

# Microbicides for HIV prevention: reality or hope?

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## Purpose of review

This review discusses recent developments within the field of microbicide development and considers whether there are grounds to be hopeful that it will be possible to develop a microbicide for the prevention of HIV infection.

## Recent findings

Phase 2B/3 effectiveness studies of surfactant and polyanion vaginal microbicides have demonstrated modest or no effect against HIV infection and in the case of nonoxynol-9 and cellulose sulfate the potential to increase the risk of HIV acquisition. However, newer antiretroviral microbicide candidates, such as tenofovir, have shown good safety and significant efficacy in animal models and human tissue explant systems and are currently being evaluated in human effectiveness studies. New formulation platforms, such as vaginal rings, are being developed to optimize product acceptability and adherence, and far greater scrutiny of candidate microbicides is happening at both the preclinical and early clinical phase of development.

## Summary

Drug development is an inherently high-risk activity and many promising candidates are discarded due to safety issues or lack of efficacy. Lessons learned over the last two decades have helped to improve the microbicide development pathway and provide hope that it will be possible to develop a safe and effective microbicide for HIV prevention.

## Keywords

drug development, HIV prevention, microbicide

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## Introduction

Microbicides are products that can be applied to vaginal or rectal mucosa with the intent of preventing, or at least significantly reducing, the transmission of sexually transmitted infections including HIV-1. The last decade has seen a movement away from the development of broad-spectrum microbicide products with relatively nonspecific mechanisms of action, such as surfactants, to antiretroviral microbicides that target specific steps in the viral life cycle. Other recent innovations include the development of slow release delivery systems, such as vaginal rings impregnated with antiretroviral drugs, improved preclinical evaluation of candidate microbicides, sophisticated multicompartmental pharmacokinetic characterization of product distribution, and the use of tissue explant systems, to provide preliminary data on product efficacy. Despite these technological advances, fundamental questions remain unanswered. These include defining the criteria to move products from preclinical to clinical studies, the optimal phase 1 evaluation of candidate microbicides, and the use of nonhuman primate (NHP) efficacy studies in the development pathway. The effectiveness phase of drug evaluation

also remains problematic. In the absence of a robust surrogate for HIV infection, phase 2B/3 microbicide studies require thousands of participants from populations with a high annual seroincidence of new HIV infections. The contemporary design of phase 2B/3 studies necessitates inclusion of a comprehensive portfolio of HIV prevention measures including safer sex counseling, diagnosis and treatment of sexually transmitted infections (STIs), provision of male and female condoms, and potentially offering circumcision to male partners. These interventions all lower the risk of acquiring HIV infection and increase the difficulty of demonstrating microbicide efficacy. More recent challenges include the potential risk of resistance associated with the use of antiretroviral microbicides and the provision of study product once studies have been completed.

## Microbicide pipeline

It is estimated that there are approximately 50 candidate microbicides currently in development. A complete listing can be obtained at the Alliance for Microbicide Development website ([www.microbicide.org](http://www.microbicide.org)). It is

unlikely that the majority of these candidates will progress to clinical studies. Many products will fail to demonstrate an adequate preclinical safety/efficacy profile or prove refractory to attempts to formulate the product. Unfortunately, many development teams are simply unable to generate sufficient funds to develop good manufacturing practice (GMP) grade clinical trial material and/or conduct the necessary preclinical toxicology required to undertake subsequent human phase 1 studies.

### New preclinical microbicides

Cyanovirin-N (CV-N) is an 11-kDa protein originally extracted from cyanobacterium (*Nostoc ellipsosporum* [1]) that was found to have antiretroviral properties. CV-N works by binding to HIV envelope glycoproteins and preventing fusion with host cell membranes, and is active in the nanomolar range *in vitro*. The efficacy of CV-N has been demonstrated in SHIV<sub>89.6P</sub> challenge models as well as human cervical explant studies [2,3]. Development of lactobacilli that can produce CV-N [4] and transgenic plants that synthesize CV-N may provide an economically viable way to produce CV-N for microbicide development [5,6\*\*]. Griffithsin (GRFT) is another product that targets HIV envelope glycoproteins. Derived from red algae, GRFT has extremely potent inhibitory activity with an EC<sub>50</sub> of 40 pmol/l, activity in the cervical explant model, and against multiple viral clades. Importantly, GRFT does not appear to induce cell activation or proliferation, a common feature of lectins. Similar to CV-N, GRFT has been manufactured using transgenic plant technology [7\*]. PSC-RANTES is a synthetic CCR5 antagonist. As these molecules target the host coreceptor and not the HIV-1 envelope, it is hoped that they will retain activity against all HIV clades, and *in vitro* studies using peripheral blood mononuclear cell targets confirm this [8]. Concern that manufacturing costs might limit the utility of PSC-RANTES in the developing world has led to the production of two PSC-RANTES derivatives (5P12-RANTES and 6P4-RANTES) that appear stable, lack the ability to induce cell proliferation [9\*], and are active in the macaque challenge model [10].

