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# Non-Antiretroviral Microbicides for HIV Prevention

*Yanille Scott[1] and Charlene S. Dezzutti[2]*

*[1]University of Pittsburgh, Graduate School of Public Health; [2]University of Pittsburgh, School of Medicine; Magee-Womens Research Institute. Pittsburgh, USA*

## Abstract

***Non-antiretroviral microbicide candidates were previously explored as a female-controlled method of preventing sexual transmission of HIV. These products contained non-HIV specific active compounds that were ultimately found to disrupt the vaginal epithelium, cause increased immune activation in the female genital tract, disturb vaginal flora, and/or cause other irritation that precluded their use as vaginal microbicides. Due to the failure of these first-generation candidates, there was a shift in focus to developing HIV pre-exposure prophylaxis and microbicides containing small-molecule antiretrovirals. Even with the limited success of the antiretroviral-based microbicides in clinical evaluations and no commercially available products, there has been significant progress in microbicide research. The lessons learned from previous trials have given rise to more rigorous preclinical evaluation that aims to be better at predicting microbicide efficacy and safety and to novel formulation and delivery technologies. These advances have resulted in renewed interest in developing non-antiretroviral-based microbicides, such as broadly neutralizing antibodies (for example, VRC01) and anti-viral proteins (for example, Griffithsin), as options for persons not wanting to use antiretroviral drugs, and for their potential to prevent multiple sexually transmitted infections.*** **(AIDS Rev. 2016;18:145-50)**

*Corresponding author: Charlene S. Dezzutti, cdezzutti@mwri.magee.edu*

## Key words

***Pre-exposure prophylaxis. HIV antibodies. Broadly neutralizing monoclonal antibodies. Plant lectins. Multi-purpose technologies.***

## Introduction

The women of sub-Saharan Africa bear the greatest burden of incident HIV infections in the world. This is due in part to social factors that restrict their ability to negotiate condom use in their sexual relationships; intravaginal hygiene practices that affect the vaginal mucosa[1,2]; lack of perceived HIV risk[3-5]; and innate biological factors that may make them more susceptible to HIV infection[6,7]. To address this disparity, research has been ongoing from the early days of the HIV epidemic toward the goal of producing self-administered vaginal microbicides for women[8]. Microbicides are products designed to prevent the sexual transmission of HIV and potentially other sexually transmitted diseases. The first products were primarily gels because vaginal gel and cream products, such as spermicides and vaginal yeast medications, existed and the technology for their manufacture was readily accessible. However, since then the dosage forms and delivery technologies used for microbicide products have expanded. This review provides an overview of the evolution of microbicide products whose active compounds are not small molecule HIV inhibitors, herein referred to as non-antiretroviral (non-ARV) microbicides, and discusses progress in the development and formulation of these new non-ARV microbicide candidates.

**Correspondence to:**
Charlene S. Dezzutti
E-mail: cdezzutti@mwri.magee.edu

**Table 1. Outcomes of clinical trials evaluating first-generation non-antiretroviral microbicides**

| Microbicide candidate | Clinical trial outcome | References |
|---|---|---|
| N-9<br>(Surfactant) | No reduction in HIV incidence; higher incidence of genital ulcers<br>No reduced incidence of HIV, gonorrhea or chlamydia<br>Enhanced HIV acquisition | Kreiss, et al.[10]<br>Roddy, et al.[11]<br>Van Damme, et al.[12] |
| SAVVY<br>(Surfactant) | Did not prevent male-to-female HIV transmission<br>Higher incidence of reproductive tract adverse events | Feldblum, et al.[13]<br>Peterson, et al.[14] |
| Carraguard<br>(Carrageenan derivative) | Did not prevent HIV transmission | Skoler-Karpoff, et al.[21] |
| BufferGel<br>(Carbomer polyacrylic acid) | Did not prevent HIV transmission<br>Did not prevent other sexually transmitted infections | Abdool Karim, et al.[15]<br>Guffey, et al.[16] |
| PRO 2000<br>(Naphthalene sulfonate) | Ineffective in reducing HIV acquisition | McCormack, et al.[17] |
| Cellulose Sulfate<br>(Sulfated polymer) | Increased HIV incidence with cellulose sulfate use, trial being halted early | Ramjee, et al.[9] |
| VivaGel<br>(Dendrimer) | Mild-to-moderate perturbations of genital and urinary tract microflora<br>Poor acceptability | Carballo-Dieguez, et al.[18],<br>McGowan, et al.[19]<br>O'Loughlin, et al.[20] |
| ACIDFORM<br>(Buffering gel) | Mild-to-moderate genitourinary irritation<br>Inconclusive microbicidal efficacy | Keller, et al.[22]<br>Keller, et al.[22] |

