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Non-Specific Microbicide Product Development: Then and Now

Joseph W. Romano[*,1], **Melissa Robbiani**[2], **Gustavo F. Doncel**[3], and **Thomas Moench**[4]

[1]NWJ Group, LLC, 101 Oakford Circle, Wayne, PA 19087, USA

[2]Center for Biomedical Research, Population Council, 1230 York Avenue, New York, NY 10065, USA

[3]CONRAD, Eastern Virginia Medical School, 601 Colley Ave., Norfolk, VA 23507, USA

[4]ReProtect, Inc., Baltimore, MD 21286, USA

Abstract

Despite the identification of HIV-1 as the etiological agent responsible for AIDS nearly 30 years ago, a sterilizing vaccine capable of preventing transmission of the virus remains elusive. In response to struggles on the vaccine development front, significant effort has been devoted to preventing the transmission of HIV with alternative products, technologies, and strategies. One of the early alternative HIV prevention strategies was microbicides, which are topical products that can be used to prevent sexual transmission of HIV either vaginally or rectally. First generation microbicide products were designed to be simple gel formulations comprised of readily available active agents that were inexpensive and broadly active (i.e., non-specific). Unfortunately, despite the clinical investigation of multiple product concepts satisfying these requirements, none were shown to be efficacious in pivotal trials. More recently, microbicide and oral prevention strategies involving highly specific and potent anti-retroviral (ARV) drugs have shown to be efficacious in trials. Although building on these successes continues, these products have a number of issues including potential toxicity with long term use, selection of HIV resistance, and cost. Further, all of the original justifications for non-specific microbicide products remain valid. This review provides a brief history of non-specific microbicide development, outlines the evolution to, and limitations of, ARV based microbicides, and summarizes the current activity on non-specific microbicide product development.

Keywords

Microbicides; HIV; prevention; product development

[*]Address correspondence to this author at the NWJ Group, LLC, 101 Oakford Circle, Wayne, PA 19087, USA; Tel: 610-225-0478; Jromano.nwjgroup@gmail.com.

CONFLICTS OF INTEREST

As an employee of the non-profit organization, The Population Council, MR is involved in the research on the ZA-containing formulations, but has no financial interest in any of the organization's research or products.

INTRODUCTION

The need for HIV prevention strategies dates back to the earliest days of the epidemic. Identification of HIV as the pathogenic cause of AIDS [1,2] spawned hopes for a speedy path to a preventive vaccine. Nearly 30 years later, despite some recent positive results in vaccine development [3], a highly efficacious vaccine for HIV remains elusive. Over that time period, highly effective treatment strategies have emerged that can largely control HIV infection and have greatly enhanced survivability and quality of life for HIV infected patients [4]. However, these treatment strategies can be costly and have been challenging to fund and apply broadly in low resource countries. With neither a highly potent vaccine or effective treatment readily available in resource poor settings, the epidemic has progressed to a devastating level in parts of the world least equipped to address the situation. Today more than 30 million people are living with HIV, and there were more than 2.6 million new infections in 2009, most of which occur in sub-Saharan Africa [5]. Further, the incidence of new infections in women continues to be a major health problem in these settings, and is likely based in both biological and socioeconomic factors.

Nearly 20 years ago, the notion of an alternative prevention strategy based on the use of vaginally dosed products to inhibit HIV infection in women was formalized [6,7,8]. The concept of a “microbicide” product was born out of the unavailability of a vaccine and the limited capacity for women to negotiate the use of condoms by their male partners, which compounds their apparently elevated biological susceptibility to sexual transmission of HIV. Women in resource poor parts of the world desperately needed a means of controlling their risk of infection and to be empowered to make their own decisions regarding their sexual and reproductive health. Consequently, to best serve this population, microbicide products would need to be safe and effective at preventing sexual transmission of HIV, acceptable to the at risk populations, inexpensive and easily distributed, and women initiated. Further, in view of the rapidly expanding nature of the epidemic (particularly in women) and slow progress on vaccine development, microbicide products needed to be available as soon as possible. In the earliest days of microbicide development options that satisfied the microbicide target product profile were extremely limited. Anti-retroviral (ARV) agents used in treatment were expensive, and generally, their developer pharmaceutical companies had limited interest in pursuing their use for prevention. Therefore, the earliest microbicide product development efforts attempted to take advantage of inexpensive and available agents that could be readily formulated for vaginal (and eventually rectal) use, and had the additional potential to serve as inhibitors of other sexually transmitted infections (STI) and as contraceptives. The prevention of other STI and pregnancy remain important to the base goal of HIV prevention, given that the former are co-factors increasing susceptibility to HIV infection [e.g., 9], and that contraceptive efficacy provides a non-stigmatized additional motivation for microbicide uptake and use, which does not require women to self-identify as “high risk”. This review summarizes the early attempts to develop such microbicide products and how those first generation product development efforts led to the current state of the microbicide field. Table 1 provides a summary of the early generation, non-specific microbicide candidate products.

