent HIV from binding to or entering target cells. Examples of entry inhibitors that are being, or have been, studied for use as microbicides include polyanions, CCR5 inhibitors and fusion inhibitors. Polyanions, such as PRO2000, cellulose sulphate, carraguard and the dendrimer SPL7013 (vivagel) have been shown in vitro to interact with the HIV-1 surface envelope glycoprotein (gp120) and block the attachment of HIV to target cells [75, 76]. At least in the case of PRO2000, this may reflect binding of polyanions to multiple sites on gp120 [77], including the positively charged, exposed V3 loop domain [78]. These compounds have been extensively tested in phase III trials, but have repeatedly failed to show in-vivo efficacy against HIV transmission [79, 80]. This may be due to the complexity of the mucosal environment, as well as the ability of semen to interfere with the antiviral activity of PRO2000 and cellulose sulphate [44, 45].

CCR5 inhibitors block the interaction of HIV with CCR5, the co-receptor for R5 strains of HIV, which are almost exclusively the strains transmitted during sexual intercourse [81]. Several different CCR5 inhibitors have shown efficacy as microbicides in the simian immunodeficiency virus (SIV)/macaque model and are poised to enter clinical trials [82-84]. Maraviroc, a CCR5 inhibitor already in use as an oral antiretroviral, has been effective in both the macaque and humanized mouse models [82, 85], and a phase I safety study of maraviroc in coformulation with the reverse transcriptase inhibitor dapivirine is pending [86].

Fusion inhibitors, as their name implies, block fusion of HIV virions with target cells. C52L, a peptide inhibitor of gp41, and Cyanovirin, a lectin that binds to high mannose residues on gp120, are two compounds under development that have shown efficacy in the SHIV macaque model [84, 87].

Vaginal milieu protectors are designed to maintain or enhance the protective acidic pH of the vaginal environment. Buffergel is a low pH aqueous gel based on the widely used buffering agent Carbopol 974, which can rapidly buffer twice its volume of semen to a pH of 5 [88]. Unfortunately, it failed to show efficacy in phase IIIB trials [89]. An alternative strategy is the use of lactobacilli, the commensal species that maintain the acidic vaginal pH. Exogenous colonization has been demonstrated in macaques, as well as in a pilot human study [81, 90, 91]. Furthermore, preclinical research is being done in which lactobacilli are being genetically engineered to express antiviral proteins such as cyanovirin [92], fusion inhibitors [93] and entry/CCR5 antagonists [94, 95].

Reverse transcriptase inhibitors, specifically the nucleotide analogue tenofovir, are currently the most promising microbicide candidates for licensure. This is due to the results of the CAPRISA 004 trial, a phase IIb (proof of concept) trial which showed that a 1% tenofovir gel applied before and after sex was 39% effective overall in preventing HIV infection in women, and 54% effective among highly adherent users of the gel [96]. This was the first microbicide trial to demonstrate statistically significant efficacy. Expanded studies of tenofovir are planned or already underway. The VOICE (MTN003) trial is looking at tenofovir gel in comparison to oral pre-exposure prophylaxis using tenofovir alone or tenofovir plus emtricitabine (Truvada) [97]. Trials to examine the safety and acceptability of tenofovir as a rectal microbicide are also underway [98, 99].

Non-nucleoside reverse transcriptase inhibitors are also microbicide candidates. The NNRTI dapivirine has been shown to be safe in phase I trials, and is being studied in a variety of formulations, including vaginal rings [100, 101]. The NNRTI UC781 was also shown to be safe in phase I clinical trials, but development as a microbicide was discontinued due to formulation difficulties [102].

Future of the field

Formulation improvement

Much work is being done to create improved formulations of current microbicide candidates, with enhanced efficacy and acceptability. The traditional gel formulation for microbicides has low user acceptability, principally because they can be difficult to apply and messy [103, 104]. Quick dissolve vaginal films are an alternative formulation with greater anticipated acceptability [105]. These films are a solid dry delivery system which dissolves rapidly once in contact with vaginal fluids [105]. Another priority for formulation improvement is the development of a vehicle, such as a vaginal ring, that will allow long term release of drug at the proper location. Vaginal rings are inserted by the user, and are flexible polymeric devices in which drug diffuses through the polymer and into the surrounding vaginal fluid and tissues. Different ring designs alter the kinetics of drug release from the polymer; shell or reservoir style rings are able to provide a constant daily release rate [106]. Such rings are already used for contraception, and are currently being studied with antiviral compounds - including dapivirine, which was tested in vaginal ring form and found to be safe and well tolerated, and to release high concentrations of drug, in phase I trials [107, 108]. Further trials are planned. With a sustained release vehicle, a microbicide would not have to be applied before every exposure, greatly increasing adherence. A recent study found that female users and their male partners preferred vaginal rings to both a vaginal gel applicator and use of a diaphragm to deliver a microbicide [109].

Another strategy being studied to improve formulation is the use of nanoparticles coated with low molecular weight polyethylene glycol [110]. These particles are able to move through cervical mucus more freely, and therefore are able to penetrate into the inner layers, which have slower turnover. As a result, they may provide better biodistribution and more long lasting protection [111].

The development of a triggered release system to improve gel formulation is also being studied. Kiser *et al.* recently reported the development of inhibitor containing microgel particles which are degraded by prostate specific antigen, leading to the release of viral inhibitor in the presence of semen [112]. This approach would protect the antiviral inhibitor from the mucosal environment, limit diffusion of the drug, and allow the release of a high concentration of drug at the appropriate time and location.

Microbicides in combination

Much like the use of combination antiretroviral drugs, it is likely that the development of an effective microbicide will require more than one antiviral ingredient. With the high rate of HIV mutations, the use of a single microbicide ingredient makes it likely that resistance will arise, or that the microbicide will fail to protect against some of the variants present during a virus exposure. Targeting the virus with more than one drug and/or by more than one mechanism makes this less likely. Also, for a microbicide to be effective worldwide, it will have to protect against a very broad range of circulating viruses – including different clades/subgroups.

Development of combination microbicides is especially important because of the probability that tenofovir will become the first licensed microbicide. Since it has been widely used in oral antiretroviral therapy, tenofovir resistant strains are already known [113, 114]. Therefore there is a risk that the widespread use of tenofovir alone as a microbicide might increase the spread of these resistant strains.

Combining inhibitors also offers the possibility of synergy between the different ingredients, in which the activity of two ingredients together is stronger than the sum of the two alone. This effect has been seen in vitro with combinations such as polyanions plus the neutralizing antibody b12, or polyanions plus the CCR5 inhibitor TAK779 [115], as well as combinations of CCR5 inhibitors and fusion inhibitors in macaques [84].

Indirect approaches

HIV can also be targeted indirectly, for example by enhancing the endogenous antiviral state of the mucosa, or by inhibiting the proviral effects of semen. This approach may be especially valuable for the development of compounds to be used in combination with antiretrovirals, as these inhibitors might show synergy with antiviral agents by decreasing the ability of HIV to cross the mucosal barrier, allowing additional time for antiviral inhibitors to act.