## First-generation candidates

The first-generation microbicide candidates were non-ARV compounds that demonstrated non-specific HIV inhibitory activity. These included cellulose sulfate[9], an array of surfactants (N-9[10-12], SAVVY[13,14]), polyanions (PRO 2000[15-17]), dendrimers (VivaGel[18-20]), carrageenan derivatives (Carraguard[21]), and buffering agents (BufferGel[15-17], ACIDFORM[22]) designed to inactivate the virus prior to infection of local immune cells and/or to bolster natural vaginal defenses to HIV infection. These early products were also shown to have some broad-spectrum antimicrobial activity, partly due to their non-specific nature, which was desirable for simultaneous prevention of several sexually transmitted infections. An additional benefit of using non-ARV compounds was their inability to contribute to the development of drug resistance mutations that could potentially arise with long-term use of ARV drugs. Non-ARV microbicides would also be effective against wild-type and circulating drug-resistant strains of HIV. Despite their promise, the first-generation microbicide products were not successful in clinical evaluations due to lack of efficacy or poor safety indicators (Table 1).

## New non-antiretroviral microbicides

With the lack of success of the first-generation non-HIV specific microbicide candidates, the field moved toward ARV drugs. Although ARV microbicide candidates have had some success in clinical trials, efforts have recently been refocused on developing non-ARV alternatives. This is amid concerns of increased transmission of drug-resistant HIV due to larger scale use of ARVs for prevention of mother-to-child transmission and increased access to combination ARV therapy. It has also been acknowledged that there is not a "one-size-fits-all" microbicide product that meets the needs of every susceptible niche population. Hence, non-ARV candidates would provide additional options for HIV prevention. The new non-ARV microbicide candidates are highly HIV-specific in comparison to the first-generation microbicides, and include active biologics like algal and bacterial lectins, as well as broadly neutralizing monoclonal antibodies specific for HIV envelope epitopes.

Cyanovirin-N, a mannose-binding lectin isolated from the cyanobacterium *Nostoc ellipsosporum,* binds to HIV gp120 in a manner that is independent of CD4 receptor or coreceptor interactions[23]. This activity

inhibits HIV binding and entry, thus reducing HIV transmission. Cyanovirin-N has been shown to prevent vaginal acquisition of SHIV in nonhuman primate models[24] and in human *ex vivo* tissues[25]. Another mannose-specific lectin, Griffithsin, was isolated from the marine red alga, *Griffithsia sp.* This protein binds to HIV virions, preventing viral adsorption to target cells and causing an irreversible inhibition of HIV infectivity. Griffithsin has shown potent cross-clade anti-HIV activity[26] and broad-spectrum antiviral activity against HSV-2[27], hepatitis C[28], and coronaviruses[29]. The lectins generally have favorable safety profiles *in vitro*[30,31] and in macaque studies[24], although an *in vitro* study showed that cyanovirin-N had mitogenic effects and caused increased expression of inflammatory markers in PBMC cultures[32]. Development of a cyanovirin-N microbicide is ongoing and work is in progress to define clinical safety and efficacy metrics of a rectal-specific griffithsin gel product.

Broadly neutralizing monoclonal antibodies to HIV were isolated from chronically HIV-infected individuals and demonstrate cross-reactivity to multiple HIV strains. Neutralizing antibodies arise during acute infection, but develop increasingly broad neutralizing capacity over time through a process of somatic hypermutation[33]. HIV neutralizing antibodies were previously the focus of vaccine researchers; however, their potential as HIV microbicides is now being explored. These antibodies bind to key regions on the HIV gp120 envelope protein, preventing interactions with entry receptors and co-receptors on host target cells, thereby reducing viral entry.