THE USE OF SURFACE ACTIVE AGENTS

Nonoxynol 9 (N-9) is a non-ionic surfactant that has been used as an over-the-counter (OTC) spermicide to prevent pregnancy for decades. The spermicidal effect of N-9 is achieved *via* solubilization of the sperm cell membrane [10]. A number of contraceptive vaginal formulations of N-9 (2–12%) are commercially available. In addition to its spermicidal activity, a number of *in vitro* studies have demonstrated that not only could N-9 inhibit HIV [11,12], but it was also effective against several other STIs [13,14] including Herpes Simplex Virus (HSV) [15]. Further, an animal model had been used to demonstrate that N-9 could inhibit transmission of SIV *in vivo* [16]. The *in vitro* and *in vivo* anti-microbial and anti-viral activity associated with N-9, along with the fact that vaginal formulations for contraception were available as OTC products suggested that evaluation of N-9 containing formulations for the prevention of HIV transmission in the clinic was reasonable. This was supported by other safety data for N-9 available at the time [17].

Vaginal gel formulations with N-9 were evaluated in a number of safety, acceptability, and efficacy studies as a potential microbicide product for the prevention of HIV transmission and STI [18–22]. Perhaps the most relevant of these trials was the evaluation of the COL-1492 formulation of N-9 (3.5%, 52.5 mg dose) in female sex workers [22]. This study was conducted in four countries (three in sub-Saharan Africa and Thailand) with 765 women randomized 1:1 with a placebo gel control. HIV incidence in the N-9 group was 16% versus 12% in the placebo group (hazard ratio adjusted for center 1.5; 95% CI 1.0–3.2; p=0.047). Thirty-two percent of the women reported mean use of more than 3.5 applicators per day (instructions were to use within one hour prior to sex), and the HIV infection rate in this group was nearly twice that in placebo users. Thus, not only did this N-9 product fail to show efficacy against HIV transmission in this high risk population, intensive use of the product was likely increasing the risk of HIV infection. Although previous studies had shown this formulation to be both safe [19] and acceptable [18] in similar populations of women, the results from the efficacy study clearly demonstrated that N-9 was not a viable candidate for microbicide development. In rectal safety studies, N-9 formulations were shown to cause severe epithelial damage, indicating this compound was not appropriate for rectal use [23].

Another surface active drug, C31G, was also developed as a vaginal microbicide and contraceptive product. Savvy® is a vaginal gel formulated with 1% C31G, an amphoteric surfactant that functioned similar to N-9 in terms of cellular membrane disruption. C31G was shown to be a potent inhibitor of HIV *in vitro* [24] and had an acceptable cytotoxicity profile [25]. Like N-9, C31G was also inexpensive, potentially broad spectrum [26], and contraceptive [27]. Therefore, a series of phase 1 safety studies were conducted with the final vaginal gel formulation of C31G, including male and female tolerance and acceptability trials [28,29]; a post-coital testing study [30], and a daily use dose escalation study [31]. These studies demonstrated that C31G gel formulation was generally safe and well tolerated. Consequently, the Savvy gel was progressed into two HIV prevention phase 3 efficacy studies (one in Ghana [32] and one in Nigeria [33]), and a third contraceptive efficacy trial [34]. In the Ghana study, a total of 17 seroconversions occurred; eight in the Savvy group and nine in the placebo group. In the Nigeria study, there were 21

seroconversions in the Savvy group and 12 in the placebo group. The number of adverse events (AE), including reproductive tract and pelvic findings were similar in the two groups in the Nigeria study, however more reproductive tract AE were observed in the Savvy arm versus placebo in the Ghana trial (13.0% versus 9.4%, respectively). Both of these studies failed to reach the required number of HIV endpoints to achieve power to determine an effect by Savvy, and both studies were consequently stopped early for futility.