Some consideration has been given to the use of immune activators to induce an antiviral state in the mucosal tissues. However, the innate immune response also functions to recruit new target cells to the mucosa, facilitating local propagation of the virus. Thus, the net effect of immune activators may be to increase HIV infection, as a consequence of mucosal inflammation [2, 3]. In keeping with this, topical TLR7 and TLR9 agonists have been shown to increase the efficiency of vaginal SIV infection in rhesus macaques, despite stimulating production of antiviral cytokines and chemokines [116].

Preclinical experience with anti-inflammatory mediators underscores the concern that immune activation may enhance virus transmission. Thus, topical application of glycerol monolaurate (GML), which blocks T cell activation, resulted in decreased MIP-3 α /CCL20 levels in vaginal fluid of treated macaques, and a subsequent reduction in the recruitment of SIV target cells; vaginal delivery of GML was also able to protect macaques from repeated high dose intravaginal challenge with SIV [7].

Better assessment of preclinical efficacy

Given the need for additional microbicides, and the failure of several prior candidates in late stage clinical trials, one of the biggest priorities is the development of a better algorithm to assess microbicide safety and efficacy in a preclinical setting. This includes the development of a model system that provides information on both efficacy and toxicity, as well as the identification of biomarkers that will allow accurate prediction of these parameters. Models for preclinical testing will also need to be able to evaluate the impact of mucosal

environmental factors such as seminal plasma and cervicovaginal secretions on microbicide safety and efficacy [117]. One cell culture model for early evaluation of candidate microbicides is being developed by Herold *et al.* using a dual chamber system with human endometrial cells [2]. Polarized endometrial epithelial cells in the top layer provide a relatively impervious epithelial barrier, blocking the migration of HIV virions to target T cells in the lower chamber. Exposure to nonoxynol-9 and cellulose sulfate resulted in changes in transepithelial resistance and/or expression of junctional proteins, which allowed HIV to cross the epithelial barrier and infect the target T cells [2].

The standard model for in vivo preclinical assessment of candidate microbicides has been the non-human primate model. However, this model is prohibitively expensive, and requires the use of SIV or hybrid SIV-HIV viruses (SHIV), rather than HIV-1 itself. Thus, there is considerable interest in alternative models – including humanized mice. These are transgenic immune deficient mice that are reconstituted with human hematopoietic stem cells or lymphocytes. The bone marrow/liver/thymus (BLT) model, in which an immune deficient mouse is reconstituted with human fetal liver, thymus and CD34+ hematopoietic cells, is especially promising for microbicide research. Denton *et al.* have shown that the female reproductive tract of BLT mice is populated with human CD4+ T cells, macrophages and dendritic cells, and that these mice are susceptible to both vaginal and rectal transmission of HIV-1 [118-120]. Furthermore, they were able to replicate the results of the CAPRISA 004 trial in the BLT mouse model, suggesting that humanized mice may serve as a valid preclinical assessment tool for microbicide candidates [121]. However, there are significant differences in the physiology and physical characteristics of the murine and human vaginal and rectal mucosae, and it is therefore unclear whether this model can be used to predict which microbicides will cause inflammatory toxicity in the human reproductive tract.

There is also a need for ex vivo models, to assess the efficacy of novel microbicidal approaches. One such model involves administration of a candidate microbicide to vaginal or rectal tissue in human volunteers, followed by biopsy of the administration site and establishment of a tissue explant. This explant can then be exposed to HIV, and the efficiency of virus infection compared pre and post-treatment [117]. Conceptual advantages of this approach include its use of primary human tissue, as well as the presence of endogenous local microflora, cervicovaginal secretions and semen [45].

Tissue explants can also be used to study safety and efficacy in an entirely ex-vivo approach. Ectocervical and colorectal explants have been used to study effects of microbicides both on inhibiting HIV transmission and on the viability of cells and endogenous microflora, as well as to examine the ability of microbicide formulations to penetrate through tissue [122, 123]. However, explant systems have some limitations, including the fact that explant tissues undergo relatively rapid deterioration due to physical manipulation and removal from the blood supply [124]. Moreover, protocols for establishing and using explants are not standardized, and the tissues are often treated during surgery and culture with compounds such as betadine and amphotericin-B, an antifungal drug, which could have confounding effects on HIV infection [125, 126]. Nonetheless, tissue explants remain a promising preclinical model for assessing microbicide safety and efficacy.

In order for these model systems to be used successfully, an algorithm to predict safe and effective microbicides must be available. Markers for safety include assessment of cell viability and proliferation, epithelial integrity, release of inflammatory mediators and enhancement of infection [117]. Some biomarkers currently being examined for their ability to predict toxicity include inflammatory cytokines, such as MIP-2, which was increased in response to N-9 and PRO 2000 [127], markers of leukocyte activation such as the

transcription factors NF κ B and AP-1, which have been shown to be induced by N-9, benzalkonium chloride and SDS in the rabbit vaginal irritation (RVI) model, and soluble markers of vascular permeability. The latter include the presence of soluble E-selectin, vascular adhesion molecule-1 (VCAM-1), CD14 and myeloperoxidase in vaginal fluid, which were also induced by toxic microbicides in the RVI model - and may indicate the ability of lymphocytes to extravasate into the tissue due to an inflammatory response [128, 129].

Pharmacokinetic profiles are also critical to predicting the effectiveness of microbicide candidates, and the local tissue concentration of an antiretroviral may be an especially important biomarker of efficacy. Consistent with this, a study of tenofovir in the macaque model found that plasma tenofovir concentration measured 15 min after rectal administration was associated with the degree of protection from rectal infection [130]. Furthermore, as one might expect, increasing the local concentration of a drug may result in improved efficacy. Thus, one recent study found that a combination microbicide gel containing zinc acetate dehydrate and the NNRTI MIV-150 resulted in increased levels of MIV-150 in cervical tissue, when compared to a gel containing MIV-150 alone, and afforded better protection from vaginal SHIV infection [131].

Development of Rectal Specific Microbicides

Most of the work on microbicide safety and formulation has been specific to the vaginal environment, and many of these studies may need to be repeated for the rectal mucosa. The single layered rectal epithelium is especially vulnerable to damage, and therefore may be especially sensitive to microbicide induced toxicity. Another factor that affects development of a rectal microbicide is the potential for GI side effects. A recent clinical trial (RMP-02/MTN-006) tested the vaginal formulation of tenofovir for rectal use and concluded that “rectal dosing with the vaginal formulation of 1% TFV was neither entirely safe nor fully acceptable” [132]; MTN-006 also reported GI side effects, including diarrhea and lower abdominal cramps. This may be due to the glycerol in the vaginal formulation, as Fuchs *et al.* have reported that hyperosmolar gels can cause damage to the rectal epithelium and luminal secretion [133]. A new clinical trial, MTN-007, is testing a lower glycerol formulation of tenofovir gel. This trial also includes a nonoxynol-9 condition, in order to determine an “upper barrier” of inflammatory markers in the rectal epithelium, as the inflammatory markers that indicate epithelial toxicity have not been well studied in the rectal environment [99].