Neutralizing antibodies were shown to inhibit HIV infection *in vitro*[34-36], and in mouse[37] and macaque models[38-40] of infection. Importantly, animal studies involving topical vaginal application of neutralizing antibodies with subsequent vaginal viral challenge have provided proof-of-concept data that antibodies can retain their function in mucosal secretions and are a viable microbicide candidate[37,41]. Ongoing preclinical evaluation of topically applied neutralizing antibodies showed that they reduced HIV transmission in polarized human ectocervical and colonic explants *ex vivo*[42]. Neutralizing antibodies evaluated for efficacy in the presence of semen also retained their potency[42], supporting their suitability for use in preventing sexual HIV transmission. This is in comparison to polyanionic gel microbicides, which showed reduced potency in the presence of semen[43]. Use of combinations of HIV neutralizing antibodies could provide increased neutralization breadth compared to single antibodies as multiple HIV epitopes would be targeted simultaneously. This principle may also be applied to designing a broad-spectrum neutralizing antibody microbicide composed of a cocktail of antibodies that targets a range of other sexually transmitted pathogens. Another benefit of using neutralizing antibodies, as well as the lectins, is that they are both expected to retain efficacy against HIV strains that have developed drug resistance mutations to HIV enzyme inhibitors used in ARV therapy.

Traditionally, antibodies have been produced in cell lines or expressed in humanized mice. However, it has become possible to produce large quantities of human antibodies at lower cost in *Nicotiana sp.* plant expression systems[44]. The use of transient plant expression systems makes it relatively easy to customize and optimize antibodies. Additionally, the ability to reverse engineer antibodies to key HIV antigens, and optimize *Fc* and complementarity-determining regions to improve neutralization breadth and potency[45], broadens the scope of potential neutralizing antibody diversity and would be indispensable to generating a diverse range of antibody-based microbicides. The promise of neutralizing antibodies is their potential to offer a continuous supply of new microbicide candidates.

With this new generation of protein-based microbicide agents, the issues of rapid, large-scale, low-cost production may be potential barriers to their accessibility as microbicides. Like the monoclonal neutralizing HIV antibodies, lectins are being produced on a commercial scale in non-mammalian expression systems. Both griffithsin and cyanovirin-N production utilizes a *Nicotiana sp.* transient expression system[26,46], and scalable production of cyanovirin-N is also being optimized in *Escherichia coli*[47], *Lactobacillus jensenii*[48], and soya bean[49] expression systems. Another factor that may be critical to the success of lectins or neutralizing antibodies as microbicides is the formulation strategy used. As luminally active products, these proteins are required to be present in sufficient quantities at every possible surface of contact on the mucosa of the female reproductive tract or lower gastrointestinal tract at the time of semen ejaculation. Additionally, the formulation must support the optimal homeostatic conditions of the vagina or rectum while maintaining the integrity of the functional immunoglobulins and lectins. Hence, appropriate formulation and delivery technologies are pivotal for the success of the new generation of non-ARV microbicides.

## New microbicide formulation and delivery technologies

The focus of microbicide design has shifted from coital-dependent aqueous gels designed exclusively for vaginal use to generating multiple microbicide options that use various formulation and delivery platforms to appeal to as many vulnerable populations as possible. These also include the first products designed specifically for rectal use[50].

Advances in vaginal drug pharmaceutics have generated new solid (e.g. quick dissolving films and tablets) and semi-solid dosage forms (e.g. suppositories) that can deliver active molecules with diverse physical properties, including hydrophobic or hydrophilic molecules and proteins that have proven challenging to deliver in aqueous-based formulations[51]. This expands the repertoire of potential microbicide candidate compounds to include those that may have had unfavorable release profiles in an aqueous gel format. Solid dosage forms would also circumvent common user acceptability issues often associated with vaginal gels such as messiness or leakage[52]. Moreover, these solid dosage forms may be used to co-formulate active compounds with incompatible physicochemical properties into a single delivery platform[51]. This technology could also lend itself well to developing multipurpose products that incorporate multiple active compounds to prevent simultaneous infection by various pathogens or concomitantly provide contraception.