With multiple phase 3 trials showing lack of effectiveness and possible harm, and a series of *in vitro* and animal studies linking surfactants to inflammatory changes in the cervicovaginal mucosa [35–39], the microbicide field was ready to move away from surfactant based product options.

DEVELOPMENT AND CLINICAL EVALUATION OF BUFFERING AGENTS

Microbicides based on acid buffering were developed in light of reports based on experiments indicating that HIV-1 was inactivated at acid pH [40,41], and that lymphocytes and macrophages, potential vectors for entry of HIV [42–46], can be immobilized and killed [46] by acidity. In addition, HSV-2, certain bacterial STI pathogens, and sperm can also be inhibited at acid pH and their transmission inhibited by acid buffering microbicides [47–51]. Further supporting the potential utility of vaginal acidification are the consistent epidemiological observations that women with lactobacillus-dominated flora and vaginal acidity are at reduced risk of HIV, HSV, and other STIs compared to women with bacterial vaginosis (BV), a condition characterized by the loss of lactobacilli and vaginal acidity [52–56].

BufferGel® [51], Amphora™ (formerly Acidform™) [57], and lime juice are acid buffering agents that have been studied clinically. Development of lime juice as a microbicide has been abandoned due to cervicovaginal epithelial toxicity [58, 59]. Amphora has shown protection against vaginal transmission of *Neisseria gonorrhoeae* [49] and HSV [50] in animal models, and has been evaluated in human safety studies [60,61]. Amphora is being studied in a Phase 3 contraceptive trial (ClinicalTrials.gov Identifier NCT0130633), but has not entered HIV/STI efficacy studies.

BufferGel is the most extensively studied of the acid buffering products. BufferGel was protective against *Chlamydia trachomatis*, HSV-2, and cottontail rabbit papilloma virus in mouse and rabbit models [47,48], and was contraceptive in the rabbit [47]. In the pigtailed macaque vaginal model, BufferGel had a good safety profile, but did not protect against chlamydia challenge [62]. It was effective in blocking vaginal infection with an HIV-infected-cell challenge in the huSCID mouse model [46] but did not protect macaques from cell-free SIV challenge (CC Tsai, University of Washington unpublished).

In human Phase 1 safety trials, BufferGel was safe and acceptable [63,64]. Moreover, BufferGel significantly reduced BV prevalence in women with BV at enrollment [64]. BufferGel was chosen for inclusion in a Phase 2b HIV effectiveness trial (HPTN 035) due to its theoretical safety (reinforcing a natural vaginal protective mechanism), its observed safety in animal models [62] and Phase 1 trials [63,64], and its activity in some vaginal challenge models. It was also chosen for inclusion in the trial because its mechanism of

action was distinct from the second agent studied in the trial, the polyanion PRO 2000. Finally, BufferGel's contraceptive potential (later confirmed in a Phase 3 efficacy trial [65]) was considered a potential benefit in providing multipurpose protection, and providing a non-stigmatized motivation for product use.

However, BufferGel was ineffective in reducing the incidence of HIV and other STIs in the HPTN 035 trial [66]. What factors might explain it's failure? Recent studies of non-clade B HIV-1 have been reported showing lower sensitivity to acid inactivation [67] than those of clade B virus [40,41]. BufferGel has a relatively short duration of action [47], thus likely requiring close adherence to precoital dosing timing, and thus increasing the difficulty of adherence. Duration may be particularly limited in women lacking an intrinsically acidic vaginal environment, since here BufferGel's acidity would more rapidly decline. And, unlike the outcome during twice-daily BufferGel dosing in Phase 1 where BV prevalence was substantially reduced [64], BufferGel did *not* reduce BV prevalence in HPTN 035, where dose frequency was <3/week. Finally, BufferGel effectiveness is lost after modest dilution [51]. Challenge studies with a variety of microbicide candidates indicate that *in vivo* efficacy often require microbicide concentrations two to three orders of magnitude higher than those effective *in vitro* [68,17]. These factors, individually or in combination likely are responsible for the lack of BufferGel effectiveness against HIV/STI in HPTN 035.