A better understanding is also needed of the area of distribution a rectal microbicide needs to cover. The rectal epithelium is continuous with the GI tract, and reports have suggested that HIV may be able to travel as far as the splenic flexure [134]. However, it is unclear if this is actually a regular occurrence. Also, as discussed above, a recent study by Garcia-Lerma *et al.* showed that the level of dNTPs in rectal lymphocytes is very high [41]. This raises the concern that the effectiveness of nucleotide analog reverse transcriptase inhibitors, such as tenofovir, might be more limited in the rectal environment. The factors unique to the rectal environment are being incorporated into microbicide development in the hopes of developing a product that will function effectively in both the vaginal and rectal compartments.

Other Priorities

One important unanswered question is whether cell-associated virus (i.e., HIV contained within seminal leukocytes) plays an important role in the sexual transmission of HIV [135]. It is relevant to microbicide design and selection, because many microbicide candidates are designed principally to target cell free virions. Moreover, most preclinical assessments of

microbicide candidates rely upon non-human primate challenge models that use cell free virus. Therefore it is extremely relevant to understand whether cell associated virus does in fact have an important role in transmission.

In order to optimize the efficacy of microbicides, it is also important to understand the effect that hormones may have on both the efficacy of microbicides and the transmissibility of HIV. Natural hormone fluctuation, as well as the use of hormonal contraceptives is associated with changes in the thickness of the vaginal epithelium, and alterations to immunological signaling [24, 32]. For example, as discussed earlier, progesterone has been shown to exert an immunosuppressive effect on both the humoral and cellular immune response, decreasing production and transport of IgG and IgA, inhibiting the cytolytic T-cell response, and biasing the T cell response towards a Th2 phenotype [26, 136]. Thus, susceptibility to HIV infection may vary throughout the menstrual cycle, or if a woman is using hormonal birth control [32]. Microbicide development and testing must account for these changes in order to ensure protection throughout the menstrual cycle and for women taking hormonal contraception.

Another interesting question is whether the implementation of a successful microbicide may result in “chemovaccination”, in which exposures to HIV that are not infectious (due to the use of a microbicide) may result in a protective immune response against the virus. This would improve the efficacy of a microbicide, even if adherence were less than perfect. A recent study found a chemovaccination effect, as measured by the development of SHIV specific T cells, in macaques that were treated with a pre-exposure prophylaxis (PrEP) regimen [130, 137]. However, this response was short lived and no longer protective when animals were challenged after an extended (28 week) rest period [137]. Further studies will obviously be needed, but this work highlights the potential for synergy between traditional vaccination approaches, PrEP and microbicides.

Future perspective

Microbicide development remains one of the most promising hopes for controlling the spread of HIV. With the recent success of tenofovir as a microbicide in the CAPRISA 004 trial, a licensed microbicide to protect against HIV infection is likely to be a reality within the next few years. The development of additional microbicide candidates is still an important priority, as the use of multiple drugs in a microbicide will increase efficacy and decrease resistance. In addition to direct antivirals, agents that enhance mucosal protection against HIV, or inhibit proviral factors, are a promising strategy to augment microbicide effectiveness.

There are many pressing issues in the microbicide field beyond the development of new microbicide candidates. Optimization of microbicide formulas will make microbicides more acceptable and therefore improve adherence. Development of a sustained release vehicle, such as a vaginal ring, or optimized gel technology will allow “coital independence” of microbicides. One of the hopes for a microbicide is that it will free women from being dependent on their partners for protection, unlike the use of condoms. Therefore, a microbicide that allows application to be separated from intercourse would offer important advantages. Due to the differences in the anatomy and structure of mucosal surfaces in the reproductive and gastrointestinal tracts, multiple sustained release technologies may be needed to optimize both vaginal and rectal microbicides.

Finally, several early microbicide candidates that reached late phase clinical trials were found to be ineffective, or worse, to increase the risk of infection. This highlights the need for more predictive preclinical models to improve assessment of microbicide safety and

efficacy, as well as improved approaches to early phase clinical testing, such that ineffective or unsafe candidates can be eliminated earlier in the evaluation process.

Key Terms

Stratified squamous epithelium	The type of epithelium found in the vagina and ectocervix. It consists of multiple layers, with the outer layer continuously sloughed off and replaced by cells from the deeper layers.
Transformation zone	The region where the stratified squamous epithelium of the ectocervix meets the single layered columnar epithelium of the endocervix. This is a region of high cell turnover and is frequently where the initial focus of HIV infection is found.
“Resting” CD4 cells	The initial target cells in an HIV infection. These CD4 ⁺ CCR5 ⁺ cells have a resting phenotype, meaning they lack markers of T cell activation. However, since they express CCR5 and are able to support virus replication, they are not truly resting T cells.
PrEP	Preexposure prophylaxis. The use of oral antiretrovirals by HIV negative patients as a strategy to prevent HIV infection.
Chemovaccination	An effect where repeated sub-infectious exposures to a virus result in a vaccine-like effect by stimulating a protective immune response to the virus.
Mucoadhesive polymers	A drug delivery system in which the vehicle adheres to the epithelial mucous. This allows for prolonged and targeted drug delivery.

References

1. UNAIDS: Report on the Global AIDS Epidemic. 2010
2. Mesquita PM, Cheshenko N, Wilson SS, et al. Disruption of tight junctions by cellulose sulfate facilitates HIV infection: model of microbicide safety. *The Journal of infectious diseases*. 2009; 200(4):599–608. [PubMed: 19586414]
3. Stafford MK, Ward H, Flanagan A, et al. Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998; 17(4):327–331. [PubMed: 9525433]
4. Kaushic C, Ferreira VH, Kafka JK, Nazli A. HIV infection in the female genital tract: discrete influence of the local mucosal microenvironment. *American journal of reproductive immunology*. 2010; 63(6):566–575. [PubMed: 20384619]
5. Wira CR, Grant-Tschudy KS, Crane-Godreau MA. Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection. *American journal of reproductive immunology*. 2005; 53(2):65–76. [PubMed: 15790340]
6. 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(6):1253–1263. [PubMed: 16093359]
7. Li Q, Estes JD, Schlievert PM, et al. Glycerol monolaurate prevents mucosal SIV transmission. *Nature*. 2009; 458(7241):1034–1038. [PubMed: 19262509]
8. Kell PD, Barton SE, Edmonds DK, Boag FC. HIV infection in a patient with Meyer-Rokitansky-Kuster-Hauser syndrome. *J R Soc Med*. 1992; 85(11):706–707. [PubMed: 1474562]
9. Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. *Nat Rev Immunol*. 2008; 8(6):447–457. [PubMed: 18469831]