The first-generation microbicides were hyperosmolar gels and optimized to preserve the low pH vaginal environment and the *Lactobacillus*-dominant vaginal microbiome[53]. However, the colorectal compartment has a distinctly different homeostatic environment. The lower gastrointestinal tract has a pH closer to neutral and is sensitive to low pH and hyperosmolar conditions[54-56]. Previous studies showed that rectal application of hyperosmolar products resulted in reduced epithelial barrier function and caused epithelial disruptions in the rectal lumen that could enhance HIV infection[57]. Hence, new formulations of non-ARV microbicides are being explored to address the need for rectal-specific microbicides. A topically applied cyanovirin-N gel was effective in preventing rectal SHIV transmission in macaques[58], and a griffithsin gel is currently being investigated as the first rectal-specific non-ARV microbicide (K. Palmer, personal communication).

Multipurpose products were conceptualized early on in microbicide research, and microbicide candidates that also had purported contraceptive efficacy (N-9, SAVVY[59], Cellulose sulfate[60] and BufferGel[61]) were highly desirable. In this new era of non-ARV microbicide development, products with both microbicidal and contraceptive properties are needed to meet the demand for polyfunctional products. More progress has been made with ARV-based multipurpose technologies, which include co-formulations with the hormonal contraceptive, levonorgestrel, or the anti-herpetic, acyclovir[62,63]. However, non-ARV multipurpose options are also being investigated. Engineered *Lactobacillus jensenii,* which produce cyanovirin-N, are being developed to prevent bacterial vaginosis and HIV[64]. These engineered bacteria are intended to colonize the vagina and promote a healthy vaginal microbiota, while producing sustained protective concentrations of cyanovirin-N. Also, a vaginal film designed to deliver a combination of the broadly neutralizing HIV antibody, VRC01, and the herpes simplex virus-1/2 neutralizing antibody, HSV8, is also in the early stages of clinical evaluation (K. Whaley, personal communication).

Alternative delivery formats are also being explored for non-ARV-based HIV prevention products. Studies are ongoing to determine the safety and pharmacokinetic metrics of a passive infusion of VRC01 in children and cohorts of HIV-positive and -negative adults[65-67]. Sustained-release or long-acting formulations and delivery platforms are being investigated as options to help improve adherence to the product. A long-acting injectable nanosuspension formulation of VRC01 is also being developed for use as pre-exposure prophylaxis (PrEP). Additionally, studies conducted to evaluate the sustained release of HIV-neutralizing antibodies achieved through the intramuscular administration of adenoviral vectors showed that mice were protected from subsequent HIV infection[68]. This novel delivery platform would provide longer lasting protection than a typical gel format and represents a trend toward designing HIV prophylactics with more prolonged protective effects than traditional on-demand microbicide products. The potential benefit of using a long-acting formulation was observed in the results of the VOICE and FACTS 001 trials, which evaluated the efficacy of daily or pericoital applied tenofovir 1% gel in high-risk young women. Poor efficacy of tenofovir gel was attributed to poor adherence in both trials, while the subset of women who used the product more often achieved greater reduction in HIV transmission risk[69,70]. Hence, new delivery formats that do not rely on user compliance are expected to improve adherence, and contribute to improved efficacy metrics for new non-ARV products. Non-ARV microbicides may be more

successful, with longer acting formulations or sustained delivery methods that would allow the user a larger window of protection. That leeway may result in more effective non-ARV microbicides.

## Conclusion

The new generation of active biologic non-ARV microbicides represents a departure from the non-HIV specific compounds that had been considered previously. They are highly specific for HIV and are expected to inhibit transmission of viral strains that are resistant to HIV reverse transcriptase, integrase, and protease inhibitors. These non-ARVs present alternatives for individuals who may not benefit from ARV-based microbicide products. Antibody and lectin-based microbicides provide options where approved PrEP strategies are not effective due to drug resistance, such as in serodiscordant partnerships where infected partner has failed ARV therapy with drug-resistant virus. These microbicides may also be a more attractive non-chemotherapeutic option for individuals who want to avoid the potential side effects of taking a drug or the stigma of taking an ARV for HIV prevention. Preclinical data for neutralizing antibodies and lectins indicate they would be successful as topical microbicides. And although clinical evaluations of these new non-ARV microbicide candidates are just beginning, these compounds provide hope for a marketable non-ARV microbicide product in the near future.

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