### Microbicides in clinical development

As can be seen from Table 1, the vast majority of microbicides in clinical development are reverse transcriptase (RT) inhibitors. RT inhibitors, whether delivered topically or orally, work by delivering sufficient mucosal concentrations to abort nascent viral infection. In the case of UC781, it has also been suggested that the drug can also act as a 'tight binder', which might allow viral inactivation in the lumen prior to infection. VivaGel is one of the two non-RT inhibitors currently in clinical development and is a lysine dendrimer that has shown HIV activity in preclinical studies [11,12] including a macaque challenge study [13].

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### Microbicide formulation

Vaginal rings have been used to deliver contraception and estrogen replacement therapy and are being developed to provide slow release of intravaginal RT inhibitors such as TMC-102 [14\*]. Vaginal rings that could be left *in situ* for weeks or months would hopefully increase patient adherence. Jay *et al.* [15] have described a pH-sensitive hydrogel that undergoes reversible conformational change at varying pH. In an elegant experiment they demonstrated significant retardation in the movement of HIV or nanoparticles in a gel as the pH moved from 4.3 to 4.8.

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### Preclinical development of microbicides

Clinical development of candidate microbicides is expensive and time consuming, and it is critical that the products moving from the preclinical to clinical phase of evaluation are safe, effective, and economically viable. Unfortunately, the current preclinical process is imperfect, and efforts are underway to develop new safety biomarkers and efficacy models [16]. It is also now recognized that product evaluation should include exposing candidate microbicides to relevant biological matrices (such as semen or cervicovaginal fluid), physiologically relevant pH, and the types of bacterial flora found in the vaginal or rectal compartment, as all these parameters have the potential to significantly reduce product efficacy [17]. Mesquita *et al.* [18\*\*] have described an enhanced approach to the preclinical evaluation of candidate microbicides. The components include a dual chamber transwell model to evaluate microbicide induced epithelial toxicity combined with a broad range of cell-based assays.

The development of humanized murine models has been used to conduct vaginal and rectal HIV efficacy challenge studies [19\*\*]. These models recapitulate HIV pathogenesis [20] and may provide greater flexibility in characterizing dose response of microbicide candidates. Such studies, due to the large sample sizes needed, would be prohibitively expensive in the NHP model.

The NHP has been used extensively to study the safety and efficacy of vaginal and rectal microbicides [21,22]. The rhesus macaque (*Macaca mulatta*) is the most widely used NHP model, but studies have also been conducted with pigtailed and cynomolgus macaques. The majority of studies have used a large viral challenge to ensure uniform infection rates in the placebo arm of microbicide viral challenge experiments [10]. This single high-dose model may also include the use of progestins to synchronize menstrual cycles and to thin the vaginal epithelium to increase the efficiency of infection further. Clearly this approach, although valuable to screen microbicide candidates, does not recapitulate human transmission or acquisition of HIV infection. The recent development of

**Table 1 Current clinical microbicide trials**

Phase	Drug(s)	Route	Study title	N	Countries	Study design
2B	Tenofovir gel, oral tenofovir, Truvada	V/O	MTN-003 (VOICE)	5000	Malawi, South Africa, Zimbabwe, Zambia, Uganda	Safety and effectiveness study with daily dosing of study product
2B	Tenofovir gel	V	CAPRISA-004	980	South Africa	Safety and effectiveness study: participants will be asked to apply study product within 12 h prior to coitus and within 12 h after coitus.
2	Tenofovir gel, oral tenofovir	V/O	MTN-001	144	South Africa, USA, Uganda	Safety, acceptability, and pharmacokinetic study (blood, cervicovaginal, and tissue levels)
1	TMC-120 gel (4759 and 4789)	V	IPM020	180	USA	Safety, acceptability, and pharmacokinetic study (blood, cervicovaginal, and tissue levels)
1	TMC-120 gel (4759)	V	IPM014A	320	Kenya, Malawi, Rwanda, South Africa, Tanzania	Safety and acceptability study with evaluation of daily monitored adherence
1	TMC-120 gel (4789)	V	IPM014B	320	Kenya, Malawi, Rwanda, South Africa, Tanzania	Safety and acceptability study with evaluation of daily monitored adherence
1	TMC-120 ring	V	IPM024	16	Belgium	Safety, acceptability, and pharmacokinetic study (blood and cervicovaginal levels)
1	VivaGel	V	MTN-004	61	USA	Safety and acceptability study
1	Tenofovir gel	V	MTN-002	16	USA	Single-dose pharmacokinetics study in healthy term gravidas with collection of blood, placental, cord, and cervicovaginal levels
1	Tenofovir gel	V	NIAID/DAIDS/AECOM	24	USA	Changes in cytokines, chemokines, and other mediators of innate immunity through examination of cervicovaginal secretions
1	Tenofovir gel, oral tenofovir	R/O	RMP-02/MTN-006	18	USA	Placebo-controlled rectal microbicide safety, acceptability, and pharmacokinetic study including explant challenge studies
1	Tenofovir gel	R	MTN-007	60	USA	Placebo-controlled rectal safety and acceptability study including N-9 comparator arm
1	Acidform gel	V	AF 020, AECOM	36	USA	Safety and acceptability study