10. King AE, Critchley HO, Kelly RW. Presence of secretory leukocyte protease inhibitor in human endometrium and first trimester decidua suggests an antibacterial protective role. *Molecular human reproduction*. 2000; 6(2):191–196. [PubMed: 10655462]
11. Mcneely TB, Dealy M, Dripps DJ, Orenstein JM, Eisenberg SP, Wahl SM. Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. *The Journal of clinical investigation*. 1995; 96(1):456–464. [PubMed: 7615818]
12. Lai SK, Hida K, Shukair S, et al. Human immunodeficiency virus type 1 is trapped by acidic but not by neutralized human cervicovaginal mucus. *J Virol*. 2009; 83(21):11196–11200. [PubMed: 19692470]
13. Moriuchi M, Moriuchi H. A milk protein lactoferrin enhances human T cell leukemia virus type I and suppresses HIV-1 infection. *Journal of immunology*. 2001; 166(6):4231–4236.
14. Sun L, Finnegan CM, Kish-Catalone T, et al. Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection. *Journal of virology*. 2005; 79(22):14318–14329. [PubMed: 16254366]
15. Iqbal SM, Ball TB, Levinson P, et al. Elevated elafin/trappin-2 in the female genital tract is associated with protection against HIV acquisition. *AIDS*. 2009; 23(13):1669–1677. [PubMed: 19553806]
16. Ghosh M, Shen Z, Fahey JV, Cu-Uvin S, Mayer K, Wira CR. Trappin-2/Elafin: a novel innate anti-human immunodeficiency virus-1 molecule of the human female reproductive tract. *Immunology*. 2010; 129(2):207–219. [PubMed: 19824918]
17. Trabattoni D, Caputo SL, Maffei G, et al. Human alpha defensin in HIV652 exposed but uninfected individuals. *J Acquir Immune Defic Syndr*. 2004; 35(5):455–463. [PubMed: 15021310]
18. Kuhn L, Trabattoni D, Kankasa C, et al. Alpha-defensins in the prevention of HIV transmission among breastfed infants. *J Acquir Immune Defic Syndr*. 2005; 39(2):138–142. [PubMed: 15905728]
19. Hill JA, Anderson DJ. Human vaginal leukocytes and the effects of vaginal fluid on lymphocyte and macrophage defense functions. *Am J Obstet Gynecol*. 1992; 166(2):720–726. [PubMed: 1536258]
20. Klebanoff SJ, Coombs RW. Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. *The Journal of experimental medicine*. 1991; 174(1):289–292. [PubMed: 1647436]
21. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev*. 2005; 206:306–335. [PubMed: 16048557]
22. Cocchi F, Devico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV668 suppressive factors produced by CD8+ T cells. *Science*. 1995; 270(5243):1811–1815. [PubMed: 8525373]
23. Abdelwahab SF, Cocchi F, Bagley KC, et al. HIV-1-suppressive factors are secreted by CD4+ T cells during primary immune responses. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(25):15006–15010. [PubMed: 14657379]
24. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nature reviews. Immunology*. 2008; 8(9):737–744. [PubMed: 18728636]
25. White HD, Crassi KM, Givan AL, et al. CD3+ CD8+ CTL activity within the human female reproductive tract: influence of stage of the menstrual cycle and menopause. *Journal of immunology*. 1997; 158(6):3017–3027.
26. Kutteh WH, Prince SJ, Hammond KR, Kutteh CC, Mestecky J. Variations in immunoglobulins and IgA subclasses of human uterine cervical secretions around the time of ovulation. *Clin Exp Immunol*. 1996; 104(3):538–542. [PubMed: 9099941]
27. Cherpes TL, Busch JL, Sheridan BS, Harvey SA, Hendricks RL. Medroxyprogesterone acetate inhibits CD8+ T cell viral-specific effector function and induces herpes simplex virus type 1 reactivation. *Journal of immunology*. 2008; 181(2):969–975.
28. Laskarin G, Strbo N, Sotosek V, et al. Progesterone directly and indirectly affects perforin expression in cytolytic cells. *American journal of reproductive immunology*. 1999; 42(5):312–320. [PubMed: 10584987]

29. Smith SM, Baskin GB, Marx PA. Estrogen protects against vaginal transmission of simian immunodeficiency virus. *The Journal of infectious diseases*. 2000; 182(3):708–715. [PubMed: 10950763]
30. Gupta S, Kumar N, Singhal N, Manektala U, Jain S, Sodhani P. Cytohormonal and morphological alterations in cervicovaginal smears of postmenopausal women on hormone replacement therapy. *Diagn Cytopathol*. 2006; 34(10):676–681. [PubMed: 16955474]
31. Marx PA, Spira AI, Gettie A, et al. Progesterone implants enhance SIV vaginal transmission and early virus load. *Nature medicine*. 1996; 2(10):1084–1089.
32. Hel Z, Stringer E, Mestecky J. Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection. *Endocr Rev*. 2010; 31(1):79–97. [PubMed: 19903932]
33. Miller L, Patton DL, Meier A, Thwin SS, Hooton TM, Eschenbach DA. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. *Obstet Gynecol*. 2000; 96(3):431–439. [PubMed: 10960638]
34. Wieser F, Hosmann J, Tschugguel W, Czerwenka K, Sedivy R, Huber JC. Progesterone increases the number of Langerhans cells in human vaginal epithelium. *Fertil Steril*. 2001; 75(6):1234–1235. [PubMed: 11384659]
35. Iqbal SM, Kaul R. Mucosal innate immunity as a determinant of HIV susceptibility. *American journal of reproductive immunology*. 2008; 59(1):44–54. [PubMed: 18154595]
36. Johnson LF, Lewis DA. The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. *Sex Transm Dis*. 2008; 35(11):946–959. [PubMed: 18685546]
37. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS*. 2006; 20(1):73–83. [PubMed: 16327322]
38. Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1 infection. *Nat Rev Microbiol*. 2003; 1(1):25–34. [PubMed: 15040177]
39. Neutra MR. Interactions of viruses and microparticles with apical plasma membranes of M cells: implications for human immunodeficiency virus transmission. *The Journal of infectious diseases*. 1999; 179(Suppl 3):S441–443. [PubMed: 10099115]
40. Lackner AA, Mohan M, Veazey RS. The gastrointestinal tract and AIDS pathogenesis. *Gastroenterology*. 2009; 136(6):1965–1978. [PubMed: 19462506]
41. Garcia-Lerma JG, Aung W, Cong ME, et al. Natural substrate concentrations can modulate the prophylactic efficacy of nucleotide HIV reverse transcriptase inhibitors. *Journal of virology*. 2011
42. Tevi-Benissan C, Belec L, Levy M, et al. In vivo semen-associated pH neutralization of cervicovaginal secretions. *Clin Diagn Lab Immunol*. 1997; 4(3):367–374. [PubMed: 9144379]
43. Munch J, Rucker E, Standker L, et al. Semen-derived amyloid fibrils drastically enhance HIV infection. *Cell*. 2007; 131(6):1059–1071. [PubMed: 18083097]
44. Patel S, Hazrati E, Cheshenko N, et al. Seminal plasma reduces the effectiveness of topical polyanionic microbicides. *The Journal of infectious diseases*. 2007; 196(9):1394–1402. [PubMed: 17922405]
45. Keller MJ, Mesquita PM, Torres NM, et al. Postcoital bioavailability and antiviral activity of 0.5% PRO 2000 gel: implications for future microbicide clinical trials. *PLoS One*. 2010; 5(1):e8781. [PubMed: 20107502]
46. Berlier W, Cremel M, Hamzeh H, et al. Seminal plasma promotes the attraction of Langerhans cells via the secretion of CCL20 by vaginal epithelial cells: involvement in the sexual transmission of HIV. *Human reproduction*. 2006; 21(5):1135–1142. [PubMed: 16531471]
47. Sharkey DJ, Macpherson AM, Tremellen KP, Robertson SA. Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells. *Mol Hum Reprod*. 2007; 13(7):491–501. [PubMed: 17483528]
48. Mcneely TB, Shugars DC, Rosendahl M, Tucker C, Eisenberg SP, Wahl SM. Inhibition of human immunodeficiency virus type 1 infectivity by secretory leukocyte protease inhibitor occurs prior to viral reverse transcription. *Blood*. 1997; 90(3):1141–1149. [PubMed: 9242546]