THE USE OF POLYANIONS FOR THE PREVENTION OF HIV INFECTION

Early in the HIV epidemic, polyanions, electronegatively charged polymers, were shown to inhibit HIV-1 replication, primarily by preventing viral entry through interference with gp120-cell receptor interactions [69–71]. *In vitro* and animal studies proved that, in general, these compounds were safe, particularly for topical use [72,73]. With varying degrees of activity, most of them also inhibited other sexually transmitted viruses such as HSV-2 and HPV [74,75]. Higher potency against HIV-1 and HSV-2, better safety profiles, ease of manufacturing in gels and lack of restrictive patents made polyanions reasonable candidates to follow surfactants in the quest for a successful microbicide. Among all the compounds with demonstrated anti-HIV activity, three were fully developed and entered clinical effectiveness trials. These were cellulose sulfate (UsherCell), carrageenan (Carraguard), and a sulfonated naphthalene derivative (PRO 2000).

Cellulose sulfate (CS) was developed by Rush University in collaboration with CONRAD and Polydex, Inc. CS is a sulfate ester of β1,4-glucose polymer with an average molecular weight around $2x10^6$ Daltons, which displays inhibitory activity against HIV-1/2, HSV-2, HPV, *N. gonorrhoeae*, *C. trachomatis*, and other STI-causing pathogens [49, 75–77]. Numerous phase 1/2 clinical studies support CS cervicovaginal and penile safety [78–80]. CS is the only polyanion with clinically proven contraceptive efficacy [81]. Two clinical HIV effectiveness trials were run concomitantly, sponsored by CONRAD and FHI360, respectively [82,83]. The final, intent-to-treat analysis of the results of the CONRAD trial, which was stopped early due to the independent data monitoring committee's recommendation, revealed 25 and 16 new infections in the CS and placebo arms, respectively, with a relative risk (RR) of 1.61 (0.86–3.01), which was not statistically significant (p=0.1). Acting on the possibility of harm, the FHI360 trial was also stopped

early, showing 10 and 13 new seroconversions in the CS and placebo arms, respectively, with a RR=0.8 (0.3–1.9; p=0.56).

Carraguard, developed by the Population Council, is a mixture of lambda and kappa carrageenans, which are seaweed-derived compounds with demonstrated antiviral and antibacterial activity and a track-record of safety as food additives [49, 72, 84–87]. Recent macaque studies of Carraguard have shown statistically significant partial protection against RT-SHIV [88]. These results seem to contradict *in vitro* data suggesting weak potency against R5 viruses and a possible enhancement of infection of RT-SHIV at low concentrations [88,89]. A randomized, placebo-controlled trial of Carraguard in South Africa revealed similar HIV incidence in both Carraguard and placebo groups with a hazard ratio of 0.87 (0.69–1.09) and no significant difference in the time to seroconversion (p=0.30) [90]. Gel use based on applicator testing was about 42% on average, suggesting poor protocol adherence.

PRO 2000, developed successively by Procept, Indevus and Endo Pharmaceutical Solutions, is a synthetic naphthalene sulfonate derivative (~5 kDa) displaying antiviral and antibacterial activities [49,91,92]. Macaque studies revealed partial protection against X4 and R5 SHIV [93,94]. Phase 1/2 clinical studies demonstrated safety for PRO 2000 0.5 and 2.0% gels [95–97]. Two randomized, placebo-controlled studies were performed to test the effectiveness and safety of PRO 2000 gel against HIV-1 [98,99]. The HPTN-035 (phase 2b) compared PRO 2000 0.5% gel and BufferGel against placebo and no gel (condom-only), while the MDP-301 compared PRO 2000 0.5 and 2.0% gels with placebo gel. Although the HIV incidence was lower for PRO 2000 0.5% in the HPTN trial (HR=0.7; p=0.1), the more powerful phase 3 MDP trial did not show any statistically significant difference, demonstrating no protection against HIV-1 acquisition.