AECOM, Albert Einstein College of Medicine; IPM, International Partnership for Microbicides; MTN, Microbicide Trials Network; O, Oral; R, Rectal; V, Vaginal.

a low-dose repeat challenge macaque model with human/simian immunodeficiency virus (SHIV) may provide a more biologically relevant model and has been used with success to screen a range of RT inhibitors including tenofovir gel [23,24\*\*].

It has been suggested that incorporation of a macaque challenge model as a gatekeeper to regulate transition of products from preclinical to clinical development would increase the likelihood of success in phase 2B/3 efficacy studies [25\*,21]. Others have argued that the macaque model is too variable, unvalidated, and uses simian immunodeficiency virus (SIV) or a pathogenic chimeric SHIV rather than HIV. However, Keele *et al.* [26] have demonstrated that rectal inoculation of rhesus macaques with SIV<sub>smE660</sub> or SIV<sub>mac251</sub> recapitulates human mucosal HIV infection, and the majority of candidates currently in clinical development have been evaluated in the macaque model.

The human explant challenge model provides a less contentious and important bridge between the preclinical and clinical phase of microbicide evaluation. Tissue explants (cervical, rectal, foreskin, or tonsil) collected during elective surgery or, in the case of rectal explants, via endoscopy are exposed to combinations of drug and HIV *in vitro* to determine whether the candidate microbicide has activity in the relevant tissue [27,28,29\*,30]. Explant production of HIV-1 p24 is monitored over 2–3 weeks for evidence of viral inhibition. Elliott *et al.* [31\*\*] have incorporated this approach into the phase 1 evaluation of UC781. A recent multisite study demonstrated that different laboratories can provide consistent measurements of anti-HIV efficacy in the explant model when standardized endpoints are used; drugs, reagents, and virus are centrally sourced; and similar explant tissue techniques are employed [29\*].

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### Clinical development of microbicides

Phase 1/2 microbicide studies are used to generate pharmacokinetic and clinical safety data, and as mentioned above may provide preliminary efficacy data. In the absence of a specific safety biomarker, phase 1/2 studies try to use clinical symptoms and signs to identify harm. Unfortunately, in the case of N-9 and cellulose sulfate, this was inadequate and the design of these studies has been expanded to include biomarkers such as cytokines [16]. It has also been argued that the size and duration of current phase 1/2 studies may be inadequate to even identify conventional clinical safety signals [32].

The success or failure of a microbicide is likely to be determined by the complex interaction between product pharmacokinetics, viral kinetics, and possible product induced toxicity [33]. With regard to antiretroviral drugs,

there is considerable variability in genital tract concentration following oral administration [34]. As one example, the cervicovaginal fluid concentration of the CCR5 antagonist maraviroc is almost two-fold higher than the blood plasma level [35]. These data emphasize the importance of developing compartmental pharmacokinetic profiles for microbicide candidates that encompass plasma and tissue levels. These assays are technically demanding but are beginning to be included in phase 1 studies.

To date, phase 2B/3 efficacy studies have been conducted on six microbicides (N-9, C31G, Carraguard, cellulose sulfate, BufferGel, and PRO-2000) without evidence of a significant reduction in HIV incidence [36–39,40\*\*,41] (S.S. Karim, unpublished data). Indeed, the use of N-9 and cellulose sulfate may have increased the risk of HIV acquisition. These very public failures have encouraged some to question the direction of microbicide research [25\*]. It is clear that we need to improve the preclinical and phase 1/2 evaluation of candidate microbicides to prevent unsafe products moving into phase 2B/3 evaluation. In addition, it will be important to determine whether the four current RT microbicides (tenofovir, UC781, TMC-120, and MIV-150) have sufficiently different profiles to warrant moving them all into phase 2B/3 evaluation.