49. Ohlsson K, Bjartell A, Lilja H. Secretory leucocyte protease inhibitor in the male genital tract: PSA-induced proteolytic processing in human semen and tissue localization. *Journal of andrology*. 1995; 16(1):64–74. [PubMed: 7539415]
50. Roan NR, Munch J, Arhel N, et al. The cationic properties of SEVI underlie its ability to enhance human immunodeficiency virus infection. *J Virol*. 2009; 83(1):73–80. [PubMed: 18945786]
51. Kim KA, Yolamanova M, Zirafi O, et al. Semen-mediated enhancement of HIV infection is donor-dependent and correlates with the levels of SEVI. *Retrovirology*. 2010; 7:55. [PubMed: 20573198]
52. Clumeck N, Taelman H, Hermans P, Piot P, Schoumacher M, De Wit S. A cluster of HIV infection among heterosexual people without apparent risk factors. *The New England journal of medicine*. 1989; 321(21):1460–1462. [PubMed: 2811959]
53. Neildez O, Le Grand R, Cheret A, et al. Variation in virological parameters and antibody responses in macaques after atraumatic vaginal exposure to a pathogenic primary isolate of SIVmac251. *Res Virol*. 1998; 149(1):53–68. [PubMed: 9561564]
54. Miller CJ, Marthas M, Torten J, et al. Intravaginal inoculation of rhesus macaques with cell-free simian immunodeficiency virus results in persistent or transient viremia. *Journal of virology*. 1994; 68(10):6391–6400. [PubMed: 8083977]
55. Norvell MK, Benrubi GI, Thompson RJ. Investigation of microtrauma after sexual intercourse. *J Reprod Med*. 1984; 29(4):269–271. [PubMed: 6716372]
56. Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. *Nature medicine*. 1997; 3(1):42–47.
57. Dezzutti CS, Guenther PC, Cummins JE Jr. et al. Cervical and prostate primary epithelial cells are not productively infected but sequester human immunodeficiency virus type 1. *The Journal of infectious diseases*. 2001; 183(8):1204–1213. [PubMed: 11262202]
58. Wu Z, Chen Z, Phillips DM. Human genital epithelial cells capture cell-free human immunodeficiency virus type 1 and transmit the virus to CD4+ Cells: implications for mechanisms of sexual transmission. *The Journal of infectious diseases*. 2003; 188(10):1473–1482. [PubMed: 14624372]
59. Yeaman GR, Asin S, Weldon S, et al. Chemokine receptor expression in the human ectocervix: implications for infection by the human immunodeficiency virus-type I. *Immunology*. 2004; 113(4):524–533. [PubMed: 15554931]
60. Furuta Y, Eriksson K, Svennerholm B, et al. Infection of vaginal and colonic epithelial cells by the human immunodeficiency virus type 1 is neutralized by antibodies raised against conserved epitopes in the envelope glycoprotein gp120. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91(26):12559–12563. [PubMed: 7809077]
61. Turville SG, Cameron PU, Handley A, et al. Diversity of receptors binding HIV on dendritic cell subsets. *Nature immunology*. 2002; 3(10):975–983. [PubMed: 12352970]
62. Bouschbacher M, Bomsel M, Verronese E, et al. Early events in HIV transmission through a human reconstructed vaginal mucosa. *AIDS*. 2008; 22(11):1257–1266. [PubMed: 18580604]
63. Hu J, Gardner MB, Miller CJ. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. *Journal of virology*. 2000; 74(13):6087–6095. [PubMed: 10846092]
64. Li Q, Duan L, Estes JD, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. *Nature*. 2005; 434(7037):1148–1152. [PubMed: 15793562]
65. Gupta P, Collins KB, Ratner D, et al. Memory CD4(+) T cells are the earliest detectable human immunodeficiency virus type 1 (HIV-1)-infected cells in the female genital mucosal tissue during HIV-1 transmission in an organ culture system. *Journal of virology*. 2002; 76(19):9868–9876. [PubMed: 12208964]
66. Zhang ZQ, Wietgreffe SW, Li Q, et al. Roles of substrate availability and infection of resting and activated CD4+ T cells in transmission and acute simian immunodeficiency virus infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(15):5640–5645. [PubMed: 15064398]
67. Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature*. 2010; 464(7286):217–223. [PubMed: 20220840]