Other polyanionic compounds were developed as microbicides but never made it past preclinical stages or phase 1 clinical trials [100]. Notwithstanding their differences, all three polyanions tested in effectiveness trials failed to protect women against HIV-1. Reasons for this failure may be found in their low potency (in comparison to antiretrovirals), negligible absorption with the consequent need to act at the surface of the mucosa, reduction of activity in seminal plasma, short duration of effect, and possible induction of mucosal and microflora changes facilitating HIV-1 initial infection.

THE EVOLUTION TO ARV BASED MICROBICIDES

The early generation non-specific microbicides were a diverse collection of compounds and formulations that shared some fundamental attributes as microbicide candidate products. They were all inexpensive, readily available, potentially broad spectrum, and were shown to be safe and acceptable in early stage trials. However, low potency, short duration of action, challenges to consistent adherence to product use, and in some cases product alteration of epithelial barrier function all contributed to the conclusion that this set of early microbicide compounds was not viable for continued development. Efforts with these early compounds led to initiation of development of ARV based microbicide products, which were far more potent and specific, and likely to be safe based on extensive clinical experience with

systemic use. Fortunately, there have been a number of successes recently reported with ARV based prevention strategies [e.g., 101,102], including the results in the CAPRISA 004 trial with tenofovir gel, which showed a statistically significant protective effect [103]. However ARV based prevention products are not without their own issues. For example, there are concerns regarding the emergence of resistance associated with the use of such products, especially for those already in use in front line HIV therapies. This concern will likely dictate the need for regular testing for HIV status in women using ARVs. Thus, a higher level of health care management will be associated with their use and there is likely a higher hurdle to reach OTC status with these products. There are also concerns regarding toxicity with long term use of these compounds used orally, or after absorption following topical use, consistent with what has been seen in therapy, but less acceptable in this new role of prophylaxis for healthy individuals. These compounds are more expensive than the early generation non-specific compounds, and the individual physical-chemical properties of ARV can limit the available formulation options. Thus, the original justifications for the non-specific microbicide products remain valid today, even in the context of emerging successful ARV based prevention options.

CURRENT STATE OF THE “NON-SPECIFIC” MICROBICIDE PIPELINE

There remains a critical need to identify broad-acting microbicide formulations that (i) are potent against HIV, as well as other STIs, (ii) are safe when used repeatedly and/or intensively (as will occur in real life), (iii) have a long duration of activity to help circumvent adherence issues and provide users with a more flexible dosing regimen, (iv) limit the development and/or transmission of drug-resistant HIV, and (v) are effective both vaginally and rectally to provide greater protection for women and men.

A partial summary of the current non-specific product pipeline is provided in Table 2. This includes formulations that are in clinical or preclinical testing, as well as novel agents that may represent the next APIs advancing for formulation and more extended testing.

Clinical Testing

VivaGel® is the SLP7013 dendrimer-containing gel (3% or 30mg/ml SLP7013) developed by Starpharma, which has activity against HIV and HSV-2 (*in vitro*) [104,105]). Macaque testing demonstrated that the 3% gel protected 5 of 6 macaques (16.7% infection) from vaginal infection when applied 20 min prior to challenge with SHIV89.6P (compared to 100% infection in the controls and 66.7% infection in animals treated with the 1% gel) [106]. Initial clinical testing of VivaGel following penile [107] or vaginal [108] application once a day for 7d suggested that the gel was safe. However, more recent Phase 1 studies of twice-daily vaginal application for 14d revealed evidence of mild irritation after this more extensive repeated vaginal use [109,110]. Notably, Phase 2 testing demonstrated VivaGel is effective in the treatment of bacterial vaginosis (BV), which has been linked to HIV [52]. Phase 3 testing of VivaGel’s efficacy against BV is being planned.