It is likely that an HIV infected individual repeatedly exposed to an antiretroviral microbicide will develop resistance to that product and so use of this class of microbicide will have to be linked to prospective voluntary counseling and HIV testing. The virological and clinical sequelae of an individual becoming infected while using an antiretroviral microbicide are far less clear as are the ramifications at a community level. Data on seroconverters are almost nonexistent but are being collected in ongoing oral and topical antiretroviral HIV prevention trials. Combination therapy is routinely used in the management of HIV infection to prevent treatment failure and the development of antiviral resistance. Combinations of antiretroviral agents have been evaluated in the macaque model [42,23] and colorectal explants [43]. Dual and triple combinations appear to be more potent than single agents but it is unclear if combinations would reduce the development of resistance if individuals became infected while using multiple agents. Conversely, Parikh *et al.* [24\*\*] have shown that tenofovir gel alone protected macaques from 20 exposures to SHIV<sub>SF162p3</sub>.

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### Rectal microbicide development

To date, the primary focus of microbicide research has been the development of a safe and effective vaginal microbicide. Although this should remain a key scientific priority, emerging epidemiological data provide a rationale

for a parallel programme to develop rectal microbicides. Since the beginning of the HIV pandemic, men who have sex with men (MSM) have been the main focus of HIV infection in the developed world. Unprotected receptive anal intercourse (URAI) is the primary risk factor for HIV acquisition in MSM. The unique vulnerability of the intestinal mucosa to HIV transmission results in a per act exposure risk approximately 20-fold greater than unprotected vaginal intercourse. Increasingly, it is apparent that women in both the developed and developing world practice URAI [44,45]. Although the absolute frequency of URAI in women may be low, the increased risk per act is such that URAI may play an important role in propagating HIV infection in women as well as MSM. Another recent important development is the recognition of sexually active MSM in sub-Saharan Africa [46\*\*]. These men have a high prevalence of HIV infection, often have male and female partners, and may play an important bridging role in disseminating HIV infection. Even with these limited epidemiological data, there is clearly a need for both rectal and vaginal microbicides and even better a product that is safe and effective in both compartments.

In contrast to vaginal microbicide development, the field of rectal microbicide development is relatively new. In some respects this had been advantageous because the field has had the opportunity to incorporate lessons learned from vaginal microbicide development into the preclinical and clinical development of rectal microbicides [47]. Recent phase 1 rectal microbicide studies have incorporated detailed assessment of mucosal injury [48,49], product distribution [50], and acceptability [51].

### Integration of microbicides into the HIV prevention 'tool box'

The HIV prevention research portfolio incorporates multiple types of intervention including behavioral modification, voluntary counseling and HIV testing, circumcision, diagnosis and treatment of STIs, vaccines, oral pre and postexposure prophylaxis, treatment of serodiscordant partners, broader 'test and treat' strategies, and microbicides. These various interventions do not exist in isolation and there is a growing interest in integrating multiple modalities into the design of HIV prevention trials [52]. This reality presents opportunities and challenges for both research and implementation and should not be ignored. In the context of microbicides, there is a need to evaluate the differential safety and efficacy of oral versus topical administration of antiretrovirals for HIV prevention and to explore whether certain high-risk populations, such as MSM, might benefit from using both routes of administration. As one example, the VOICE study (Vaginal and Oral Interventions to Control the Epidemic) being conducted by the NIH sponsored Microbicide Trials Network ([www.mtnstopsHIV.org](http://www.mtnstopsHIV.org))

will evaluate tenofovir gel, tenofovir, and Truvada in approximately 5000 women in Sub-Saharan Africa.

### Conclusion

There is now breadth and depth to the microbicide development pipeline. More sophisticated preclinical and phase 1/2 evaluation will hopefully prevent the movement of inferior products into efficacy studies. New formulation platforms offer the possibility of enhanced acceptability and adherence. Potent antiretroviral microbicides are being evaluated in effectiveness trials and it is hoped that these studies will finally demonstrate significant efficacy against HIV-1. Rectal microbicide development has been recognized as a priority within the biomedical prevention research agenda and multiple phase 1 studies are ongoing or planned. Microbicides are not yet a reality but there is certainly hope that they will be in the near future.

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