68. Cutler B, Justman J. Vaginal microbicides and the prevention of HIV transmission. *The Lancet infectious diseases*. 2008; 8(11):685–697. [PubMed: 18992405]
69. Degruittola V, Seage GR 3rd, Mayer KH, Horsburgh CR Jr. Infectiousness of HIV between male homosexual partners. *J Clin Epidemiol*. 1989; 42(9):849–856. [PubMed: 2789269]
70. Downs AM, De Vincenzi I. Probability of heterosexual transmission of HIV: relationship to the number of unprotected sexual contacts. European Study Group in Heterosexual Transmission of HIV. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1996; 11(4):388–395. [PubMed: 8601226]
71. Morrow KM, Hendrix C. Clinical evaluation of microbicide formulations. *Antiviral Res*. 2010; 88(Suppl 1):S40–46. [PubMed: 21109067]
72. Hoffman IF, Taha TE, Padian NS, et al. Nonoxynol-9 100 mg gel: multi-site safety study from sub-Saharan Africa. *AIDS*. 2004; 18(16):2191–2195. [PubMed: 15577653]
73. Peterson L, Nanda K, Opoku BK, et al. SAVVY (C31G) gel for prevention of HIV infection in women: a Phase 3, double-blind, randomized, placebo-controlled trial in Ghana. *PLoS One*. 2007; 2(12):e1312. [PubMed: 18091987]
74. 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(1):e1474. [PubMed: 18213382]
75. 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(4852):646–649. [PubMed: 2452480]
76. O'loughlin J, Millwood IY, McDonald HM, Price CF, Kaldor JM, Paull JR. Safety, tolerability, and pharmacokinetics of SPL7013 gel (VivaGel): a dose ranging, phase I study. *Sex Transm Dis*. 2010; 37(2):100–104. [PubMed: 19823111]
77. Sachdev DD, Zerhouni-Layachi B, Ortigoza M, et al. The differential binding and activity of PRO 2000 against diverse HIV-1 envelopes. *J Acquir Immune Defic Syndr*. 2009; 51(2):125–129. [PubMed: 19349871]
78. Callahan LN, Phelan M, Mallinson M, Norcross MA. Dextran sulfate blocks antibody binding to the principal neutralizing domain of human immunodeficiency virus type 1 without interfering with gp120-CD4 interactions. *Journal of virology*. 1991; 65(3):1543–1550. [PubMed: 1995952]
79. McCormack S, Ramjee G, Kamali A, et al. PRO2000 vaginal gel for prevention of HIV-1 infection (Microbicides Development Programme 301): a phase 3, randomised, double-blind, parallel-group trial. *Lancet*. 2010; 376(9749):1329–1337. [PubMed: 20851460]
80. Van Damme L, Govinden R, Mirembe FM, et al. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. *The New England journal of medicine*. 2008; 359(5):463–472. [PubMed: 18669425]
81. Keele BF, Giorgi EE, Salazar-Gonzalez JF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(21):7552–7557. [PubMed: 18490657]
82. Veazey RS, Ketas TJ, Dufour J, et al. Protection of rhesus macaques from vaginal infection by vaginally delivered maraviroc, an inhibitor of HIV-1 entry via the CCR5 co-receptor. *The Journal of infectious diseases*. 2010; 202(5):739–744. [PubMed: 20629537]
83. Lederman MM, Veazey RS, Offord R, et al. Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5. *Science*. 2004; 306(5695):485–487. [PubMed: 15486300]
84. 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(7064):99–102. [PubMed: 16258536]
85. Neff CP, Kurisu T, Ndolo T, Fox K, Akkina R. A Topical Microbicide Gel Formulation of CCR5 Antagonist Maraviroc Prevents HIV-1 Vaginal Transmission in Humanized RAG-hu Mice. *PLoS One*. 2011; 6(6):e20209. [PubMed: 21673796]
86. Schader SM, Colby-Germinario SP, Schachter JR, Xu H, Wainberg MA: Synergy against drug resistant HIV-1 with the microbicide antiretrovirals, dapivirine and tenofovir, in combination. *AIDS*. 2011

87. Tsai CC, Emau P, Jiang Y, et al. Cyanovirin-N gel as a topical microbicide prevents rectal transmission of SHIV89.6P in macaques. *AIDS research and human retroviruses*. 2003; 19(7): 535–541. [PubMed: 12921090]
88. 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(4):687–693. [PubMed: 10731526]
89. Abdool Karim SS, Richardson BA, Ramjee G, et al. Safety and effectiveness of BufferGel and 0.5% PRO2000 gel for the prevention of HIV infection in women. *AIDS*. 2011; 25(7):957–966. [PubMed: 21330907]
90. 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(5):1881–1887. [PubMed: 12734221]
91. Patton DL, Cosgrove Sweeney YT, Antonio MA, Rabe LK, Hillier SL. *Lactobacillus crispatus* capsules: single-use safety study in the Macaca nemestrina model. *Sex Transm Dis*. 2003; 30(7): 568–570. [PubMed: 12838085]
92. Liu X, Lagenaur LA, Simpson DA, et al. Engineered vaginal *Lactobacillus* strain for mucosal delivery of the human immunodeficiency virus inhibitor cyanovirin-N. *Antimicrobial agents and chemotherapy*. 2006; 50(10):3250–3259. [PubMed: 17005802]
93. Pusch O, Kalyanaraman R, Tucker LD, Wells JM, Ramratnam B, Boden D. An anti-HIV microbicide engineered in commensal bacteria: secretion of HIV-1 fusion inhibitors by *Lactobacilli*. *AIDS*. 2006; 20(15):1917–1922. [PubMed: 16988512]
94. 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. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(20):11672–11677. [PubMed: 12972635]
95. Vangelista L, Secchi M, Liu X, et al. Engineering of *Lactobacillus jensenii* to secrete RANTES and a CCR5 antagonist analogue as live HIV-1 blockers. *Antimicrobial agents and chemotherapy*. 2010; 54(7):2994–3001. [PubMed: 20479208]
96. Abdool Karim Q, Abdool Karim SS, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science*. 2010; 329(5996):1168–1174. [PubMed: 20643915]
97. Microbicidetrialsnetwork: MTN-003. May 28.2011 2011
98. Microbicidetrialsnetwork: RMP-02/MTN-006. Jun 1.2011 2011
99. Microbicidetrialsnetwork: MTN-007. Jun 8.2011 2011
100. Woolfson AD, Malcolm RK, Morrow RJ, Toner CF, McCullagh SD. Intravaginal ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide. *Int J Pharm*. 2006; 325(1-2): 82–89. [PubMed: 16884869]
101. Malcolm RK, Woolfson AD, Toner CF, Morrow RJ, McCullagh SD. Long-term, controlled release of the HIV microbicide TMC120 from silicone elastomer vaginal rings. *J Antimicrob Chemother*. 2005; 56(5):954–956. [PubMed: 16155060]
102. Clinical Trials:CONRAD. Jun 1.2011 2011
103. Malonza IM, Mirembe F, Nakabiito C, et al. Expanded Phase I safety and acceptability study of 6% cellulose sulfate vaginal gel. *AIDS*. 2005; 19(18):2157–2163. [PubMed: 16284466]
104. Bentley ME, Fullem AM, Tolley EE, et al. Acceptability of a microbicide among women and their partners in a 4-country phase I trial. *Am J Public Health*. 2004; 94(7):1159–1164. [PubMed: 15226137]
105. Rohan LC, Sassi AB. Vaginal drug delivery systems for HIV prevention. *Aaps J*. 2009; 11(1):78–87. [PubMed: 19194802]
106. Malcolm RK, Edwards KL, Kiser P, Romano J, Smith TJ. Advances in microbicide vaginal rings. *Antiviral Res*. 2010; 88(Suppl 1):S30–39. [PubMed: 21109066]
107. Nel A, Smythe S, Young K, et al. Safety and pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to HIV-negative women. *J Acquir Immune Defic Syndr*. 2009; 51(4):416–423. [PubMed: 19623693]