Praneem is a polyherbal tablet being developed in India as a multipurpose prevention technology for the prevention of HIV and pregnancy. Initial *in vitro* studies also suggest that it may have activity against HSV [111]. Phase 1 and 2 testing of Praneem suggests that it is

safe, acceptable for use [112–115], and efficacious in relieving abnormal vaginal discharge (caused by various STIs) [116]. However, two of the Phase 2 studies reported that participants experienced transient genital discomfort (though there were no discontinuations of product use) [114], and revealed a concern with adherence (and, therefore, ultimately efficacy) [115].

Preclinical Testing of Established Formulations

The next most advanced products are the Population Council's zinc acetate-containing gels, which are in the final stages of preclinical testing, with Phase 1 testing slated to commence in 2012. Although hampered by zinc's toxic effects in cell assays, *in vitro* studies to date suggest it has anti-viral and virucidal activity against HIV and other viruses, including HSV-2 [117–125] (and MR, unpublished). It is likely that the immunomodulatory properties of zinc [126–127] also contribute to limiting HIV spread, by rendering the mucosal milieu more resistant to infection and/or impeding virus-driven responses that are critical to the onset of HIV infection [128]. As a result, zinc is expected to provide protection against wild type and drug-resistant HIV, underscoring its potential as a broad-acting API, also potentiating the potent activity of MIV-150 (a non-nucleotide reverse transcriptase inhibitor) when used together. Moreover, zinc has been reported to have activity against other STI pathogens such as *T. vaginalis* [129,130].

Carrageenan was chosen as a safe and acceptable vehicle [90,131–136], with excellent properties to deliver APIs [137]. This formulation was found to be safe and provided mice significant protection against high-dose HSV-2 challenge *via* the vaginal and rectal routes, where carrageenan is ineffective; (Fernández-Romero *et al.*, 2011, in press). Zinc in solution or formulated in HEC was also ineffective. Macaque studies also revealed that zinc acetate-containing gels are extremely effective at preventing immunodeficiency virus infection (Table **3**) [138]. Repeated application of 14 mM (0.3% or 3mg/ml) zinc acetate dihydrate in carrageenan which is effective against HSV-2 in mice, was shown to be safe and provide significant (70%) protection against vaginal SHIV-RT infection. Protection is further enhanced when zinc acetate is combined with only 50 μM MIV-150 (0.00185% or 18.5μg/ml; ~90% protection, compared to the carrageenan vehicle controls). Notably, the protection afforded by these zinc acetate-containing gels lasts for at least 24 h, unlike other preparations tested in macaques that often only provide partial protection for minutes to a few hours, even when using considerably higher doses of drugs [106,139–145].

There is emerging evidence that carrageenan can reduce HPV infection in mice [87,146] and reduces the incidence of HPV infection in women [147]. A Phase 2b study being carried out by researchers at the Albert Einstein School of Medicine in collaboration with the Population Council should provide the first insight as to whether carrageenan is effective against HPV in humans. While clinical testing is necessary to verify the safety and activity of zinc-containing gels, they represent an exciting new generation formulation that could be used repeatedly by women (and potentially men) independent of the time of intercourse (if desired) to protect them against HIV, HSV-2, and possibly other STIs. While multicomponent formulations pose regulatory challenges, both carrageenan and zinc acetate are generally recognized as safe (GRAS), MIV-150 has completed all preclinical toxicology

testing, and the final preclinical testing of the zinc-containing formulations is underway – thus positioning these gels to advance for clinical testing.

To provide an alternative delivery vehicle for the promising zinc acetate/MIV-150 API combination, a vaginal ring loaded with these two API is currently being developed by Population Council researchers. Proof-of-concept macaque studies have confirmed that MIV-150 delivered from a vaginal ring is effective at preventing SHIV-RT infection (Singer *et al.,* in preparation). Preclinical formulation research is also underway to explore the potential of combining the zinc acetate-containing formulations with a contraceptive levonorgestrel (LNG; in gels and/or vaginal rings), similar to the strategies being pursued elsewhere in the field [148].