108. Romano J, Variano B, Coplan P, et al. Safety and availability of dapivirine (TMC120) delivered from an intravaginal ring. *AIDS research and human retroviruses*. 2009; 25(5):483–488. [PubMed: 19388819]
109. Hardy E, Hebling EM, Sousa MH, Almeida AF, Amaral E. Delivery of microbicides to the vagina: difficulties reported with the use of three devices, adherence to use and preferences. *Contraception*. 2007; 76(2):126–131. [PubMed: 17656182]
110. Cu Y, Saltzman WM. Controlled surface modification with poly(ethylene)glycol enhances diffusion of PLGA nanoparticles in human cervical mucus. *Mol Pharm*. 2009; 6(1):173–181. [PubMed: 19053536]
111. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev*. 2009; 61(2):75–85. [PubMed: 19135107]
112. Clark MR, Aliyar HA, Lee CW, et al. Enzymatic triggered release of an HIV-1 entry inhibitor from prostate specific antigen degradable microparticles. *Int J Pharm*. 2011; 413(1-2):10–18. [PubMed: 21511017]
113. Sluis-Cremer N, Moore K, Radzio J, Sonza S, Tachedjian G. N348I in HIV-1 reverse transcriptase decreases susceptibility to tenofovir and etravirine in combination with other resistance mutations. *AIDS*. 2010; 24(2):317–319. [PubMed: 20010074]
114. Hachiya A, Kodama EN, Schuckmann MM, et al. K70Q adds high-level tenofovir resistance to “Q151M complex” HIV reverse transcriptase through the enhanced discrimination mechanism. *PLoS One*. 2011; 6(1):e16242. [PubMed: 21249155]
115. Gantlett KE, Weber JN, Sattentau QJ. Synergistic inhibition of HIV-1 infection by combinations of soluble polyanions with other potential microbicides. *Antiviral Res*. 2007; 75(3):188–197. [PubMed: 17408760]
116. Wang Y, Abel K, Lantz K, Krieg AM, Mcchesney MB, Miller CJ. The Toll-like receptor 7 (TLR7) agonist, imiquimod, and the TLR9 agonist, CpG ODN, induce antiviral cytokines and chemokines but do not prevent vaginal transmission of simian immunodeficiency virus when applied intravaginally to rhesus macaques. *Journal of virology*. 2005; 79(22):14355–14370. [PubMed: 16254370]
117. Doncel GF, Clark MR. Preclinical evaluation of anti-HIV microbicide products: New models and biomarkers. *Antiviral Res*. 2010; 88(Suppl 1):S10–18. [PubMed: 21109063]
118. Wege AK, Melkus MW, Denton PW, Estes JD, Garcia JV. Functional and phenotypic characterization of the humanized BLT mouse model. *Curr Top Microbiol Immunol*. 2008; 324:149–165. [PubMed: 18481459]
119. Denton PW, Estes JD, Sun Z, et al. Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. *PLoS Med*. 2008; 5(1):e16. [PubMed: 18198941]
120. Denton PW, Krisko JF, Powell DA, et al. Systemic administration of antiretrovirals prior to exposure prevents rectal and intravenous HIV-1 transmission in humanized BLT mice. *PLoS One*. 2010; 5(1):e8829. [PubMed: 20098623]
121. Denton PW, Othieno F, Martinez-Torres F, et al. Topically Applied 1% Tenofovir in Humanized BLT Mice Using the CAPRISA 004 Experimental Design Demonstrates Partial Protection from Vaginal HIV Infection Validating the BLT Model for the Evaluation of New Microbicide Candidates. *Journal of virology*. 2011
122. Lackman-Smith C, Osterling C, Luckenbaugh K, et al. Development of a comprehensive human immunodeficiency virus type 1 screening algorithm for discovery and preclinical testing of topical microbicides. *Antimicrobial agents and chemotherapy*. 2008; 52(5):1768–1781. [PubMed: 18316528]
123. Rohan LC, Moncla BJ, Kunjara Na Ayudhya RP, et al. In vitro and ex vivo testing of tenofovir shows it is effective as an HIV-1 microbicide. *PLoS One*. 2010; 5(2):e9310. [PubMed: 20174579]
124. 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. *Journal of virology*. 2000; 74(12):5577–5586. [PubMed: 10823865]

125. Bestman-Smith J, Desormeaux A, Tremblay MJ, Bergeron MG. Targeting cell978 free HIV and virally-infected cells with anti-HLA-DR immunoliposomes containing amphotericin B. *AIDS*. 2000; 14(16):2457–2465. [PubMed: 11101055]
126. Kaplan JC, Crawford DC, Durno AG, Schooley RT. Inactivation of human immunodeficiency virus by Betadine. *Infect Control*. 1987; 8(10):412–414. [PubMed: 3667119]
127. Galen BT, Martin AP, Hazrati E, et al. A comprehensive murine model to evaluate topical vaginal microbicides: mucosal inflammation and susceptibility to genital herpes as surrogate markers of safety. *The Journal of infectious diseases*. 2007; 195(9):1332–1339. [PubMed: 17397004]
128. Trifonova RT, Bajpai M, Pasieczny JM, Chandra N, Doncel GF, Fichorova RN. Biomarkers of leukocyte traffic and activation in the vaginal mucosa. *Biomarkers*. 2007; 12(6):608–622. [PubMed: 17852080]
129. Cummins JE Jr, Doncel GF. Biomarkers of cervicovaginal inflammation for the assessment of microbicide safety. *Sex Transm Dis*. 2009; 36(3 Suppl):S84–91. [PubMed: 19218890]
130. Cranage M, Sharpe S, Herrera C, et al. Prevention of SIV rectal transmission and priming of T cell responses in macaques after local pre-exposure application of tenofovir gel. *PLoS Med*. 2008; 5(8):e157. discussion e157. [PubMed: 18684007]
131. Kenney J, Aravantinou M, Singer R, et al. An antiretroviral/zinc combination gel provides 24 hours of complete protection against vaginal SHIV infection in macaques. *PLoS One*. 2011; 6(1):e15835. [PubMed: 21246052]
132. Anton, P.; Cranston, R.; Carballo-Dieguez, A.; Kashuba, A.; Khanukhova, E.; Elliott, J.; Janocko, L.; Cumberland, W.; Mauck, C.; McGowan, I. RMP-02/MTN- 006: A Phase 1 Placebo-controlled Trial of Rectally Applied 1% Vaginal TFV Gel with Comparison to Oral TDF; Conference on Retroviruses and Opportunistic Infections; Boston, MA. 2011.
133. Fuchs EJ, Lee LA, Torbenson MS, et al. Hyperosmolar sexual lubricant causes epithelial damage in the distal colon: potential implication for HIV transmission. *The Journal of infectious diseases*. 2007; 195(5):703–710. [PubMed: 17262713]
134. Hendrix CW, Fuchs EJ, Macura KJ, et al. Quantitative imaging and sigmoidoscopy to assess distribution of rectal microbicide surrogates. *Clin Pharmacol Ther*. 2008; 83(1):97–105. [PubMed: 17507921]
135. Anderson DJ, Politch JA, Nadolski AM, Blaskewicz CD, Pudney J, Mayer KH. Targeting Trojan Horse leukocytes for HIV prevention. *AIDS*. 2010; 24(2):163–187. [PubMed: 20010071]
136. Szekeres-Bartho J, Wegmann TG. A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. *Journal of reproductive immunology*. 1996; 31(1-2):81–95. [PubMed: 8887124]
137. Kersh EN, Adams DR, Youngpairoj AS, et al. T cell chemo-vaccination effects after repeated mucosal SHIV exposures and oral pre-exposure prophylaxis. *PLoS One*. 2011; 6(4):e19295. [PubMed: 21541293]

Executive Summary

- The development of an effective means of preventing virus transmission is a key priority in the effort to stem the HIV pandemic.
- Microbicide development should focus on protecting against both vaginal and rectal transmission of HIV. The formulation of a successful rectal microbicide may have to be different than a vaginal one.
- Many promising microbicide candidates in vitro failed to protect against infection in vivo. This was often related to effects on the mucosal environment.
- The cervicovaginal and rectal mucosa provide both physical and biological protection from HIV infection.
- Semen has numerous proviral effects, including neutralization of the acidic vaginal environment, recruitment of HIV target cells, and inactivation of antiviral peptides.
- During transmission of HIV, the virus crosses the epithelial barrier, infects resting CD4 T cells in the submucosa, and establishes a small founder population of infected cells. Expansion of the infection occurs by recruitment of new target cells to the initial cluster.
- A successful microbicide must be efficacious, safe, acceptable and affordable. A key attribute for a safe microbicide is the lack of any proinflammatory or toxic effects to the epithelium.
- Results from the CAPRISA 004 trial, in which a 1% tenofovir gel applied before and after sex provided 39% overall protection from infection, validates the use of microbicides to prevent transmission.
- Improved preclinical models are needed to test potential microbicides. This would allow better early detection of microbicide toxicity, as well as easier identification of the most promising candidates.

1. . Potential mechanisms of HIV infection across the mucosal surface

HIV must penetrate the outer mucus layer in order to reach the surface of the mucosal epithelium. In binding to mucosal epithelial cells, the virus induces “outside-in signaling” that results in the release of chemokines that can recruit new virus target cells to the subepithelium - setting the stage for local amplification of the “founder” infection. It is possible that this process of virus binding, and “outside-in signaling” may be further amplified as a consequence of SEVI-enhanced virus binding to epithelial cells. Once the virus has reached the mucosal epithelial surface, it must cross the epithelial barrier in order to initiate infection of target CD4⁺ T cells in the subepithelial tissue. This may occur via an abraded, inflamed or injured area of epithelium, via transmigration or transcytosis, or possibly via uptake of virus particles by dendritic cell processes that protrude into the luminal space. After crossing the epithelial barrier, the virus then establishes a localized infection in a small population of “founder” cells (“resting” CD4⁺ T cells). These initial steps of virus passage across the mucosal barrier represent a key first level for therapeutic intervention, at which microbicides can act to block transmission. Following these earliest events, little virus is produced for the next several days. This *in vivo* “eclipse” phase (defined by the lack of detectable new virus production) represents a second, important window of opportunity for therapeutic intervention (such as vaccine-mediated protection).

Finally, at about 7-10 days following initial transmission, the virus establishes a disseminated infection and begins to replicate exponentially throughout the body.

2. . HIV Transmission via the Stratified Squamous Epithelium of the Vagina and Ectocervix

The stratified squamous epithelium found in the vagina and ectocervix provides extensive protection against HIV infection. HIV is deposited on the mucosal layer and must cross through the thick mucin network and withstand innate immune defenses that include lysozymes, lactoferrin, secretory leukocyte peptidase inhibitor (SLPI), and human beta defensins (HBDs). Disruption of the intact epithelium through abrasion, infection with pathogenic microorganisms (e.g., bacterial vaginosis) or changes induced by hormonal status can impair the protection provided by this multilayered epithelial barrier, allowing the virus to more easily access target cells below the basement membrane. Finally, by analogy to other mucosal surfaces, it is possible that the dendrites of interdigitating Langerhans cells may project into the vaginal lumen. If so, HIV may also be able to circumvent the epithelial barrier by attaching to these exposed dendrites, and then undergoing intracellular uptake and transport across the intact epithelial barrier.

3. . HIV Transmission via the Simple Columnar Epithelium of the Endocervix and Rectum

The single layered simple columnar epithelium in the endocervix and rectum provides comparably little defense against HIV, when compared to the stratified squamous epithelium of the vagina and ectocervix. Abrasion, microbial infection or any event that alters the integrity of the single layer can enhance the accessibility of underlying target cells to HIV infection. The gut mucosa is also known to contain lamina propria dendritic cells (DC) which sample the gastrointestinal lumen - potentially providing a means of transportation for HIV virions across an intact mucosal epithelium. The rectum and possibly the endocervical epithelium may also contain such cells. Finally, the rectum also contains Microfold cells (M cells) which can bind and transport HIV to underlying target cells.

Table 1

Selected Clinical Trials of HIV Microbicide Candidates

	Trial Name/Number	Current status	Composition/Mechanism of Action	Notes	Ref.
Surfactants					
Nonoxonyl (N-9)	NCT00000926	Failed Phase III trials	Detergent; disrupts virion integrity	Caused vaginal ulceration and inflammation	(3,53)
SAVVY® gel (C31G)	NCT00129532, NCT00130078	Inconclusive in Phase III trials	Detergent; disrupts virion integrity	Low rate of HIV infection in both arms of the study eliminated the ability to draw statistically significant differences between the groups.	(54,55)
Entry Inhibitors					
PRO 2000	MDP301	Failed Phase III trials	Anionic polymer; binds gp120	Safe but ineffective	(60)
Cellulose Sulphate	NCT00153777	Failed Phase III trials	Anionic polymer; binds gp120	Enhanced HIV infection, caused damage to epithelial barrier	(61)
Vivagel® (SPL7013)	MTN-004	Inconclusive safety profile (phase I)	Polyanionic dendrimer; binds gp120	Generally well tolerated but did induce mild epithelial irritation and inflammation in some users	(57)
Carraguard®		Failed Phase III trials	Polyanion derived from carrageenan; binds gp120	Safe but ineffective Being studied as microbicide vehicle	(62)
Vaginal Milieu Protectors					
BufferGel®	HPTN 035	Failed Phase II trials	Maintains acidic vaginal pH	Safe but ineffective	(70)
Reverse Transcriptase inhibitors					
Tenofovir (1% gel formulation)	HPTN 050, HPTN 059, CAPRISA 004 MTN-003 (VOICE)	Effective in Phase IIb trials	Nucleotide reverse transcriptase inhibitor (NRTI), with a prolonged intracellular half-life	39% effective in preventing HIV infection overall, and 54% effective in highly adherent persons	(76)
Dapivirine (TMC-120)	IPM020, IPM024, IPM003, IMP005B, MTN-013/IPM-026	Safe in phase I/II studies. Further Phase II and Phase III trials planned	Non nucleoside reverse transcriptase inhibitor (NNRTI)	Being tested in both gel and vaginal ring form, for extended release	(80,81)