Next Generation Non-Specific Drugs in Preclinical Testing

Although in much earlier stages of investigation, several promising non-specific API candidates are emerging in the field (most recent references relevant to microbicides research are cited in Table 2). These include a variety of agents derived from natural sources (e.g. praneem, carragaurd combinations), novel synthesized agents, and neutralizing monoclonal antibodies expressed in plants, and used in combinations for expanded spectrum against HIV-1, HSV, and potentially other pathogens). All have activity against HIV and some also against HSV. To date, all of these have demonstrated potent anti-HIV activity *in vitro* and some have even been shown to have activity *in vivo* (macaques or mice), thereby providing the proof-of-concept for advancing their development. However, most of these products have not yet been formulated and need further testing as formulated products (stability, efficacy, safety, etc). It will be critical to determine which will advance further as a promising formulation.

SUMMARY

Despite recent success with highly specific ARV based HIV prevention strategies and products, the original drivers for non-specific microbicide product development remain valid. Products which have broad spectrum activity, are less toxic, have reduced risks associated with the emergence of resistance, and are inexpensive remain important to the HIV prevention field. Although there is significant focus and investment today in ARV based HIV prevention products, there remain a number of non-specific product candidates in development. Hopefully, the development of these new non-specific products can be empowered by the lessons learned from earlier generation non-specific products, and the successes of recent generation ARV based products.

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Table 1

Classes and Mechanism of Action of Non-Specific, Broad Spectrum Microbicide Candidates Evaluated in Phase 3 Efficacy Trials

Product	Agent Class	Mechanism of Action
Nonoxynol-9	Detergent	Virucide/Disrupts viral lipid envelope
C31G (Savvy)	Detergent	Virucide/Disrupts viral lipid envelope
Carraguard	Polyanion	Entry Inhibitor/Blocks virus-target cell interaction
Cellulose Sulfate	Polyanion	Entry Inhibitor/Blocks virus-target cell interaction
PRO 2000	Polyanion	Entry Inhibitor/Blocks virus-target cell interaction
BufferGel	Acidifier	Virucide/Presumed to denature viral proteins

Table 2

Non-Specific Microbicide Candidates Currently in Development*

Product	Mechanism of Action/Targets	Stage of Development	References
VivaGel (SPL7013 dendrimer)	Attachment/Entry Inhibitor; HIV/HSV	Clinical	[104–110]
Praneen (Polyherbal Tablet)	HIV/Pregnancy; Possibly HSV	Clinical	[111–116]
Zinc Acetate (±MIV-150) in Carrageenan Gel	Antiviral/Virucidal/Immunomodulatory; HIV/HSV	Pre-Clinical	[138]
Aptamers	Attachment/Entry Inhibitor; HIV/HSV	Pre-Clinical	[149,150]
Aptamers-siRNA Hybrid	CCR5 knockdown; HIV/HSV	Pre-Clinical	[151]
Sydencan-Fc Hybrid	Attachment/Entry Inhibitor; HIV/HSV	*In vitro*	[152]
Monoclonal Antibodies	Broadly Neutralizing; HIV/HSV	Pre-Clinical	[153–155]

* This is a partial listing of non-specific, broad spectrum microbicide candidates currently in development, and does not include non-ARV, anti-HIV compounds also in development (e.g., [156,157]).

Table 3

Zinc Acetate (ZA)-Containing Gels Provide 24h of Significant Protection Against SHIV-RT Infection

Gel	Time of Virus Challenge After the Last Gel (Infected/Challenged)			Total (Infected/Challenged)	Protection *vs* Carrageenan	P Value *vs* Carrageenan
	4h	8h	24h			
MIV-150/ZA	0/7	0/7	2/14*	2/28	89%	<0.0002
ZA	ND	1/7	3/14*	4/21	70%	<0.012
MIV-150	ND	2/7	4/7	6/14	33%	<0.5
Carrageenan				9/14^		

*Data from animals receiving a daily dosing of gel for 2 weeks and dosing every other day for 4 weeks has been pooled.

^Data from real time controls for each challenge are pooled with data from historical controls.

Depo-Provera-treated Chinese rhesus macaques were challenged with 10^3 $TCID_{50}$ SHIV-RT at the indicated times relative to repeated gel application. This virus dose routinely infects 60–65% of placebo/untreated animals.

ND = not done.

From reference [138].